Breeding for High Leaf Yield and Minerals Content in *Gynandropsis gynandra* (L.) Briq.

by

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THESIS ABSTRACT

Gynandropsis gynandra (Spider plant) is an African leafy vegetable rich in minerals, vitamins, and health-promoting compounds with great potential in addressing malnutrition. The species is used as food and medicine and provides substantial incomes for smallholder's farmers with an increasing interest for its cultivation in Africa. Spider plant is also an important resource for pharmaceutical industries. However, its production is still hampered by low leaf yield, early flowering, pests and disease and poor seed germination, resulting from the lack of improved cultivars. Our study intended to develop high yielding and nutrient-dense cultivars for farmers through merging modern molecular and classical plant breeding tools to increase income generation and improve nutrition and health. Specifically, the study: i) assessed the phenotypic variability among advanced lines of spider plant using biomass and related traits; ii) profiled the leaf mineral content among advanced lines of *G. gynandra*; iii) determined the combining ability, gene action and heterosis of mineral content in spider plant; iv) identified the genetics of the inheritance of biomass and related traits in spider plant; and v) deciphered genomic regions associated with combining ability and heterosis of biomass and related traits in *G. gynandra*.

The evaluation of 71 advanced lines of spider plant derived from accessions originating from Asia, East, Southern and West Africa using biomass and related traits revealed significant difference among lines and principal component analysis grouped them into three clusters: Asia (Cluster 1), West Africa (Cluster 2), and East/Southern Africa (Cluster 3). The West and East/Southern African groups were comparable in biomass productivity and superior to the Asian group. Specifically, the West African group had high dry matter content and flowered early while the East/Southern African group was characterized by broad leaves and late flowering. The maintenance of lines' membership to their group of origin strengthens the hypothesis of geographical signature in cleome diversity as genetic driver of the observed variation.

The leaf mineral profile of 70 advanced lines of spider plant derived from accessions originating from Asia, East, Southern and West Africa revealed significant variation among lines and zinc, calcium, phosphorus, copper, magnesium, and manganese as landmark elements in the genotypes. East and Southern African genotypes were clustered together in group 1 with higher phosphorus, copper and zinc contents than Asian and West African lines, which clustered in group 2 and were characterized by higher calcium, magnesium and manganese

contents. An additional outstanding group 3 of six genotypes (three, two, and one from Asia, Southern Africa and Eastern Africa, respectively) was identified with high iron, zinc, magnesium, manganese and calcium contents and potential candidates for cultivar release.

Significant differences ($P < 0.001$) were observed among and between experimental hybrids and parents for the levels of all mineral contents. Significant general and specific combining ability effects together with variance components analysis revealed that both additive and nonadditive gene action controlled mineral content with a predominance of nonadditive gene action. Mid- and best-parent heterosis ranged from -84.98 and 404.79% for minerals. Parents with good general combining ability were identified, as well as crosses with high specific combining ability and heterosis. There were significant and moderate to strong correlations between mean hybrid performance, specific combining ability effects and heterosis levels and low to moderate correlations between general combining ability and mean parents' performance.

Similar to leaf mineral content, significant differences ($P < 0.001$) were observed among and between hybrids and parents for fourteen agronomic traits. Hybrids outperformed their parents with more than 50% for total and edible fresh biomass, showing the existence of hybrid vigour. Mid- and best-parent heterosis varied between -51.89% and 192.10% with only positive heterosis effects for leaf area and total fresh biomass, characterized by an average mid-parent heterosis greater than 50%. Significant general and specific combining ability (GCA and SCA) effects together with variance component analysis revealed that both additive and nonadditive gene action, controlled biomass and related traits in the species with the predominance of additive gene action. Moderate to high broad- and narrow-sense heritability was observed for most agronomic traits, except for dry matter content. The environment significantly interacted with genotype, GCA and SCA. Parents with good GCA and crosses with high SCA and heterosis were identified. There were significant changes from parents to hybrids in the association of harvest index and time to 50% flowering with biomass per plant and leaf traits on the one hand and between harvest index and dry matter content on the other hand.

A core set of 594 diversity array technology sequencing (DArt-seq) markers were identified and differentiated the 38 parental lines into three clusters linked with the provenance of the original accession. Using this set of markers, a genome-wide association analysis revealed two markers linked to heterosis level for flowering time, a single marker for edible biomass, one marker for total fresh biomass and one marker for the number of primary branches.

Specifically, the marker MABiomLa1 was a pleiotropic marker and was associated with heterosis level for biomass and leaf area. In contrast, no consistent markers associated with combining ability were observed for general combining ability and might be due to the low number of parents and the density of markers used.

The study thus revealed that reciprocal recurrent selection would be a sound breeding strategy for *G. gynandra* improvement with the development of hybrid cultivars to exploit heterosis. These findings showed that *G. gynandra* could be used as a model plant to study the genetic mechanism underlying heterosis in orphan leafy vegetables. The identified markers open room for implementing marker-assisted selection in the species for better exploitation of heterosis.

DECLARATION: PLAGIARISM

I, Aristide Carlos Houdegbe, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

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a) their words have been re-written but the general information attributed to them has been referenced;

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(v) this dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

Signed  .....

Aristide Carlos Houdegbe

As the candidate's supervisors we agree to the submission of this thesis

.....  .....

Prof Julia Sibiya (Supervisor)

.....  .....

Prof Enoch G. Achigan-Dako (Co-Supervisor)

DECLARATION: PUBLICATIONS

Publications pertaining to this thesis, include:

Aristide Carlos Houdegbe; Enoch G. Achigan-Dako; E. O. Dêêdi Sogbohossou; M. Eric Schranz; Alfred O. Odindo; Julia Sibiya. Phenotypic variation in biomass and related traits among four generations advanced lines of Cleome (*Gynandropsis gynandra* L. (Briq.)): Accepted in PLOS One: PONE-D-22-01944R1 - [EMID:657e3924fcfd2262] (Chapter 3)

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DEDICATION

To

The Almighty Father for your glory.

My late mother Marie Assou for all your sacrifices for me and your children. You could not see the end of the journey, but you started another one. May you be proud of this wherever you are. May your soul rest in peace!

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CHAPTER 1
General Introduction

1.1 Background

In sub-Saharan Africa, approximately 264.2 million people were undernourished in 2020 (FAO et al. 2021) and this affected mainly women and children (Black et al. 2013). The rate of undernourished people is especially increasing in Sub-Saharan Africa, with a 20.27% increase from 2019 to 2020 (FAO et al. 2021). This situation of undernourished people is responsible for many acute and chronic diseases and will thus, affect the workforce of future generations. Better nutrition is, therefore, indispensable to the economic productivity of individuals and societies (Black et al. 2013). This situation is also a result of food habit changes with low diet diversification. Overcoming this big challenge requires an integrated approach that merges agriculture, education, society, politics, and economy (Shrimpton and Rokx 2012; Bain et al. 2013). Most of the people suffering from malnutrition are in rural areas and depend on agriculture for their subsistence. In addition, agriculture is the main source of food for humans. Improving agriculture through its supply of micronutrients will significantly enhance the diet of the population and reduce malnutrition if adequately managed. Currently, many options are available and include increasing the nutrient content of staple crops (biofortification) and crop diversification with diet diversification (Dawson et al. 2018).

Crop diversification implies the cultivation and consumption of diverse nutrient-rich crops and appears to be a cost-effective method to overcome malnutrition (Smith 2013). This leads to dietary diversity and the intake of specific nutrient types resulting from the increase in species diversity in the farming systems (Pandey et al. 2016). This calls for the valorisation of orphan crops for better nutrition (Dawson et al. 2018; Padulosi et al. 2013). Orphan crops, also called neglected and underutilized crops, have a great potential to provide substantial and quality nutrients to human diets (Padulosi et al. 2013; Heywood 2013). They contain several micronutrients deficient in the main staple crops in terms of quality and quantity. Moreover, many of them are indigenous to the regions where they are found and are therefore important for climate change adaptation (Heywood 2013; Padulosi et al. 2013). Orphan crops include traditional fruit tree species, legumes, cereals, roots, nuts, vegetables and many African leafy vegetables.

African leafy vegetables are important sources of vitamins and minerals (Schönfeldt and Pretorius 2011; Smith and Eyzaguirre 2007; van Jaarsveld et al. 2014). They have a high potential to efficiently reduce hidden hunger and malnutrition compared to major staple crops (Yang and Keding 2009). Adapted to local agro-ecological conditions, they are a prime source of income mainly for rural populations and have been viewed for a long time as the poor man's food (Padulosi et al., 2013). This perception is quickly changing, and there is an increasing interest in African leafy vegetables as they have the ability to grow in low fertility soils, have high relative drought tolerance and faster growth rates; hence, they can be harvested within a short period of time (van Jaarsveld et al. 2014). African leafy vegetables are diverse, and more than 280 species have been recorded in sub-Saharan Africa (Grubben and Denton 2004). Those that are widely represented in sub-Saharan Africa include *Amaranthus* spp. (Amaranth), *Gynandropsis gynandra* (L.) Briq (spider plant), *Solanum* spp (African vegetable nightshades), *Vigna unguiculata* (L.) Walp (cowpea), *Corchorus olitorius* L. (jute mallow), *Moringa oleifera* L. (moringa), *Solanum macrocarpon* L. (African eggplant), *Brassica carinata* A. Braun (Ethiopian kale), *Celosia argentea* L. (Celosia), *Crassocephalum rubens* (Juss. ex Jacq.) S. Moore, *Talinum* spp., *Vernonia* spp, *Bidens pilosa* L. (blackjack), *Ocimum* spp. (Grubben and Denton 2004; Smith and Eyzaguirre 2007; Maundu et al. 2009; Nono-Womdim et al. 2012; Weinberger and Pichop 2009; Grubben et al. 2014).

Gynandropsis gynandra (L.) Briq. (Syn. *Cleome gynandra* L.) is one of the most promising African leafy vegetables due to its wide range of utilisation and values. The species has been for a long time restricted or mostly valorised in Eastern and Southern Africa, where its weed status was quickly converted as cultivated species (Maundu et al. 1999; Schippers 2004). The species thrives in tropical and subtropical regions in Africa, South America, Southeast Asia and Oceania, where it grows abundantly during the rainy season (Sogbohossou 2019; Feodorova et al. 2010; Vandebroek and Voeks 2018). It is found near human settlements or roadsides in wild populations but is also cultivated in home gardens or in urban and peri-urban agriculture (Achigan-Dako et al. 2010; Chweya and Mnzava 1997; Kiebre et al. 2015b; Weinberger and Pichop 2009). The potential of the species to effectively contribute to overcoming hidden hunger and malnutrition is due to its nutrient content. The leaves of the species contain high amounts of minerals, including potassium, calcium, magnesium, phosphorus, iron, manganese, sodium and zinc (Moyo et al. 2018; Omondi et al. 2017b; van Jaarsveld et al. 2014; Koua et al. 2015; Schönfeldt and Pretorius 2011; Moyo and Aremu 2021). *Gynandropsis gynandra* is also a rich source of vitamins C, A, E, B1, B2, B9 and β-carotene

(Neugart et al. 2017; Moyo et al. 2018; Schönfeldt and Pretorius 2011; van Jaarsveld et al. 2014; Sogbohossou et al. 2019). The leaves are also an important source of proteins and fatty acids (Glew et al. 2009; Yuan et al. 2021), including essential amino acids (histidine, isoleusine, leucine, lysine, methionine, phenylalanine, threonine, valine) (Glew et al. 2009). Maundu et al. (1999) and Omondi et al. (2017b) reported significant nutritional differences among morphotypes of *Gynandropsis gynandra*.

Gynandropsis gynandra is an annual herb that belongs to the Cleomaceae family. The species is used in food and medicine mainly by rural populations and has recently been introduced in some urban and peri-urban areas of Africa. Leaves constitute the main part of the species that are used as vegetables, but young shoots and flowers have also been utilised (Dickson 2007; Chweya and Mnzava 1997; Maroyi 2013; Mnzava and Chigumira 2004). Two main forms of consumption were distinguished by local communities; mainly the leaves are directly cooked or dried. In addition, the species is used in the treatment of more than 50 diseases (Maundu et al. 1999; Sogbohossou et al. 2018b; Onyango et al. 2013b; Weinberger and Msuya 2004). The most common are malaria, stomach-ache, headache, diarrhoea, toothache, microbial diseases, earache, oral problems and treatment of worms, anaemia, fever, and haemorrhoids. All these applications are a result of the concentration of phytochemicals and health-promoting compounds in leaves and inflorescence (Moyo et al. 2018; Neugart et al. 2017; Omondi et al. 2017b).

The numerous secondary metabolites found in the species include flavonoids, terpenoids, tannins, glucosinolates, aldehydes, ketones, sesquiterpenes and many other phenolic compounds (Sogbohossou et al. 2020; Omondi et al. 2017b; Neugart et al. 2017; Moyo et al. 2018; Moyo and Aremu 2021; Chataika et al. 2021) with diverse pharmaceutical applications (plant extracts, drugs, etc.) (Achigan-Dako et al. 2021). The species is therefore a prime resource for the pharmaceutical industry, as its extracts have several biological and pharmacological effects (Chand et al. 2022; Achigan-Dako et al. 2021; Moyo and Aremu 2021; Singh et al. 2018), including antimicrobial (fungi and bacteria), anthelmintic (Ajaiyeoba et al. 2001), antimalarial (Igoli et al. 2016), hepatoprotective (Narsimhulu et al. 2019), antiarthritic (Narendhirakannan et al. 2005), antioxidant, anti-inflammatory (Chandradevan et al. 2020), immunomodulatory (Kori et al. 2009), antinociceptive (Ghogare et al. 2009), anticancer (Bala et al. 2010), antidiabetic (Ravichandra et al. 2014) and vasodilatory (Runnie et al. 2004) activities. Promoting this vegetable will, therefore, contribute to fighting malnutrition, health promotion and income generation for stakeholders, including pharmaceutical companies and

local communities. Seeds have been reported to have anthelmintic properties, and oil is used as a fish poison. Stems are used as an analgesic and anti-inflammatory agent (Gupta and Chakravarty 1957). The species also has several cultural uses, particularly during wedding ceremonies and lobola negotiations and other forms of religious rituals, such as naming of a child and funerals as well as in welcoming an important visitor (Maundu et al. 1999; Onyango et al. 2013b).

Gynandropsis gynandra is harvested in the wild in some communities, while in others, the species is cultivated and sold in local markets. In Kenya, *G. gynandra* is sold in open air markets, supermarkets and green grocer stores (Onyango et al. 2013a). The selling price varies from US$ 0.41 (Weinberger and Pichop 2009) to US$ 0.5 (Onyango et al. 2013a) per kg in Kenya during the rainy season and increases by almost double during the dry season when vegetables become rare (Onyango et al. 2013a). The average profit margin was estimated at 30 to 40% of the selling price (Weinberger and Pichop 2009). Moreover, *G. gynandra* contributed to 15-40% of the total income of the farmers that produce it for sale. The low supply of vegetables and their limited shelf life are among the main constraints encountered by vegetable sellers. The longest storage period was two days in the outlets. Postharvest losses due to wilting, rotting and lesions during harvesting and transportation contribute to the reduction in the profitability of the vegetable (Onyango et al. 2013a). In Burkina-Faso, the species is used as a famine, flood or dry food (Kiebre et al. 2015b).

Extending the production and exploiting the potential of the species requires the development of a value chain that includes the selection of desired varieties for farmers. A better understanding of the constraints encountered by farmers involved in its cultivation is crucial for the establishment of a good breeding programme. These constraints include seed quality, particularly low seed germination, low leaf yield, early flowering, pests and diseases (Kwarteng et al. 2018; Onyango et al. 2013b; Sogbohossou et al. 2018a). Sogbohossou et al. (2018a) have established a breeding pipeline to address these constraints.

1.2 Rationale of the study

Early flowering, low leaf or biomass yield, poor germination, small leaves, insect pests and seed availability are important constraints hindering the cultivation of *G. gynandra* (Onyango et al. 2013a; Sogbohossou et al. 2018a; Matro 2015; Abukutsa-Onyango 2007). Flowering time and biomass yield are linked because early flowering limits vegetative growth and therefore

severely decreases yield (Schiessl et al. 2017). To overcome these constraints, previous studies in *G. gynandra* have focused on evaluating the effect of agronomic practices such as shoot and flower removal, fertilization, and harvest techniques on the extent of its vegetative period (Wangolo et al. 2015; Mavengahama 2013; Seeiso and Materechera 2012; Masinde and Agong 2011; Mutua et al. 2015; Houdegbe et al. 2018). However, these developed practices could become difficult and time-consuming in practice when a large area of the crop is planted. Thus, an alternative and efficient approach is to screen the genetic diversity in the species and to select for late flowering and high biomass yielding genotypes that would benefit the farmers.

Assessment of genetic diversity revealed significant and origin-driven variation in *Gynandropsis gynandra* for plant morphology (Wu et al. 2018; Sogbohossou et al. 2019), secondary metabolite concentrations (Sogbohossou et al. 2020), seed germination, mineral composition and morphology (Blalogoe et al. 2020), leaf vitamin contents (Sogbohossou et al. 2019), antioxidant activity (Chataika et al. 2021), and photosynthesis traits (Reeves et al. 2018). Morphological traits with significant variation were related to plant architecture (plant height, number of primary branches, plant habit, stem hairiness and colour), leaf size (leaf area, leaflet length and width, petiole length, leaflet shape), leaf colour, time to 50% flowering, germination (percentage and mean time), pod characteristics (pod length and width, number of seeds per pod), seed size (length, width, perimeter, area), 1000-seed weight, flower traits (androphore length, filament length, pedicel length, gynophore length), and biomass (total shoot fresh and dry weight, leaf fresh and dry weight) (Sogbohossou et al. 2019; Wu et al. 2018; Omondi et al. 2017b; Blalogoe et al. 2020). In addition, phenotypic differentiation among diverse accessions of *G. gynandra* was found to be associated with the genetic makeup of the genotypes (Sogbohossou 2019; Omondi et al. 2017a). While Omondi et al. (2017a) differentiated advanced lines and genebanks' accessions from farmer cultivars using simple sequence repeats (SSR) markers, Sogbohossou (2019) observed genomic differentiation among accessions from West Africa, East/Southern Africa and Asia. This important diversity represents an important resource for a successful breeding program.

However, most studies assessing morphological diversity in *G. gynandra* did not include leaf biomass yield, although leaf biomass yield is the most important trait for farmers and breeding programs. Those that included it were limited to regional accessions and advanced lines (Omondi et al. 2017b) and countrywide accessions (Kiebre et al. 2017a; Mosenda et al. 2020). Therefore, assessing the biomass yield potential of large and worldwide collections is required to select elite genotypes for breeding programs.

Developing high-yielding cultivars requires knowledge of the inheritance of target traits, which is still lacking in *G. gynandra* (Sogbohossou et al. 2018a). Consequently, using mating designs such as diallel and North Carolina design will help to understand the gene action controlling farmers' preferred traits such as biomass yield and related traits (plant height, number of primary branches, stem diameter), leaf area, days to flowering and dry matter content. Additionally, determining the combining ability of selected genotypes to identify elite ones that have the ability to transfer their desirable traits to their progenies is key in speeding up the breeding process. To date, there are no reports on the combining ability potential of *G. gynandra* breeding lines. Another important genetic parameter is heritability, which includes broad- and narrow-sense heritability. Only broad-sense heritability was reported for the species (Kiebre et al. 2017b; Kangai Munene et al. 2018; Omondi 1989; Sogbohossou 2019) and this was based on the natural and F2 biparental populations. Thus, it is important to determine narrow sense heritability of farmers' desired traits, as this is the component that is passed from parent to offspring and plays an important role in breeding. Overall information on quantitative genetic parameters in *G. gynandra* is needed not only for agronomic traits but also for nutritional traits such as minerals and secondary metabolites to support the development of high-yielding and nutrient-dense cultivars.

Moreover, combining farmer-preferred agronomic and nutritional traits in one elite cultivar is a great challenge in the species breeding program due to their quantitative nature and sometimes negative correlations combined with low heritability (Kiebre et al. 2015a; Omondi 1989; Wasonga 2014; Wenyika et al. 2015). There is a need, therefore, to implement efficient and cost-effective methods that use the available tools through molecular breeding. Molecular breeding methods include marker-assisted selection (MAS) and genomic selection (GS). The implementation of the MAS program requires the identification of molecular markers or quantitative trait loci (QTL) linked with the traits of interest (Varshney et al. 2014). For that purpose, association mapping (AM) based on phenotypic and genotypic data is crucial and achievable through genome-wide association studies (GWAS) (Varshney et al. 2014; Huang and Han 2014).

The GWAS is an efficient method to dissect several complex traits and has been implemented in several crops, including rice (Mogga et al. 2018), maize (Yang et al. 2014; Xiao et al. 2017), sorghum (Boyles et al. 2016) and other crops (Liu et al. 2017). It has become the most popular approach to identify genes controlling any trait and has been facilitated by the availability of the reference genomes (Xiao et al. 2017). The method has the advantage of covering the entire

genome of the species and, hence, includes genes with minor effects that are mostly difficult to cover in biparental QTL mapping (Varshney et al. 2014; Burghardt et al. 2017). To date, no GWAS have been reported in *G. gynandra* and this is needed to accelerate cultivar development.

Based on the abovementioned findings and gaps, this study will contribute to the knowledge of the genetics of the inheritance of biomass and related traits and mineral content in the species. The knowledge generated will guide the breeding program on the type of cultivars to be developed for the species and the efficient breeding strategies that can be used to accelerate high-yield and nutrient-dense cultivar development.

1.3 Research questions

1.3.1 Central question

The study sought to answer the following broad question:

How can modern molecular and classical breeding tools be used to accelerate cultivar development for African orphan leafy vegetables for wider cultivation and adoption in the context of malnutrition and climate change?

1.3.2 Research questions

The following research questions were formulated:

(i) What is the phenotypic diversity associated with biomass and related traits in *Gynandropsis gynandra*?
(ii) What is the genetic variability associated with mineral content in *G. gynandra*?
(iii) What is the genetic mechanism controlling the inheritance of element composition in *G. gynandra*?
(iv) What are the quantitative genetic parameters and the level of heterosis associated with biomass and related traits in *G. gynandra*?
(v) What are the genomic regions controlling the heterosis and combining ability of biomass and related traits in *Gynandropsis gynandra*?

1.4 Objectives

1.4.1 Breeding objective/overall objective

The overall objective of the study was to select high biomass yielding and mineral-dense cultivars of spider plant (*G. gynandra*) desired by African smallholder farmers to improve their livelihoods and for better nutrition.

1.4.2 Specific objectives

Specifically, the present study aimed to:

(i) assess the phenotypic variability in biomass and related traits in a collection of *G. gynandra* advanced lines to select elite genotypes for improved cultivar development;
(ii) profile the leaf elemental composition of *G. gynandra* and depict any potential geographical signature using a collection of 70 advanced lines derived from accessions originating from Asia and Eastern, Southern and West Africa;
(iii) investigate the mineral profile of experimental hybrids of spider plant and determine the level of heterosis and combining ability effects and gene action governing the inheritance of mineral content to select high-yielding and nutritious hybrids for cultivar development and release in *G. gynandra*;
(iv) to investigate the genetics of the inheritance of biomass and related traits in *G. gynandra* to inform breeding programs on the type of cultivars to be developed; and
(v) dissect genomic regions associated with the level of heterosis and combining the ability effects of biomass and related traits in *G. gynandra* to contribute to uncovering the genetic mechanism controlling heterosis in plants using a genome-wide association study.

1.5 Thesis structure

This thesis is composed of five research chapters in accordance with the above-mentioned objectives, one literature review chapter and the introductory and general discussion chapters. The thesis is in the form of discrete research chapters, each following the format of a stand-alone research paper (whether or not the chapter has already been published or is under review or revision). Due to their interdependence, there are some unavoidable overlaps and repetitions of introduction, discussions and references sections between chapters.

Chapter 1 presents the context/background, rationale, research questions, the objectives underlying the present study, and the structure of the thesis. It highlights the need of breeding high-yielding and nutrient-dense cultivars for spider plant for better nutrition. **Chapter 2**, as a literature review, summarizes the current knowledge on spider plant with focus on farmer's preferred traits, genetics, genomics and breeding achievements in spider plant. This chapter specifically points out the lack of improved varieties and knowledge gaps on the genetics of farmers preferred traits and nutrients, which is vital for developing improved varieties. As the genetic variability is key in filling this gap, **Chapter 3** investigates the phenotypic variation in biomass and related traits and **Chapter 4** profile the leaf mineral diversity among advanced lines of spider plant as the first step. **Chapter 5** determines the combining ability and heterosis for mineral content, and **Chapter 6** analyses the genetics mechanism governing the biomass and related traits in the species, and both discover the importance of heterosis with hybrid as the type of cultivars to be developed. As Chapter 6 highlighted the importance of combining ability and heterosis for biomass and related traits, **Chapter 7** uncovers the genomics regions controlling the heterosis level for biomass and related traits in the species. Lastly, **Chapter 8** brings together important findings in the stand-alone chapters 2-7 and discusses them in view of our overall objective as well as provides an overall conclusion and implications for future activities in the species breeding.

The outline of the thesis is presented as follows:

Chapter 1. Introduction

Chapter 2. Literature review

Chapter 3. Phenotypic variation in biomass and related traits among four generations of advanced lines of Cleome (*Gynandropsis gynandra* L. (Briq.))

Chapter 4. Leaf ionome analysis in spider plant (*Gynandropsis gynandra* L. (Briq.)) differentiates three nutritional groups

Chapter 5. Combining ability and heterosis analysis for mineral content in the leafy vegetable *Gynandropsis gynandra* (L.) Briq.

Chapter 6. Genetic analysis of biomass and related traits provides insights into hybrid breeding in the leafy vegetable *Gynandropsis gynandra* (L.) Briq.

Chapter 7. Genomic dissection of combining ability and heterosis of mineral content, biomass yield and related traits in *Gynandropsis gynandra* (L.) Briq.

Chapter 8. General discussion and implications of the study

References

Abukutsa-Onyango MO (2007) The diversity of cultivated African leafy vegetables in three communities in western Kenya. Afr J Food Agric Nutr Dev 7 (3):1-15. 10.18697/ajfand.14.IPGRI1-3

Achigan-Dako EG, Pasquini MW, Assogba-Komlan F, N'danikou S, Yédomonhan H, Dansi A, Ambrose-Oji B (2010) Traditional vegetables in Benin. Institut National des Recherches Agricoles du Bénin. Imprimeries du CENAP, Cotonou

Achigan-Dako EG, Sogbohossou DEO, Houdegbe CA, Salaou MA, Sohindji FS, Blalogoe J, Chataika BY, Zohoungbogbo HF, Adje CAO, Fassinou Hotegni NV, Francisco R, Abukutsa-Onyango MO, Schranz ME (2021) Ten years of *Gynandropsis gynandra* research for improvement of nutrient-rich leaf consumption: lessons learnt and way forwards. Annu Rev Plant Biol 4 (3):767-812. 10.1002/9781119312994.apr0774

Ajaiyeoba EO, Onocha PA, Olarenwaju OT (2001) In vitro anthelmintic properties of *Buchholzia coriaceae* and *Gynandropsis gynandra* extracts. Pharm Biol 39 (3):217-220. 10.1076/phbi.39.3.217.5936

Bain LE, Awah PK, Geraldine N, Kindong NP, Siga Y, Bernard N, Tanjeko AT (2013) Malnutrition in Sub–Saharan Africa: burden, causes and prospects. Pan African Medical Journal 15 (1)

Bala A, Kar B, Haldar PK, Mazumder UK, Bera S (2010) Evaluation of anticancer activity of *Cleome gynandra* on Ehrlich's Ascites Carcinoma treated mice. J Ethnopharmacol 129 (1):131-134. 10.1016/j.jep.2010.03.010

Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, De Onis M, Ezzati M, Grantham-McGregor S, Katz J, Martorell R (2013) Maternal and child undernutrition and overweight in low-income and middle-income countries. The lancet 382 (9890):427-451

Blalogoe JS, Odindo AO, Sogbohossou EOD, Sibiya J, Achigan-Dako EG (2020) Origin-dependence of variation in seed morphology, mineral composition and germination percentage in *Gynandropsis gynandra* (L.) Briq. accessions from Africa and Asia. BMC Plant Biol 20 (1):168. 10.1186/s12870-020-02364-w

Boyles RE, Cooper EA, Myers MT, Brenton Z, Rauh BL, Morris GP, Kresovich S (2016) Genome-wide association studies of grain yield components in diverse sorghum germplasm. The plant genome 9 (2)

Burghardt LT, Young ND, Tiffin P (2017) A Guide to Genome-Wide Association Mapping in Plants. Current Protocols in Plant Biology:22-38

Chand J, Panda SR, Jain S, Murty USN, Das AM, Kumar GJ, Naidu VGM (2022) Phytochemistry and polypharmacology of cleome species: A comprehensive Ethnopharmacological review of the medicinal plants. J Ethnopharmacol 282:114600. 10.1016/j.jep.2021.114600

Chandradevan M, Simoh S, Mediani A, Ismail IS, Abas F (2020) 1H NMR-based metabolomics approach in investigating the chemical profile, antioxidant and anti-inflammatory activities of *Gynura procumbens* and *Cleome gynandra*. Plant Foods Hum Nutr 75 (2):243-251. 10.1007/s11130-020-00805-3

Chataika BY, Akundabweni LS-M, Houdegbe AC, Achigan-Dako EG, Sibiya J, Masamba K (2021) Dietary phytochemical screening of spider plant (*Gynandropsis gynandra* (L.) Briq.) accessions from Africa and Asia to identify genotypes for use in nutraceutical breeding. Front Sustain Food Syst 5 (344). 10.3389/fsufs.2021.733909

Chweya JA, Mnzava NA (1997) Cat's whiskers. *Cleome gynandra* L. Promoting the conservation and use of underutilized and neglected crops. 11. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy

Dawson IK, Hendre P, Powell W, Sila D, McMullin S, Simons T, Revoredo-Giha C, Odeny DA, Barnes AP, Graudal L, Watson CA, Hoad S, Burnett F, Muchugi A, Roshetko JM, Hale IH, Van Deynze A, Mayes S, Kindt R, Prabhu R, Cheng S, Xu X, Guarino L, Jamnadass R (2018) Supporting human nutrition in Africa through the integration of new and orphan crops into food systems: placing the work of the African Orphan Crops Consortium in context. ICRAF Working Paper No 276 Nairobi, World Agroforestry

Centre http://dx.doi.org/10.5716/WP18003.PDF. http://dx.doi.org/10.5716/WP18003.PDF

Dickson N (2007) Opportunities for increased production, utilization and income generation from African leafy vegetables in Zambia. Afr J Food Agric Nutr Dev 7 (1):1-20

FAO, IFAD, UNICEF, WFP, WHO (2021) The State of Food Security and Nutrition in the World 2021. Transforming food systems for food security, improved nutrition and affordable healthy diets for all. FAO, Rome. https://doi.org/10.4060/cb4474en

Feodorova TA, Voznesenskaya EV, Edwards GE, Roalson EH (2010) Biogeographic patterns of diversification and the origins of C4 in Cleome (Cleomaceae). Syst Bot 35 (4):811-826

Ghogare UR, Nirmal SA, Patil RY, Kharya MD (2009) Antinociceptive activity of *Gynandropsis gynandra* leaves. Nat Prod Res 23 (4):327-333. 10.1080/14786410802047862

Glew RS, Amoako-Atta B, Ankar-Brewoo G, Presley J, Chuang L-T, Millson M, Smith BR, Glew RH (2009) Non-cultivated plant foods in West Africa: Nutritional analysis of the leaves of three indigenous leafy vegetables in Ghana. Food 3 (1):39-42

Grubben G, Klaver W, Nono-Womdim R, Everaarts A, Fondio L, Nugteren JA, Corrado M (2014) Vegetables to combat the hidden hunger in Africa. Chron Hortic 54:24-32

Grubben GJH, Denton OA (2004) Plant resources of tropical Africa 2. Vegetables, vol. 668 pp. PROTA Foundation, Wageningen, Netherlands / Backhuys Publishers, Leiden, Netherlands/CTA, Wageningen, Netherlands,

Gupta AS, Chakravarty MM (1957) Studies on the seed for composition of desert plants. The component fatty acids of *Gynandropsis pentaphylla* seed fat. Sci Cul 23:306-307

Heywood VH (2013) Overview of agricultural biodiversity and its contribution to nutrition and health. In: Diversifying Food and Diets. Routledge, pp 67-99

Houdegbe CA, Sogbohossou EOD, Achigan-Dako EG (2018) Enhancing growth and leaf yield in *Gynandropsis gynandra* (L.) Briq. (Cleomaceae) using agronomic practices to accelerate crop domestication. Sci Hortic 233:90-98. 10.1016/j.scienta.2018.01.035

Huang X, Han B (2014) Natural Variations and Genome-Wide Association Studies in Crop Plants. Annual Review of Plant Biology 65 (1):531-551. 10.1146/annurev-arplant-050213-035715

Igoli JO, Masao CA, Orkpeh U, Nakamatte E, Yakumbur DT, Nnadozie T, Yunusa S, Vihior B, Manyi MM, Tor-Anyiin TA (2016) In vivo antimalarial activity of *Cleome gynandra* extracts. J Nat Prod Res Updates 2:19-29

Kangai Munene A, Nzuve F, Ambuko J, Odeny D (2018) Heritability Analysis and Phenotypic Characterization of Spider Plant (*Cleome gynandra* L.) for Yield. Adv Agric 2018:11. 10.1155/2018/8568424

Kiebre Z, Bationa Kando P, Sawadogo N, Kiebre M, Sawadogo B, Traore RE, Sawadogo M, Zongo J-D (2017a) Évaluation de la diversité agromorphologique d'une collection de *Cleome gynandra* L. du Burkina Faso. J Appl Biosci 118:11768-11780

Kiebre Z, Bationo Kando P, Barro A, Sawadogo B, Kiebre M, Ouedraogo MH, Sawadogo M, Zongo JD (2017b) Estimates of genetic parameters of spider plant (*Cleome gynandra* L.) of Burkina Faso. Int J Agric Policy Res 5 (9):138-144. 10.15739/IJAPR.17.016

Kiebre Z, Bationo Kando P, Nanema RK, Sawadogo M, Zongo J-D (2015a) Caractérisation agromorphologique du caya blanc (*Cleome gynandra* L.) de l'Ouest du Burkina Faso. International Journal of Innovation and Applied Studies 11 (1):156-166

Kiebre Z, Bationo Kando P, Sawadogo N, Sawadogo M, Zongo J-D (2015b) Selection phenotypic interests for the cultivation of the plant *Cleome gynandra* L. in the vegetable garderns in Burkina-Faso. J Exp Biol Agric Sci 3 (3):288-297. 10.18006/2015.3(3).288.297

Kori M, Gaur K, Dixit V (2009) Investigation of immunomodulatory potential of *Cleome gynandra* Linn. Asian J Pharm Clin Res 2 (1):35-39

Koua GAY, Zoue LT, Ouattara S, Niamke SL (2015) Studies on nutritive nalue and antioxidant properties of three neglected leafy vegetable of Côte d'Ivoire. Pak J Nutr 14 (12):877-884

Kwarteng AO, Abogoom J, Adu Amoah R, Nyadanu D, Ghunney T, Nyam KC, Ziyaaba JZ, Danso EO, Asiedu DD, Whyte T (2018) Current knowledge and breeding perspectives for the spider plant (*Cleome gynandra* L.): a potential for enhanced breeding of the plant in Africa. Genet Resour Crop Evol 65 (5):1529-1550. 10.1007/s10722-018-0626-5

Liu Y, Lin Y, Gao S, Li Z, Ma J, Deng M, Chen G, Wei Y, Zheng Y (2017) A genome-wide association study of 23 agronomic traits in Chinese wheat landraces. The Plant Journal 91 (5):861-873. doi:10.1111/tpj.13614

Maroyi A (2013) Use of weeds as traditional vegetables in Shurugwi District, Zimbabwe. J Ethnobiol Ethnomed 9:60

Masinde P, Agong S (2011) Plant growth and leaf N of spiderplant (*Cleome gynandra* L.) genotypes under varying nitrogen supply. Afr J Hortic Sci 5:36-49

Matro SCAX (2015) Analyse du fonctionnement des jardins de case à *Cleome gynandra* dans le département du couffo. University of Abomey-Calavi, Abomey-Calavi

Maundu P, Achigan-Dako E, Morimoto Y (2009) Biodiversity of African Vegetables. In: Shackleton CM, Pasquini MW, Drescher AW (eds) African Indigenous Vegetables in Urban Agriculture. Earthscan, UK and USA, pp 65-104

Maundu PM, Njiro ES, Chweya J. A., Imungi JK, Seme EN (1999) Kenya. In: Chweya JA, Eyzaguirre PB (eds) The biodiversity of traditional leafy vegetables. International Plant Genetic Resources Institute, Rome, Italy, pp 51-84

Mavengahama S (2013) Yield response of bolted spider plant (*Cleome gynandra*) to deflowering and application of nitrogen top dressing. J Food Agric Environ 11 (3&4):1372-1374

Mnzava NA, Chigumira FN (2004) *Cleome gynandra* L. In: Grubben GJH, Denton OA (eds) Plant Resources of Tropical Africa 2. Vegetables. PROTA Foundation, Wageningen, Netherlands/Backhuys Publishers, Leiden, Netherlands/CTA, pp 191-194

Mogga M, Sibiya J, Shimelis H, Lamo J, Yao N (2018) Diversity analysis and genome-wide association studies of grain shape and eating quality traits in rice (Oryza sativa L.) using DArT markers. PLOS ONE 13 (6):e0198012. 10.1371/journal.pone.0198012

Mosenda E, Chemining'wa G, Ambuko J, Owino W (2020) Assessment of agronomic traits of selected spider plant (*Cleome gynandra* L.) accessions. J Med Active Plants 9 (4):222-241. 10.7275/n1wf-6y77

Moyo M, Amoo SO, Aremu A, Gruz J, Šubrtová M, Jarošová M, Tarkowski P, Dolezal K (2018) Determination of mineral constituents, phytochemicals and antioxidant qualities of *Cleome gynandra*, compared to *Brassica oleracea* and *Beta vulgaris*. Front Chem 5:128. 10.3389/fchem.2017.00128

Moyo M, Aremu AO (2021) Nutritional, phytochemical and diverse health-promoting qualities of *Cleome gynandra*. Crit Rev Food Sci Nutr:1-18. 10.1080/10408398.2020.1867055

Mutua CM, Mulwa RS, Ogweno JO (2015) NPK fertilization and deflowering increases leaf yield and extends the vegetative phase of *Cleome gynandra* L. Int J Plant Soil Sci 8 (6):1-8

Narendhirakannan RT, Kandaswamy M, Subramanian S (2005) Anti-inflammatory activity of *Cleome gynandra* L. on hematological and cellular constituents in adjuvant-induced arthritic rats. J Med Food 8 (1):93-99. 10.1089/jmf.2005.8.93

Narsimhulu BL, Suresh Y, Rajasekar G, Lavanya T, Philip GH, Mohiyuddin SS, Reddy SR (2019) Evaluation of hepatoprotective and nephroprotective activity of methanolic extract of *Cleome viscosa* and *Cleome gynandra* in STZ-induced diabetic rats. Pharma Innov J 8 (2):574-581

Neugart S, Baldermann S, Ngwene B, Wesonga J, Schreiner M (2017) Indigenous leafy vegetables of Eastern Africa—A source of extraordinary secondary plant metabolites. Food Res Int 100:411-422. 10.1016/j.foodres.2017.02.014

Nono-Womdim R, Ojiewo C, Abang M, Oluoch MO (2012) Good Agricultural Practices for African Indigenous Vegetables. ISHS, Gent-Oostakker, Belgium

Omondi CO (1989) Variation and yield prediction analyses of some morphological traits in six Kenyan landrace populations of spiderflower (gynandropsis gynandra (L) briq. University of Nairobi,

Omondi EO, Debener T, Linde M, Abukutsa-Onyango M, Dinssa FF, Winkelmann T (2017a) Mating biology, nuclear DNA content and genetic diversity in spider plant (*Cleome gynandra*) germplasm from various African countries. Plant Breed 136:578-589. 10.1111/pbr.12485

Omondi EO, Engels C, Nambafu G, Schreiner M, Neugart S, Abukutsa-Onyango M, Winkelmann T (2017b) Nutritional compound analysis and morphological characterization of spider plant (*Cleome gynandra*)-an African indigenous leafy vegetable. Food Res Int 100:284-295. 10.1016/j.foodres.2017.06.050

Onyango C, Kunyanga C, Ontita E, Narla R, Kimenju J Production, utilisation and indigenous knowledge of spider plant in Kenya. In: African Crop Science Conference Proceedings, 2013a. pp 925-930

Onyango CM, Kunyanga CN, Ontita EG, Narla RD, Kimenju JW (2013b) Current status on production and utilization of spider plant (*Cleome gynandra* L.) an underutilized leafy vegetable in Kenya. Genet Resour Crop Evol 60:2183-2189. 10.1007/s10722-013-0036-7

Padulosi S, Thompson J, Rudebjer P (2013) Fighting poverty, hunger and malnutrition with neglected and underutilized species (NUS): needs, challenges and the way forward. Bioversity International, Rome

Pandey VL, Dev SM, Jayachandran U (2016) Impact of agricultural interventions on the nutritional status in South Asia: A review. Food policy 62:28-40

Ravichandra B, Ram PS, Saritha C, Shankaraiah P (2014) Anti diabetic and anti dyslipidemia activities of *Cleome gynandra* in alloxan induced diabetic rats. J Pharmacol Toxicol 9 (1):55-61. 10.3923/jpt.2014.55.61

Reeves G, Singh P, Rossberg TA, Sogbohossou EOD, Schranz ME, Hibberd JM (2018) Natural variation within a species for traits underpinning C4 photosynthesis. Plant Physiol 177 (2):504. 10.1104/pp.18.00168

Runnie I, Salleh MN, Mohamed S, Head RJ, Abeywardena MY (2004) Vasorelaxation induced by common edible tropical plant extracts in isolated rat aorta and mesenteric vascular bed. J Ethnopharmacol 92 (2):311-316. 10.1016/j.jep.2004.03.019

Schiessl SV, Huettel B, Kuehn D, Reinhardt R, Snowdon RJ (2017) Flowering Time Gene Variation in Brassica Species Shows Evolutionary Principles. Frontiers in Plant Science 8:1742. 10.3389/fpls.2017.01742

Schippers RR (2004) Légumes africaines indigènes - Présentattion des espèces cultivees. Margraf Publishers GmbH, Scientifics Books, Wageningen, Pays-Bas

Schönfeldt HC, Pretorius B (2011) The nutrient content of five traditional South African dark green leafy vegetables - A preliminary study. J Food Compos Anal 24:1141-1146. 10.1016/j.jfca.2011.04.004

Seeiso MT, Materechera SA (2012) Yield response of the African indigenous leafy vegetable *Cleome gynandra* to application of cattle and goat kraal manure and harvesting techniques. J Food Agric Environ 10 (3&4):789-794. 10.1234/4.2012.3514

Shrimpton R, Rokx C (2012) The double burden of malnutrition: a review of global evidence vol HNP Discussion Paper Series. The International Bank for Reconstruction and Development/The World Bank, Washington, DC

Singh H, Mishra A, Mishra AK (2018) The chemistry and pharmacology of *Cleome* genus: A review. Biomed Pharmacother 101:37-48. 10.1016/j.biopha.2018.02.053

Smith IF (2013) Sustained and integrated promotion of local, traditional food systems for nutrition security. Diversifying food and diets: Using agricultural biodiversity to improve nutrition and health:122-139

Smith IF, Eyzaguirre P (2007) African leafy vegetables: their role in the World Health Organization's global fruit and vegetable initiative. Afr J Food Agric Nutr and Dev 7 (3&4):1-8

Sogbohossou DEO (2019) Orphan no more : Ethnobotany and genetic analysis of leaf yield and secondary metabolites content in *Gynandropsis gynandra* (Cleomaceae). PhD Thesis, Wageningen University, Wageningen

Sogbohossou EOD, Achigan-Dako EG, Maundu P, Solberg S, Deguenon EMS, Mumm RH, Hale I, Van Deynze A, Schranz ME (2018a) A roadmap for breeding orphan leafy vegetable species: a case study of *Gynandropsis gynandra* (Cleomaceae). Hortic Res 5 (1):2. 10.1038/s41438-017-0001-2

Sogbohossou EOD, Achigan-Dako EG, Mumm R, de Vos RCH, Schranz ME (2020) Natural variation in specialised metabolites production in the leafy vegetable spider plant (*Gynandropsis gynandra* L. (Briq.)) in Africa and Asia. Phytochemistry 178:112468. 10.1016/j.phytochem.2020.112468

Sogbohossou EOD, Achigan-Dako EG, van Andel T, Schranz ME (2018b) Drivers of management of spider plant (*Gynandropsis gynandra*) across different socio-linguistic groups in Benin and Togo. Econ Bot:1-25. 10.1007/s12231-018-9423-5

Sogbohossou EOD, Kortekaas D, Achigan-Dako EG, Maundu P, Stoilova T, Van Deynze A, de Vos RCH, Schranz ME (2019) Association between vitamin content, plant morphology and geographical origin in a worldwide collection of the orphan crop *Gynandropsis gynandra* (Cleomaceae). Planta 250:933–947. 10.1007/s00425-019-03142-1

van Jaarsveld P, Faber M, van Heerden I, Wenhold F, van Rensburg WJ, van Averbeke W (2014) Nutrient content of eight African leafy vegetables and their potential contribution to dietary reference intakes. J Food Compos Anal 33:77-84. 10.1016/j.jfca.2013.11.003

Vandebroek I, Voeks R (2018) The Gradual Loss of African Indigenous Vegetables in Tropical America: A Review. Econ Bot 72 (4):543-571. 10.1007/s12231-019-09446-3

Varshney RK, Terauchi R, McCouch SR (2014) Harvesting the Promising Fruits of Genomics: Applying Genome Sequencing Technologies to Crop Breeding. PLOS Biology 12 (6):e1001883. 10.1371/journal.pbio.1001883

Wangolo EE, Onyango CM, Gachene CKK, Mong'are PN (2015) Effects of shoot tip and flower removal on growth and Yield of spider plant (*Cleome gynandra* L.) in Kenya. American Journal of Experimental Agriculture 8 (6):367-376

Wasonga DO (2014) Phenotypic characterization of Kenyan and South African spider plant (*Cleome gynandra* L.) ecotypes. Master of Science, University of Nairobi, Nairobi

Weinberger K, Msuya J (2004) Indigenous Vegetables in Tanzania- Significance and Prospects. Technical Bulletin No. 31. AVRDC-The World Vegetable Center, Shanhua, Taiwan

Weinberger K, Pichop GN (2009) Marketing of African indigenous vegetables along urban and peri-urban supply chains in sub-Saharan Africa. In: Shackleton C, Pasquini M, Drescher A (eds) African indigenous vegetables in urban agriculture. Earthscan, UK,, pp 225-244

Wenyika P, Gasura E, Kafesu N, Goss M, Matikiti A, Kujeke G (2015) Discriminating phenotypic markers reveal low genetic diversity in spider plant. Afr Crop Sci J 23 (4):305-310

Wu T-h, Solberg SO, Yndgaard F, Chou Y-Y (2018) Morphological patterns in a world collection of *Cleome gynandra*. Genet Resour Crop Evol 65 (1):271-283. 10.1007/s10722-017-0529-x

Xiao Y, Liu H, Wu L, Warburton M, Yan J (2017) Genome-wide Association Studies in Maize: Praise and Stargaze. Molecular Plant 10 (3):359-374. 10.1016/j.molp.2016.12.008

Yang N, Lu Y, Yang X, Huang J, Zhou Y, Ali F, Wen W, Liu J, Li J, Yan J (2014) Genome Wide Association Studies Using a New Nonparametric Model Reveal the Genetic

Architecture of 17 Agronomic Traits in an Enlarged Maize Association Panel. PLOS Genetics 10 (9):e1004573. 10.1371/journal.pgen.1004573

Yang R-Y, Keding GB (2009) Nutritional contributions of important African indigenous vegetables. In: Shackleton CM, Pasquini MW, Drescher AW (eds) African Indigenous Vegetables in Urban Agriculture. Earthscan, UK and USA, pp 105-143

Yuan B, Lyu W, Dinssa FF, Simon JE, Wu Q (2021) Free amino acids in African indigenous vegetables: Analysis with improved hydrophilic interaction ultra-high performance liquid chromatography tandem mass spectrometry and interactive machine learning. J Chromatogr A 1637:461733. 10.1016/j.chroma.2020.461733

CHAPTER 2

Enhancing genetic gain in the leafy vegetable *Gynandropsis gynandra* (L.) Briq. at the era of genomics: A review

Abstract

African leafy vegetables, including *Gynandropsis gynandra*, are a rich source of micronutrients, an important resource for climate change adaptation and a sustainable source of income for local farmers, especially women. *G. gynandra* has received great attention over the last decade, as shown by the increasing number of studies on phenotypic variability, germination, agronomic practices, molecular genetic diversity, selection of improved cultivars, reproductive biology, gene discovery and participatory varietal selection. Significant variations were reported for leaf vitamins, plant morphology, secondary metabolite concentrations, seed germination, genome variation, mineral composition, and photosynthesis traits, which were all found to be origin dependent. Another important achievement has been the release of the chloroplast reference genome and more importantly, another milestone achievement is the soon to be the released reference genome. However, despite the availability of all these important resources, genetic gain in the species is still low due to limited knowledge on the genetic mechanism controlling farmers' desired traits and genes associated with farmers' preferred traits. Efforts are, thus, needed to generate key information on the genetics of inheritance of desired traits and exploit the recent advances in molecular biology tools to speed up the breeding program for improved genetic gain.

Keywords: Spider plant, *Cleome gynandra*, genomics, selection, leaf yield, food security, smallholder farmer, genetic improvement, orphan crops

2.1 Introduction

African leafy vegetables (ALVs) have been reported to have great potential in ensuring food and nutritional security and adaptation to climate change as well as serving as a sustainable source of income generation for local farmers. These vegetables are rich in minerals and vitamins that are mostly deficient in staple crops (Yang and Keding 2009; Padulosi et al. 2021). In addition, ALVs contain several healthy compounds or phytonutrients, such as phenolics, carotenoids, antioxidants, flavonoids and glucosinolates that are essential for human well-being (Neugart et al. 2017; Moyo et al. 2021). Increasing the consumption of ALVs is thus crucial and will contribute to combating malnutrition and hidden hunger in the world, especially in sub-Saharan Africa (SSA), where approximately 264.2 million people were reported in 2020 to be undernourished (FAO et al. 2021). A prerequisite to achieve this is to ensure a year-round production and consumption of ALVs. Nevertheless, the production of ALVs does not meet the demand due to several constraints, including a lack of improved cultivars and appropriate agricultural practices. Consequently, providing farmers with improved varieties and best agricultural practices is key in a context where few sustainable orphan leafy vegetable crop breeding programs are established.

The diversity of ALVs is large and more than 280 species have been recorded in SSA (Grubben and Denton 2004). The most nutritious and widely represented ALVs in SSA include amaranth (*Amaranthus* spp), spider plant (*Gynandropsis gynandra* L. Briq.), African vegetable nightshades (*Solanum* spp.), cowpea (*Vigna unguiculata* (L.) Walp), jute mallow (*Corchorus olitorius* L.), moringa (*Moringa oleifera* L.), African eggplant (*Solanum macrocarpon* L.), Ethiopian kale (*Brassica carinata* A. Braun), celosia (*Celosia argentea* L.), blackjack (*Bidens pilosa* L.), *Crassocephalum rubens* (Juss. ex Jacq.) S. Moore, *Talinum* spp., *Vernonia* spp., and *Ocimum* spp. (Grubben and Denton 2004; Smith and Eyzaguirre 2007; Maundu et al. 2009; Nono-Womdim et al. 2012; Weinberger and Pichop 2009; Grubben et al. 2014).

The spider plant (*G. gynandra* syn. *Cleome gynandra* L.), belonging to the Cleomaceae family, is an annual herb with increasing interest because of its high vitamin, mineral and secondary metabolite contents (Sogbohossou et al. 2019; Omondi et al. 2017b; Gowele et al. 2019; Moyo et al. 2018; Neugart et al. 2017; Schönfeldt and Pretorius 2011; Sogbohossou et al. 2020; Chataika et al. 2021; Thovhogi et al. 2021a). Minerals found in the most consumed part, the leaves, include iron, zinc, calcium, copper, potassium, magnesium, manganese, phosphorus and sodium (Omondi et al. 2017b; Thovhogi et al. 2021a; Jiménez-Aguilar and Grusak 2015;

Gowele et al. 2019). The leaves are also rich in vitamins C, A, E, B1, B2, and B9 (Sogbohossou et al. 2019; Moyo and Aremu 2021; Omondi et al. 2017b; van Jaarsveld et al. 2014; Schönfeldt and Pretorius 2011), amino acids and fatty acids (Glew et al. 2009; Yuan et al. 2021). In addition, spider plant contains several dietary phytochemicals, including flavonoids, glucosinolates, aldehydes, ketones, terpenoids, tannins, sesquiterpenes and many other phenolic compounds (Sogbohossou et al. 2020; Omondi et al. 2017b; Neugart et al. 2017; Moyo et al. 2018; Moyo and Aremu 2021; Chataika et al. 2021), with diverse pharmaceutical applications (plant extracts, drugs, etc.) (Achigan-Dako et al. 2021).

Given the potential of *G. gynandra* for food security and better nutrition, increased investigations have been carried out to understand the genetic diversity, reproductive biology, agronomic practices, taxonomy, indigenous knowledge, seed germination, nutritional values, crop physiology, genetic resource collection and evaluation (Achigan-Dako et al. 2021). Some large and comprehensive reviews on *G. gynandra* include Achigan-Dako et al. (2021) on achievements over the last decade, Moyo and Aremu (2021) on the nutritional, phytochemical and health-promoting qualities, Shilla et al. (2019) on the origin, taxonomy and morphology and Sogbohossou et al. (2018a) on a general genomic integrated breeding roadmap. To avoid repetition, this review focuses on detailed breeding strategies to speed up genetic gain and cultivar release in *G. gynandra*, considering the recently generated knowledge for the complete exploitation of the full potential of the species. Therefore, the review (i) summarizes the constraints associated with the cultivation of *G. gynandra*; (ii) identifies farmers' desired traits and other important research traits; (iii) identifies knowledge gaps associated with these important traits; and (iv) proposes pathways and strategies for improved genetic gain in the species for cultivar release.

2.2 Description of *Gynandropsis gynandra*

Gynandropsis gynandra (L.) Briq. is a semi to erect annual herb (Figure 2.1 A-F), which grows up to 170 cm tall on average and is strongly branched (4 to 12 branches) (Chweya and Mnzava 1997; Wasonga 2014; Sogbohossou et al. 2019). The species has a long taproot and few secondary roots. The stem is densely glandular, may be green, violet or pink in colour and hairy or not (Figure 2.1 A-F). Leaves are alternate, palmately compound with 3, 5 or 7 leaflets (Figure 2.1 A-F). The petiole is glandular and varies from 2 to 23 cm in length. Leaflets almost sessile are obovate to elliptical or lanceolate, acute or acuminate at apex (Chweya and Mnzava 1997; Mnzava and Chigumira 2004) (Figure 2.1 A-F).

The inflorescence, bracteate up to 30 cm long, has terminated and axillary determinate racemes with many flowers (Figure 2.1 G). The bract is 3-foliolate to simple, resembling the leaves but smaller and sessile. Two types of flowers were observed in *Gynandropsis gynandra*: staminate consisting of residual ovary devoid of ovules and bisexual consisting of functional ovary and fertile stamens (Raju and Rani 2016). Both flowers have four sepals, four petals and six stamens. Sepals are free, green, ovate to lanceolate and up to 8 mm long. Petals are also free but white and sometimes fade to rose pink, rounded at the apex, and abruptly narrowed to a basal claw (Chweya and Mnzava 1997; Mnzava and Chigumira 2004). The stamens have long purple filaments and green exserted dithecous anthers. The ovary is bicarpellary, syncarpous, superior, and unilocular with numerous ovules on parietal placentation (Raju and Rani 2016). Among bisexual flowers, three flowers and three types of flowers can be observed based on anther shedding and stigma receptivity allowing protandry and protogyny mechanisms (Silué 2009).

Gynandropsis gynandra is sexually propagated (Chweya and Mnzava 1997; Onyango et al. 2013b). Based on the flower morphology, the stamens can coil and contact the stigma, facilitating autogamy, and the powdery pollen can be driven away by wind. Flowers of the species are visited by insects, mainly bees showing high cross-pollination (Raju and Rani 2016). This shows that the species is both self- and cross-pollinated. The evaluation of self-incompatibility in *G. gynandra* revealed that the species is self- and cross compatible (Omondi et al. 2017a). Evaluation of mating systems in the species showed that open pollination and cross-hand pollination led to higher fruit and seed set (Zohoungbogbo et al. 2018).

The fruit is a capsule dehiscent from below with two valves (Figure 2.1 H) and up to 15 cm long and 1 cm wide (Chweya and Mnzava 1997; Mnzava and Chigumira 2004; Onyango et al. 2013b). The fruit is either large and rough or thin and smooth (Kiebre et al. 2015a). The capsules are green or violate and turn yellow when ripe and brown when dried (Figure 2.1 H). They are linear and suberect to spreading with persistent style (Onyango et al. 2013b). There are approximately 230 seeds/fruit (Omondi et al. 2017a). Seeds are small (1.5 mm diameter), subglobose or circular and have rough or irregular ribbeds with narrow clefts (Chweya and Mnzava 1997; Mnzava and Chigumira 2004; Onyango et al. 2013b). They are black when harvested from yellow pods and brown or grey when dried (Figure 2.1 I).

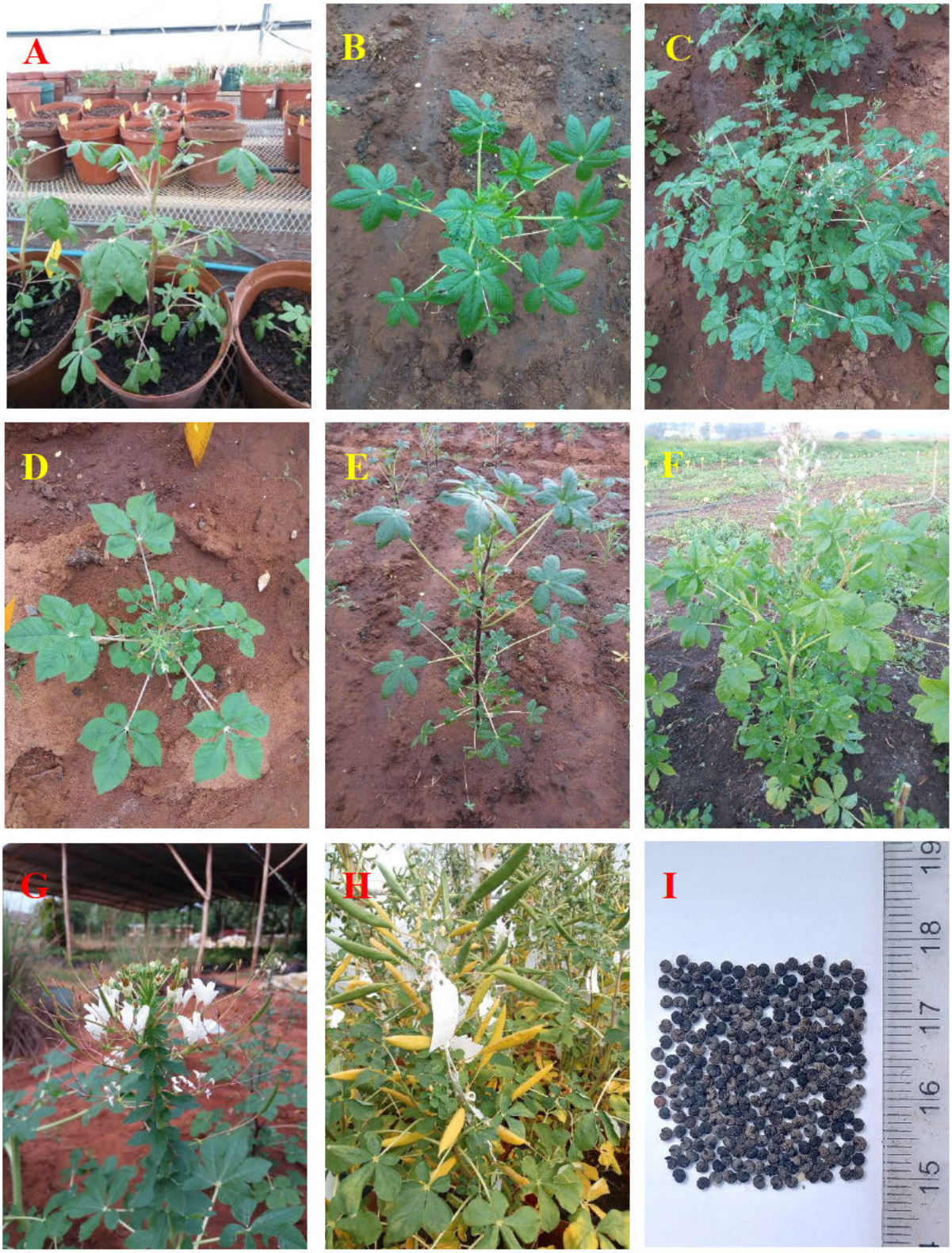


Figure 2.1. Description of *Gynandropsis gynandra*. A-F: Diversity in plant growth habit, leaves and petiole colour. G: Inflorescence. H: Green and yellow (mature) pods. I: Seeds. © Aristide Carlos Houdegbe

2.3 Distribution and uses of *Gynandropsis gynandra*

The species thrives in tropical and subtropical regions of the world, mainly in Africa, South America, Southeast Asia and Oceania, where it grows abundantly during the rainy season (Sogbohossou 2019; Feodorova et al. 2010; Vandebroek and Voeks 2018). It is found near human settlements or roadsides in wild populations but is also cultivated in home gardens or in urban and peri-urban agriculture (Achigan-Dako et al. 2010; Chweya and Mnzava 1997; Kiebre et al. 2015b; Weinberger and Pichop 2009). *Gynandropsis gynandra* is used in food and medicine mainly by rural populations. In Burkina-Faso, the species is used as a famine, flood or dry food (Kiebre et al. 2015c).

Leaves are the main part of the species used as vegetables, but young shoots and flowers have also been utilised (Dickson 2007; Chweya and Mnzava 1997; Maroyi 2013; Mnzava and Chigumira 2004). Leaves and young shoots are used as leafy vegetables. Two main forms of leaf consumption distinguished by local communities include direct cooking or drying. The leaves contain several micronutrients. Minerals include calcium (Ca), iron (Fe), magnesium (Mg), phosphorus (P), sodium (Na), potassium (K), copper (Cu), zinc (Zn) and manganese (Mn), while vitamins include thiamin (vitamin B1), vitamin E, riboflavin (vitamin B2), folate, vitamin C (ascorbic acid) and vitamin A (Sogbohossou et al. 2019; Omondi et al. 2017b; Moyo et al. 2018; Moyo and Aremu 2021; Schönfeldt and Pretorius 2011). Significant variation was reported among accessions and inbred lines of *G. gynandra* for minerals and vitamins (Omondi et al. 2017b; Sogbohossou et al. 2019).

For medicinal purposes, *G. gynandra* is consumed by breast-feeding mothers to stimulate milk production as reported amongst the Kisii community in Kenya. The vegetable has been recommended to mothers soon after child birth and to boys and girls after circumcision for quick regain after loss of blood (Maundu et al. 1999). The species is also used to promote male potency and provide energy (Onyango et al. 2013b). *Gynandropsis gnandra* has been used in the treatment of more than 50 diseases (Maundu et al. 1999; Sogbohossou et al. 2018b; Onyango et al. 2013b; Weinberger and Msuya 2004). The most common are malaria, stomach-ache, headache, diarrhoea, toothache, microbial diseases, earache, oral problems, and treatment of worms, anaemia, fever and haemorrhoids. It has also been used as a cure for constipation, while leaves in boiling water are used to treat diarrhoea (Onyango et al. 2013b) and root infusion is used for chest pain (Chweya and Mnzava 1997).

In addition, the leaves and seeds are used as rubefacients and vesicants and to treat conjunctivitis and rheumatism both externally and internally (Chweya and Mnzava 1997; Mnzava and Chigumira 2004). Leaves are pounded with a little water and the extract drank to treat chira (a condition with symptoms similar to those of AIDS but associated with a curse or punishment from the spirits) (Onyango et al. 2013b). Seeds have been reported to have anthelmintic properties and oil is used as a fish poison. Stems are used as an analgesic and anti-inflammatory agent (Gupta and Chakravarty 1957). All these applications are due to the concentration of phytochemicals and health-promoting compounds in leaves and inflorescence (Moyo et al. 2018; Neugart et al. 2017; Omondi et al. 2017b).

The numerous secondary metabolites found in the species include flavonoids, terpenoids, tannins, glucosinolates, aldehydes, ketones, sesquiterpenes and many other phenolic compounds (Sogbohossou et al. 2020; Omondi et al. 2017b; Neugart et al. 2017; Moyo et al. 2018; Moyo and Aremu 2021; Chataika et al. 2021) with diverse pharmaceutical applications (plant extracts, drugs, etc.) (Achigan-Dako et al. 2021). The species is therefore a prime resource for the pharmaceutical industry, as its extracts have several biological and pharmacological effects (Chand et al. 2022; Achigan-Dako et al. 2021; Moyo and Aremu 2021; Singh et al. 2018), including antimicrobial (fungi and bacteria), anthelmintic (Ajaiyeoba et al. 2001), antimalarial (Igoli et al. 2016), hepatoprotective (Narsimhulu et al. 2019), antiarthritic (Narendhirakannan et al. 2005), antioxidant, anti-inflammatory (Chandradevan et al. 2020), immunomodulatory (Kori et al. 2009), antinociceptive (Ghogare et al. 2009), anticancer (Bala et al. 2010), antidiabetic (Ravichandra et al. 2014) and vasodilatory (Runnie et al. 2004) activities.

However, some of these metabolites are antinutrient factors and included tannins and total polyphenols among others. Specifically, the level of tannins was found to be correlated with bitterness level in spider plant (Kutsukutsa et al. 2014). The desire for bitter taste in spider plant is variable and depends on the regions of the consumers. For instance, farmers in Kenya (East Africa) preferred no bitterness, while those in Benin (West Africa) preferred slight bitterness (Sogbohossou et al. 2018a). In Limpopo Province in South Africa, the appreciation of spider plant's bitter taste is variable among households and communities with some desire the bitterness without an increase while others want a decrease in bitterness (Thovhogi et al. 2021b). Thus, bitterness of the spider plant deters some end-users from its consumption. Depending on the target environment and group, breeding program should consider the bitterness to develop varieties/cultivars with no bitterness and slight bitterness. This is

achievable as significant variation in tannins concentration (41 – 464 mg TAE/g) was observed among accessions of spider plant (Chataika et al. 2021). More importantly, genotypes CGKEX and CGSKP (Kutsukutsa et al. 2014), TOT6439, TOT7197 and TOT8926 were identified with low tannins content (Chataika et al. 2021). In contrast, accessions ODS-15-037, ODS-15-053, ODS-15-121 and TOT4976 were reported with high level of tannins (Chataika et al. 2021). These identified genotypes could be use in breeding program and identification of genomic regions controlling tannins content in spider plant. Another important factor affecting bitterness is the leaf age with leaves more bitter with growing age (Thovhogi et al. 2021b), which should be considered in assessing tannins content in spider plant. Furthermore, further studies should assess the variability in other antinutrients factors, such as phytic acid, acid oxalic, among genotypes using a large collection. Association between antinutrients factors and others important nutraceutical and nutritional elements is needed to inform breeding program for not compromising the nutraceutical and nutritional properties of new select cultivars of spider plant.

The species also has several cultural uses, for example, in Kisii (Kenya), a mixture of *G. gynandra* and blood is used in wedding ceremonies and during lobola negotiations and other forms of religious rituals such as naming of a child and during funerals (Maundu et al. 1999). In Luo and Kisii communities (Kenya), the species is cooked and offered to important visitors such as in-laws as a sign of respect (Onyango et al. 2013b).

Gynandropsis gynandra is harvested in the wild in some communities, while in others, the species is cultivated and sold in local markets. The cultivation and commercialization of the species are mainly performed by women and provide substantial income for households (Matro 2015; Onyango et al. 2013b; Sogbohossou et al. 2018b). The leaves are sold in open markets in many African countries (e.g., Kenya, Namibia, Benin, Tanzania, South Africa, Togo, Ghana, Burkina-Faso and Uganda) but also in supermarkets (e.g., Kenya) (Onyango et al. 2013b; Matro 2015; Kiebre et al. 2015b; Chweya and Mnzava 1997). The species production from home gardens generated a profit margin between 40% and 57% and production efficiency (benefit cost ratio) between 1.66 and 2.33 during the rainy season in the Adja community of Benin (Matro 2015). In Kenya, the selling price varies from US$0.41 (Weinberger and Pichop 2009) to US$0.5 (Onyango et al. 2013a) per kg during the rainy season and increases by almost double during the dry season when vegetables become rare (Onyango et al. 2013a). The average profit margin was estimated at 30 to 40% of the selling price (Weinberger and Pichop 2009). Moreover, *G. gynandra* contributed to 15-40% of the total income of the farmers

producing it for sale. The low supply of vegetables and their limited shelf life are among the main constraints encountered by vegetable sellers. The longest period of storage was two days in the outlets. Postharvest losses due to wilting, rotting and lesions during harvesting and transportation contribute to decreasing the profitability of the vegetable (Onyango et al. 2013a). The demand for spider plant consumption is increasing across sub-Saharan African countries. For instance, spider plant production increased from 19 428 metric tons to 21 507 metric tons between 2012 and 2013, with an average increase of 50% in the cultivated area in Kenya (HCDA 2014).

2.4 Constraints associated with cultivation of *Gynandropsis gynandra*

Farmers' production constraints include poor germination, early flowering, low yield, insect pests and diseases, and seed availability (Onyango et al. 2013b; Matro 2015; Abukutsa-Onyango 2007; Sogbohossou et al. 2018a). Poor germination refers to erratic germination leading to nonuniform seedling establishment, therefore affecting plant density and yield. The poor germination was due the fact that most spider plant seeds were harvested much later after physiological maturity and therefore the seeds had developed secondary dormancy (Ochuodho, 2005). Flowering time is associated with early development of reproductive organs, limiting vegetative growth and severely decreasing yield (Schiessl et al. 2017). The reported pests associated with *Gynandropsis gynandra* include aphids, leaf miners, webbers and defoliators (caterpillars, beetles), nematodes, and stem borers, among others (Sithanantham et al. 2005; Mnene 2021). Reported nematodes belong to *Meloidogyne* genus, while pests include *Brevicoryne brassicae* L., *Aphis gossypii* Glover, *Nezara viridula* L., *Nezara* spp., *Helicoverpa armigera* Hübner, *Phyllotreta mashonana* L., *Phyllotreta* spp., *Athalia* spp., *Empoasca* spp. *Bagrada* spp., and *Phyllobrotica elegans* Kraatz among others (Schippers 2004; Mnene 2021). The reported diseases include those caused by *Sphaerotheca fuliginea* (Schltdl.) U. Braun et S. Takam. and *Oidiopsis Taurica* (Léveillé) E.S. Salmon, wet rot (*Choanephora cucurbitarum*), Fusarium wilt, and root rot (*Fusarium oxysporum*). Seed availability is associated with a lack of improved cultivars and a shattered pod nature as well as it long viewed as semi-wild and a vegetable for the poor in the society (Shilla et al. 2019) leading to lack of interest in seed business. Insects' attack was reported responsible for about 17% yield loss on a station in Kenya (Sithanantham et al. 2004). Cabbage aphid (*Brevicoryne brassicae* L.) causes wrinkling of the growing tips and leaves as well stunted growth with total crop failure (Mnzava and Chigumira, 2004). *Phyllotreta* spp. devour leaves and make them not suitable for consumption

(Schippers, 2004). The rank of these constraints varied between countries and regions, indicating the potential influence of biophysical and socio-economic factors (Chataika et al. 2022).

2.5 Traits preferred by farmers and consumers

For the cultivation of *G. gynandra*, farmers selected cultivars based on certain traits with a slight difference between regions. In Burkina-Faso (West Africa), farmers prefer cultivars with green stem, giant plant, green leaves, long cycle, high leaf biomass, a high number of primary branches and a small number of flowers (Kiebre et al. 2015c). In Kenya (East Africa), farmers' preferred traits include tall plants, good germination, high yield, resistance to pests and diseases, broad leaves, and many branches (Mutoro 2019; Ndinya et al. 2020). It is clear that common farmer-preferred traits in *G. gynandra*, include high leaf yield and related traits (plant height and the number of leaves), broad leaves, late flowering, good germination and resistance to pests and diseases (Ndinya et al. 2020; Kiebre et al. 2015b; Mutoro 2019; Cleome Consortium 2017). Late flowering implies a long vegetative growth period, which increases the species biomass production. A high number of primary branches and leaves play a key role in vegetable species and are significantly correlated with biomass yield. Leaf area is also significant for farmers but mainly for consumers, as it makes it easy to remove leaves from stems for cooking. In addition, organoleptic characteristics play a major role for farmers but varies from one region to another. For instance, farmers in Kenya (Eastern Africa) preferred no bitterness, while those in Benin (West Africa) preferred strong aroma, slight bitterness and spiciness (Sogbohossou et al. 2018a). Consumer or buyer preferences include appearance, colour, flavour/aroma and medicinal attributes (Mutoro 2019). Additionally, Sogbohossou et al. (2018a) pointed out that good appearance, superior taste and aroma, high nutritional value, long shelf life and affordability were amongst the popular traits sought after by retailers and consumers. The observed farmers' preferred traits were tightly associated with the constraints of the species production. These traits showed the farmers' willingness to ensure good field establishment and higher productivity to ensure high income and profitability. The difference in consumers' preferences might be associated with food habits and cultural behaviours. The knowledge of the desired traits is key, particularly in plant breeding as it guides the development of new cultivars easily adopted by users.

2.6 Progress in addressing production constraints and farmer-preferred traits

2.6.1 Seed germination

Several studies addressed the observed poor germination and pointed out that dormancy was responsible for erratic germination (Blalogoe et al. 2020; Muasya et al. 2009; Ekpong 2009). Specifically, Ochuodho (2005) found that most spider plant seeds were harvested much later after physiological maturity and therefore the seeds had developed secondary dormancy. The type of dormancy in the species is a physiological dormancy (Baskin and Baskin 2014) and the acquisition of dormancy depends on stage where seeds were harvested (Ochuodho 2005; Ochuodho and Modi 2007; Kamotho et al. 2014). Black seeds harvested from green mature pods germinated better (more than 90%) than black seeds from yellow and brown pods sowing directly (Ochuodho and Modi 2007). In contrast, Kamotho et al. (2014) found that seeds from yellow pods germinated more than seeds from yellow-green and green pods but there was no precision on seeds colours. Blalogoe (2019) showed that the germination potential according to seed and pod colours is genotype dependent. Therefore, seeds and pods colours are indicators of relative physiological maturity that is reached in seeds of spider plant just before the yellow pod stage (Ochuodho 2005) and genotype dependent (Blalogoe, 2019). Methods to effectively overcome these problems include treating seeds with gibberellic acid (0.5% or 1%), preheating at 40°C and storage (at least three months) (Kamotho et al. 2014; Blalogoe et al. 2020; Ekpong 2009; Muasya et al. 2009). While significant progress has been made in improving seed germination in the species, efforts are still needed to understand the causal factors associated with erratic germination. One important finding is the differential germination ability observed among Asian and African genotypes whereby Asian genotypes had higher germination percentage values and West African genotypes had a lower germination percentage (Blalogoe et al. 2020). More importantly, seed size was found to be associated with germination ability. Small seeds displayed higher germination percentage compared to large seeds, showing origin-driven germination ability and seed size in the species. Additionally, seed mineral contents were shown to be origin-dependent (Blalogoe et al. 2020). The exploitation of this variability calls for assessment of the hormonal, genetic and metabolomic factors responsible for germination in the species. Therefore, breeding populations, including biparental populations, Multiparent Advanced Generation Inter-Cross (MAGIC) lines and association mapping panels could play important roles in improving germination in the species. Additionally, backcrossing

methods can be implemented to transfer germination ability from genotypes with higher germination ability to high-performing genotypes with low germination ability. It is essential that new cultivars possess a higher germination percentage if successful adoption by farmers has to be realised.

2.6.2 Leaf biomass yield

Leaf biomass yield is the most important trait for farmers and can be improved by developing appropriate agronomic practices and improved cultivars. Previous studies have focused on establishing the best agronomic practices for improved yield, including optimal planting density, type and fertilizer application rates, planting date, stage of transplanting, harvesting frequency and techniques (cutting, uprooting whole plants, defoliation), deflowering, sowing depth and net cover colour (Houdegbe et al. 2018; Gonye et al. 2017; Ayua et al. 2016; Mavengahama 2013; Seeiso and Materechera 2012; Masinde and Agong 2011; Wangolo et al. 2015). In addition, many studies have assessed morphological diversity in *G. gynandra* using countrywide collections [e.g., Ghana (Kwarteng et al. 2018), Burkina-Faso (Kiebre et al. 2017a), Kenya (Adeka 2020; Mosenda et al. 2020b)], regionwide germplasm [e.g., Kenya and South Africa (Kangai Munene et al. 2018), East and Southern Africa (Omondi et al. 2017b)] and worldwide collections (Wu et al. 2018; Sogbohossou et al. 2019). The authors reported significant variations among genotypes/accessions. Key outcomes included the origin-dependence of plant morphology and geographical origins (Sogbohossou et al. 2019; Wu et al. 2018). However, few studies have assessed the yield potential of the accessions and these include that of Omondi et al. (2017b), Mosenda et al. (2020b) and Kiebre et al. (2017a).

Traits related to biomass that have been investigated include total shoot fresh and dry weight and leaf fresh and dry weight (Mosenda et al. 2020a; Kiebre et al. 2017a; Omondi et al. 2017b). Although several studies did not include leaf biomass yield, some included the number of leaves per plant, which can be considered a proxy for biomass estimation. Studies focusing on biomass yield were based on countrywide accessions and Eastern/Southern African genotypes. There is a need to fill this gap in knowledge of leaf biomass productivity in the species using worldwide collections, as the worldwide collection provides insights into the origin dependence of plant morphology.

2.6.3 Flowering time

Flowering time is linked to biomass yield because early flowering limits vegetative growth and thus, severely decreases yield (Schiessl et al. 2017). Morphological characterization of germplasm studies revealed significant variation among accessions of *G. gynandra* (Kiebre et al. 2017a; Omondi et al. 2017b; Sogbohossou et al. 2019; Kangai Munene et al. 2018; Wu et al. 2018). Flowering time ranged from 42 to 73 days after emergence (Sogbohossou et al. 2019). Flowering time was also found to be origin dependent (Wu et al. 2018; Sogbohossou et al. 2019). Wu et al. (2018) observed that African accessions flowered an average 33 days after sowing, while Asian accessions flowered an average 25 days after sowing. The use of a more comprehensive germplasm revealed significant variation within African accessions, with West African accessions characterized by early flowering and East and Southern African accessions by late flowering (Sogbohossou et al. 2019). Moreover, Zorde et al. (2020) screened 4536 plants of *G. gynandra* developed from nine different advanced lines of spider plant and showed that flowering time is accession specific and highly heritable. High broad-sense heritability was reported for flowering time from the germplasm collection (0.70-0.9) (Kangai Munene et al. 2018; Kiebre et al. 2017b) and from the F2 population (0.82) (Sogbohossou 2019). Spider plant was reported to be daylength sensitive. In fact, Zorde et al. (2020) observed significant variation in days to flowering between the greenhouse (10-182 days) and field (20-57) trials in Arusha due to the differential day length and light intensity. The authors observed an early flowering of the plants grown under daylight conditions between 11:52-12:17 hours of daylight as opposed to late flowering of plants grown under 14 hours in the greenhouse. In addition, Imbamba and Tieszen (1977) found that photosynthesis rate in spider plant increase with light intensity (from 200 to 2000 μmol m^{-2} s^{-1}) and that 2000 μmol m^{-2} s^{-1}, which is close to full sunlight, does not saturate photosynthesis in *G. gynandra* due to the C4 plant nature of spider plant. Similar observations were done by Kocacinar (2015) with an increase in net photosynthetic rate and stomatal conductance with increasing light intensity.

2.6.4 Plant height

Significant variations were associated with plant height in *G. gynandra*. While Sogbohossou et al. (2019) observed a range of 19.00-168.00 cm, Wu et al. (2018) observed a range of 50–199 cm. In addition, a range of 53.3-77.4 cm was observed by Omondi et al. (2017b), and 22.1-111.7 cm was observed by Kangai Munene et al. (2018). This trait was also origin dependent (Wu et al. 2018; Sogbohossou et al. 2019). East-Southern African accessions had taller plants,

while Asian and West African accessions had shorter plants (Sogbohossou et al. 2019). The variation observed between researchers' outcomes could be attributed to the genotypes used as well as the various environments of evaluation ranging from greenhouse to the field. Plant height had a significant and positive association with flowering time. This variation represents an important resource for plant breeding.

2.6.5 Number of primary branches

Significant variation among genotypes of spider plant was observed for the number of primary branches (NPB). This ranged from 4 to 16 branches (Kiebre et al. 2017a; Omondi et al. 2017b; Mosenda et al. 2020b). Sometimes number of branches refers to branching habit and include sparse (≤ 5), intermediate (6-10) and abundant (> 10). Therefore, Asian and East-Southern accessions were mostly intermediate, while West African genotypes were sparse.

2.6.6 Leaf area and related traits

Geographical signatures were observed in leaf area and related traits in spider plant. The Asian and East-Southern African accessions displayed broad leaves and West African accessions exhibited small leaves (Sogbohossou et al. 2019). Related traits to leaf area include central leaflet length and width, leaf width and petiole length. Significant and positive correlations were reported between leaf area and related traits (Sogbohossou et al. 2019; Kangai Munene et al. 2018; Kiebre et al. 2017b). This offers opportunities for indirect selection for leaf area based on related traits.

2.6.7 Other qualitative and quantitative morphological traits

Other morphological traits investigated in the species were related to plant architecture (plant habit, stem hairiness and colour), leaf colour, pod characteristics (pod length and width, number of seeds per pod), seed size (length, width, perimeter, area), 1000-seed weight, and flower traits (androphore length, filament length, pedicel length, gynophore length) (Sogbohossou et al. 2019; Wu et al. 2018; Omondi et al. 2017b; Blalogoe et al. 2020; Mosenda et al. 2020b). Significant variations were observed among traits. Qualitative traits such as leaf colour, petiole colour, stem colour and stem hairiness were associated with medicinal and nutritional values in the species.

2.7 Nutritional and phytochemical compounds diversity in *Gynandropsis gynandra*

Nutrients found in *G. gynandra* include minerals, vitamins, proteins, and dietary phytochemicals. Vitamins reported in the species are vitamins C, A, E, B1, B2, and B9, while minerals are iron, zinc, copper, potassium, magnesium, sodium, calcium, phosphorus and manganese (Sogbohossou et al. 2019; Moyo and Aremu 2021; Omondi et al. 2017b; van Jaarsveld et al. 2014; Schönfeldt and Pretorius 2011). *Gynandropsis gynandra* leaves are also an important source of proteins and fatty acids (Glew et al. 2009; Yuan et al. 2021), including essential amino acids (histidine, isoleusine, leucine, lysine, methionine, phenylalanine, threonine, valine) (Glew et al. 2009). Dietary phytochemicals include numerous secondary metabolites, such as flavonoids, terpenoids, tannins, glucosinolates, aldehydes, ketones, sesquiterpenes and many other phenolic compounds (Sogbohossou et al. 2020; Omondi et al. 2017b; Neugart et al. 2017; Moyo et al. 2018; Moyo and Aremu 2021; Chataika et al. 2021). The origin of accessions drove the significant variation in secondary metabolite concentrations (Sogbohossou et al. 2020), leaf vitamin contents (Sogbohossou et al. 2019) and antioxidant activity (Chataika et al. 2021). Significant variation was observed among genotypes for minerals (Omondi et al. 2017b) and amino acids (Yuan et al. 2021). However, there has been no investigation of the variation in minerals, proteins and fatty acids in the species using accessions from different continents.

2.8 Ability to withstand biotic and abiotic stresses

In the current changing climate, biotic and abiotic stresses have become important traits for breeding programs. Assessing the ability of the species to withstand biotic and abiotic stresses is key towards the full exploitation of the crop potential. Current biotic stresses affecting the species include pests and diseases and these have been discussed in the section under constraints. To date, efforts towards addressing biotic stresses are still in the early stages with the inventory of entomofauna associated with the species (Mnene 2021). Abiotic stresses include heat and drought stress. Mosenda et al. (2020a) assessed the drought tolerance potential of 14 selected Kenyan spider plant genotypes by comparing their potential under varying water regimes of 40%, 60%, and 80% field capacity. The authors observed a reduction in growth and leaf yield ranging from 25.7% to 74.2. The drought-tolerant genotypes identified were Kakamega, Kuria, Baringo, GBK040449, Homabay and GBK-032210. Further studies are needed to assess drought tolerance in the species using a large collection of the species.

2.9 Natural variation in photosynthesis traits in spider plant

Gynandropsis gynandra is a C4 plant species with significant variation in photosynthesis performance (Reeves et al. 2018). This variation was also observed to be origin dependent and two major groups; the East/Southern African and Asian/West African groups were identified based on DNA sequencing, phylogenetic reconstruction and photosynthesis traits of nine accessions from Asia and East, Southern and West Africa (Reeves et al. 2018). The authors reported the East/Southern African genotypes to have lower core gene transcripts encoding C4 enzymes and water use efficiency, higher stomatal conductance and transpiration, higher vein and stomatal density, and smaller bundle sheath cell and area sizes compared to Asia/West African genotypes. Photosynthesis is an important trait for improving crop productivity and thus, exploiting this significant variation through an integrated breeding approach will increase crop productivity. This variation represents an important basis for photosynthesis breeding in the species.

2.10 Gene discovery for various traits in *Gynandropsis gynandra*

To date, only one study has reported quantitative trait loci (QTLs) associated with morphological and nutritional traits in *Gynandropsis gynandra* (Sogbohossou 2019). Using an F2 population of 219 individuals, Sogbohossou (2019) generated the first genetic map for the species using 269 segregating SNPs. Two quantitative trait loci were identified for flowering time and were located on linkage groups 7 and 9. A single QTL was identified for plant height on linkage group 3. Similarly, a single QTL was found on linkage groups 6 and 1 for ascorbic acid and lutein content, respectively. Moreover, two QTLs for leaf area on linkage groups 3 and 7 with the single plant height QTL and one QTL for leaf area were colocalized on linkage group 3. Three QTLs were reported for alpha-tocopherol and were located on linkage groups 9, 13 and 16. Regarding the ratio of carotenoids and tocopherols, QTLs on linkage groups 14 and 16 were identified. It is important to highlight that some of these identified QTLs had pleiotropic effects and were localized on linkage groups 3, 7, 9 and 16. These QTLs were found to be associated with known genes in *Arabidopsis thaliana* for flowering time, plant height leaf area, and tocopherol and carotenoid biosynthesis (Sogbohossou 2019). However, these identified QTLs need validation. Consequently, using a large natural population collection and developed advanced mapping populations (RILs, MAGIC, etc.) and applying genome-wide association studies (GWAS) and QTL mapping methods would be useful to validate these QTLs and to decipher genes associated with functional and farmers' preferred traits.

2.11 Ploidy and genomic resources

Genomic resources are key tools for accelerating genetic gain and cultivar release. An important resource generated includes the complete chloroplast genome sequence of *G. gynandra* (Shi et al. 2021). The size of the genome is 158,152 bp with 35.81% GC content. The genome provided insights into 131 genes, including seven rRNA, 37 tRNA, and 87 protein-coding genes (Shi et al. 2021). Another important resource is the reference genome under development through the initiative of the African Orphan Crops Consortium (Hendre et al. 2019). *Gynandropsis gynandra* is a diploid species whose number of chromosomes is 2n=34. The size of the spider plant genome was reported to be approximately 1 Gb (van den Bergh et al. 2014), which was later confirmed by an average genome size of 2.38 pg/2C obtained by Omondi et al. (2017a). Recently, with the improved draft genome, a size of approximately 750 Mb was reported by Sogbohossou (2019) with 1693 scaffolds characterized by an N50 of 1.4 Mbp. A total of 27 154 genes were identified and clustered into 15 955 gene families. The genome of *G. gynandra* is characterized by whole-genome duplication (Sogbohossou 2019), which facilitated the evolution of C4 photosynthesis in the species (Huang et al. 2021). With genome sequencing, Sogbohossou (2019) observed an association between genomic variation and geographical origins and suggested the African origin of the species, with Asian and West African populations being closed and recently divergent from East and Southern African populations. More investigations are needed to clarify the origin of the species as well as its route of colonization.

2.12 Participatory varietal selection

Efforts are ongoing to select improved varieties for farmers. These led to the identification of different stakeholders (consumers, farmers, and retailers) in the value chain preferences (Mutoro 2019; Sogbohossou et al. 2018a; Kiebre et al. 2015b; Ndinya et al. 2020; Cleome Consortium 2017). Participatory evaluation of nine genotypes for farmers' and consumers' preferences identified genotypes UGSF36, MLSF17, UGSF9 UGSF14, and Control for adoption by farmers. In addition, genotypes such as commercial UG-15 and UG-23 from the Word Vegetable Centre, Eastern and Southern Africa were selected from cultivation by farmers in Kenya (Ndinya et al. 2020).

2.13 Breeding spider plant: current status, knowledge gap and future perspective

Numerous efforts are ongoing to improve the species. However, most cultivars used by farmers are landraces with seeds collected from on-farm protected plants, bought from local markets, borrowed from neighbours or collected from the wild (Sogbohossou et al. 2018a; Muasya et al. 2009; Thovhogi et al. 2021b). However, there are reports of some lines but not improved being commercialized by some seed companies in Tanzania and Kenya (Shilla et al. 2019). These lines were selected from some advanced lines developed by the World Vegetable Center – East and Southern Africa through mass selection from their collection from various African countries (Shilla et al. 2019). Additionally, the Department of Horticulture of Jomo Kenyatta University of Agriculture and Technology (Kenya), through the Professor Mary Abukutsa-Onyango, has released some cultivars selected from the evaluation of local Kenyan accessions (Shilla et al. 2019). A commercial variety named Saga was reported in Kenya and sold by the Simlaw seed company (Mosenda et al. 2020b). Recent genetic diversity analysis of 30 entries, including farmers' cultivars, gene bank accessions and advanced breeding lines of the World Vegetable Center (Tanzania), and lines of Jomo Kenyatta University of Agriculture and Technology (Kenya) revealed two major groups; group 1 comprising farmers' cultivars and JKUAT lines and group 2 including WorldVeg gene bank accessions and advanced lines (Omondi et al. 2017a). This shows a clear genetic differentiation between farmers' cultivars and genebank's accessions and advanced lines. The authors observed a high heterozygosity level in the genotypes. In addition, Thovhogi et al. (2021c) observed a high level of heterozygosity in South African accessions compared to WorldVeg genotypes. Farmers' cultivars are highly heterozygous, which might be due to the cross-pollinated nature of the species as the flowers are visited by numerous pollinators. Farmers' cultivars are not pure, and continuous breeding efforts are needed to assist farmers.

To the best of our knowledge, research activities toward breeding *G. gynandra* are ongoing at the University of Abomey-Calavi (Benin), University of KwaZulu-Natal (South Africa), Joseph KI-ZERBO University (Burkina-Faso), Wageningen University (The Netherlands), University of Cambridge (United Kingdom), Jomo Kenyatta University of Agriculture and Technology (Kenya), World Vegetable Center – East and Southern Africa (Tanzania), Egerton University (Kenya), University of Namibia (Namibia), Marondera University of Agricultural Sciences and Technology (Zimbabwe). Luckily, Sogbohossou et al. (2018a) developed a

roadmap for breeding *Gynandropsis gynandra* and highlighted seven key steps in ensuring genome-assisted breeding after farmers and consumers' consultation, including: (i) germplasm collection and management; (ii) product target definition and refinement; (iii) characterization of the genetic control of key traits; (iv) design of the process for cultivar development; (v) integration of genomic data to optimize that process; (vi) multi-environmental participatory testing and end-user evaluation; and (vii) crop value chain development. More importantly, the authors detailed each step with focus on improving farmers' preferred traits (leaf yield, resistance to biotic and abiotic stresses) as well as retailers and consumers desired traits (phytonutrient content, organoleptic quality, and post-harvest management).

Based on this roadmap, significant progress has been made in steps 1 and 2 with very limited in other steps, which is concentrated in some African countries (Benin, Kenya, South Africa, Namibia and Malawi). However, these steps need to be reinforced especially in expanding the germplasm collection and characterisation across the species' distribution area, including African and Asian countries, America and Oceania. Efforts are still needed for other steps. Regarding step 2, numerous types of cultivars could be developed and cultivars exploiting out-crossing could benefit farmers but their cost-effectiveness needs to be assessed considering the socio-economic context of the target environment. Regarding trait preferences, it is clear that leaf yield is the ultimate trait. Other farmers preferred traits include leaf yield related traits (plant height and the number of leaves), broad leaves, late flowering, good germination and resistance to pests and diseases (Ndinya et al. 2020; Kiebre et al. 2015; Mutoro 2019; Cleome Consortium 2017). These traits should be refined in the target environment. Retailers and consumers traits should be considered and were highlighted in section 2.5.

In step 3, knowledge associated with quantitative genetic parameters of key and functional traits in the species is still lacking. This includes understanding the combining ability (general and specific combining ability), narrow sense heritability, genetic gain, and gene action controlling the identified traits. To this end, exploiting the worldwide collection available is required and using various mating designs, namely diallel and North Carolina Design. This knowledge will influence the types of cultivars that can be developed for the species to maximize productivity. However, considering the fact that the species is predominantly outcrossing, heterosis or hybrid vigour might occur and this needs to be quantified. This step could be associated with steps 4 and 5. Therefore, the generated populations in step 3 could serve as inputs for step 4 to develop improved cultivars (pure lines, hybrid, synthetic cultivars). Specifically, lines and crosses showing good combining ability will be used in selecting

improved cultivars for release. The generated populations in step 3 and 4 could employed in gene discovery (step 5). Genes associated with functional traits would be important findings for future studies to drive the increase in genetic gain. This is achievable through the improvement of natural population collection and the development of advanced mapping populations (RILs, MAGIC, etc.) and the use of genome-wide association studies (GWAS) and QTL mapping methods. This is part of step 6 of the roadmap. Increased genetic gain in the species could be realized by taking advantage of existing knowledge on major crops on genomic selection. Single trait genomics models such as Bayes A, Bayes B (Meuwissen et al. 2001), Bayes C (de los Campos et al. 2013), Bayesian LASSO, Bayesian Ridge Regression (de los Campos et al. 2013; Pérez and de Los Campos 2014), ridge regression–best linear unbiased prediction (rrBLUP), Reproducing Kernel Hilbert Spaces (RKHS) regression, mixed model using Newton-Raphson algorithm (NR), and genomic best linear unbiased prediction (GBLUP) could be investigated. More importantly, genomic selection methods such as the multi-traits random forest (RF), the multi-traits genomic best linear unbiased predictor (GBLUP), and the multi-traits partial least squares (PLS) could be of interest giving their advantage to exploit the correlation between traits. In genomic selection, training population is key and depends on the type of cultivars. For instance, multi-hybrid populations will be useful when hybrid is the end product. The best training population could be determined using the TrainSel R Package (Akdemir et al. 2021).

Knowledge of the genotype x environment interaction effects in the species is important to develop stable genotypes for wide adoption. This could be integrated into steps 3, 4 and 5 and was part of the step 7 of the roadmap. Various methods including AMMI and GGE Biplot (Yan 2014; Yan et al. 2007) could be investigated, particularly in evaluating genotypes in various environment in their geographical origin. The genotype x environment interaction could also be included in the genomic selection using the EnvRtype R package (Costa-Neto et al. 2021). With the recent development of machine and deep learning methods, exploring the applicability of these methods in the species is plausible. Deep learning methods include Multilayer Perceptron (MLP) and Convolutional Neural Network (CNN) while the machine learning methods comprise Support Vector Machine (SVM) and Random Forests (RF). Speed breeding will enhance the genetic gain in the species given its cropping cycle of 3 to 4 months. This will increase the number of generations per year and therefore reduce the breeding cycle. However, given each species' specific requirements, the speed breeding protocol should be optimized for the species. Gene editing is another technology that will foster the breeding of the species,

particularly for traits with undesirable associations. Given the high cost of nutritional value assessment, establishing an association between morphological and nutritional characteristics would help in simultaneous selection for high yield and nutritional values. The natural variation in photosynthesis of the species offers the opportunity to improve the photosynthesis traits, including leaf gas exchange and chlorophyll fluorescence parameters, in the species for improved productivity.

2.14 Conclusion

This review pointed out the evidence of geographical signature in the genetic variability in *G. gynandra* for morphological traits, leaf vitamins and health-promoting compounds. This variability represents an important resource for improving species productivity. However, knowledge of quantitative genetic parameters and genes controlling functional traits in species is still lacking. Understanding the genotype-by-environment interaction in the species is needed to guide breeding for specific or broad adaptation. Taking advantage of recent advances in molecular breeding (GWAS, GS), gene editing, high-throughput phenotyping and speed breeding will enhance genetic gain in the species.

References

Abukutsa-Onyango MO (2007) The diversity of cultivated African leafy vegetables in three communities in western Kenya. Afr J Food Agric Nutr Dev 7 (3):1-15. 10.18697/ajfand.14.IPGRI1-3

Achigan-Dako EG, Pasquini MW, Assogba-Komlan F, N'danikou S, Yédomonhan H, Dansi A, Ambrose-Oji B (2010) Traditional vegetables in Benin. Institut National des Recherches Agricoles du Bénin. Imprimeries du CENAP, Cotonou

Achigan-Dako EG, Sogbohossou DEO, Houdegbe CA, Salaou MA, Sohindji FS, Blalogoe J, Chataika BY, Zohoungbogbo HF, Adje CAO, Fassinou Hotegni NV, Francisco R, Abukutsa-Onyango MO, Schranz ME (2021) Ten years of *Gynandropsis gynandra* research for improvement of nutrient-rich leaf consumption: lessons learnt and way forwards. Annu Rev Plant Biol 4 (3):767-812. 10.1002/9781119312994.apr0774

Adeka RK (2020) Determination of variation in morphology and leaf bitterness in spider plant (*Gynandropsis gynandra* (L.) Briq. across aix agroecological zones in Kenya. MSc Thesis, University of Nairobi,

Ajaiyeoba EO, Onocha PA, Olarenwaju OT (2001) In vitro anthelmintic properties of *Buchholzia coriaceae* and *Gynandropsis gynandra* extracts. Pharm Biol 39 (3):217-220. 10.1076/phbi.39.3.217.5936

Akdemir D, Rio S, Isidro y Sánchez J (2021) TrainSel: An R Package for Selection of Training Populations. Front Genet 12:655287. doi:10.3389/fgene.2021.655287

Ayua E, Mugalavai V, Simon J, Weller S, Obura P, Nyabinda N (2016) Ascorbic acid content in leaves of Nightshade (*Solanum* spp.) and spider plant (*Cleome gynandra*) varieties grown under different fertilizer regimes in Western Kenya. Afr J Biotechnol 15 (7):199-206. 10.5897/AJB2015.14936

Bala A, Kar B, Haldar PK, Mazumder UK, Bera S (2010) Evaluation of anticancer activity of *Cleome gynandra* on Ehrlich's Ascites Carcinoma treated mice. J Ethnopharmacol 129 (1):131-134. 10.1016/j.jep.2010.03.010

Baskin CC, Baskin JM (2014) Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination. 2nd edn. Elsevier Inc., San Diego, USA

Blalogoe SJ (2019) The physiology of germination and dormancy in seeds of *Gynandropsis gynandra* L. Briq syn *Cleome gynandra* L.(Cleomaceae). MSc Thesis, University of KwaZulu-Natal

Blalogoe JS, Odindo AO, Sogbohossou EOD, Sibiya J, Achigan-Dako EG (2020) Origin-dependence of variation in seed morphology, mineral composition and germination percentage in *Gynandropsis gynandra* (L.) Briq. accessions from Africa and Asia. BMC Plant Biol 20 (1):168. 10.1186/s12870-020-02364-w

Chand J, Panda SR, Jain S, Murty USN, Das AM, Kumar GJ, Naidu VGM (2022) Phytochemistry and polypharmacology of cleome species: A comprehensive Ethnopharmacological review of the medicinal plants. J Ethnopharmacol 282:114600. 10.1016/j.jep.2021.114600

Chandradevan M, Simoh S, Mediani A, Ismail IS, Abas F (2020) 1H NMR-based metabolomics approach in investigating the chemical profile, antioxidant and anti-inflammatory activities of *Gynura procumbens* and *Cleome gynandra*. Plant Foods Hum Nutr 75 (2):243-251. 10.1007/s11130-020-00805-3

Chataika BY, Akundabweni LS-M, Houdegbe AC, Achigan-Dako EG, Sibiya J, Masamba K (2021) Dietary phytochemical screening of spider plant (*Gynandropsis gynandra* (L.)

Briq.) accessions from Africa and Asia to identify genotypes for use in nutraceutical breeding. Front Sustain Food Syst 5 (344). 10.3389/fsufs.2021.733909

Chataika BY, Akundabweni LS-M, Sibiya J, Achigan-Dako EG, Sogbohossou DEO, Kwapata K, Awala S (2022) Major production constraints and spider plant [*Gynandropsis gynandra* (L.) Briq.] traits preferences amongst smallholder farmers of Northern Namibia and Central Malawi. Front Sustain Food Syst 6:831821. doi:10.3389/fsufs.2022.831821

Chweya JA, Mnzava NA (1997) Cat's whiskers. *Cleome gynandra* L. Promoting the conservation and use of underutilized and neglected crops. 11. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy

Cleome Consortium (2017) Utilizing the genome of *Cleome gynandra* for the development of improved cultivars for West and East African markets - Annual progress report 2016. Cleome Consortium, Cotonou

Costa-Neto G, Galli G, Carvalho HF, Crossa J, Fritsche-Neto R (2021) EnvRtype: a software to interplay enviromics and quantitative genomics in agriculture. G3 11 (4):jkab040

de los Campos G, Pérez P, Vazquez AI, Crossa J (2013) Genome-Enabled Prediction Using the BLR (Bayesian Linear Regression) R-Package. In: Gondro C, van der Werf J, Hayes B (eds) Genome-Wide Association Studies and Genomic Prediction. Humana Press, Totowa, NJ, pp 299-320. doi:10.1007/978-1-62703-447-0_12

Dickson N (2007) Opportunities for increased production, utilization and income generation from African leafy vegetables in Zambia. African Journal of Food, Agriculture, Nutrition and Development 7 (1):1-20

Ekpong B (2009) Effects of seed maturity, seed storage and pre-germination treatments on seed germination of cleome (*Cleome gynandra* L.). Sci Hortic 119:236-240. 10.1016/j.scienta.2008.08.003

FAO, IFAD, UNICEF, WFP, WHO (2021) The State of Food Security and Nutrition in the World 2021. Transforming food systems for food security, improved nutrition and affordable healthy diets for all. FAO, Rome. https://doi.org/10.4060/cb4474en

Feodorova TA, Voznesenskaya EV, Edwards GE, Roalson EH (2010) Biogeographic patterns of diversification and the origins of C4 in Cleome (Cleomaceae). Syst Bot 35 (4):811-826

Ghogare UR, Nirmal SA, Patil RY, Kharya MD (2009) Antinociceptive activity of *Gynandropsis gynandra* leaves. Nat Prod Res 23 (4):327-333. 10.1080/14786410802047862

Glew RS, Amoako-Atta B, Ankar-Brewoo G, Presley J, Chuang L-T, Millson M, Smith BR, Glew RH (2009) Non-cultivated plant foods in West Africa: Nutritional analysis of the leaves of three indigenous leafy vegetables in Ghana. Food 3 (1):39-42

Gonye E, Kujeke G, Edziwa X, Ncube A, Masekesa R, Icishahayo D, Matikiti A, Chabata I (2017) Field performance of spider plant (*Cleome gynandra* L.) under different agronomic practices. Afr J Food Agric Nutr Dev 17 (3):12179-12197. 10.18697/ajfand.79.15985

Gowele VF, Kinabo J, Jumbe T, Kirschmann C, Frank J, Stuetz W (2019) Provitamin A carotenoids, tocopherols, ascorbic acid and minerals in indigenous leafy vegetables from Tanzania. Foods 8 (1):35. 10.3390/foods8010035

Grubben G, Klaver W, Nono-Womdim R, Everaarts A, Fondio L, Nugteren JA, Corrado M (2014) Vegetables to combat the hidden hunger in Africa. Chron Hortic 54:24-32

Grubben GJH, Denton OA (2004) Plant resources of tropical Africa 2. Vegetables, vol. 668 pp. PROTA Foundation, Wageningen, Netherlands / Backhuys Publishers, Leiden, Netherlands/CTA, Wageningen, Netherlands,

Gupta AS, Chakravarty MM (1957) Studies on the seed for composition of desert plants. The component fatty acids of *Gynandropsis pentaphylla* seed fat. Sci Cul 23:306-307

HCDA (2014) Horticulture data 2011-2013 Validation report. Horticultural Crops Development Authority, Nairobi, Kenya

Hendre PS, Muthemba S, Kariba R, Muchugi A, Fu Y, Chang Y, Song B, Liu H, Liu M, Liao X, Sahu SK, Wang S, Li L, Lu H, Peng S, Cheng S, Xu X, Yang H, Wang J, Liu X, Simons A, Shapiro H-Y, Mumm RH, Van Deynze A, Jamnadass R (2019) African Orphan Crops Consortium (AOCC): status of developing genomic resources for African orphan crops. Planta 250 (3):989-1003. 10.1007/s00425-019-03156-9

Houdegbe CA, Sogbohossou EOD, Achigan-Dako EG (2018) Enhancing growth and leaf yield in *Gynandropsis gynandra* (L.) Briq. (Cleomaceae) using agronomic practices to accelerate crop domestication. Sci Hortic 233:90-98. 10.1016/j.scienta.2018.01.035

Huang C-F, Liu W-Y, Lu M-YJ, Chen Y-H, Ku MSB, Li W-H (2021) Whole-Genome Duplication facilitated the evolution of C4 photosynthesis in *Gynandropsis gynandra*. Molecular Biology and Evolution 38 (11):4715-4731. 10.1093/molbev/msab200

Igoli JO, Masao CA, Orkpeh U, Nakamatte E, Yakumbur DT, Nnadozie T, Yunusa S, Vihior B, Manyi MM, Tor-Anyiin TA (2016) In vivo antimalarial activity of *Cleome gynandra* extracts. J Nat Prod Res Updates 2:19-29

Imbamba SK, Tieszen LL (1977) Influence of light and temperature on photosynthesis and transpiration in some C3 and C4 vegetable plants from Kenya. Physiol Plant 39 (4):311-316. doi:10.1111/j.1399-3054.1977.tb01890.x

Jiménez-Aguilar DM, Grusak MA (2015) Evaluation of minerals, phytochemical compounds and antioxidant activity of Mexican, Central American, and African green leafy vegetables. Plant Foods Hum Nutr 70 (4):357-364. 10.1007/s11130-015-0512-7

Kamotho GN, Mathenge PW, Muasya RM, Dulloo ME (2014) Effects of maturity stage, dessication and storage period on seed quality of Cleome (*Cleome gynandra* L.). Res Desk 3 (1):419-433

Kangai Munene A, Nzuve F, Ambuko J, Odeny D (2018) Heritability Analysis and Phenotypic Characterization of Spider Plant (*Cleome gynandra* L.) for Yield. Adv Agric 2018:11. 10.1155/2018/8568424

Kiebre Z, Bationa Kando P, Sawadogo N, Kiebre M, Sawadogo B, Traore RE, Sawadogo M, Zongo J-D (2017a) Évaluation de la diversité agromorphologique d'une collection de *Cleome gynandra* L. du Burkina Faso. J Appl Biosci 118:11768-11780

Kiebre Z, Bationo Kando P, Barro A, Sawadogo B, Kiebre M, Ouedraogo MH, Sawadogo M, Zongo JD (2017b) Estimates of genetic parameters of spider plant (*Cleome gynandra* L.) of Burkina Faso. Int J Agric Policy Res 5 (9):138-144. 10.15739/IJAPR.17.016

Kiebre Z, Bationo Kando P, Nanema RK, Sawadogo M, Zongo J-D (2015a) Caractérisation agromorphologique du caya blanc (*Cleome gynandra* L.) de l'Ouest du Burkina Faso. Int J Innov Appl Stud 11 (1):156-166

Kiebre Z, Bationo Kando P, Sawadogo N, Sawadogo M, Zongo J-D (2015b) Selection phenotypic interests for the cultivation of the plant *Cleome gynandra* L. in the vegetable garderns in Burkina-Faso. J Exp Biol Agric Sci 3 (3):288-297. 10.18006/2015.3(3).288.297

Kiebre Z, Bationo Kando P, Sawadogo N, Sawadogo M, Zongo J-D (2015c) Selection phenotypic interests for the cultivation of the plant *Cleome gynandra* L. in the vegetable garderns in Burkina-Faso. Journal of Experimental Biology and Agricultural Sciences 3 (3):288-297

Kocacinar F (2015) Photosynthetic, hydraulic and biomass properties in closely related C_3 and C_4 species. Physiol Plant 153 (3):454-466. doi:10.1111/ppl.12240

Kori M, Gaur K, Dixit V (2009) Investigation of immunomodulatory potential of *Cleome gynandra* Linn. Asian J Pharm Clin Res 2 (1):35-39

Kutsukutsa RT, Gasura E, Mabasa S, Ngadze E (2014) Variability in condensed tannins and bitterness in spider plant genotypes. Afr Crop Sci 22 (4):275-280

Kwarteng AO, Abogoom J, Adu Amoah R, Nyadanu D, Nyam CK, Ghunney T, Awuah E, Ziyaaba JZ, Ogunsanya JO, Orhin EE, Asiedu DD (2018) Phenomic characterization of twenty-four accessions of spider plant (*Cleome gynandra* L.) the Upper East region of Ghana. Sci Hortic 235:124-131. 10.1016/j.scienta.2018.02.046

Maroyi A (2013) Use of weeds as traditional vegetables in Shurugwi District, Zimbabwe. Journal of Ethnobiology and Ethnomedicine 9:60

Masinde P, Agong S (2011) Plant growth and leaf N of spiderplant (*Cleome gynandra* L.) genotypes under varying nitrogen supply. Afr J Hortic Sci 5:36-49

Matro SCAX (2015) Analyse du fonctionnement des jardins de case à *Cleome gynandra* dans le département du couffo. University of Abomey-Calavi, Abomey-Calavi

Maundu P, Achigan-Dako E, Morimoto Y (2009) Biodiversity of African Vegetables. In: Shackleton CM, Pasquini MW, Drescher AW (eds) African Indigenous Vegetables in Urban Agriculture. Earthscan, UK and USA, pp 65-104

Maundu PM, Njiro ES, Chweya J. A., Imungi JK, Seme EN (1999) Kenya. In: Chweya JA, Eyzaguirre PB (eds) The biodiversity of traditional leafy vegetables. International Plant Genetic Resources Institute, Rome, Italy, pp 51-84

Mavengahama S (2013) Yield response of bolted spider plant (*Cleome gynandra*) to deflowering and application of nitrogen top dressing. J Food Agric Environ 11 (3&4):1372-1374

Meuwissen THE, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157 (4):1819-1829

Mnene A (2021) Insect diversity and their suitable management on african indigenous vegetables in Western Kenya. University of Eldoret,

Mnzava NA, Chigumira FN (2004) *Cleome gynandra* L. In: Grubben GJH, Denton OA (eds) Plant Resources of Tropical Africa 2. Vegetables. PROTA Foundation, Wageningen, Netherlands/Backhuys Publishers, Leiden, Netherlands/CTA, pp 191-194

Mosenda E, Chemining'wa G, Ambuko J, Owino W (2020a) Effect of water stress on growth and yield components of selected spider plant accessions. J Med Active Plants 9 (2):81-97

Mosenda E, Chemining'wa G, Ambuko J, Owino W (2020b) Assessment of agronomic traits of selected spider plant (*Cleome gynandra* L.) accessions. J Med Active Plants 9 (4):222-241. 10.7275/n1wf-6y77

Moyo M, Amoo SO, Aremu A, Gruz J, Šubrtová M, Jarošová M, Tarkowski P, Dolezal K (2018) Determination of mineral constituents, phytochemicals and antioxidant qualities of *Cleome gynandra*, compared to *Brassica oleracea* and *Beta vulgaris*. Front Chem 5:128. 10.3389/fchem.2017.00128

Moyo M, Aremu AO (2021) Nutritional, phytochemical and diverse health-promoting qualities of *Cleome gynandra*. Crit Rev Food Sci Nutr:1-18. 10.1080/10408398.2020.1867055

Moyo S, Serem J, Bester M, Mavumengwana V, Kayitesi E (2021) African green leafy vegetables health benefits beyond nutrition. Food Reviews International 37 (6):601-618

Muasya RM, Simiyu JN, Muui CW, Rao NK, Dulloo ME, Gohole LS (2009) Overcoming Seed Dormancy in *Cleome gynandra* L. to Improve Germination. Seed Technol 31 (2):134-143

Mutoro K (2019) Analysis of preference attributes for spider plant genotypes in Kenya: Implications for breeders and farmers. Int J Hortic Floric 7 (8):001-004. 10.46882/IJHF/1092

Narendhirakannan RT, Kandaswamy M, Subramanian S (2005) Anti-inflammatory activity of *Cleome gynandra* L. on hematological and cellular constituents in adjuvant-induced arthritic rats. J Med Food 8 (1):93-99. 10.1089/jmf.2005.8.93

Narsimhulu BL, Suresh Y, Rajasekar G, Lavanya T, Philip GH, Mohiyuddin SS, Reddy SR (2019) Evaluation of hepatoprotective and nephroprotective activity of methanolic extract of *Cleome viscosa* and *Cleome gynandra* in STZ-induced diabetic rats. Pharma Innov J 8 (2):574-581

Ndinya C, Onyango E, Dinssa F, Odendo M, Simon JE, Weller S, Thuranira E, Nyabinda N, Maiyo N (2020) Participatory variety selection of three African leafy vegetables in Western Kenya. J Med Active Plants 9 (3):145-156. 10.7275/mkq0-3p85

Neugart S, Baldermann S, Ngwene B, Wesonga J, Schreiner M (2017) Indigenous leafy vegetables of Eastern Africa—A source of extraordinary secondary plant metabolites. Food Res Int 100:411-422. 10.1016/j.foodres.2017.02.014

Nono-Womdim R, Ojiewo C, Abang M, Oluoch MO (2012) Good Agricultural Practices for African Indigenous Vegetables. ISHS, Gent-Oostakker, Belgium

Ochuodho JO (2005) Physiological basis of seed germination in Cleome gynandra L. PhD Thesis, University of KwaZulu-Natal

Ochuodho JO, Modi AT (2007) Light-induced transient dormancy in Cleome gynandra L. seeds. African Journal of Agricultural Research 2(11):587-591

Omondi EO, Debener T, Linde M, Abukutsa-Onyango M, Dinssa FF, Winkelmann T (2017a) Mating biology, nuclear DNA content and genetic diversity in spider plant (*Cleome gynandra*) germplasm from various African countries. Plant Breed 136:578-589. 10.1111/pbr.12485

Omondi EO, Engels C, Nambafu G, Schreiner M, Neugart S, Abukutsa-Onyango M, Winkelmann T (2017c) Nutritional compound analysis and morphological characterization of spider plant (*Cleome gynandra*)-an African indigenous leafy vegetable. Food Res Int 100:284-295. 10.1016/j.foodres.2017.06.050

Onyango C, Kunyanga C, Ontita E, Narla R, Kimenju J Production, utilisation and indigenous knowledge of spider plant in Kenya. In: African Crop Science Conference Proceedings, 2013a. pp 925-930

Onyango CM, Kunyanga CN, Ontita EG, Narla RD, Kimenju JW (2013b) Current status on production and utilization of spider plant (*Cleome gynandra* L.) an underutilized leafy vegetable in Kenya. Genet Resour Crop Evol 60:2183-2189. 10.1007/s10722-013-0036-7

Padulosi S, King EIO, Hunter D, Swaminathan M (2021) Orphan Crops for Sustainable Food and Nutrition Security: Promoting Neglected and Underutilized Species. Routledge,

Raju AS, Rani DS (2016) Reproductive ecology of *Cleome gynandra* and *Cleome viscosa* (Capparaceae). Phytol Balc 22 (1):15-28

Ravichandra B, Ram PS, Saritha C, Shankaraiah P (2014) Anti diabetic and anti dyslipidemia activities of *Cleome gynandra* in alloxan induced diabetic rats. J Pharmacol Toxicol 9 (1):55-61. 10.3923/jpt.2014.55.61

Reeves G, Singh P, Rossberg TA, Sogbohossou EOD, Schranz ME, Hibberd JM (2018) Natural variation within a species for traits underpinning C4 photosynthesis. Plant Physiol 177 (2):504. 10.1104/pp.18.00168

Runnie I, Salleh MN, Mohamed S, Head RJ, Abeywardena MY (2004) Vasorelaxation induced by common edible tropical plant extracts in isolated rat aorta and mesenteric vascular bed. J Ethnopharmacol 92 (2):311-316. 10.1016/j.jep.2004.03.019

Schiessl SV, Huettel B, Kuehn D, Reinhardt R, Snowdon RJ (2017) Flowering Time Gene Variation in Brassica Species Shows Evolutionary Principles. Frontiers in Plant Science 8:1742. 10.3389/fpls.2017.01742

Schippers RR (2004) Légumes africaines indigènes - Présentattion des espèces cultivees. Margraf Publishers GmbH, Scientifics Books, Wageningen, Pays-Bas

Schönfeldt HC, Pretorius B (2011) The nutrient content of five traditional South African dark green leafy vegetables - A preliminary study. J Food Compos Anal 24:1141-1146. 10.1016/j.jfca.2011.04.004

Seeiso MT, Materechera SA (2012) Yield response of the African indigenous leafy vegetable *Cleome gynandra* to application of cattle and goat kraal manure and harvesting techniques. J Food Agric Environ 10 (3&4):789-794. 10.1234/4.2012.3514

Shi D, Li J, Li Y, Li Y, Xie L (2021) The complete chloroplast genome sequence of Gynandropsis gynandra (Cleomaceae). Mitochondrial DNA Part B 6 (7):1909-1910. 10.1080/23802359.2021.1935339

Shilla O, Dinssa FF, Omondi EO, Winkelmann T, Abukutsa-Onyango MO (2019) *Cleome gynandra* L. origin, taxonomy and morphology: A review. African Journal of Agricultural Research 13 (32):1568-1583. 10.5897/AJAR2019.14064

Silué D (2009) Spider plant: An indigenous species with many uses. The world vegetable centre, AVRDC publication:09-719

Singh H, Mishra A, Mishra AK (2018) The chemistry and pharmacology of *Cleome* genus: A review. Biomed Pharmacother 101:37-48. 10.1016/j.biopha.2018.02.053

Sithanantham S, Matoka CM, Maundu M, Jakari M, Agong SG (2005) Integrated crop protection research for sustainable production of indigenous vegetable crops in Eastern Africa. Paper presented at the Proceedings of the Third Horticulture Workshop on Sustainable Horticultural Production in the Tropics, Maseno University, MSU ,Maseno, Kenya, 26th - 29th November 2003

Smith IF, Eyzaguirre P (2007) African leafy vegetables: their role in the World Health Organization's global fruit and vegetable initiative. Afr J Food Agric Nutr and Dev 7 (3&4):1-8

Sogbohossou DEO (2019) Orphan no more : Ethnobotany and genetic analysis of leaf yield and secondary metabolites content in *Gynandropsis gynandra* (Cleomaceae). PhD Thesis, Wageningen University, Wageningen

Sogbohossou EOD, Achigan-Dako EG, Maundu P, Solberg S, Deguenon EMS, Mumm RH, Hale I, Van Deynze A, Schranz ME (2018a) A roadmap for breeding orphan leafy vegetable species: a case study of *Gynandropsis gynandra* (Cleomaceae). Hortic Res 5 (1):2. 10.1038/s41438-017-0001-2

Sogbohossou EOD, Achigan-Dako EG, Mumm R, de Vos RCH, Schranz ME (2020) Natural variation in specialised metabolites production in the leafy vegetable spider plant (*Gynandropsis gynandra* L. (Briq.)) in Africa and Asia. Phytochemistry 178:112468. 10.1016/j.phytochem.2020.112468

Sogbohossou EOD, Achigan-Dako EG, van Andel T, Schranz ME (2018b) Drivers of management of spider plant (*Gynandropsis gynandra*) across different socio-linguistic groups in Benin and Togo. Econ Bot:1-25. 10.1007/s12231-018-9423-5

Sogbohossou EOD, Kortekaas D, Achigan-Dako EG, Maundu P, Stoilova T, Van Deynze A, de Vos RCH, Schranz ME (2019) Association between vitamin content, plant

morphology and geographical origin in a worldwide collection of the orphan crop *Gynandropsis gynandra* (Cleomaceae). Planta 250:933–947. 10.1007/s00425-019-03142-1

Thovhogi F, McHau GR, Gwata ET, Ntushelo N (2021a) Evaluation of leaf mineral, flavonoid, and total phenolic content in spider plant germplasm. Molecules 26 (12):3600. 10.3390/molecules26123600

Thovhogi F, Gwata ET, McHau GRA, Ntutshelo N (2021b) Perceptions of end-users in Limpopo Province (South Africa) about the Spider plant (Cleome gynandra L.). Genetic Resources and Crop Evolution 68 (2):605-614. doi:10.1007/s10722-020-01009-z

Thovhogi F, Gwata ET, McHau GRA, Safodien SS, Koopman T (2021c) Molecular characterization of spider plant (*Cleome gynandra*) accessions using SSR markers. Agronomy 11 (11):2206. doi:10.3390/agronomy11112206

van den Bergh E, Külahoglu C, Bräutigam A, Hibberd JM, Weber APM, Zhu X-G, Eric Schranz M (2014) Gene and genome duplications and the origin of C4 photosynthesis: Birth of a trait in the Cleomaceae. Current Plant Biology 1:2-9. https://doi.org/10.1016/j.cpb.2014.08.001

van Jaarsveld P, Faber M, van Heerden I, Wenhold F, van Rensburg WJ, van Averbeke W (2014) Nutrient content of eight African leafy vegetables and their potential contribution to dietary reference intakes. J Food Compos Anal 33:77-84. 10.1016/j.jfca.2013.11.003

Vandebroek I, Voeks R (2018) The Gradual Loss of African Indigenous Vegetables in Tropical America: A Review. Econ Bot 72 (4):543-571. 10.1007/s12231-019-09446-3

Wangolo EE, Onyango CM, Gachene CK, Mong'are PN (2015) Effects of shoot tip and flower removal on growth and yield of spider plant (*Cleome gynandra* L.) in Kenya. Am J Exp Agric 8 (6):367-376. 10.9734/AJEA/2015/17271

Wasonga DO (2014) Phenotypic characterization of Kenyan and South African spider plant (*Cleome gynandra* L.) ecotypes. Master of Science, University of Nairobi, Nairobi

Weinberger K, Msuya J (2004) Indigenous Vegetables in Tanzania- Significance and Prospects. Technical Bulletin No. 31. AVRDC-The World Vegetable Center, Shanhua, Taiwan

Weinberger K, Pichop GN (2009) Marketing of African indigenous vegetables along urban and peri-urban supply chains in sub-Saharan Africa. In: Shackleton C, Pasquini M, Drescher A (eds) African indigenous vegetables in urban agriculture. Earthscan, UK,, pp 225-244

Wu T-h, Solberg SO, Yndgaard F, Chou Y-Y (2018) Morphological patterns in a world collection of *Cleome gynandra*. Genet Resour Crop Evol 65 (1):271-283. 10.1007/s10722-017-0529-x

Yan W (2014) Crop variety trials: Data management and analysis. John Wiley & Sons Inc., New York. doi:10.1002/9781118688571

Yan W, Kang MS, Ma B, Woods S, Cornelius PL (2007) GGE biplot vs. AMMI analysis of genotype-by-environment data. Crop science 47 (2):643-653

Yang R-Y, Keding GB (2009) Nutritional contributions of important African indigenous vegetables. In: Shackleton CM, Pasquini MW, Drescher AW (eds) African Indigenous Vegetables in Urban Agriculture. Earthscan, UK and USA, pp 105-143

Yuan B, Lyu W, Dinssa FF, Simon JE, Wu Q (2021) Free amino acids in African indigenous vegetables: Analysis with improved hydrophilic interaction ultra-high performance liquid chromatography tandem mass spectrometry and interactive machine learning. J Chromatogr A 1637:461733. 10.1016/j.chroma.2020.461733

Zorde M, Byrnes DR, Dinssa FF, Weller S, Simon JE (2020) Selection for delayed flowering time in response to long photoperiod to increase vegetative growth and multiple harvests in spider plant (*Cleome gynandra*). Journal of Medicinally Active Plants 9 (2):60-70

CHAPTER 3

Phenotypic variation in biomass and related traits among four generations advanced lines of Cleome (*Gynandropsis gynandra* L. (Briq.))

Abstract

Gynandropsis gynandra (spider plant) is an African leafy vegetable rich in minerals, vitamins and health-promoting compounds with nutraceutical and pharmaceutical potential. However, information on biomass productivity is limited and consequently constrains breeders' ability to select high-yielding genotypes and end-users to make decisions on suitable cultivation and production systems. This study aimed to assess the phenotypic variability in biomass and related traits in a collection of *G. gynandra* advanced lines to select elite genotypes for improved cultivar development. Seventy-one advanced lines selected from accessions originating from Asia, West Africa, East Africa and Southern Africa were evaluated over two years with two replicates in a greenhouse using a 9 x 8 alpha lattice design. Significant statistical differences were observed among lines and genotype origins for all fourteen biomass and related traits. The results revealed three clusters, with each cluster dominated by lines derived from accessions from Asia (Cluster 1), West Africa (Cluster 2), and East/Southern Africa (Cluster 3). The West African and East/Southern African groups were comparable in biomass productivity and superior to the Asian group. Specifically, the West African group had a low number of long primary branches, high dry matter content and flowered early. The East/Southern African group was characterized by broad leaves, late flowering, a high number of short primary branches and medium dry matter content and was a candidate for cultivar release. The maintenance of lines' membership to their group of origin strengthens the hypothesis of geographical signature in cleome diversity and genetic driver of the observed variation. High genetic variance, broad-sense heritability and genetic gains showed the potential to improve biomass yield and related traits. Significant and positive correlations among biomass per plant, plant height, stem diameter and leaf size showed the potential of simultaneous and direct selection for farmers' desired traits. The present results provide insights into the diversity of spider plant genotypes for biomass productivity and represent key resources for further improvement in the species.

Keywords: Breeding, *Cleome gynandra*, genetic diversity, genetic gain, leaf yield, spider plant.

3.1 Introduction

Gynandropsis gynandra (L.) Briq. (Syn. *Cleome gynandra* L.), commonly known as spider plant, is an African leafy vegetable with great potential in addressing micronutrient deficiency (Moyo and Aremu 2021), which affects more than two billion people worldwide, mainly in Asia and sub-Saharan Africa (Beal et al. 2017). The leaves of the species are rich in vitamins C, A, E, B1, B2, and B9 and minerals such as iron, zinc, calcium, copper, potassium, magnesium, manganese, phosphorus and sodium (Sogbohossou et al. 2019; Moyo and Aremu 2021; Omondi et al. 2017b; van Jaarsveld et al. 2014; Schönfeldt and Pretorius 2011). *Gynandropsis gynandra* leaves are also an important source of proteins and fatty acids (Glew et al. 2009; Yuan et al. 2021), including essential amino acids (histidine, isoleusine, leucine, lysine, methionine, phenylalanine, threonine, valine) (Glew et al. 2009). In addition, spider plant has several health-promoting properties, as it contains numerous secondary metabolites, such as flavonoids, terpenoids, tannins, glucosinolates, aldehydes, ketones, sesquiterpenes and many other phenolic compounds (Sogbohossou et al. 2020; Omondi et al. 2017b; Neugart et al. 2017; Moyo et al. 2018; Moyo and Aremu 2021; Chataika et al. 2021) with diverse pharmaceutical applications (plant extracts, drugs, etc.) (Achigan-Dako et al. 2021). The species is a prime resource for the pharmaceutical industry, as its extracts have several biological and pharmacological effects (Chand et al. 2022; Achigan-Dako et al. 2021; Moyo and Aremu 2021; Singh et al. 2018), including antimicrobial (fungi and bacteria), anthelmintic (Ajaiyeoba et al. 2001), antimalarial (Igoli et al. 2016), hepatoprotective (Narsimhulu et al. 2019), antiarthritic (Narendhirakannan et al. 2005), antioxidant, anti-inflammatory (Chandradevan et al. 2020), immunomodulatory (Kori et al. 2009), antinociceptive (Ghogare et al. 2009), anticancer (Bala et al. 2010), antidiabetic (Ravichandra et al. 2014) and vasodilatory (Runnie et al. 2004) activities. Promoting this vegetable will, therefore, contribute to fighting malnutrition, health promotion and income generation for stakeholders, including pharmaceutical companies and local communities.

Gynandropsis gynandra belongs to the Cleomaceae family and is found in tropical and subtropical areas across all continents but is used mainly by local communities in Africa and Asia (Feodorova et al. 2010; Sogbohossou et al. 2019; Vandebroek and Voeks 2018). The leaves, young and tender shoots and flowers are used to prepare stews and sauces which are eaten as vegetables. The species is also used in traditional medicine. For instance, Sogbohossou et al. (2018b) reported its utilization in curing more than 40 diseases in Togo and Benin and

various uses among Ewe, Adja, Fon, Holli, Waama, Gourmantche, and Zerma socioethnic groups. These uses in local pharmacopoeia together with the scientific evidence of large variation in health promoting compounds, support the development of cleome extracts and drugs (Achigan-Dako et al. 2021). *Gynandropsis gynandra* is a semicultivated crop mainly found near human settlements, along roadsides, irrigation canals and ditches, and cultivated fields or fallows as wild populations (Chweya and Mnzava 1997; Shackleton et al. 1998). The species is cultivated in home gardens and peri-urban and urban market gardening across sub-Saharan Africa (Sogbohossou et al. 2018b; Weinberger and Pichop 2009; Kiebre et al. 2015). The cultivation and commercialisation of the species are mainly done by women and provide substantial income for households (Matro 2015; Onyango et al. 2013; Sogbohossou et al. 2018b). The leaves are sold in open markets in many African countries (e.g., Kenya, Namibia, Benin, Tanzania, South Africa, Togo, Ghana, Burkina-Faso, Uganda) but also in supermarkets (e.g., Kenya) (Onyango et al. 2013; Matro 2015; Kiebre et al. 2015; Chweya and Mnzava 1997). The species production from home gardens generated a profit margin between 40% to 57% and production efficiency (benefit cost ratio) between 1.66 and 2.33 during the rainy season in the Adja community of Benin (Matro 2015). The demand for spider consumption is increasing across sub-Saharan African countries. For instance, spider plant production increased from 19 428 metric tons to 21 507 metric tons between 2012 and 2013, with an average increase of 50% in cultivated area in Kenya (HCDA 2014).

In areas where *G. gynandra* is cultivated, production constraints faced by farmers include poor germination, early flowering, low yield, insect pests and seed availability (Onyango et al. 2013; Matro 2015; Abukutsa-Onyango 2007). Studies addressing these constraints reported that dormancy was responsible for erratic germination, and treating seeds with gibberellic acid and preheating were found to be effective (Blalogoe et al. 2020; Muasya et al. 2009; Ekpong 2009), as well as storage for three or more months (Kamotho et al. 2014). Improving leaf yield, early flowering, and insect pest resistance can be achieved by developing improved agricultural practices and high-yielding cultivars. Most previous studies focused on establishing the best agronomic practices for improved yield and included optimal planting density, type and fertilizer application rates, planting date, stage of transplanting, harvesting frequency and techniques (cutting, uprooting whole plants, defoliation), deflowering, sowing depth and net cover colour (Houdegbe et al. 2018; Gonye et al. 2017; Ayua et al. 2016; Mavengahama 2013; Seeiso and Materechera 2012; Masinde and Agong 2011; Wangolo et al. 2015). In contrast,

limited studies thus far have addressed the genetic improvement of the species (Achigan-Dako et al. 2021).

Genetic improvement requires a better understanding of the genetic diversity in the species through morphological and genetic/genomic characterization. Many studies have assessed morphological diversity in *G. gynandra* using a countrywide collection (e.g., Ghana (Kwarteng et al. 2018), Burkina-Faso (Kiebre et al. 2017a), Kenya (Adeka 2020; Mosenda et al. 2020)), regionwide germplasm (e.g., Kenya and South Africa (Kangai Munene et al. 2018), East and Southern Africa (Omondi et al. 2017b)) and worldwide collection (Wu et al. 2018; Sogbohossou et al. 2019). It is worthwhile to highlight that some of these characterization studies were extended to nutritional values, including minerals (Omondi et al. 2017b), vitamins (Sogbohossou et al. 2019), and physiological traits (Reeves et al. 2018). Significant variations were observed among accessions with a strong association between their morphology and geographical origins (Sogbohossou et al. 2019; Wu et al. 2018). East-Southern African accessions were observed to have taller plants compared to Asian and West African accessions with shorter plants (Sogbohossou et al. 2019). Additionally, West African accessions were characterized by small leaves, and Asian and East-Southern African accessions had large leaves (Sogbohossou et al. 2019). This morphological differentiation was further supported by genomic characterization (Sogbohossou 2019). Genetic differentiation was also observed between farmer's cultivars and genebank's accessions and advanced lines (Omondi et al. 2017a). The considerable diversity observed represents a valuable resource for a successful breeding program.

However, most studies assessing morphological diversity in *G. gynandra* did not include leaf biomass yield. Those that included it were limited to regional accessions and advanced lines (Omondi et al. 2017b) and countrywide accessions (Kiebre et al. 2017a; Mosenda et al. 2020). Whereas farmers prefer traits in *G. gynandra* that include high leaf yield and related traits (plant height and the number of leaves), broad leaves, late flowering, good germination and resistance to pests and diseases (Ndinya et al. 2020; Kiebre et al. 2015; Mutoro 2019; Cleome Consortium 2017). Yield is the most important trait for farmers and breeding programs. Considering farmers' preferred traits in a breeding program is vital in the successful adoption of developed cultivars. Given the availability of worldwide collections, it is, therefore, important to assess the biomass potential of large germplasm collections.

Therefore, this study aimed to assess the phenotypic diversity in biomass yield and related traits among a worldwide collection of *Gynandropsis gynandra* advanced lines to select elite genotypes for breeding programs and large-scale dissemination. Specifically, the present study: (i) assessed the phenotypic variation in biomass and related traits in *G. gynandra* using advanced lines selected from Asian, West, East and Southern African accessions; (ii) determined the relationship between biomass yield and related traits; and (iii) identified the best-performing genotypes for biomass yield.

3.2 Materials and Methods

3.2.1 Plant Material

In this study, seventy-one advanced lines (Table 3.1) selected from accessions originating from Asia (18), West Africa (19), Eastern Africa (14) and Southern Africa (20) were evaluated. The accessions were obtained from the Laboratory of Genetics, Biotechnology and Seed Science of the University of Abomey-Calavi (Republic of Benin); the World Vegetable Center (Taiwan); the Kenya Resource Center for Indigenous Knowledge (Kenya); the Lilongwe University of Agriculture and Natural Resources (Malawi); the Namibia Botanical Gardens (Namibia); the Wageningen University and Research (Netherlands) and the University of Ouagadougou (Burkina-Faso). Accessions were self-pollinated for four generations to develop the advanced lines.

Table 3.1. List of advanced lines of *Gynandropsis gynandra* used in this study and their origin.

Genotype	Genebank holding of the original accession	Country of Origin	Generation of selfing	Region
EA1	National Museums of Kenya	Kenya	S4	East Africa
EA2	National Museums of Kenya	Kenya	S4	East Africa
EA3	National Museums of Kenya	Kenya	S4	East Africa
EA4	National Museums of Kenya	Kenya	S4	East Africa
WA1	University of Ouagadougou	Burkina-Faso	S4	West Africa
WA2	University of Ouagadougou	Burkina-Faso	S4	West Africa
EA5	National Museums of Kenya	Kenya	S4	East Africa
EA6	National Museums of Kenya	Kenya	S4	East Africa
WA3	University of Ouagadougou	Burkina-Faso	S4	West Africa
WA4	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi	Benin	S4	West Africa
WA5	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi	Benin	S4	West Africa

Genotype	Genebank holding of the original accession	Country of Origin	Generation of selfing	Region
WA6	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi	Benin	S4	West Africa
WA7	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi	Benin	S4	West Africa
WA8	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi	Benin	S4	West Africa
WA9	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi	Benin	S4	West Africa
WA10	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi	Benin	S4	West Africa
WA11	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi	Togo	S4	West Africa
WA12	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi	Togo	S4	West Africa
WA13	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi	Togo	S4	West Africa
WA14	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi	Togo	S4	West Africa
WA15	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi	Togo	S4	West Africa
WA16	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi	Togo	S4	West Africa
WA17	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi	Togo	S4	West Africa
WA18	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi	Togo	S4	West Africa
WA19	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi	Ghana	S4	West Africa
AS1	World Vegetable Center	Thailand	S4	Asia
AS2	World Vegetable Center	Lao People's Democratic Republic	S4	Asia
AS3	World Vegetable Center	Lao People's Democratic Republic	S4	Asia
AS4	World Vegetable Center	Lao People's Democratic Republic	S4	Asia
AS5	World Vegetable Center	Thailand	S4	Asia
AS6	World Vegetable Center	Thailand	S4	Asia
EA7	World Vegetable Center	Kenya	S4	East Africa
SA1	World Vegetable Center	Zambia	S4	Southern Africa

Genotype	Genebank holding of the original accession	Country of Origin	Generation of selfing	Region
AS7	World Vegetable Center	Lao People's Democratic Republic	S4	Asia
AS8	World Vegetable Center	Malaysia	S4	Asia
AS9	World Vegetable Center	Malaysia	S4	Asia
AS10	World Vegetable Center	Malaysia	S4	Asia
AS11	World Vegetable Center	Malaysia	S4	Asia
AS12	World Vegetable Center	Lao People's Democratic Republic	S4	Asia
EA8	World Vegetable Center	Uganda	S4	East Africa
EA9	World Vegetable Center	Uganda	S4	East Africa
EA10	World Vegetable Center	Uganda	S4	East Africa
EA11	World Vegetable Center	Uganda	S4	East Africa
SA2	World Vegetable Center	Malawi	S4	Southern Africa
SA3	World Vegetable Center	Malawi	S4	Southern Africa
EA12	World Vegetable Center	Kenya	S4	East Africa
EA13	World Vegetable Center	Kenya	S4	East Africa
SA4	World Vegetable Center	South Africa	S4	Southern Africa
SA5	World Vegetable Center	Zambia	S4	Southern Africa
AS13	World Vegetable Center	Taiwan	S4	Asia
SA6*	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi	Mozambique	S4	Southern Africa
EA14	National Museums of Kenya	Kenya	S4	East Africa
AS14	World Vegetable Center	Malaysia	S4	Asia
AS15	World Vegetable Center	Thailand	S4	Asia
AS16	World Vegetable Center	Lao People's Democratic Republic	S4	Asia
AS17	World Vegetable Center	Lao People's Democratic Republic	S4	Asia
SA7	Okakarara	Namibia	S4	Southern Africa
SA8	Otjiwarongo	Namibia	S4	Southern Africa
SA9	Lilongwe University of Agriculture and Natural Resources	Malawi	S4	Southern Africa
SA10	Lilongwe University of Agriculture and Natural Resources	Malawi	S4	Southern Africa
SA11	Mahenene Research Station	Namibia	S4	Southern Africa
SA12	Chitedze Research Station	Malawi	S4	Southern Africa
SA13	Namibia Botanical Gardens	Namibia	S4	Southern Africa
SA14	Namibia Botanical Gardens	Namibia	S4	Southern Africa
SA16*	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi	Zimbabwe	S4	Southern Africa
AS18	Wageningen University and Research	Malaysia	S4	Asia
SA17	Okakarara	Namibia	S4	Southern Africa
SA18	Lilongwe University of Agriculture and Natural Resources	Malawi	S4	Southern Africa

Genotype	Genebank holding of the original accession	Country of Origin	Generation of selfing	Region
SA19	Lilongwe University of Agriculture and Natural Resources	Malawi	S4	Southern Africa
SA20	Chitedze Research Station	Malawi	S4	Southern Africa
SA21*	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi	Zimbabwe	S4	Southern Africa

*, Provided to the Laboratory of Genetics, Biotechnology and Seed Science (GBioS) of University of Abomey-Calavi by Mr Tomas Massingue (Mozambique) and Dr Admire Shayanowako (Zimbabwe).

3.2.2 Experimental design and growth conditions

The advanced lines were evaluated in 2020 (September to December) and 2021 (January to April) under greenhouse conditions at the Controlled Environment Facility (29°46′ S, 30°58′ E) of the University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa. Each year, the evaluation was laid out in a 9 x 8 alpha design with two replications. Seeds were pretreated by heating at 40 °C for three days to improve germination before sowing in seedling trays filled with growing media. The seedling trays were established in the greenhouse, and germination was observed three days after planting. Seedlings were grown for four weeks in a nursery and transplanted in 10 litre pots with three plants per pot. Pots were filled with composted pine bark growing media. Basal fertilizer composed of N:P:K (2:3:2) at a dose of 150 kg ha^{-1} was applied before transplanting, and limestone ammonium nitrate (28% N) was applied as topdressing two weeks after transplanting at a dose of 100 kg ha^{-1}. Automated drip irrigation was used to water the plants with 1 litre per pot daily, while weeds were controlled manually. In 2020, the average temperature and relative humidity were 28 °C day/20 °C night and 78.5%, respectively. The average temperature and relative humidity were 31 °C day/22 °C night and 77.4%, respectively, in 2021.

3.2.3 Data collection

Fourteen agronomic traits, including time to 50% flowering (DFlow), stem diameter (StDiam), plant height (PHeight), number of primary branches (NPBr), primary branch length (PBrLeng), central leaflet length (CtLleng), central leaflet width (CtLwid), leaf width (Lwid), petiole length (Ptilleng), leaf area (LfArea), total fresh biomass per plant (FBiom), edible fresh biomass per plant (EDBiom), harvest index (HI) and dry matter content (DM), were assessed four weeks after transplanting. Time to 50% flowering were recorded as the number of days from the sowing date to the day when 50% of the plants in each pot flowered. The central

leaflet length (cm), central leaflet width (cm), leaf width (cm) and petiole length (cm) were collected on a fully developed primary leaf randomly selected on each plant using a ruler. The selected leaf was scanned using a Canon PIXMA G2411 scanner (Canon INC; Tokyo, Japan), and the resultant image was used to calculate leaf area using the R package "*LeafArea*" (Katabuchi 2015). Plant height (cm) was measured from the base to the tip of the most uppermost leaf of the plant with a tape measure, while the stem diameter was measured using a digital Vernier calliper at the plant collar. Each plant was harvested by cutting at a height of 15 cm above the ground as recommended by Houdegbe et al. (2018), and the resultant biomass was weighed to determine the total fresh biomass per plant (g plant^{-1}). The edible part (edible tender tips and leaves) of the total biomass was separated and weighed to record the edible fresh biomass per plant (g plant^{-1}). The ratio of edible biomass to total fresh biomass was computed and reported as the harvest index (HI). These measurements were taken on two plants out of the three plants per pot. For dry matter content (DM), edible biomass of the plants per genotype in each replicate was bulked, and a sample of 20 g was taken and oven-dried at 65 °C until constant mass, with the first measurement taken after 72 h. DM (%) was computed as DM = (dry weight)/(fresh weight) x 100.

3.2.4 Data analysis

The quality of data was assessed for outlier detection following Bernal-Vasquez et al. (2016) using the Bonferroni–Holm test based on studentized residuals at the significance level of 5%. The mean, minimum, maximum, coefficient of variation and standard deviation were generated to characterize the plant material using the function *describe* of the R package *"psych"* (Revelle 2019). The difference among regions of origin was tested using an analysis of variance or Kruskal–Wallis test, when necessary. Variance components across years were estimated by fitting a linear mixed-effect model using the restricted maximum likelihood (REML) implemented in the ASReml-R package version 4.1.0.160 (Butler et al. 2017) according to the following statistical model:

$$y_{ijkl} = \mu + Y_j + R_k(Y_j) + B_l[R_k(Y_j)] + G_i + GY_{ij} + \varepsilon_{ijkl} \quad (1)$$

in which y_{ijkl} was the phenotypic observation of the i^{th} line in the l^{th} incomplete block within the k^{th} replicate at the j^{th} year, μ was the overall mean, Y_j was the random effect of the j^{th} year, $R_k(Y_j)$ was the random effect of the k^{th} replicate within the j^{th} year, $B_l[R_k(Y_j)]$ was the random effect of the l^{th} incomplete block within the k^{th} replicate at the j^{th} year, G_i was the random effect

of the i^{th} line, GY_{ij} was the random effect of the interaction between the i^{th} line and the j^{th} year, and ε_{ijkl} was the random residual. Heterogeneous variances were assumed for residual effects in different years. The likelihood ratio test (Self and Liang 1987) was used to test the significance of the variance components using the function *lrt* implemented in the ASREML-R package. Standard broad-sense heritability (Holland et al. 2003) was calculated as follows:

$$H^2 = \sigma_G^2/(\sigma_G^2 + \sigma_{G\times Y}^2/n + \sigma_e^2/nr) \quad (2)$$

where σ_G^2 is the genotypic variance of the lines, $\sigma_{G\times Y}^2$ is the line × year interaction variance, σ_e^2 is the residual variance, r is the number of replications, and n is the number of years.

The phenotypic best linear unbiased predictors (BLUPs) were generated from model 1. BLUPs were used because they have good predictive accuracy over the best linear unbiased estimators (BLUEs) due to their high correlation with the true values and their ability to handle environmental effects and have been recommended for phenotypic selection in plant breeding (Piepho et al. 2008; Molenaar et al. 2018; Kleinknecht et al. 2013). The values refer to mean genotypic values and were used in further analyses. Pearson's correlation coefficients among all traits and their level of significance were calculated using the function *corr* from the R package *"Hmisc"* (Harrell Jr and Dupont 2021). Genotypic correlations among traits were estimated using META-R software (Alvarado et al. 2020). Both genotypic and phenotypic correlations were plotted using the *"metan"* R package (Olivoto and Lúcio 2020). A principal component analysis was performed using the *PCA* function implemented in the R *"FactoMineR"* package (Lê et al. 2008) to assess the relationship among the lines and the biomass and related traits. Furthermore, we performed hierarchical clustering on principal components (HCPC) to group the genotypes based on the measured traits, and the results were visualized using the *fviz_cluster* function of the R package *"factoextra"* (Kassambara and Mundt 2020).

The genetic advance (GA) for each trait was computed as $GA = i \times H^2 \times \sigma_P$, where σ_P was the phenotypic standard deviation, H^2 was the broad-sense heritability, and i was the standardized selection differential at the selection intensity of 5% (i = 2.06) (Singh and Chaudhary 1985). Genetic advance over mean (GAM) was further computed as $GAM = (GA/\mu) \times 100$, where μ was the overall mean and *GA* was the genetic advance of the trait. Genotypic, phenotypic and error coefficients of variation (GCV, PCV and ECV, respectively) were estimated according to Burton and DeVane (1953) as follows:

$$GCV\ (\%) = \frac{\sqrt{\sigma_G^2}}{\mu} \times 100 \quad (3)$$

$$PCV\ (\%) = \frac{\sqrt{\sigma_P^2}}{\mu} \times 100 \quad (4)$$

$$ECV\ (\%) = \frac{\sqrt{\sigma_e^2}}{\mu} \times 100 \quad (5)$$

in which σ_G^2 was the genotypic variance, σ_P^2 was the phenotypic variance, σ_e^2 was the residual variance, and μ was the overall mean. R software version 4.1.1 (R Core Team 2021) was used to perform all statistical analyses.

3.3 Results

3.3.1 Quantitative variation in biomass and related traits

A highly significant variation ($p < 0.001$) was observed among genotypes for all agronomic traits (Tables 3.2 and 3.3). The coefficient of variation evolved between 14.01% and 82.48%. Overall, lower values for dry matter content and higher values for primary branch length were observed. The average plant total fresh biomass and edible fresh biomass were 67.19 ± 2.67 g and 28.34 ± 1.08 g, respectively. As the second most variable trait, the plant total fresh biomass (CV = 63.59%) ranged from 2.10 g to 248.40 g, while the edible fresh biomass (CV = 61.08%) ranged between 1.20 g and 101.90 g per plant. The harvest index was 0.47 ± 0.01 on average with a range of 0.24-0.91. The spider plant genotypes flowered on average 60.14 ± 0.90 days after sowing, and time to 50% flowering ranged between 32 and 95 days after sowing. The plant height ranged from 13 cm to 117.5 cm, with an average of 70.6 ± 1.31 cm. The average number of primary branches was 10.7 ± 0.26 per plant and varied between 2.5 and 23.5. The single leaf area ranged from 5.64 to 147.76 cm^2 with an average of 53.22 ± 1.66 cm^2. The dry matter content was 10.67 ± 0.09% on average, with a range of 7.60-15.42.

The distribution frequency of all agronomic traits according to the regions of origin of the lines is presented in Figure 3.1. Significant differences ($p < 0.05$) were observed among the regions of origin for all fourteen investigated traits. East African genotypes followed by the Southern African genotypes outperformed West African and Asian genotypes in stem diameter, number of primary branches, petiole length, total fresh biomass and edible fresh biomass, and time to 50% flowering. The Southern African genotypes had longer central leaflet and broader leaf. In

contrast, the West African genotypes had longer primary branch and higher dry matter content, whereas the Asian genotypes had broader central leaflet and a higher harvest index (Figure 3.1).

Table 3.2. Descriptive statistics of biomass and related traits investigated in 71 advanced lines of *Gynandropsis gynandra*

Traits	Mean	Minimum	Maximum	Standard deviation	Coefficient of variation (%)
StDiam: stem diameter (mm)	9.94	2.27	18.72	2.85	28.63
PHeight: plant height (cm)	70.6	13	117.5	20.99	29.74
NPBr: number of primary branches	10.7	2.5	23.50	4.19	39.19
PBrLeng: primary branch length (cm)	31.04	0.2	106	25.6	82.48
CtLleng: central leaflet length (cm)	7.17	2.5	12.35	1.77	24.64
CtLwid: central leaflet width (cm)	3.17	1	5.50	0.71	22.43
Lwid: leaf width (cm)	10.9	4	19.60	3.14	28.78
Ptillen: petiole length (cm)	10.95	4.50	20.35	3.11	28.39
LfArea: leaf area (cm^2)	53.22	5.64	147.76	26.59	49.97
FBiom: total fresh biomass per plant (g)	67.19	2.10	248.40	42.73	63.59
EDBiom: edible fresh biomass per plant (g)	28.34	1.20	101.90	17.31	61.08
HI: harvest index	0.47	0.24	0.91	0.12	25.78
DM: dry matter content (%)	10.67	7.60	15.42	1.5	14.01
DFlow: time to 50% flowering (days)	60.14	32	95	13.88	23.07

3.3.2 Variance components, heritability and genetic gain estimates of biomass and related traits

Significant genotypic variances (σ_G^2) were observed for all traits, while genotype × year interaction variances ($\sigma_{G\times Y}^2$) were significant for stem diameter, primary branch length, number of primary branches, leaf width and area, petiole length, harvest index and time to 50% flowering (Table 3.3). For all traits, genotype × year interaction variances were lower than genotypic variances (σ_G^2). The broad-sense heritability was high for all traits and ranged between 0.64 ± 0.09 (edible biomass per plant) and 0.87 ± 0.03 (petiole length) (Table 3.3). Genetic gains at 5% selection intensity were variable (Table 3.3). Estimates of genetic gains over the mean of the current population were low for dry matter content (13.75%) and high for

primary branch length (117.36%). Specifically, significant genetic gains (> 50%) were observed for the number of primary branches, leaf area, and total and edible fresh biomass.

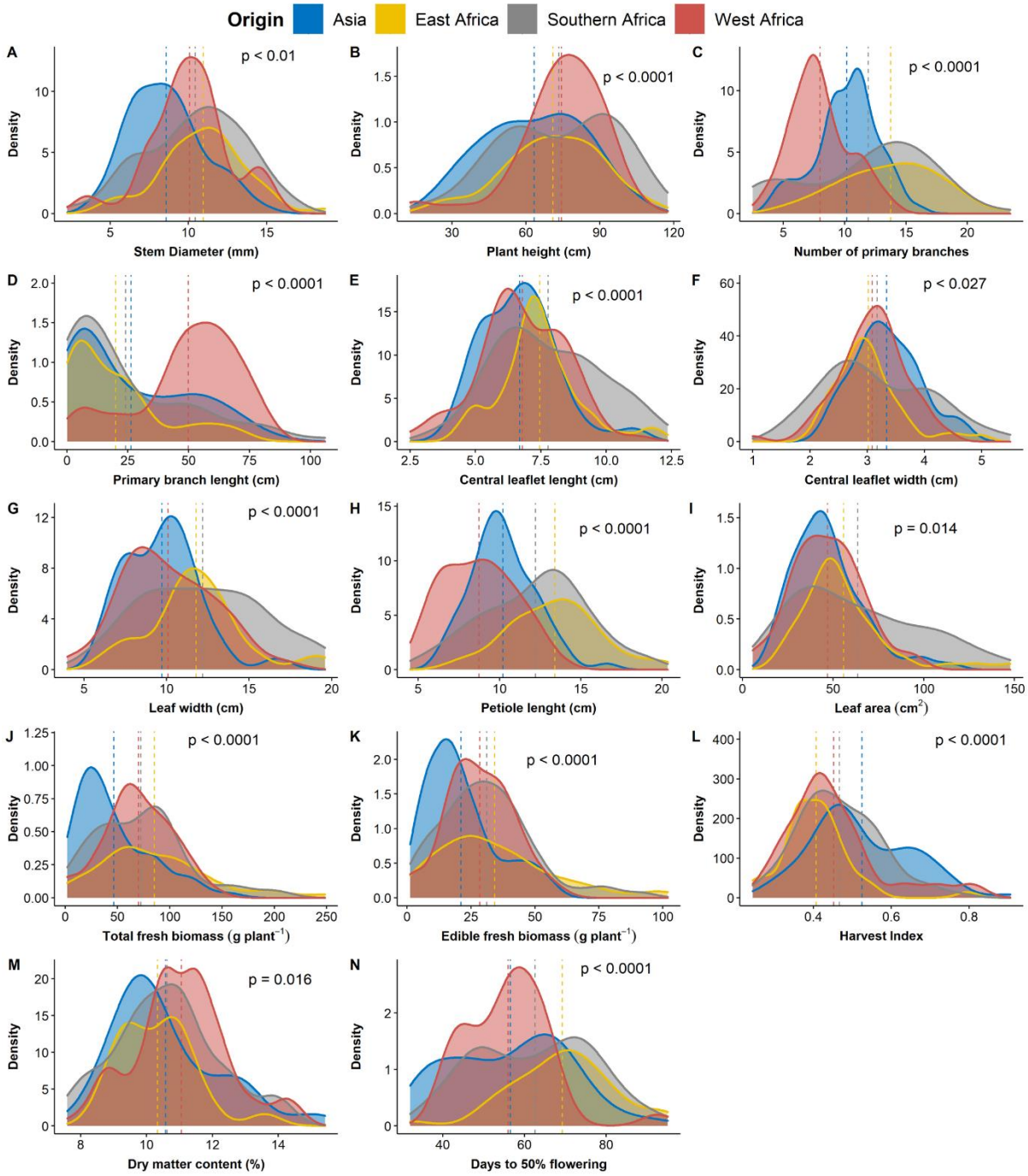


Figure 3.1. Distribution of phenotypic values for fourteen agronomic traits among regions of origin of 71 advanced lines of *Gynandropsis gynandra*. (A) stem diameter (mm). (B) Plant height (cm). (C) Primary branch length (cm). (D) Number of primary branches. (E) Central leaflet length (cm). (F) Central leaflet width (cm). (G) Leaf width (cm). (H) petiole length (cm). (I) Leaf area (cm^2). (J) Total fresh biomass per plant (g). (K) Edible fresh biomass per plant (g). (L) Harvest index. (M) Dry matter content (%). (N) Time to 50% flowering (days). The mean of each population of regions of origin is indicated by a dotted line.

Table 3.3. Estimates of genetic parameters for biomass and related traits in 71 advanced lines of *Gynandropsis gynandra* evaluated over two years.

Traits	σ_G^2	$\sigma_{G\times Y}^2$	σ_e^2	H^2	σ_P^2	**GA**	**GAM**	**GCV**	**PCV**	**ECV**
StDiam	3.95 ± 0.95 ***	1.03 ± 0.56 *	3.14 ± 0.58	0.75 ± 0.06	5.25 ± 0.91	3.55	35.85	20.07	23.14	17.90
PHeight	204.79 ± 48.23 ***	32.13 ± 29.50	188.05 ± 36.63	0.76 ± 0.06	267.86 ± 46.02	25.78	36.68	20.36	23.29	19.51
PBrLeng	394.96 ± 86.71 ***	70.72 ± 37.40 *	223.93 ± 40.54	0.81 ± 0.05	486.30 ± 84.26	36.89	117.36	63.22	70.15	47.60
NPBr	11.79 ± 2.38 ***	1.37 ± 0.80 *	4.91 ± 0.91	0.86 ± 0.04	13.70 ± 2.35	6.56	62.06	32.48	35.01	20.96
CtLleng	1.85 ± 0.39 ***	0.19 ± 0.17	1.10 ± 0.21	0.83 ± 0.04	2.22 ± 0.38	2.56	35.86	19.07	20.88	14.68
CtLwid	0.27 ± 0.06 ***	0.03 ± 0.03	0.21 ± 0.04	0.80 ± 0.05	0.33 ± 0.06	0.96	30.26	16.40	18.31	14.50
Lwid	5.59 ± 1.25 ***	1.50 ± 0.60 **	2.83 ± 0.53	0.79 ± 0.05	7.04 ± 1.21	4.34	39.79	21.69	24.35	15.44
Ptillen	6.52 ± 1.31 ***	0.84 ± 0.40 **	2.34 ± 0.44	0.87 ± 0.03	7.52 ± 1.29	4.90	45.04	23.48	25.23	14.07
LfArea	424.98 ± 92.24 ***	106.21 ± 41.98 **	186.06 ± 35.78	0.81 ± 0.05	524.60 ± 89.59	38.22	71.90	38.78	43.08	25.66
FBiom	732.44 ± 188.27 ***	180.28 ± 138.26	895.93 ± 171.22	0.70 ± 0.08	1046.56 ± 175.74	46.64	69.13	40.11	47.95	44.37
EDBiom	97.52 ± 27.31 ***	25.87 ± 22.96	170.46 ± 30.26	0.64 ± 0.09	153.07 ± 24.92	16.24	57.58	35.02	43.87	46.30
HI	0.006 ± 0.002 ***	0.002 ± 0.001 **	0.005 ± 0.001	0.70 ± 0.08	0.01 ± 0.00	0.13	27.91	16.19	19.36	15.18
DM	0.76 ± 0.21 ***	0.20 ± 0.16	1.10 ± 0.20	0.67 ± 0.09	1.13 ± 0.20	1.46	13.75	8.17	10.00	9.85
DFlow	50.68 ± 13.03 ***	28.63 ± 7.57 ***	20.29 ± 4.32	0.72 ± 0.07	70.07 ± 12.34	12.47	20.75	11.85	13.93	7.50

StDiam: stem diameter (mm), PHeight: plant height (cm), PBrLeng: primary branch length (cm), NPBr: number of primary branches, CtLleng: central leaflet length (cm), CtLwid: central leaflet width (cm), Lwid: leaf width (cm), Ptillen: petiole length (cm), LfArea: leaf area (cm^2), FBiom: total fresh biomass per plant (g), EDBiom: edible fresh biomass per plant (g), HI: harvest index, DM: dry matter content (%), DFlow: time to 50% flowering (days), σ_e^2 = residual variance, σ_G^2 = genotypic variance, $\sigma_{G\times Y}^2$ = genotype × year variance, σ_P^2 = phenotypic variance, H^2 = broad-sense heritability, GA: Genetic advance; GAM: genetic advance over mean, GCV: coefficient of genotypic variation; PCV: coefficient of phenotypic variation, ECV: residual coefficient of variation. ***, **, *: significantly different from zero at the 0.001, 0.01, and 0.05 probability level, respectively. *ns*: not significantly different from zero at the 0.05 level of probability.

Variable genotypic and phenotypic coefficients of variation were observed for all fourteen traits. Dry matter content had low phenotypic and genotypic coefficients of variation (< 10%), while time to 50% flowering, central leaflet width and harvest index had medium phenotypic and genotypic coefficients of variation (ranging between 10 and 20%). Other traits displayed high phenotypic and genotypic coefficients of variation. In comparison, trends in error coefficients of variation for all traits were similar to those of phenotypic and genotypic coefficients of variation (Table 3.3).

3.3.3 Association among plant biomass and related traits

Significant phenotypic and genotypic correlation coefficients were observed among the fourteen agronomic traits (Figure 3.2). While the phenotypic correlation coefficients ranged from -0.77 to 0.95, the genotypic correlation coefficients varied between -0.89 and 0.99. Similar trends were observed for the two types of correlation. For instance, a highly significant and positive correlation was observed between edible and total fresh biomass per plant at both phenotypic ($r = 0.94$, $p < 0.001$) and genotypic ($r = 0.95$, $p < 0.001$) levels (Figure 3.2). Total and edible biomass per plant had strong and positive correlations with plant height and stem diameter and positive and moderate correlations with all leaf-related traits (central leaflet length, central leaflet width, leaf width, petiole length and leaf area) and primary branch length. There were moderate to strong positive correlations among leaf traits, with leaf area being strongly and positively correlated with central leaflet length, central leaflet width and leaf width. Time to 50% flowering had moderate and positive correlations with the number of primary branches and petiole length but had a strong and negative correlation with the primary branch length and a moderate and negative correlation with dry matter content (Figure 3.2). The harvest index had negative and significant correlations with most traits, with strong correlations with stem diameter, plant height, and total fresh biomass. Additionally, the harvest index had moderate and negative correlations with edible plant biomass, dry matter content, primary branch length and leaf traits (central leaflet length, leaf width, and leaf area). Dry matter content had moderate and positive correlations with plant height, stem diameter, number of primary branches, total and edible fresh biomass per plant and leaf traits. The number of primary branches had a strong and negative correlation with primary branch length. A strong and positive correlation was observed between stem diameter and plant height. In addition, stem diameter and plant height had a moderate to strong positive correlation with leaf traits (Figure 3.2).

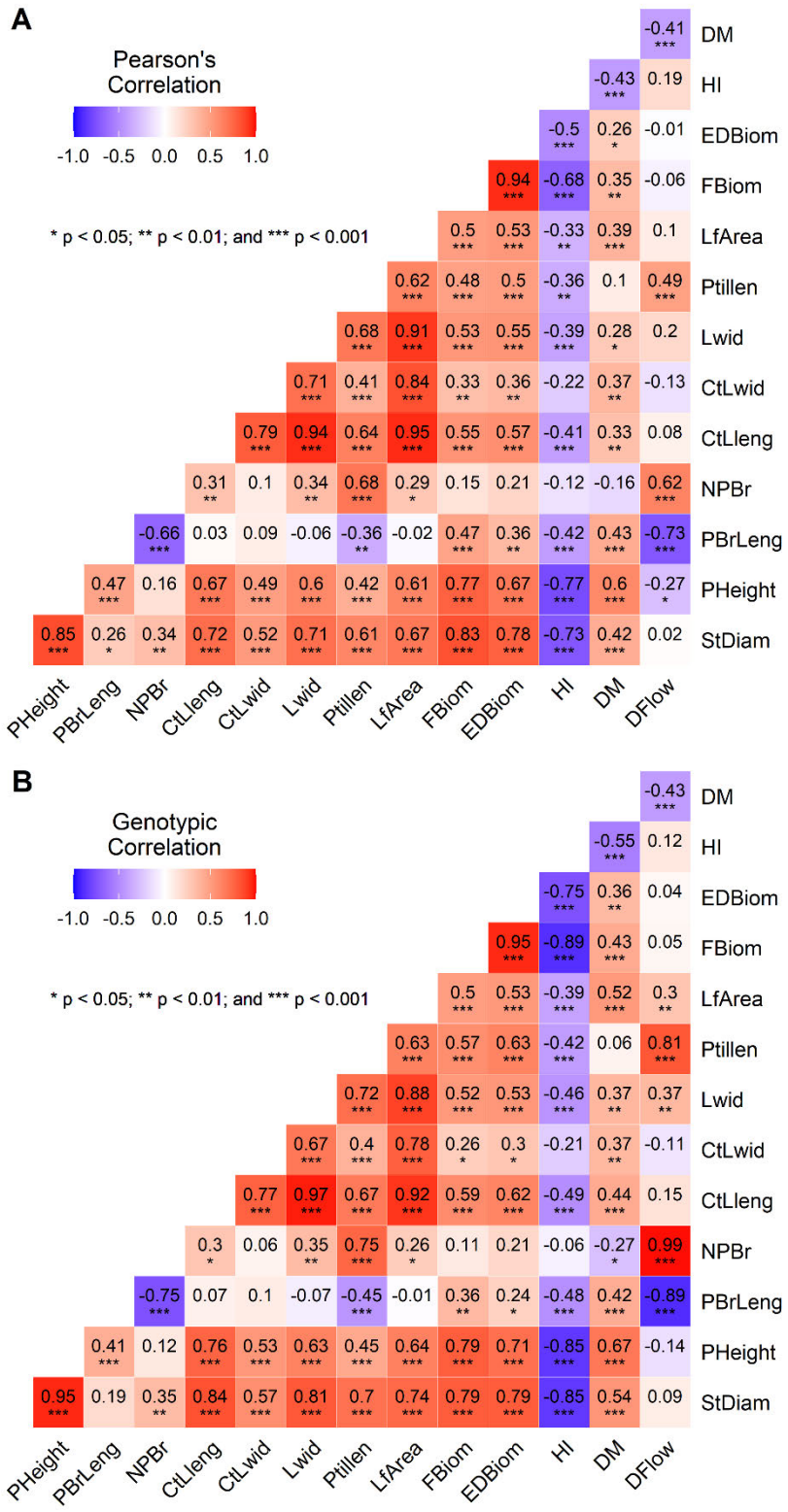


Figure 3.2. Plots of Pearson's phenotypic (A) and genotypic (B) correlation coefficients for fourteen agronomic traits of 71 advanced lines of *Gynandropsis gynandra*. StDiam: stem diameter (mm), PHeight: plant height (cm), PBrLeng: primary branch length (cm), NPBr: number of primary branches, CtLleng: central leaflet length (cm), CtLwid: central leaflet width (cm), Lwid: leaf width (cm), Ptillen: petiole length (cm), LfArea: leaf area (cm^2), FBiom: total fresh biomass per plant (g), EDBiom: edible fresh biomass per plant (g), HI: harvest index, DM: dry matter content (%), DFlow: time to 50% flowering (days).

Multivariate analysis of biomass and related traits in spider plant

To assess the relationship among genotypes, we first performed a principal component analysis. The results of the principal component analysis revealed that the first three components explained 83.02% of the total variation in the biomass and related traits (Table 3.4, Figure 3.3A). More importantly, the first two components explained 72.43% of the total variation and correlated with most traits (Figure 3.3A). Traits significantly associated with the first principal component (explaining 49.79% of the total variation) included stem diameter, plant height, leaf traits (central leaflet length, central leaflet width, leaf width, petiole length and leaf area), biomass (total and edible fresh biomass par plant) and harvest index. Principal component 1 was negatively correlated with harvest index but positively correlated with all other traits. Principal component 2 was positively and significantly associated with time to 50% flowering and the number of branches but negatively correlated with the primary branch length (Table 3.4, Figure 3.3A).

Table 3.4 Correlations between variables and the first three principal components in 71 advanced lines of *Gynandropsis gynandra* based on biomass and related traits.

Variables	**Dimension 1 (49.79%)**	**Dimension 2 (22.64%)**	**Dimension 3 (10.59%)**
StDiam: stem diameter (mm)	**0.92**	-0.05	-0.22
PHeight: plant height (cm)	**0.87**	-0.32	-0.12
NPBr: number of primary branches	0.23	**-0.92**	-0.08
PBrLeng: primary branch length (cm)	0.33	**0.77**	-0.30
CtLleng: central leaflet length (cm)	**0.89**	0.19	0.33
CtLwid: central leaflet width (cm)	**0.70**	0.04	0.61
Lwid: leaf width (cm)	**0.86**	0.28	0.27
Ptillen: petiole length (cm)	**0.69**	0.57	-0.16
LfArea: leaf area (cm^2)	**0.86**	0.21	0.42
FBiom: total fresh biomass per plant (g)	**0.82**	-0.23	-0.42
EDBiom: edible fresh biomass per plant (g)	**0.79**	-0.12	-0.36
HI: harvest index	**-0.67**	0.32	0.39
DM: dry matter content (%)	*0.49*	-0.48	0.22
DFlow: time to 50% flowering (days)	0.001	**0.86**	-0.25

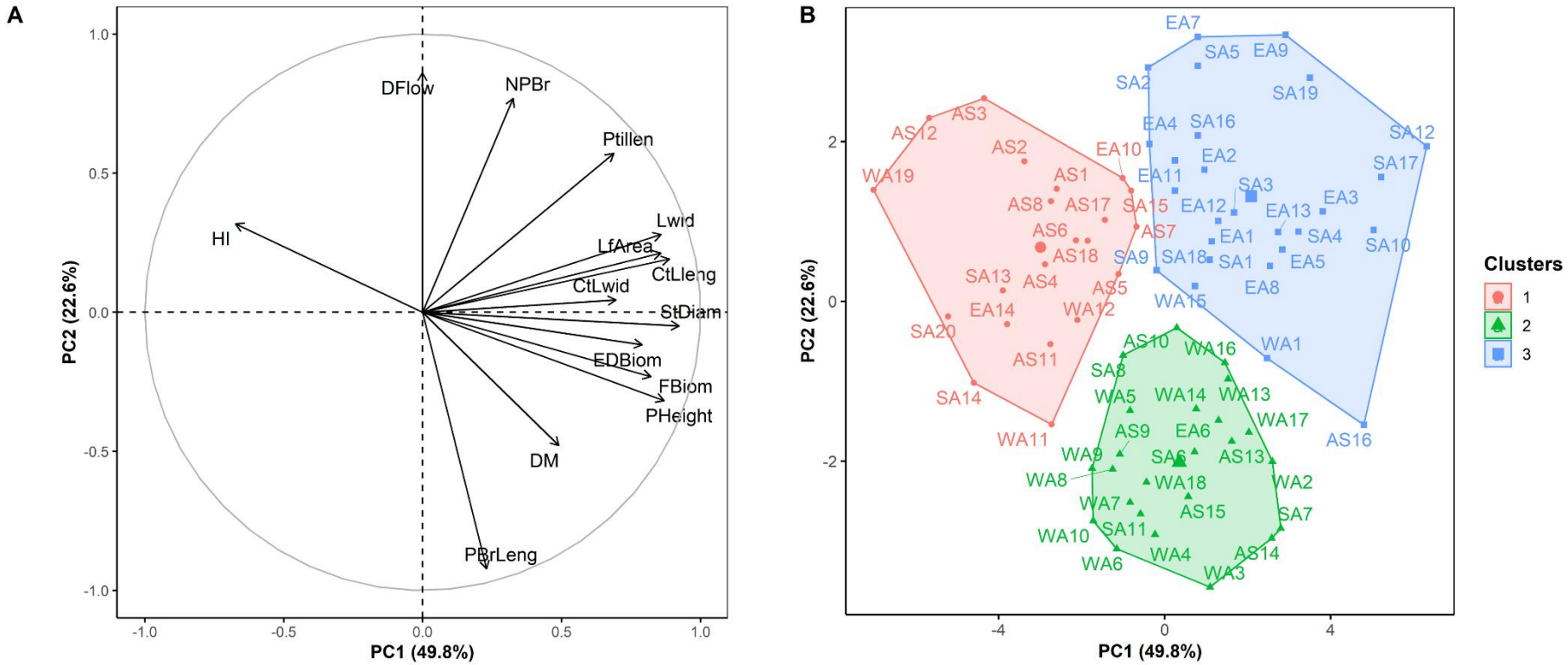


Figure 3.3. Correlation circle (A) and factor map (B) showing the clustering pattern of 71 advanced lines of *Gynandropsis gynandra* based on the hierarchical clustering on principal components analysis (HCPC). Cluster 1 (n = 21), Cluster 2 (n = 24) and Cluster 3 (n = 26). StDiam: stem diameter (mm), PHeight: plant height (cm), PBrLeng: primary branch length (cm), NPBr: number of primary branches, CtLleng: central leaflet length (cm), CtLwid: central leaflet width (cm), Lwid: leaf width (cm), Ptillen: petiole length (cm), LfArea: leaf area (cm^2), FBiom: total fresh biomass per plant (g), EDBiom: edible fresh biomass per plant (g), HI: harvest index, DM: dry matter content (%), DFlow: time to 50% flowering (days). AS: Asia; EA: East Africa; SA: Southern Africa; WA: West Africa.

Clustering pattern analysis using hierarchical clustering on principal components classified the lines into three clusters (Figure 3.3B). A significant difference was observed among the clusters for all traits (Table 3.5). Cluster 1 (29.58% of all lines) encompassed mainly Asian lines (66% of all Asian lines) with some from other regions and was characterized by less vigorous plants, with a moderate number of short primary branches, low biomass productivity and dry matter content, relatively late flowering time, small leaves, and high harvest index (Table 3.5). Cluster 2 included mainly lines originating from West Africa (73.68% of all West African lines) and some from other regions. Genotypes in cluster 2 had high dry matter content, long primary branches, high biomass productivity, low number of primary branches, moderate vigor, medium leaf size and flowered early. Cluster 3, mainly composed of lines from East and Southern Africa (88.46% of all lines in the cluster), was characterized by late flowering and vigorous plants, a high number of short primary branches, high biomass productivity, broad leaves, moderate dry matter content and a low harvest index (Table 3.5).

3.4 Discussion

Genetic variation is the foundation of any plant breeding program. Significant and origin-driven variation has been reported in *Gynandropsis gynandra* for plant morphology (Wu et al. 2018; Sogbohossou et al. 2019), secondary metabolite concentrations (Sogbohossou et al. 2020), seed germination, mineral composition and morphology (Blalogoe et al. 2020), leaf vitamin contents (Sogbohossou et al. 2019), antioxidant activity (Chataika et al. 2021), and photosynthesis traits (Reeves et al. 2018). Morphological traits with significant variation were related to plant architecture (plant height, number of primary branches, plant habit, stem hairiness and colour), leaf size (leaf area, leaflet length and width, petiole length, leaflet shape), leaf colour, time to 50% flowering, germination (percentage and mean time), pod characteristics (pod length and width, number of seeds per pod), seed size (length, width, perimeter, area), 1000-seed weight, flower traits (androphore length, filament length, pedicel length, gynophore length), and biomass (total shoot fresh and dry weight, leaf fresh and dry weight) (Sogbohossou et al. 2019; Wu et al. 2018; Omondi et al. 2017b; Blalogoe et al. 2020). In addition, phenotypic differentiation among diverse accessions of *G. gynandra* was found to be associated with the genetic makeup of the genotypes (Sogbohossou 2019; Omondi et al. 2017a). While Omondi et al. (2017a) differentiated advanced lines and genebank's accessions from farmer cultivars using simple sequence repeats (SSR) markers, Sogbohossou (2019)

Table 3.5. Phenotypic descriptors of *Gynandropsis gynandra*'s clusters.

Phenotypic descriptors	**Cluster 1 (n = 21)** **Asia n = 12** **East Africa n = 2** **Southern Africa n = 4** **West Africa n = 3**	**Cluster 2 (n =24)** **Asia n = 5** **East Africa n = 1** **Southern Africa n = 4** **West Africa n = 14**	**Cluster 3 (n = 26)** **Asia n = 1** **East Africa n = 11** **Southern Africa n = 12** **West Africa n = 2**	**F Value**	**All the germplasm** **Asia n = 18** **East Africa n = 14** **Southern Africa n = 20** **West Africa n = 19**
StDiam: stem diameter (mm)	*7.84 ± 0.21* c	10.29 ± 0.20 b	**11.21 ± 0.21 a**	69.53 ***	9.9 ± 0.2
PHeight: plant height (cm)	*56.64 ± 2.07* b	**75.67 ± 1.51 a**	**76.31 ± 1.86 a**	35.52 ***	70.27 ± 1.47
NPBr: number of primary branches	10.02 ± 0.42 b	*7.97 ± 0.38* c	**13.42 ± 0.51 a**	40.12 ***	10.57 ± 0.37
PBrLeng: primary branch length (cm)	*19.21 ± 2.13* b	**52.12 ± 1.88 a**	*22.22 ± 2.02* b	81.74 ***	31.44 ± 2.09
CtLleng: central leaflet length (cm)	*6.18 ± 0.18* c	6.99 ± 0.16 b	**8.06 ± 0.24 a**	21.90 ***	7.14 ± 0.15
CtLwid: central leaflet width (cm)	*2.94 ± 0.10* b	3.19 ± 0.06 ab	**3.30 ± 0.10 a**	4.14 *	3.16 ± 0.05
Lwid: leaf width (cm)	*9.23 ± 0.27* c	10.41 ± 0.28 b	**12.69 ± 0.36 a**	31.68 ***	10.9 ± 0.25
Ptillen: petiole length (cm)	*9.57 ± 0.36* b	*9.53 ± 0.31* b	**13.16 ± 0.32 a**	42.14 ***	10.87 ± 0.28
LfArea: leaf area (cm^2)	*40.00 ± 2.32* c	49.72 ± 2.06 b	**66.97 ± 4.00 a**	20.13 ***	53.16 ± 2.18
FBiom: total fresh biomass per plant (g)	*40.01 ± 2.08* b	**77.67 ± 2.63 a**	**80.23 ± 3.57 a**	54.99 ***	67.47 ± 2.69
EDBiom: edible fresh biomass per plant (g)	*19.42 ± 0.84* b	**30.96 ± 0.98 a**	**32.75 ± 1.48 a**	35.38 ***	28.2 ± 0.95
HI: harvest index	**0.54 ± 0.01 a**	*0.44 ± 0.01* b	*0.44 ± 0.01* b	37.52 ***	0.47 ± 0.01
DM: dry matter content (%)	*10.27 ± 0.10* b	**10.92 ± 0.13 a**	10.69 ± 0.16 ab	5.61 **	10.64 ± 0.08
DFlow: time to 50% flowering (days)	61.00 ± 1.05 a	*54.73 ± 0.52* b	**64.33 ± 1.03 a**	30.72 ***	60.1 ± 0.7

Values in bold and italics indicate clusters' means that are significantly greater and lower than the overall means for all accessions, respectively, and describe the given cluster. Values within a row followed by the different letters are significantly different according to Tukey's HSD at $P \leq 0.05$.

***, **, * indicate significance at the 0.001, 0.01, and 0.05 probability level, respectively.

observed genomic differentiation among accessions from West Africa, East/Southern Africa and Asia. Our study revealed that four generations of selfing maintained significant variation and membership in their group of origin, strengthening the hypothesis of geographical signature in cleome genetic diversity. We observed highly significant variation among advanced lines for biomass productivity, growth traits, leaf traits and flowering time in *Gynandropsis gynandra*. Similar observations for morphological traits have also been reported for worldwide accessions (Wu et al. 2018; Sogbohossou et al. 2019), East and Southern African accessions and cultivars (Omondi et al. 2017b), and accessions from South Africa and Kenya (Kangai Munene et al. 2018), Ghana (Kwarteng et al. 2018), and Burkina-Faso (Kiebre et al. 2017a). This significant variation represents a valuable resource for sustainable and successful breeding programs for the species.

On the other hand, the average and the highest total fresh biomass in the present study were higher than those reported by Omondi et al. (2017b) in East-Southern African genotypes but slightly lower than those of Kiebre et al. (2017a) for accessions from Burkina-Faso. The difference might be attributable to the genotypes, agricultural practices, and environment since those authors evaluated their germplasm in the field. For instance, agronomic practices such as planting density, type and fertilizer application rates, planting date, stage of transplanting, harvesting frequency and techniques (cutting, uprooting whole plants, defoliation) significantly affect growth and biomass yield in *G. gynandra* (Houdegbe et al. 2018; Gonye et al. 2017; Ayua et al. 2016; Mavengahama 2013; Seeiso and Materechera 2012; Masinde and Agong 2011; Wangolo et al. 2015). Therefore, genotype performance should be investigated under different agricultural practices considering farmers' practices in target environments.

The clustering analysis identified three groups, each dominated by lines derived from accessions originating from different geographical regions. Clusters 1, 2 and 3 were dominated by lines derived from Asian, West African, and East/Southern African accessions, respectively. The clustering results were supported by the significant differences observed among regions of origin for all fourteen investigated traits. These results align with previous reports on the association between the geographical origin and the morphology of the accessions of *G. gynandra* (Wu et al. 2018; Sogbohossou et al. 2019). Specifically, Sogbohossou et al. (2019) identified three distinct groups similar to those of this study: East-Southern African accessions (tall plants with broad leaves), Asian accessions (short plants with broad leaves) and West African accessions (short plants with small leaves). Furthermore, the genetic constitution could be the main driver of this clustering, as Sogbohossou (2019) reported genomic differentiation

between Asian, West African and East/Southern African accessions. This clustering pattern might reflect the local adaptation of the species in response to environmental/climatic factors and different uses by local communities.

Farmers' preferred traits in *G. gynandra* include high leaf yield and related traits (plant height and the number of leaves), broad leaves and late flowering (Ndinya et al. 2020; Mutoro 2019; Kiebre et al. 2015; Cleome Consortium 2017). We observed that East and Southern African lines combined several farmers' preferred traits such as broad leaves, late flowering and high biomass, while West African genotypes had high biomass and dry matter content. Based on biomass productivity, East, Southern and West African genotypes were similar and outperformed the Asian accessions, which could be in response to ancient domestication or advanced selection for biomass occurring in these regions compared with Asia. Intensive utilization of the species as a leafy vegetable has been reported in Africa rather than Asia. In several Asian countries, the species was mainly reported as weeds and rarely cultivated (Bhattacharya et al. 2019; Rajendrudu and Das 1982) and primarily used in traditional medicine (Narendhirakannan et al. 2005; Ghogare et al. 2009). In contrast, although the species still grows as weeds, it is cultivated in many African countries for its leaves as vegetables (Chweya and Mnzava 1997). In Africa, the semi-cultivated status of *G. gynandra* was reported earlier in the 1950s (Irvine 1956). The domestication of the species might have first started in Eastern and Southern Africa, as its weed status was quickly converted to cultivated species (Schippers 2004). West African genotypes had similar biomass yields as the East and Southern African genotypes, suggesting West Africa as a secondary domestication hotspot for the species, while domestication and selection are still at the earlier stage or might not have started in Asia. Feodorova et al. (2010) support these findings, by suggesting that the speciation event of *G. gynandra* might have occurred in South Africa. Using genome sequencing, Sogbohossou (2019) suggested the African origin of the species, with Asian and West African populations being closed and recently divergent from East and Southern African populations. More investigations are needed to clarify the origin of the species as well as its route of colonization.

Heritability is important in breeding, as it helps in predicting the efficiency of the selection. Broad-sense heritability (H^2) measures the proportion of the total phenotypic variation attributable to the variance of genetic values (Visscher et al. 2008). High broad-sense heritability estimates (> 0.60) were observed for all investigated traits, showing that phenotypic variation observed among genotypes is mostly due to genotypic variation. More importantly, we also observed low genotype × year interaction variance compared with genotypic variance.

We therefore hypothesize that phenotypes can accurately predict genotypes, but this should be confirmed with multi-environmental trials. Similarly, high broad-sense heritability estimates were reported for stem diameter, plant height, number of primary branches, leaf biomass, leaf area, leaflet length and width, and time to 50% flowering in the species (Kangai Munene et al. 2018; Kiebre et al. 2017b). This suggests that high genetic advancement is achievable for biomass and related traits in the species. As a consequence, we observed significant expected genetic gain at a selection intensity of 5%, showing that significant improvement would be possible through direct phenotypic selection, particularly for total fresh biomass, edible fresh biomass, the number of primary branches and leaf area. These findings concur with earlier reports in *G. gynandra* for biomass yield and related traits (Kiebre et al. 2017b). The low genetic gain observed for dry matter content might suggest that selecting this trait might be difficult, as low variability was also observed. More genetic material is needed to broaden the available variability.

Genotype × year interaction variances were significant for stem diameter, primary branch length, number of primary branches, leaf width and area, petiole length, harvest index and days to 50% flowering. This is showing that these traits were influenced not only by the genotype but also the interaction between genotype and year. As agronomic practices were the same during the two years, the differential environmental conditions between 2020 and 2021 could play a significant role in the significance of genotype × year interaction. Potential environmental factors that might influence these traits could include the temperature, the relative humidity and the light intensity (photoperiods). Imbamba and Tieszen (1977) found that photosynthesis rate in spider plant increase with light intensity (from 200 to 2000 μmol m^{-2} s^{-1}) and that 2000 μmol m^{-2} s^{-1}, which is close to full sunlight, does not saturate photosynthesis in *G. gynandra* due to the C4 plant nature of spider plant. Similar observations were done by Kocacinar (2015) with an increase in net photosynthetic rate and stomatal conductance with increasing light intensity. Zorde et al. (2020) observed significant variation in days to flowering between the greenhouse (10-182 days) and field (20-57) trials in Arusha due to the differential day length and light intensity, as the plants were grown under daylight conditions between 11:52-12:17 hours of daylight as opposed to 14 hours in the greenhouse. The leaf temperature also significantly influences the rates of CO_2 assimilation, and that the species requires high temperature (30-40 °C) to attain the maximum photosynthesis, playing a key role in the species growth and biomass productivity. On the other hand, the year significantly affected these traits, implying that these traits might vary with year. In addition, the significant genotype × year

interaction indicated that the genotypes' performance was not consistent across environments, and selection should consider the interaction effect when selecting genotypes. However, evaluation in additional environments, particularly in field conditions, is required to better decipher the genotype by environment interaction in the species.

Understanding the association between traits offers an opportunity for efficient and simultaneous selection. Both phenotypic and genotypic correlations showed similar trends. In the present study, the correlation between total fresh biomass and edible fresh biomass was strong, positive and significant. In addition, these two traits were highly and positively correlated with plant height and stem diameter, suggesting that selection for vigorous and tall plants will lead to high-yielding cultivars. This might be accompanied by broad leaves resulting from the positive and moderate association between biomass and leaf-related traits (central leaflet length, central leaflet width, leaf width, petiole length, leaf area). Previous findings corroborated these results as a positive and strong correlation of leaf biomass with plant height, stem diameter, leaf length and width and petiole length (Kiebre et al. 2017b). Similarly, Kangai Munene et al. (2018) and Mosenda et al. (2020) observed a positive and strong association between the number of leaves per plant and plant height (Kangai Munene et al. 2018). Such a positive association between these traits imply that simultaneous and direct selection for such farmers' desired traits would be possible. This association could result from pleiotropic or linked genes controlling biomass, plant height, stem diameter, and leaf traits in the species. Using an F2 population, Sogbohossou (2019) found a single QTL for plant height and two for leaf area, and this plant height QTL and one QTL for leaf area were colocalized on the same linkage group, with potential pleiotropic effects of a candidate gene, although the author recommended the validation of the QTLs.

The number of primary branches was positively correlated with time to 50% flowering, suggesting that late flowering plants had more branches. In contrast, primary branch length had a negative and significant correlation with time to 50% flowering and number of branches, showing the existence of a trade-off between the number of primary branches, the primary branch length and time to 50% flowering in the species. After flowering, plants allocate resources for lateral branch growth, therefore, the plant can achieve higher biomass either by flowering early and developing long branches or delaying flowering to produce more branches. This might explain why West African genotypes had similar biomass yields to East/Southern genotypes, which are late flowering with a high number of short branches. This calls for an in-depth investigation to understand resource allocation in the species and genes involved in

flowering time, branch development, and plant architecture. To this end, developing mapping populations using genotypes from all clusters will be insightful.

In this study, the harvest index was negatively associated with plant biomass and most other agronomic traits, suggesting that selection for the harvest index might be difficult. However, using appropriate agronomic traits, such as early harvesting, could help improve the harvest index. Frequent harvesting (e.g., every week or two weeks) might increase biomass productivity and extend the harvesting period. This would strongly depend on the regrowth ability of the genotype. An evaluation of the germplasm under different agronomic practices, including harvesting techniques and frequency, is required, as suggested by Houdegbe et al. (2018). Assessing the regrowth ability would be crucial, particularly in West Africa, where cutting is the frequent harvesting technique employed by farmers and genotypes with several cuttings are desired (Sogbohossou et al. 2018a). In this case, the ability to predict yield for the subsequent harvest should be investigated through genetic correlation analysis.

Dry matter content is associated with shelf life and determines the vegetable's post-harvest behaviour (Gogo et al. 2017; Min et al. 2021; Valverde-Miranda et al. 2021). The moderate and significant association of dry matter content with plant biomass, growth traits and leaf traits suggested that increasing the leaf area might not affect dry matter content in the species. In contrast, the negative association between time to 50% flowering and dry matter content showed that late flowering plants might have low dry matter content with reduced shelf life, suggesting plausible linkage drag between flowering time and dry matter accumulation in the species. Similarly, a negative correlation was observed between dry matter content and days to silking in maize for biogas production (Grieder et al. 2012). Such an association could be investigated using mapping populations developed between West African genotypes and East/Southern Africa. In addition, broadening the narrow genetic variation for dry matter content is needed through extensive germplasm collections, introductions and characterization.

Overall, considering farmers' preferred traits, genotypes in cluster 3 and somewhat cluster 2 are prime resources for cultivar release but require intensive field evaluation through multi-environment trials within each region. This would help in understanding the genotype-by-environment interaction in the species and whether to breed for specific or broad adaptation. Furthermore, establishing the link between the phenotype and genotype is required to help implement marker-assisted selection in the species. Genome-wide association studies (GWAS) can be implemented to decipher genes associated with functional and farmers' preferred traits

and would serve in the validation of QTLs reported by Sogbohossou (2019) on flowering time, plant height, and leaf area. The best genotypes from each cluster could be involved in studies to estimate the narrow-sense heritability and determine gene action controlling the key traits using factorial mating designs such as diallel and North Carolina mating designs. In addition, assessing the potential hybrid vigour in the species would help design efficient breeding strategies for ideal cultivar development. Association of these traits with nutritional traits is needed. Evaluation of these genotypes under different disease and pest pressures and biotic stresses is required, particularly in the current changing climate.

3.5 Conclusion

The present study revealed the biomass potential and genetic variation in a diverse set of advanced lines of *G. gynandra*. The four selfed generations of advanced lines from diverse origins clustered into three groups and maintained their origin group membership, thus strengthening the hypothesis of geographical signature in cleome diversity. Group 1 mainly encompassed lines derived from Asian accessions and was characterized by low biomass productivity and a high harvest index. Groups 2 and 3, dominated by West and East/Southern African lines, respectively, had similar biomass productivity. Group 2 lines had high dry matter content, a low number of long primary branches and flowered early. Group 3 genotypes differ from group 2 with broad leaves, vigorous plants with a high number of short branches and late flowering. The high biomass productivity of West, East and Southern African genotypes suggested advanced selection and domestication in Africa than Asia for biomass. The observed significant variation offers a solid basis for the species improvement. On the other hand, plant biomass exhibited a positive association with most related traits, providing the opportunity for positive and simultaneous selection for several traits, especially farmers' preferred traits such as biomass yield, leaf size, flowering time and the number of branches. High broad-sense heritability and significant genetic gain observed at 5% selection intensity showed the positive effect of selection in improving species performance. Further studies should target multi-environment trials to determine genotype by environment interaction effect, determine the genotypes' response to different agronomic practices such as cutting, fertilization considering the locally available resources, identify gene action and genes controlling farmers preferred traits and evaluate the germplasm tolerance to biotic and abiotic stress. Additionally, the association of plant biomass and related traits with key nutritional traits such as minerals is required to ensure the quality of the end products for users.

References

Abukutsa-Onyango MO (2007) The diversity of cultivated African leafy vegetables in three communities in western Kenya. Afr J Food Agric Nutr Dev 7 (3):1-15. 10.18697/ajfand.14.IPGRI1-3

Achigan-Dako EG, Sogbohossou DEO, Houdegbe CA, Salaou MA, Sohindji FS, Blalogoe J, Chataika BY, Zohoungbogbo HF, Adje CAO, Fassinou Hotegni NV, Francisco R, Abukutsa-Onyango MO, Schranz ME (2021) Ten years of *Gynandropsis gynandra* research for improvement of nutrient-rich leaf consumption: lessons learnt and way forwards. Annu Rev Plant Biol 4 (3):767-812. 10.1002/9781119312994.apr0774

Adeka RK (2020) Determination of variation in morphology and leaf bitterness in spider plant (*Gynandropsis gynandra* (L.) Briq. across aix agroecological zones in Kenya. MSc Thesis, University of Nairobi,

Ajaiyeoba EO, Onocha PA, Olarenwaju OT (2001) In vitro anthelmintic properties of *Buchholzia coriaceae* and *Gynandropsis gynandra* extracts. Pharm Biol 39 (3):217-220. 10.1076/phbi.39.3.217.5936

Alvarado G, Rodríguez FM, Pacheco A, Burgueño J, Crossa J, Vargas M, Pérez-Rodríguez P, Lopez-Cruz MA (2020) META-R: A software to analyze data from multi-environment plant breeding trials. Crop J 8 (5):745-756. 10.1016/j.cj.2020.03.010

Ayua E, Mugalavai V, Simon J, Weller S, Obura P, Nyabinda N (2016) Ascorbic acid content in leaves of Nightshade (*Solanum* spp.) and spider plant (*Cleome gynandra*) varieties grown under different fertilizer regimes in Western Kenya. Afr J Biotechnol 15 (7):199-206. 10.5897/AJB2015.14936

Bala A, Kar B, Haldar PK, Mazumder UK, Bera S (2010) Evaluation of anticancer activity of *Cleome gynandra* on Ehrlich's Ascites Carcinoma treated mice. J Ethnopharmacol 129 (1):131-134. 10.1016/j.jep.2010.03.010

Beal T, Massiot E, Arsenault JE, Smith MR, Hijmans RJ (2017) Global trends in dietary micronutrient supplies and estimated prevalence of inadequate intakes. PLoS ONE 12 (4):e0175554. 10.1371/journal.pone.0175554

Bernal-Vasquez A-M, Utz H-F, Piepho H-P (2016) Outlier detection methods for generalized lattices: a case study on the transition from ANOVA to REML. Theor Appl Genet 129 (4):787-804. 10.1007/s00122-016-2666-6

Bhattacharya E, Bose R, Mandal Biswas S (2019) A comprehensive study on occurrence records of African neglected and underutilized weed species, *Cleome gynandra* L. (cat's whiskers) validating the ecogeographical range expansion in West Bengal, India. Weed Biol Manag 19 (4):129-134. 10.1111/wbm.12189

Blalogoe JS, Odindo AO, Sogbohossou EOD, Sibiya J, Achigan-Dako EG (2020) Origin-dependence of variation in seed morphology, mineral composition and germination percentage in *Gynandropsis gynandra* (L.) Briq. accessions from Africa and Asia. BMC Plant Biol 20 (1):168. 10.1186/s12870-020-02364-w

Burton GW, DeVane EH (1953) Estimating heritability in tall fescue (*Festuca Arundinacea*) from replicated clonal material. Agron J 45 (10):478-481. 10.2134/agronj1953.00021962004500100005x

Butler D, Cullis B, Gilmour A, Gogel B, Thompson R (2017) ASReml-R Reference Manual Version 4. . VSN International Ltd Hemel Hempstead, HP1 1ES. UK

Chand J, Panda SR, Jain S, Murty USN, Das AM, Kumar GJ, Naidu VGM (2022) Phytochemistry and polypharmacology of cleome species: A comprehensive Ethnopharmacological review of the medicinal plants. J Ethnopharmacol 282:114600. 10.1016/j.jep.2021.114600

Chandradevan M, Simoh S, Mediani A, Ismail IS, Abas F (2020) 1H NMR-based metabolomics approach in investigating the chemical profile, antioxidant and anti-inflammatory activities of *Gynura procumbens* and *Cleome gynandra*. Plant Foods Hum Nutr 75 (2):243-251. 10.1007/s11130-020-00805-3

Chataika BY, Akundabweni LS-M, Houdegbe AC, Achigan-Dako EG, Sibiya J, Masamba K (2021) Dietary phytochemical screening of spider plant (*Gynandropsis gynandra* (L.) Briq.) accessions from Africa and Asia to identify genotypes for use in nutraceutical breeding. Front Sustain Food Syst 5 (344). 10.3389/fsufs.2021.733909

Chweya JA, Mnzava NA (1997) Cat's whiskers. *Cleome gynandra* L. Promoting the conservation and use of underutilized and neglected crops. 11. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy

Cleome Consortium (2017) Utilizing the genome of *Cleome gynandra* for the development of improved cultivars for West and East African markets - Annual progress report 2016. Cleome Consortium, Cotonou

Ekpong B (2009) Effects of seed maturity, seed storage and pre-germination treatments on seed germination of cleome (*Cleome gynandra* L.). Sci Hortic 119:236-240. 10.1016/j.scienta.2008.08.003

Feodorova TA, Voznesenskaya EV, Edwards GE, Roalson EH (2010) Biogeographic patterns of diversification and the origins of C4 in Cleome (Cleomaceae). Syst Bot 35 (4):811-826

Ghogare UR, Nirmal SA, Patil RY, Kharya MD (2009) Antinociceptive activity of *Gynandropsis gynandra* leaves. Nat Prod Res 23 (4):327-333. 10.1080/14786410802047862

Glew RS, Amoako-Atta B, Ankar-Brewoo G, Presley J, Chuang L-T, Millson M, Smith BR, Glew RH (2009) Non-cultivated plant foods in West Africa: Nutritional analysis of the leaves of three indigenous leafy vegetables in Ghana. Food 3 (1):39-42

Gogo EO, Opiyo AM, Hassenberg K, Ulrichs C, Huyskens-Keil S (2017) Postharvest UV-C treatment for extending shelf life and improving nutritional quality of African indigenous leafy vegetables. Postharvest Biol Technol 129:107-117. 10.1016/j.postharvbio.2017.03.019

Gonye E, Kujeke G, Edziwa X, Ncube A, Masekesa R, Icishahayo D, Matikiti A, Chabata I (2017) Field performance of spider plant (*Cleome gynandra* L.) under different agronomic practices. Afr J Food Agric Nutr Dev 17 (3):12179-12197. 10.18697/ajfand.79.15985

Grieder C, Dhillon BS, Schipprack W, Melchinger AE (2012) Breeding maize as biogas substrate in Central Europe: I. Quantitative-genetic parameters for testcross performance. Theor Appl Genet 124 (6):971-980. 10.1007/s00122-011-1761-y

Harrell Jr FE, Dupont C (2021) Hmisc: Harrell Miscellaneous. R package version 4.6-0 edn.,

HCDA (2014) Horticulture data 2011-2013 Validation report. Horticultural Crops Development Authority, Nairobi, Kenya

Holland JB, Nyquist WE, Cervantes-Martínez CT (2003) Estimating and interpreting heritability for plant breeding: an update. Plant Breed Rev 22:9-112. 10.1002/9780470650202.ch2

Houdegbe CA, Sogbohossou EOD, Achigan-Dako EG (2018) Enhancing growth and leaf yield in *Gynandropsis gynandra* (L.) Briq. (Cleomaceae) using agronomic practices to accelerate crop domestication. Sci Hortic 233:90-98. 10.1016/j.scienta.2018.01.035

Igoli JO, Masao CA, Orkpeh U, Nakamatte E, Yakumbur DT, Nnadozie T, Yunusa S, Vihior B, Manyi MM, Tor-Anyiin TA (2016) In vivo antimalarial activity of *Cleome gynandra* extracts. J Nat Prod Res Updates 2:19-29

Imbamba SK, Tieszen LL (1977) Influence of light and temperature on photosynthesis and transpiration in some C3 and C4 vegetable plants from Kenya. Physiol Plant 39 (4):311-316. doi:10.1111/j.1399-3054.1977.tb01890.x

Irvine FR (1956) The edible cultivated and semi-cultivated leaves of West Africa. Mater Veg 2 (1):35-42. 10.1007/BF01889772

Kamotho GN, Mathenge PW, Muasya RM, Dulloo ME (2014) Effects of maturity stage, dessication and storage period on seed quality of Cleome (*Cleome gynandra* L.). Res Desk 3 (1):419-433

Kangai Munene A, Nzuve F, Ambuko J, Odeny D (2018) Heritability Analysis and Phenotypic Characterization of Spider Plant (*Cleome gynandra* L.) for Yield. Adv Agric 2018:11. 10.1155/2018/8568424

Kassambara A, Mundt F (2020) Factoextra: extract and visualize the results of multivariate data analyses. R package version 1.0.7 edn.,

Katabuchi M (2015) LeafArea: an R package for rapid digital image analysis of leaf area. Ecol Res 30 (6):1073-1077. 10.1007/s11284-015-1307-x

Kiebre Z, Bationa Kando P, Sawadogo N, Kiebre M, Sawadogo B, Traore RE, Sawadogo M, Zongo J-D (2017a) Évaluation de la diversité agromorphologique d'une collection de *Cleome gynandra* L. du Burkina Faso. J Appl Biosci 118:11768-11780

Kiebre Z, Bationo Kando P, Barro A, Sawadogo B, Kiebre M, Ouedraogo MH, Sawadogo M, Zongo JD (2017b) Estimates of genetic parameters of spider plant (*Cleome gynandra* L.) of Burkina Faso. Int J Agric Policy Res 5 (9):138-144. 10.15739/IJAPR.17.016

Kiebre Z, Bationo Kando P, Sawadogo N, Sawadogo M, Zongo J-D (2015) Selection phenotypic interests for the cultivation of the plant *Cleome gynandra* L. in the vegetable garderns in Burkina-Faso. J Exp Biol Agric Sci 3 (3):288-297. 10.18006/2015.3(3).288.297

Kleinknecht K, Möhring J, Singh K, Zaidi P, Atlin G, Piepho H (2013) Comparison of the performance of best linear unbiased estimation and best linear unbiased prediction of genotype effects from zoned Indian maize data. Crop Sci 53 (4):1384-1391. 10.2135/cropsci2013.02.0073

Kocacinar F (2015) Photosynthetic, hydraulic and biomass properties in closely related C_3 and C_4 species. Physiol Plant 153 (3):454-466. doi:10.1111/ppl.12240

Kori M, Gaur K, Dixit V (2009) Investigation of immunomodulatory potential of *Cleome gynandra* Linn. Asian J Pharm Clin Res 2 (1):35-39

Kwarteng AO, Abogoom J, Adu Amoah R, Nyadanu D, Nyam CK, Ghunney T, Awuah E, Ziyaaba JZ, Ogunsanya JO, Orhin EE, Asiedu DD (2018) Phenomic characterization of twenty-four accessions of spider plant (*Cleome gynandra* L.) the Upper East region of Ghana. Sci Hortic 235:124-131. 10.1016/j.scienta.2018.02.046

Lê S, Josse J, Husson F (2008) FactoMineR: an R package for multivariate analysis. J Stat Softw 25 (1):1-18. 10.18637/jss.v025.i01

Masinde P, Agong S (2011) Plant growth and leaf N of spiderplant (*Cleome gynandra* L.) genotypes under varying nitrogen supply. Afr J Hortic Sci 5:36-49

Matro SCAX (2015) Analyse du fonctionnement des jardins de case à *Cleome gynandra* dans le département du couffo. University of Abomey-Calavi, Abomey-Calavi

Mavengahama S (2013) Yield response of bolted spider plant (*Cleome gynandra*) to deflowering and application of nitrogen top dressing. J Food Agric Environ 11 (3&4):1372-1374

Min Q, Marcelis LFM, Nicole CCS, Woltering EJ (2021) High light intensity applied shortly before harvest improves lettuce nutritional quality and extends the shelf life. Front Plant Sci 12 (76). 10.3389/fpls.2021.615355

Molenaar H, Boehm R, Piepho H-P (2018) Phenotypic selection in ornamental breeding: it's better to have the BLUPs than to have the BLUEs. Front Plant Sci 9:1511. 10.3389/fpls.2018.01511

Mosenda E, Chemining'wa G, Ambuko J, Owino W (2020) Assessment of agronomic traits of selected spider plant (*Cleome gynandra* L.) accessions. J Med Active Plants 9 (4):222-241. 10.7275/n1wf-6y77

Moyo M, Amoo SO, Aremu A, Gruz J, Šubrtová M, Jarošová M, Tarkowski P, Dolezal K (2018) Determination of mineral constituents, phytochemicals and antioxidant qualities of *Cleome gynandra*, compared to *Brassica oleracea* and *Beta vulgaris*. Front Chem 5:128. 10.3389/fchem.2017.00128

Moyo M, Aremu AO (2021) Nutritional, phytochemical and diverse health-promoting qualities of *Cleome gynandra*. Crit Rev Food Sci Nutr:1-18. 10.1080/10408398.2020.1867055

Muasya RM, Simiyu JN, Muui CW, Rao NK, Dulloo ME, Gohole LS (2009) Overcoming Seed Dormancy in *Cleome gynandra* L. to Improve Germination. Seed Technol 31 (2):134-143

Mutoro K (2019) Analysis of preference attributes for spider plant genotypes in Kenya: Implications for breeders and farmers. Int J Hortic Floric 7 (8):001-004. 10.46882/IJHF/1092

Narendhirakannan RT, Kandaswamy M, Subramanian S (2005) Anti-inflammatory activity of *Cleome gynandra* L. on hematological and cellular constituents in adjuvant-induced arthritic rats. J Med Food 8 (1):93-99. 10.1089/jmf.2005.8.93

Narsimhulu BL, Suresh Y, Rajasekar G, Lavanya T, Philip GH, Mohiyuddin SS, Reddy SR (2019) Evaluation of hepatoprotective and nephroprotective activity of methanolic extract of *Cleome viscosa* and *Cleome gynandra* in STZ-induced diabetic rats. Pharma Innov J 8 (2):574-581

Ndinya C, Onyango E, Dinssa F, Odendo M, Simon JE, Weller S, Thuranira E, Nyabinda N, Maiyo N (2020) Participatory variety selection of three African leafy vegetables in Western Kenya. J Med Active Plants 9 (3):145-156. 10.7275/mkq0-3p85

Neugart S, Baldermann S, Ngwene B, Wesonga J, Schreiner M (2017) Indigenous leafy vegetables of Eastern Africa—A source of extraordinary secondary plant metabolites. Food Res Int 100:411-422. 10.1016/j.foodres.2017.02.014

Olivoto T, Lúcio ADC (2020) metan: An R package for multi-environment trial analysis. Methods Ecol Evol 11 (6):783-789. 10.1111/2041-210X.13384

Omondi EO, Debener T, Linde M, Abukutsa-Onyango M, Dinssa FF, Winkelmann T (2017a) Mating biology, nuclear DNA content and genetic diversity in spider plant (*Cleome gynandra*) germplasm from various African countries. Plant Breed 136:578-589. 10.1111/pbr.12485

Omondi EO, Engels C, Nambafu G, Schreiner M, Neugart S, Abukutsa-Onyango M, Winkelmann T (2017b) Nutritional compound analysis and morphological characterization of spider plant (*Cleome gynandra*)-an African indigenous leafy vegetable. Food Res Int 100:284-295. 10.1016/j.foodres.2017.06.050

Onyango CM, Kunyanga CN, Ontita EG, Narla RD, Kimenju JW (2013) Current status on production and utilization of spider plant (*Cleome gynandra* L.) an underutilized leafy vegetable in Kenya. Genet Resour Crop Evol 60:2183-2189. 10.1007/s10722-013-0036-7

Piepho HP, Möhring J, Melchinger AE, Büchse A (2008) BLUP for phenotypic selection in plant breeding and variety testing. Euphytica 161 (1):209-228. 10.1007/s10681-007-9449-8

R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Rajendrudu G, Das VR (1982) Biomass production of two species of *Cleome* exhibiting C_3 and C_4 photosynthesis. Biomass 2 (3):223-227. 10.1016/0144-4565(82)90032-4

Ravichandra B, Ram PS, Saritha C, Shankaraiah P (2014) Anti diabetic and anti dyslipidemia activities of *Cleome gynandra* in alloxan induced diabetic rats. J Pharmacol Toxicol 9 (1):55-61. 10.3923/jpt.2014.55.61

Reeves G, Singh P, Rossberg TA, Sogbohossou EOD, Schranz ME, Hibberd JM (2018) Natural variation within a species for traits underpinning C4 photosynthesis. Plant Physiol 177 (2):504. 10.1104/pp.18.00168

Revelle W (2019) psych: procedures for psychological, psychometric, and personality research. R package version 1.9.12 edn. Northwestern University, Evanston, Illinois

Runnie I, Salleh MN, Mohamed S, Head RJ, Abeywardena MY (2004) Vasorelaxation induced by common edible tropical plant extracts in isolated rat aorta and mesenteric vascular bed. J Ethnopharmacol 92 (2):311-316. 10.1016/j.jep.2004.03.019

Schippers RR (2004) Légumes africaines indigènes - Présentattion des espèces cultivees. Margraf Publishers GmbH, Scientifics Books, Wageningen, Pays-Bas

Schönfeldt HC, Pretorius B (2011) The nutrient content of five traditional South African dark green leafy vegetables - A preliminary study. J Food Compos Anal 24:1141-1146. 10.1016/j.jfca.2011.04.004

Seeiso MT, Materechera SA (2012) Yield response of the African indigenous leafy vegetable *Cleome gynandra* to application of cattle and goat kraal manure and harvesting techniques. J Food Agric Environ 10 (3&4):789-794. 10.1234/4.2012.3514

Self SG, Liang K-Y (1987) Asymptotic Properties of Maximum Likelihood Estimators and Likelihood Ratio Tests under Nonstandard Conditions. J Am Stat Assoc 82 (398):605-610. 10.1080/01621459.1987.10478472

Shackleton S, Dzerefos C, Shackleton C, Mathabela F (1998) Use and trading of wild edible herbs in the central lowveld savanna region, South Africa. Econ Bot 52 (3):251-259

Singh H, Mishra A, Mishra AK (2018) The chemistry and pharmacology of *Cleome* genus: A review. Biomed Pharmacother 101:37-48. 10.1016/j.biopha.2018.02.053

Singh RK, Chaudhary BD (1985) Biometrical methods in quantitative genetic analysis. Third edition edn. Kalyani Publishers, New Delhi, India

Sogbohossou DEO (2019) Orphan no more : Ethnobotany and genetic analysis of leaf yield and secondary metabolites content in *Gynandropsis gynandra* (Cleomaceae). PhD Thesis, Wageningen University, Wageningen

Sogbohossou EOD, Achigan-Dako EG, Maundu P, Solberg S, Deguenon EMS, Mumm RH, Hale I, Van Deynze A, Schranz ME (2018a) A roadmap for breeding orphan leafy vegetable species: a case study of *Gynandropsis gynandra* (Cleomaceae). Hortic Res 5 (1):2. 10.1038/s41438-017-0001-2

Sogbohossou EOD, Achigan-Dako EG, Mumm R, de Vos RCH, Schranz ME (2020) Natural variation in specialised metabolites production in the leafy vegetable spider plant (*Gynandropsis gynandra* L. (Briq.)) in Africa and Asia. Phytochemistry 178:112468. 10.1016/j.phytochem.2020.112468

Sogbohossou EOD, Achigan-Dako EG, van Andel T, Schranz ME (2018b) Drivers of management of spider plant (*Gynandropsis gynandra*) across different socio-linguistic groups in Benin and Togo. Econ Bot:1-25. 10.1007/s12231-018-9423-5

Sogbohossou EOD, Kortekaas D, Achigan-Dako EG, Maundu P, Stoilova T, Van Deynze A, de Vos RCH, Schranz ME (2019) Association between vitamin content, plant morphology and geographical origin in a worldwide collection of the orphan crop *Gynandropsis gynandra* (Cleomaceae). Planta 250:933–947. 10.1007/s00425-019-03142-1

Valverde-Miranda D, Díaz-Pérez M, Gómez-Galán M, Callejón-Ferre Á-J (2021) Total soluble solids and dry matter of cucumber as indicators of shelf life. Postharvest Biol Technol 180:111603. 10.1016/j.postharvbio.2021.111603

van Jaarsveld P, Faber M, van Heerden I, Wenhold F, van Rensburg WJ, van Averbeke W (2014) Nutrient content of eight African leafy vegetables and their potential contribution to dietary reference intakes. J Food Compos Anal 33:77-84. 10.1016/j.jfca.2013.11.003

Vandebroek I, Voeks R (2018) The Gradual Loss of African Indigenous Vegetables in Tropical America: A Review. Econ Bot 72 (4):543-571. 10.1007/s12231-019-09446-3

Visscher PM, Hill WG, Wray NR (2008) Heritability in the genomics era — concepts and misconceptions. Nat Rev Genet 9 (4):255-266. 10.1038/nrg2322

Wangolo EE, Onyango CM, Gachene CK, Mong'are PN (2015) Effects of shoot tip and flower removal on growth and yield of spider plant (*Cleome gynandra* L.) in Kenya. Am J Exp Agric 8 (6):367-376. 10.9734/AJEA/2015/17271

Weinberger K, Pichop GN (2009) Marketing of African indigenous vegetables along urban and peri-urban supply chains in sub-Saharan Africa. In: Shackleton C, Pasquini M, Drescher A (eds) African indigenous vegetables in urban agriculture. Earthscan, UK,, pp 225-244

Wu T-h, Solberg SO, Yndgaard F, Chou Y-Y (2018) Morphological patterns in a world collection of *Cleome gynandra*. Genet Resour Crop Evol 65 (1):271-283. 10.1007/s10722-017-0529-x

Yuan B, Lyu W, Dinssa FF, Simon JE, Wu Q (2021) Free amino acids in African indigenous vegetables: Analysis with improved hydrophilic interaction ultra-high performance liquid chromatography tandem mass spectrometry and interactive machine learning. J Chromatogr A 1637:461733. 10.1016/j.chroma.2020.461733

Zorde M, Byrnes DR, Dinssa FF, Weller S, Simon JE (2020) Selection for delayed flowering time in response to long photoperiod to increase vegetative growth and multiple harvests in spider plant (*Cleome gynandra*). Journal of Medicinally Active Plants 9 (2):60-70

CHAPTER 4

Leaf ionome analysis in spider plant (*Gynandropsis gynandra* L. (Briq.)) differentiates three nutritional groups

Abstract

Understanding the genetic variability within a plant species is paramount in implementing a sustainable breeding program. Spider plant (*Gynandropsis gynandra*) is an orphan leafy vegetable and an extraordinary source of vitamins, secondary metabolites and minerals, representing an important resource for combatting malnutrition. However, an evaluation of the leaf ionome, using a worldwide germplasm collection to inform breeding programs and the species valorization in human nutrition is still lacking. The present study aimed to profile the leaf ionome of *G. gynandra* and depict any potential geographical signature using a collection of 70 advanced lines derived from accessions originating from Asia and Eastern, Southern and West Africa. The collection was grown in a greenhouse using a 9 x 8 alpha lattice design with two replications in 2020 and 2021. Inductively coupled plasma–optical emission spectrometry was used to profile nine minerals contents. A significant difference ($p < 0.05$) was observed among the lines for all nine minerals. Microelements such as iron, zinc, copper and manganese contents ranges were 12.59-430.72, 16.98-166.58, 19.04-955.71, 5.39-25.10 mg kg^{-1} dry weight, respectively, while the concentrations of macroelements such as potassium, calcium, phosphorus and magnesium varied in the ranges of 9992.27-49854.23, 8252.80-33681.21, 3633.55-14216.16, 2068.03-12475.60 mg kg^{-1} dry weight, respectively. Significant and positive correlations were observed between iron and zinc and calcium and magnesium. Zinc, calcium, phosphorus, copper, magnesium, and manganese represented landmark elements in the genotypes. East and Southern African genotypes were clustered together in group 1 with higher phosphorus, copper and zinc contents than Asian and West African lines, which clustered in group 2 and were characterized by higher calcium, magnesium and manganese contents. An additional outstanding group 3 of six genotypes was identified with high iron, zinc, magnesium, manganese and calcium contents and potential candidates for cultivar release. The genotype × year variance was greater than the genotypic variance, which might translate to phenotypic plasticity in the species. Broad-sense heritability ranged from low to high and was element-specific. The present results reveal the leaf ionome diversity in spider plant and represent a baseline for implementing an ionome-based breeding program and incorporating the species valorization in human nutrition.

Keywords: African leafy vegetable, breeding, *Cleome gynandra*, genetic diversity, human nutrition, local adaptation, nutrient content.

4.1 Introduction

The ionome refers to the composition of minerals as nutrients and trace elements of an organism and constitutes the inorganic component of cellular and organismal systems, such as the leaf, seed or whole plant (Salt et al. 2008). The minerals include macroelements such as carbon (C), nitrogen (N), potassium (K), oxygen (O), calcium (Ca), magnesium (Mg), phosphorus (P), hydrogen (H), and sulfur (S); microelements such as copper (Cu), zinc (Zn), manganese (Mn), iron (Fe), molybdenum (Mo), boron (B), nickel (Ni), and chlorine (Cl), which are essential for plants; and beneficial elements such as cobalt (Co), aluminum (Al), sodium (Na), selenium (Se) and silicon (Si) (Pilon-Smits et al. 2009; Kirkby 2012). The study of the ionome is called ionomics and represents the quantitative analysis of the elemental composition of an organism and its changes in relation to environments, genetic modifications, developmental stages and physiological stimuli (Salt et al. 2008). This requires the integration of high-throughput elemental analysis technologies with both genetic and bioinformatic tools.

Understanding the plant ionome is crucial for both plants and humans. In plants, macro- and micro-elements are key components of biochemical and physiological processes, including DNA synthesis, photosynthesis, chlorophyll biosynthesis, protein modifications, nitrogen fixation and sugar metabolism (Hänsch and Mendel 2009; Maathuis 2009; Singh et al. 2016). Ionomics can serve as a diagnostic tool for plant pathologists and physiologists to understand specific physiological responses to infestation and genetic or environmental perturbations (Baxter et al. 2008; Nicolas et al. 2019). In addition, knowledge of the grain and leaf ionome is paramount for breeding nutrient-dense vegetable cultivars, including biofortified ones. The plant ionome is an essential component of human nutrition, particularly in the current situation where mineral deficiencies or hidden hunger affect more than two billion people worldwide, with the majority living in low- and middle-income countries, mainly Asia and sub-Saharan Africa (FAO et al. 2019; Tulchinsky 2010). Ionome profiling is often conducted using high-throughput technologies, including inductively coupled plasma–atomic emission spectrometry (ICP–AES), inductively coupled plasma–mass spectrometry (ICP–MS), X-ray fluorescence (XRF) and neutron activation analysis (NAA) (Huang and Salt 2016; Salt et al. 2008).

The plant ionome results from the complex interaction among minerals and is controlled by genetic and physiological processes, although it is also affected by the environment (Baxter 2009). The main environmental factor driving the plant ionome is the soil because almost all the required mineral nutrients and trace elements are absorbed from the soil. For instance,

Watanabe et al. (2015) observed changes in the leaf ionome of maize under different soil conditions, and Hogan et al. (2021) observed changes in the root and leaf tissue ionome composition of plant species across a fertility gradient. Furthermore, plants grown under the same conditions or environments differ in their ionomic profile as a result of adaptation to their native environments (Huang and Salt 2016; Baxter et al. 2014). The plant ionome can inform environmental or ecological adaptation. For instance, leaf ionome profiling discriminated accessions from different European ecological regions in *Arabidopsis halleri* (L.) O'Kane and Al-Shehbaz (Stein et al. 2017), and the fruit ionome revealed geographical signatures in Indian accessions of *Artocarpus heterophyllus* Lam. (Debbarma et al. 2021). The ionome is species-specific (Neugebauer et al. 2020; Watanabe et al. 2016; White et al. 2012) but mainly driven by phylogeny (families) (Zhang et al. 2021a) and life forms (Watanabe and Azuma 2021). For instance, the leaf concentrations of many elements (K, Ca, Mg, P, Fe, Zn, Na) were significantly higher in herbaceous species than in woody species (Watanabe and Azuma 2021). Similarly, annual herbaceous species had significantly higher concentrations of K, Ca, Mg, P, Fe, Zn and Al than perennial herbaceous species (Watanabe and Azuma 2021). Zhang et al. (2021a) observed different preferences for specific element accumulation among different plant species, whereas species within the family seemed to have similar preferences for most elements. Species in the family Asteraceae had high accumulation of P, Cu, and Mo, while plants in the Dennstaedtiaceae family had the highest accumulation of Ba, Mn, Rb, and Cs. Significantly positive correlations among minerals were observed in the family Fabaceae, while weaker correlations among minerals were observed in the families Rosaceae, Poaceae and Asteraceae (Zhang et al. 2021a). In addition, the ionome is tissue-specific (Watanabe et al. 2016; Neugebauer et al. 2020) and cultivar-specific (Watanabe et al. 2016; Coulibali et al. 2020) and depends on the growth stage (Huang and Salt 2016). Consequently, understanding the natural variation of the ionome among accessions will provide insights into its adaptation to various local environments of occurrence and provide a solid basis for ionomics-based breeding programs tackling hidden hunger.

Vegetables, particularly orphan or underutilized vegetables, are an important source of micronutrients and represent an affordable source of minerals for local communities. The increasing interest in orphan leafy vegetables is particularly due to their distinct richness in minerals, vitamins, phytochemicals and antioxidants (Nyadanu and Lowor 2015; Moyo et al. 2018; Orech et al. 2007) and good adaptation to local conditions. They represent a good asset to adapt to the changing climate. Some of the most nutritious orphan African leafy vegetables

include amaranth (*Amaranthus* spp.), spider plant (*Gynandropsis gynandra* (L.) Briq.), African nightshade (*Solanum* spp.), celosia (*Celosia argentea* L.), gboma eggplant (*Solanum macrocarpon* L.), jew's mallow (*Corchorus olitorius* L.) and Ethiopian kale (*Brassica carinata* A. Braun) (Grubben et al. 2014).

Spider plant (*G. gynandra* syn. *Cleome gynandra* L.), belonging to the Cleomaceae family, is an annual herb with increasing interest because of its high vitamin, mineral and secondary metabolite contents (Sogbohossou et al. 2019; Omondi et al. 2017; Gowele et al. 2019; Moyo et al. 2018; Neugart et al. 2017; Schönfeldt and Pretorius 2011; Sogbohossou et al. 2020; Chataika et al. 2021; Thovhogi et al. 2021). The reported minerals in leaves, which are the most consumed parts of spider plant, include iron, zinc, calcium, copper, potassium, magnesium, manganese, phosphorus and sodium (Omondi et al. 2017; Thovhogi et al. 2021; Jiménez-Aguilar and Grusak 2015; Gowele et al. 2019). Most previous studies assessing elemental composition in leaves of *Gynandropsis gynandra* aimed at revealing its superiority in macro- and microelements over popular vegetables for human nutrition. Furthermore, these studies used various methods and technologies, and germplasm was limited to a country or region in Africa, with the most prominent being the study of Omondi et al. (2017). Although significant variation was observed, these authors did not attempt to identify landmark elements to differentiate accessions in the species, as has been shown in species based on morphology (Sogbohossou et al. 2019; Wu et al. 2018) and secondary metabolites (Sogbohossou et al. 2020) using worldwide accessions. Therefore, knowledge of natural ionome variation among geographically diverse accessions of *Gynandropsis gynandra* is limited.

The objectives of the present study were to: (i) profile the leaf ionome of *Gynandropsis gynandra* using worldwide assembled genotypes from Asia and Eastern, Southern and West Africa; (ii) determine the potential geographical signature of the leaf ionome; (iii) assess the relationship among the mineral element concentrations; and (iv) estimate the quantitative genetic parameters of element composition in the leaves of *G. gynandra*.

4.2 Materials and Methods

4.2.1 Plant Material

Refer to Chapter 3, Section 3.2.1. Specifically, 70 out of the 71 lines evaluated in Chapter 3 were included in this study as line W19 was removed due to limited leaf mass.

4.2.2 Experimental design and growth conditions

Refer to Chapter 3, Section 3.2.2.

4.2.3 Minerals analysis

Four weeks after transplanting, leaves were randomly collected in paper bags from all the plants in each replicate and bulked to obtain at least 20 g per genotype. The collected leaves were immediately transported to the laboratory and washed before oven-drying at 65 °C for 72 h. After cooling, dried leaves were ground using a mortar and pestle into a powder and sieved using a 1 mm screen sieve. Two independent replicates of 0.5 g each of sieved powder were weighed in porcelain crucibles using an analytical balance (D&T, ES-E200A, max=200 g, d = 0.1 mg, China). Samples were afterwards ashed in a muffle furnace at 550 °C for 2 hours. The obtained ashes were digested using 10 ml of double acid composed of nitric acid (HNO_3, 65%, Merck, Germany) and hydrochloric acid (HCl, 32%, Merck, Germany) mixed at a ratio of 1:3 (Jones 2001). The resultant mixtures were placed on a hot plate at 250 °C for 30 min and later cooled for 1 hour. Digestates were filtered using Whatman paper Grade 1 (Qualitative Filter Paper Standard Grade, circle, 125 mm, Merck, Germany) into a 100 ml volumetric flask and made up to the mark using deionized water. The resultant solutions were analyzed using an inductively coupled plasma-optical emission spectrometer (ICP–OES) (Varian 720-ES, Varian Inc., Mulgrave, Victoria, Australia) for Ca, Cu, Fe, K, Mn, Mg, Na, P and Zn at the ICP Laboratory of the School of Chemistry and Physics of the University of KwaZulu-Natal, Pietermaritzburg Campus. The wavelengths used were 317.933 nm for Ca, 324.754 nm for Cu, 259.940 nm for Fe, 766.491 nm for K, 279.078 nm for Mg, 257.610 nm for Mn, 588.995 nm for Na, 213.618 nm for P, and 213.857 nm for Zn. An ICP multielement aqueous certified reference standard (1000 µg mL^{-1} ULTRASPEC® 5% HNO_3) was purchased from De Bruyn Spectroscopic Solutions Company, South Africa and used for calibration. All mineral contents were reported in mg kg^{-1} on a dry weight basis (mg kg^{-1} DW).

4.2.4 Data analysis

All statistical analyses were conducted in R software version 4.1.1 (R Core Team 2021). Data quality was assessed for outlier detection according to Bernal-Vasquez et al. (2016) using the Bonferroni–Holm test based on studentized residuals at the level of significance of 5%. The normality of the data was assessed using the Shapiro Wilk test, and only magnesium data were normally distributed. Descriptive statistics, including distribution frequency, mean, range,

coefficient of variation, and standard error, were generated to characterize the germplasm using the function *describe()* from the R package "psych" (Revelle 2019). When necessary, the difference among genotypes and regions of origin was tested through an analysis of variance or Kruskal–Wallis test using the function *aov()* or *kruskal.test()*, respectively. Variance components for each mineral were estimated in each year and across years. Each year, data were analyzed separately by implementing a linear mixed model following this statistical model:

$$y_{ik} = \mu + R_k + G_i + \varepsilon_{ik} \quad (1)$$

in which y_{ik} was the phenotypic observation of the i^{th} genotype in the k^{th} replicate, μ was the overall mean, R_k was the random effect of the k^{th} replicate, G_i was the random effect of the i^{th} genotype, and ε_{ik} was the random residual. Broad-sense heritability was calculated according to Hallauer et al. (2010) as follows:

$$H^2 = \sigma_G^2/(\sigma_G^2 + \sigma_e^2/r) \quad (2)$$

where σ_G^2 is the total genotypic variance, σ_e^2 is the residual variance, and r is the number of replications.

Variance components across years were estimated by fitting a linear mixed-effect model according to the following statistical model:

$$y_{ijk} = \mu + Y_j + R_k(Y_j) + G_i + GY_{ij} + \varepsilon_{ijk} \quad (3)$$

in which y_{ijk} was the phenotypic observation of the i^{th} genotype in the k^{th} replicate at the j^{th} year, μ was the overall mean, Y_j was the random effect of the j^{th} year, $R_k(Y_j)$ was the random effect of the k^{th} replicate within the j^{th} year, G_i was the random effect of the i^{th} genotype, GY_{ij} was the random effect of the interaction between the i^{th} genotype and the j^{th} year, and ε_{ijkl} was the random residual. Residual variances were assumed to be heterogeneous among years. Broad-sense heritability across years was calculated according to Hallauer et al. (2010) as follows:

$$H^2 = \sigma_G^2/(\sigma_G^2 + \sigma_{G\times Y}^2/n + \sigma_e^2/nr) \quad (4)$$

where σ_G^2 is the total genotypic variance, $\sigma_{G\times Y}^2$ is the genotype × year interaction variance, σ_e^2 is the residual variance, r is the number of replications, and n is the number of years. As

adjusted means across years, the best linear unbiased estimators (BLUEs) were estimates from the across years analysis, assuming fixed genotype effects. The BLUEs were therefore used in the further analyses. All linear mixed-effects models were fitted using the restricted maximum likelihood (REML) implemented in the "ASReml-R" package version 4.1.0.160 (Butler et al. 2017). Spearman rank correlation coefficients among all leaf ionome elements and their significance level were calculated using the function *rcorr()* from the R package "Hmisc" (Harrell Jr and Dupont 2021). To assess the relationship among the genotypes and the leaf ionome elements, a principal component analysis was performed using the *PCA()* function implemented in the R "FactoMineR" package (Lê et al. 2008). Furthermore, we performed hierarchical clustering on principal components (HCPC) using the *HCPC()* function of the same R package to group the genotypes based on the minerals, and the results were visualized as a factor map using the *fviz_cluster()* function of the R package "factoextra" (Kassambara and Mundt 2020). Significant differences among clusters were tested using the Kruskal–Wallis test followed by Dunn's post-hoc test for mean separation using the function *dunn.test()* from the R package "dunn.test" (Dinno 2017). The genetic advance (GA) for each mineral was computed as:

$$GA = i \times H^2 \times \sigma_P \tag{5}$$

where σ_P is the phenotypic standard deviation, H^2 is the broad-sense heritability, and *i* is the standardized selection differential at a selection intensity of 5% (i = 2.06) (Singh and Chaudhary 1985). Genetic advance over mean (GAM) was further computed as:

$$\boldsymbol{GAM = (GA/\bar{x}) \times 100} \tag{6}$$

where $\bar{x}$ and GA are the genetic advance and the overall mean of the element content, respectively. Genotypic (GCV), phenotypic (PCV) and error (ECV) coefficients of variation were estimated as described by Burton and DeVane (1953) as follows:

$$GCV\ (\%) = \frac{\sqrt{\sigma_G^2}}{\bar{x}} \times 100 \tag{7}$$

$$PCV\ (\%) = \frac{\sqrt{\sigma_P^2}}{\bar{x}} \times 100 \tag{8}$$

$$ECV\ (\%) = \frac{\sqrt{\sigma_e^2}}{\bar{x}} \times 100 \tag{9}$$

where σ_G^2 is the genotypic variance, σ_P^2 is the phenotypic variance, σ_e^2 is the residual variance, and $\bar{x}$ is the overall mean.

4.3 Results

4.3.1 Macroelements profile of leaves in *Gynandropsis gynandra*

The macroelements detected at significant levels in the leaves of *Gynandropsis gynandra* included calcium, potassium, phosphorus and magnesium (Table 4.1). The most abundant macroelement was potassium, with content ranging from 9992.27 to 49854.23 mg kg^{-1} dry weight (DW) with a mean of 26393.85 mg kg^{-1} DW, followed by calcium (8252.8 - 33681.21 mg kg^{-1} DW), phosphorus (3633.55 - 14216.16 mg kg^{-1} DW) and magnesium (2068.03 - 12475.6 mg kg^{-1} DW), with average contents of 18539.7, 8558.29 and 6719.83 mg kg^{-1} DW, respectively. A highly significant difference ($P < 0.001$) was observed among lines overall and within the region of origin for all macroelement contents (Figure 4.1). In addition, regions of origin differed significantly ($p < 0.001$) for all macroelement contents except for potassium content (Appendix 1.1A-D). On average, lines originating from West Africa had the highest calcium and magnesium contents, followed by the Asian lines. In contrast, Eastern and Southern African genotypes had the highest phosphorus content, whereas West African genotypes had the lowest phosphorus content (Appendix 1.1A-D).

Table 4.1. Descriptive statistics of nine mineral contents in a population of 70 advanced lines of *G. gynandra* in 2020 and 2021. All minerals concentration (mg kg^{-1} dry weight).

Minerals	Mean	Minimum	Maximum	Range	Standard error	Coefficient of variation (%)
Ca	18539.7	8252.8	33681.21	25428.41	287.92	25.94
Cu	12.17	5.39	25.1	19.71	0.23	31.35
Fe	133.04	12.59	430.72	418.14	3.53	43.98
K	26393.85	9992.27	49854.23	39861.96	355.74	22.51
Mg	6719.83	2068.03	12475.6	10407.56	109.61	27.15
Mn	217.68	19.04	955.71	936.67	8.75	66.98
Na	1143.55	535.92	2165.9	1629.98	16.91	24.61
P	8558.29	3633.55	14216.16	10582.61	122.85	23.89
Zn	55.85	16.98	166.58	149.59	1.29	38.67

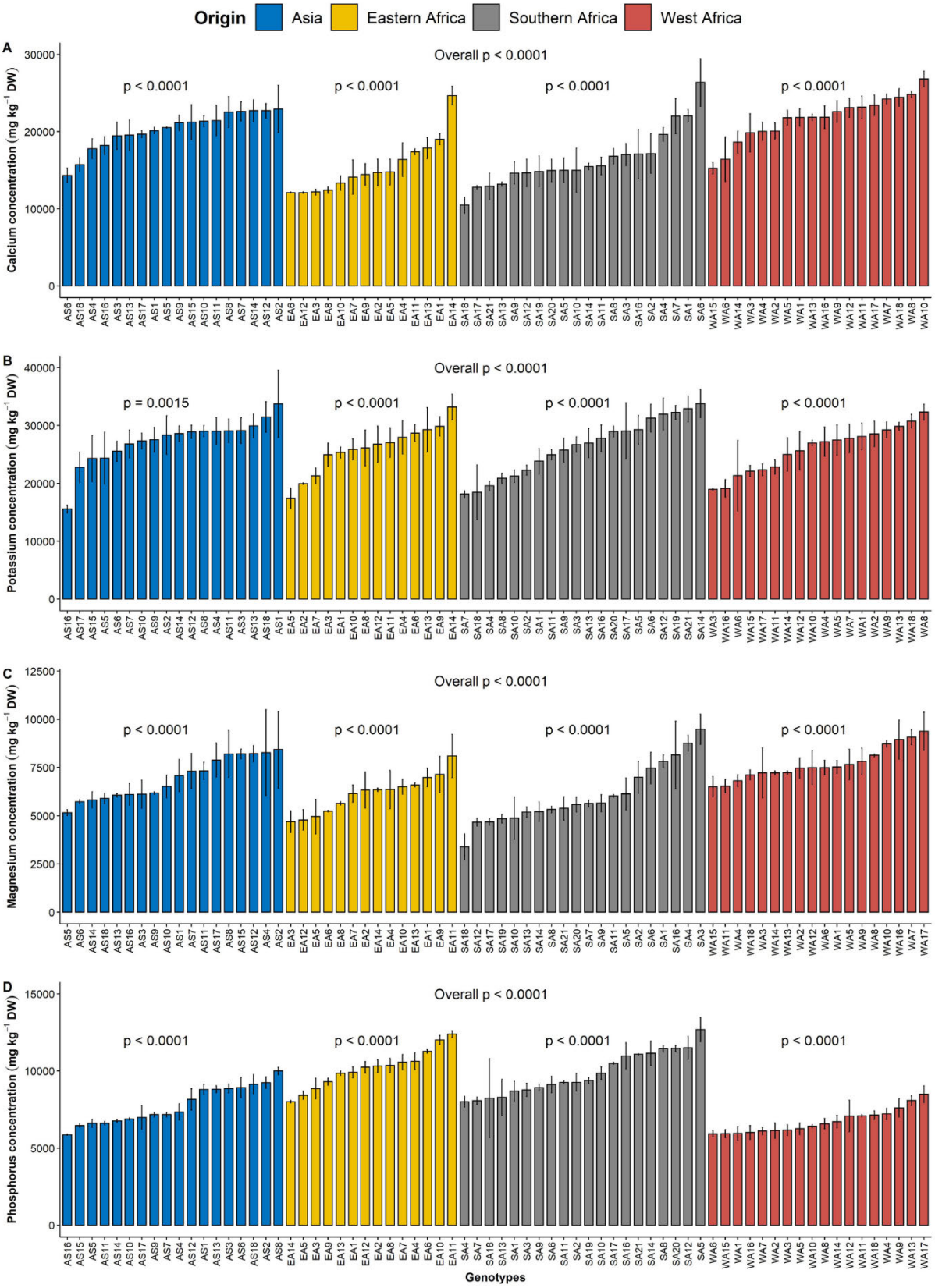


Figure 4.1. Variation in leaf macroelements content among 70 advanced lines of *G. gynandra* evaluated across years (2020 and 2021). (A) Calcium content; (B) Potassium content; (C) Magnesium content and (D) Phosphorus content. Bar plots are means and error bars represent standard errors across years (n = 4).

4.3.2 Microelements profile of leaves in *Gynandropsis gynandra*

The order of importance of microelements contents in the leaves of *Gynandropsis gynandra* was manganese > iron > zinc > copper. The manganese content varied from 19.04 to 955.71 mg kg^{-1} DW, with the highest variability (CV = 66.98%). The iron content ranged between 12.59 and 430.72 with an average of 133.04 mg kg^{-1} DW and constituted the second most variable microelement in the leaf. The zinc content had a CV of 38.67% and varied between 16.98 and 166.58, with an average of 55.85 mg kg^{-1} DW. With the lowest CV (31.35%), the copper content was 12.17 mg kg^{-1} DW on average with a range of 5.39-25.1 mg kg^{-1} DW. For iron, a significant difference ($p < 0.05$) was noticed among genotypes overall, while a high significant difference ($p < 0.01$) was observed among genotypes within each region (Figure 4.2A). Although no significant difference ($p = 0.162$) was observed among regions of origin across the two years for iron content, a significant difference ($p < 0.05$) was observed among regions of origin in each year with fluctuating performance of the regions of origin from one year to another (Appendix 4.1E).

A highly significant difference ($p < 0.001$) was observed among regions of origin across the two years for copper content, with Southern African genotypes having the highest copper content and West African genotypes having the lowest copper content (Appendix 4.1F). Manganese and zinc showed a highly significant difference among genotypes overall and within the region of origin ($p < 0.001$) (Figure 4.2C-D) but also among regions of origin (Appendix 4.1G-H). West African genotypes had the highest manganese content, followed by the Asian genotypes and the Eastern and Southern African genotypes (Appendix 4.1G). In contrast, the Southern African genotypes had the highest zinc content, while the lowest was observed for West African genotypes (Appendix 4.1H).

4.3.3 Sodium content in leaves of *Gynandropsis gynandra*

Sodium is another beneficial element investigated in the present study. The average sodium content was 1143.55 mg kg^{-1} DW with a coefficient of variation of 24.61%. A significant to highly significant difference was noticed among genotypes overall ($p < 0.001$, Figure 4.3). While a highly significant difference was observed among genotypes within each region for sodium content (Figure 4.3), no significant difference ($p = 0.17$) was depicted among the regions of origin (Appendix 4.1H).

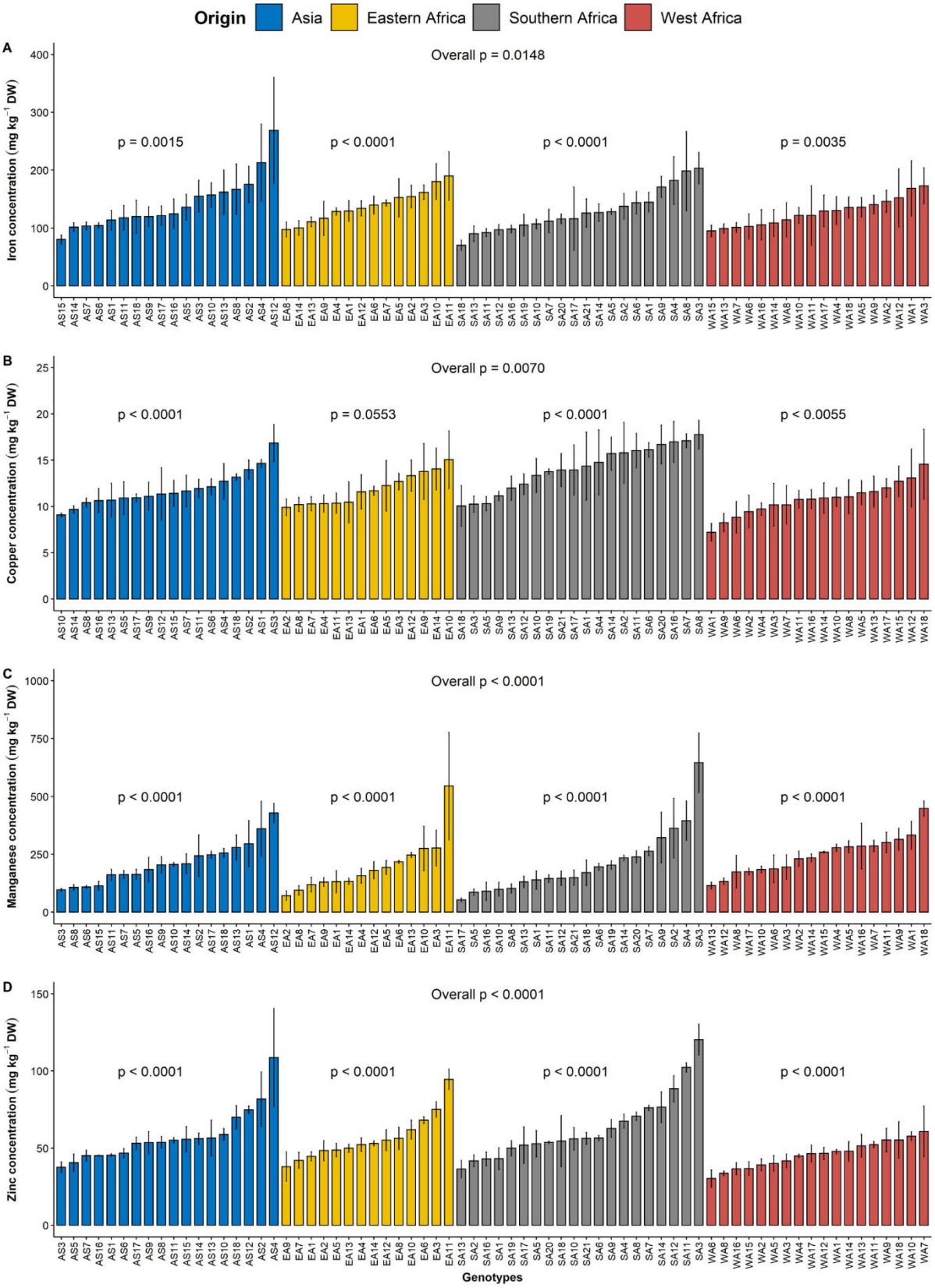


Figure 4.2. Variation in leaf microelements content among 70 advanced lines of *G. gynandra* evaluated across years (2020 and 2021). (A) Iron content; (B) Copper content; (C) Manganese content and (D) Zinc content. Bar plots are means and error bars represent standard errors across years (n = 4).

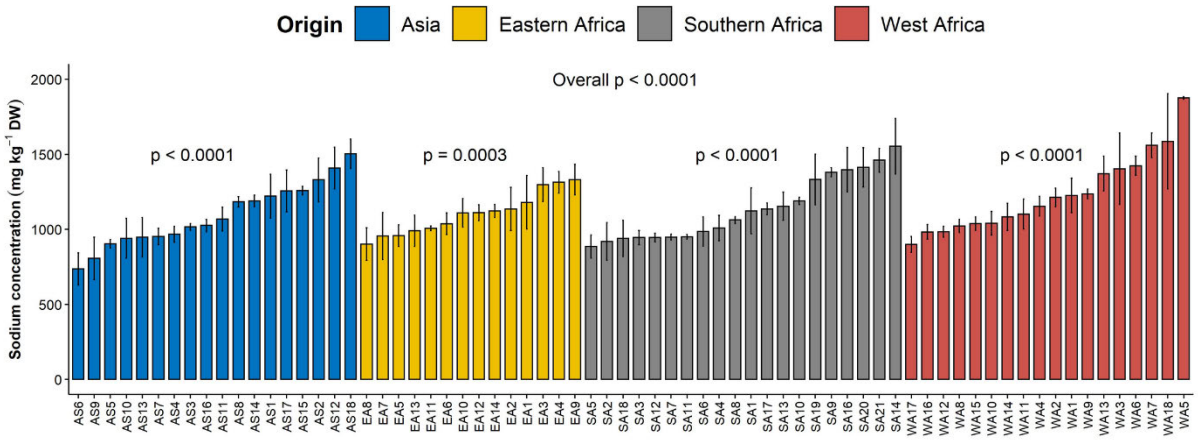


Figure 4.3. Variation in leaf sodium content among 70 advanced lines of *G. gynandra* evaluated in 2020 and 2021. Bar plots are the means, and error bars represent standard errors across years (n = 4).

4.3.4 Relationship among leaf ionome components

Correlations between the elemental composition of the leaves in *Gynandropsis gynandra* are summarized in Table 4.2 and ranged from -0.60 to 0.67. The highest, positive, and significant correlation was observed between calcium and magnesium contents (0.67, $p < 0.001$), while the highest negative and significant correlation was observed between calcium and phosphorus contents (-0.60, $p < 0.001$). A moderate, significant, and positive correlation was observed between the concentrations of copper and phosphorus (0.39, $p < 0.001$), iron and manganese (0.34, $p < 0.01$), phosphorus and zinc (0.35, $p < 0.01$), magnesium and manganese (0.32, $p < 0.01$), and potassium and sodium (0.31, $p < 0.01$). Zinc content had a weak, positive and significant correlation with iron content (0.27, $p < 0.05$) and manganese content (0.28, $p < 0.05$). Significant correlations observed between phosphorus and potassium contents (0.24, $p < 0.05$) and between the concentrations of manganese and calcium (0.26, $p < 0.05$) were weak. In contrast, there was a negative and moderate correlation between the concentration of phosphorus and magnesium ($r = -0.43$, $p < 0.001$) and between phosphorus and manganese contents ($r = -0.30$, $p < 0.01$).

Table 4.2. Spearman rank correlation coefficients among nine leaf mineral concentrations in a population of 70 advanced lines of *G. gynandra*.

Minerals	Ca	Cu	Fe	K	Mg	Mn	Na	P
Cu	-0.13							
Fe	0.06	-0.02						
K	0.13	0.14	-0.07					
Mg	**0.67*** **	-0.21	0.19	-0.02				
Mn	**0.26***	-0.12	**0.34****	0.06	**0.32****			
Na	0.04	0.08	0.01	**0.31****	0.11	0.14		
P	**-0.60*** **	**0.39*** **	0.16	**0.24***	**-0.43*** **	**-0.30***	-0.04	
Zn	-0.08	0.16	**0.27***	0.18	-0.1	**0.28***	-0.01	**0.35****

Values in bold are significant at $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

4.3.5 Clustering patterns among genotypes

Principal component analysis (PCA) showed that the first three components explained 61.02% of the total variation in the leaf ionome (Figure 4.4, Appendix 4.2). The first principal component retained 25.93% of the total variation and was positively and significantly correlated with calcium, magnesium and manganese contents (Figure 4.4, Appendix 4.2). Iron, zinc and phosphorus concentrations were positively and significantly associated with the second principal component, which explained 20.93% of the total variation (Figure 4.4, Appendix 4.2). The third principal component accounted for 14.15% of the total variation and was significantly and positively correlated with potassium and calcium contents (Figure 4.4, Appendix 4.2). Furthermore, the PCA differentiated West African genotypes from both East and Southern African genotypes, while Asian genotypes were spread between the West African and the East and Southern African genotypes (Figure 4.5). The PCA biplot based on the first two components showed that the West African genotypes were characterized by calcium and magnesium contents. In contrast, East and Southern African genotypes had substantial phosphorus and copper contents, with some genotypes having high iron, zinc and manganese contents (Figure 4.5).

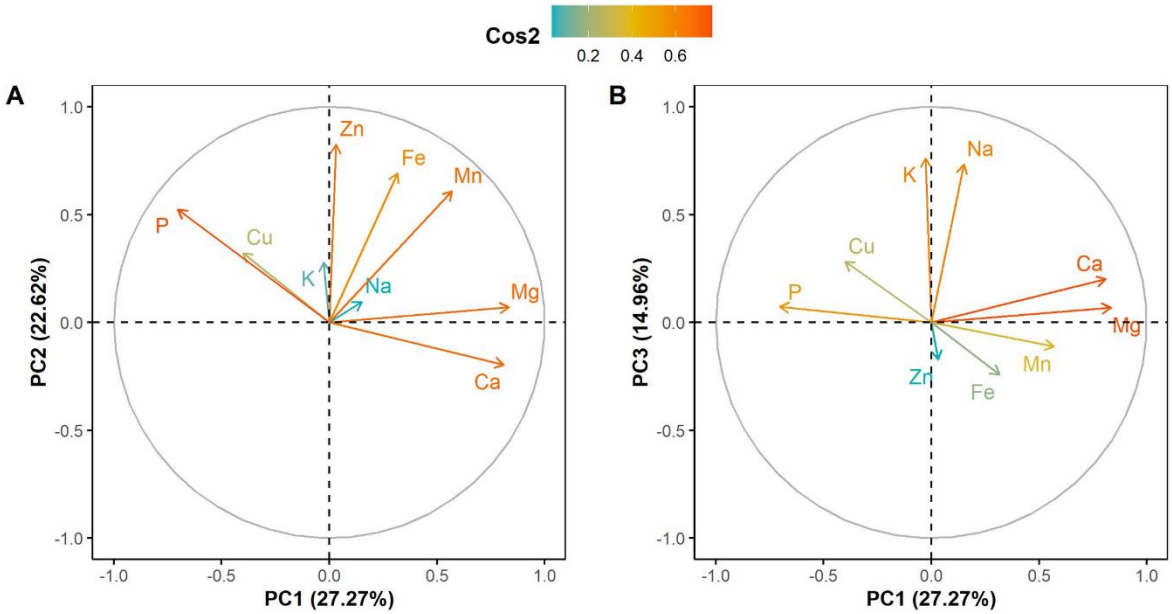


Figure 4.4. Correlation circle showing leaf ionome elements projection on (A) the first two principal components and (B) the first and third principal components. Cos2 refers to the quality of representation for variables on the principal component.

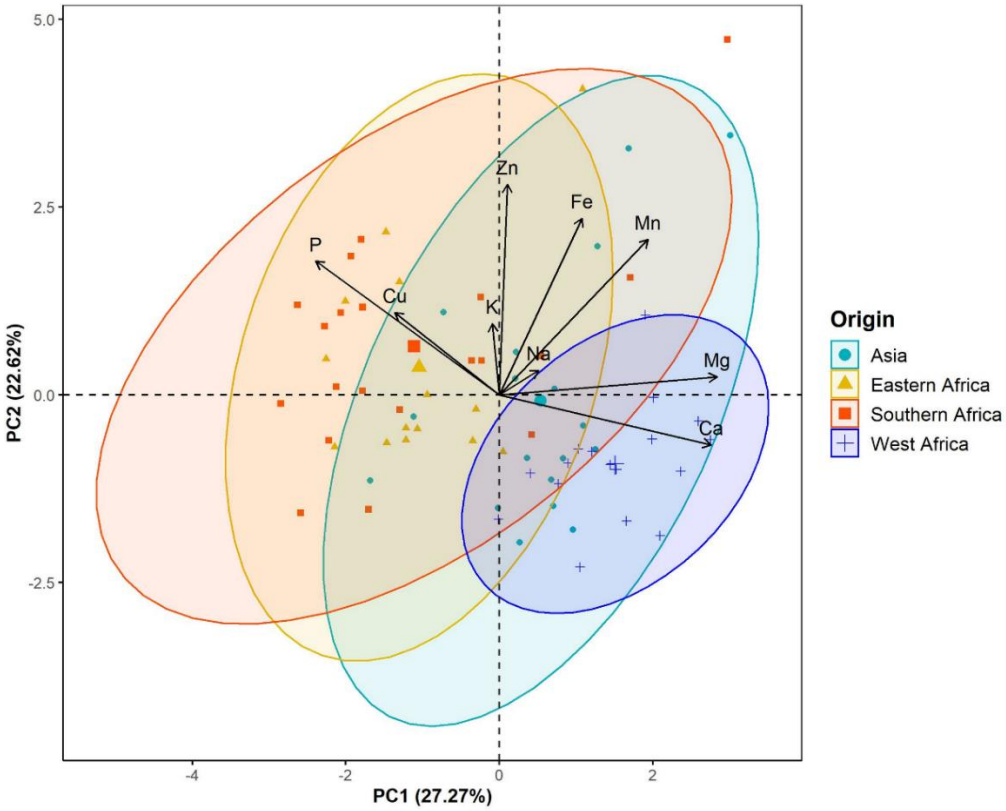


Figure 4.5. Biplots of the first principal component (PC1) versus the second principal component (PC2) for the leaf ionome in a population of 70 advanced lines of *G. gynandra*. Ninety percent bivariate ellipses were represented for lines from the same geographical origin. Asian (n = 18), East Africa (n = 14), Southern Africa (n = 20); and West Africa (n = 18).

The hierarchical clustering on principal components classified the 70 *G. gynandra* genotypes into three clusters (Figure 4.6), whose characteristics are presented in Figure 4.7. Cluster 1 consisted of 44.28% (n = 31) of all genotypes and predominantly genotypes from East (n = 12) and Southern (n = 16) Africa with three genotypes from Asia and therefore was named East/Southern African. Cluster 1 was characterized by low calcium, magnesium and manganese contents but had high phosphorus and copper contents with moderate iron and zinc contents. Genotypes in cluster 2 were mainly from West Africa (18) and Asia (12), with few from Southern (2) and East (1) Africa (Figure 4.6). Cluster 2 encompassed 47.14% of all genotypes and was called Asian/West African. High calcium content together with moderate magnesium and manganese contents and low phosphorus, copper and zinc contents described cluster 2 (Figure 4.7). The last cluster, cluster 3, was composed of six genotypes, three, two, and one from Asia, Southern Africa and Eastern Africa, respectively. Cluster 3 was the best and was characterized by high iron, zinc, magnesium and manganese contents with moderate calcium content (Figure 4.7).

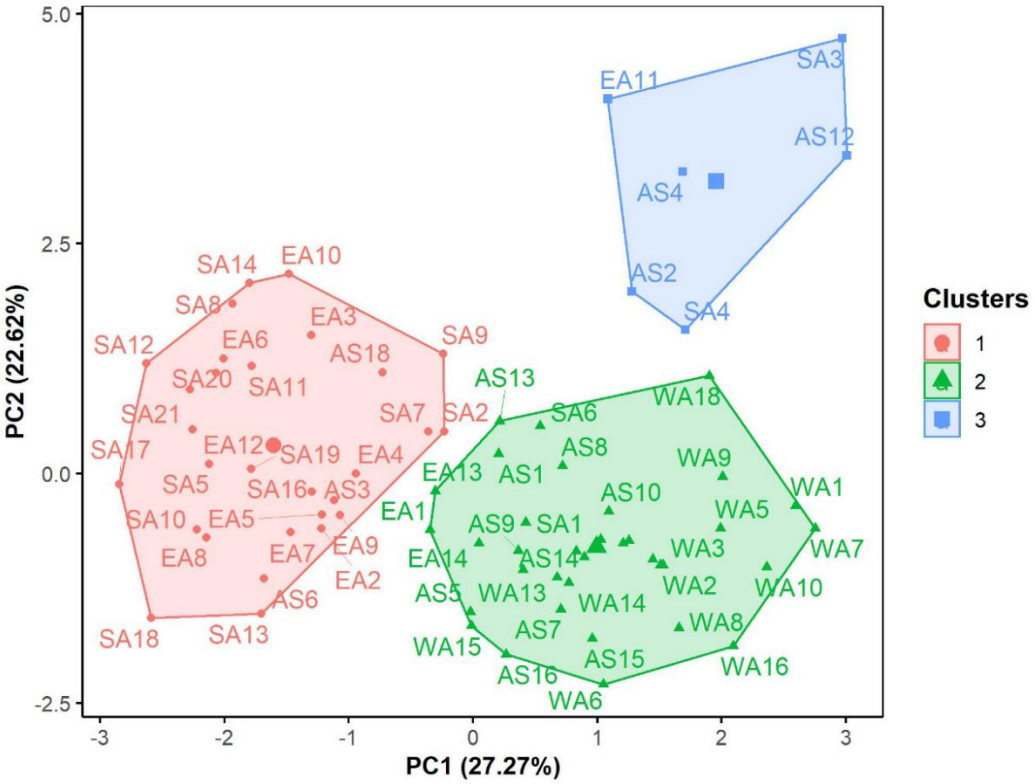


Figure 4.6. Factor map displaying the grouping pattern of 70 advanced lines of *G. gynandra* based on the hierarchical clustering on principal components analysis (HCPC). Cluster 1 (n = 31), Cluster 2 (n = 33) and Cluster 3 (n = 6). AS: Asia; EA: East Africa; SA: Southern Africa; WA: West Africa.

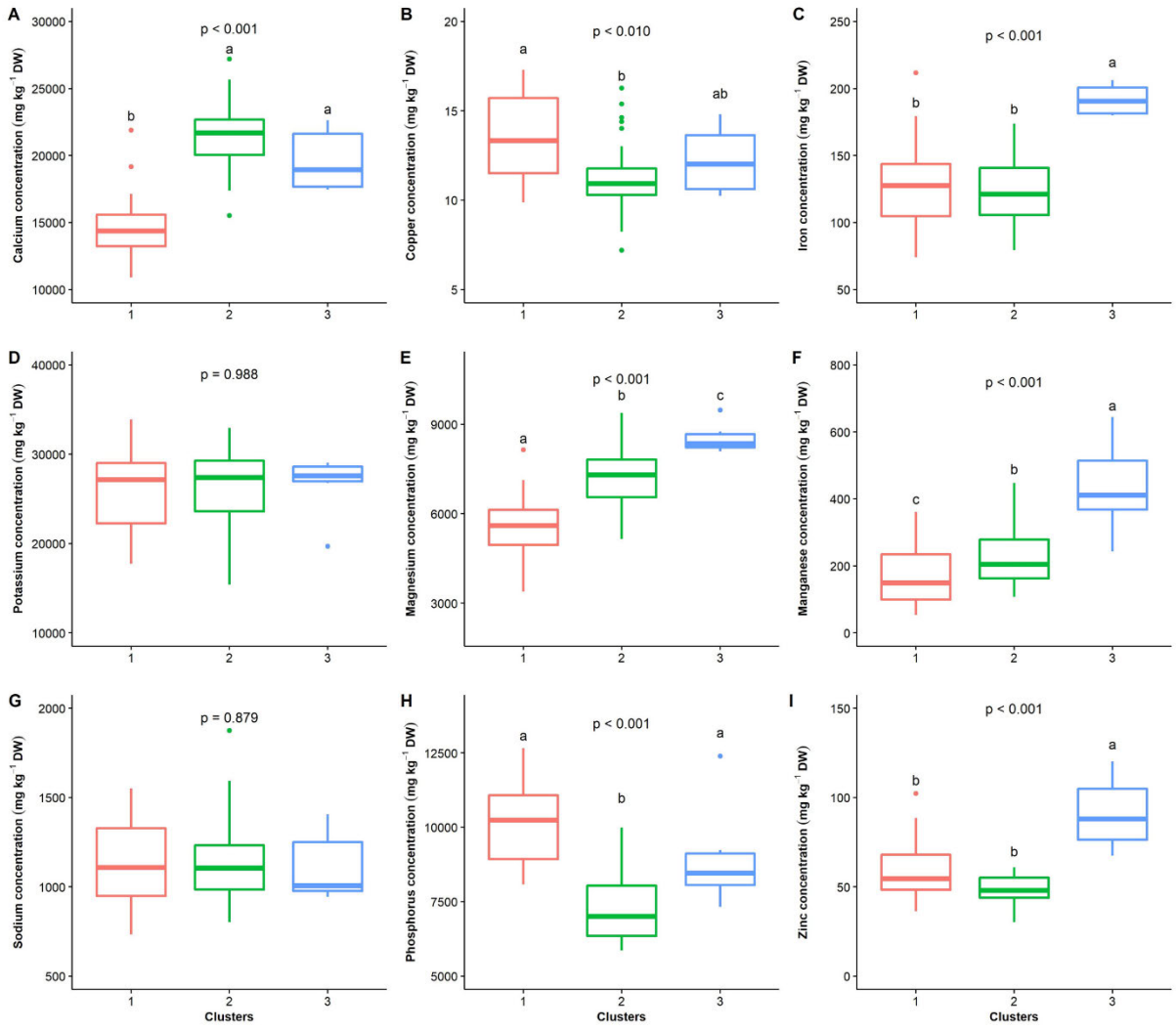


Figure 4.7. Clusters' performance comparison based on nine elemental compositions of the leaf ionome in *G. gynandra*. (A) Calcium content; (B) Copper content; (C) Iron content; (D) Potassium content; (E) Magnesium content; (F) Manganese content; (G) Sodium content; (H) Phosphorus content; and (I) Zinc content. Cluster 1 (n = 31), Cluster 2 (n = 33) and Cluster 3 (n = 6). Boxplots with the same alphabetic letter are not significantly different at $p < 0.05$ according to Dunn's post hoc test.

4.3.6 Estimates of genetic parameters of leaf ionome

Estimates of genetic parameters, including variance components, heritability, genetic gain, phenotypic (PCV), genotypic (GCV) and error (ECV) coefficient of variation, for each element per year and across years are presented in Appendix 4.3 and Table 4.3, respectively. Genotypic

variance for each leaf elemental composition of *G. gynandra* was higher than the residual variance in each year except for copper and iron in 2021 (Appendix 4.3). Consequently, high broad-sense heritability was observed and ranged from 0.62 to 0.99 for all mineral contents in both 2020 and 2021 except for copper content (0.36) in 2021. The genotypic and phenotypic coefficients of variation were moderate to high for all mineral contents each year (Appendix 4.3). Similarly, genetic gains were moderate to high for all element concentrations per year except potassium content (27.75%) in 2020 and copper content (17.95%) in 2021 (Appendix 4.3).

Across years, genotype × year interaction variance was higher than the genotypic variance for all mineral contents except for calcium and phosphorus contents. Broad-sense heritability across years varied from 0.00 to 0.78, with calcium content (0.78 ± 0.05) and phosphorus content (0.76 ± 0.06) having relatively high values. Moderate broad-sense heritability was observed for potassium (0.41 ± 0.14), magnesium (0.56 ± 0.11), manganese (0.31 ± 0.17), sodium (0.35 ± 0.16) and zinc (0.53 ± 0.11) contents. Surprisingly, genotypic variance across years for iron content was null with a heritability equal to zero, showing that leaf iron content was mostly influenced by the year or environment and that selection for iron content might be difficult. Variable genetic gains at 5% selection intensity were observed for the leaf ionome with no genetic gain for iron and the highest (35.27% over the mean of the current population) calcium content (Table 4.3). The error coefficient of variation was low (< 10%) for magnesium, manganese, sodium, phosphorus and zinc contents, moderate for calcium (11.88%) and potassium (14.18%) contents, and high for calcium (23.92%) and iron (31.34%) contents. A high (> 20%) coefficient of genotypic variation was noticed for manganese and zinc contents and moderate for calcium, potassium, magnesium, sodium and phosphorus contents (Table 4.3). The phenotypic coefficient of variation was moderate to high for all leaf ionome elements (Table 4.3).

Table 4.3. Estimates of genetic parameters for the leaf ionome in 70 advanced lines of *G. gynandra* evaluated across years (2020 and 2021).

Genetic parameters	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**P**	**Zn**
σ_G^2	12840936.71 ± 2842625.95	0.69 ± 1.20	0.00 ± NA	7035073.29 ± 3377512.82	987563.72 ± 329479.34	3639.83 ± 2458.75	16228.06 ± 9466.68	2551219.96 ± 587213.67	157.17 ± 55.79
σ_Y^2	1842256.24 ± 2748999.59	0.54 ± 1.09	15.81 ± 75.11	127852.82 ± 658317.67	1484654.60 ± 2131243.73	1977.21 ± 3129.67	0.00 ± NA	0.21 ± NA	41.57 ± 64.43
$\sigma_{G\times Y}^2$	4664094.65 ± 1238432.48	4.91 ± 1.69	1703.78 ± 330.12	13458220.41 ± 3571910.73	1534003.85 ± 266444.04	16369.10 ± 2807.40	57942.85 ± 10265.64	1547882.46 ± 272119.76	269.59 ± 47.53
σ_e^2	4847991.64 ± 639973.20	8.50 ± 1.07	1743.47 ± 217.67	14044988.71 ± 1858479.43	60650.79 ± 7327.59	234.13 ± 37.29	5487.84 ± 677.39	119358.45 ± 15600.40	18.93 ± 2.32
σ_P^2	16384981.95 ± 2774396.76	5.27 ± 0.91	1287.76 ± 155.95	17275430.67 ± 2912496.30	1769728.34 ± 301346.98	11882.91 ± 2023.28	46571.45 ± 7931.71	3355000.80 ± 571368.99	296.69 ± 50.52
H^2	0.78 ± 0.05	0.13 ± 0.21	0.00 ± 0.00	0.41 ± 0.14	0.56 ± 0.11	0.31 ± 0.17	0.35 ± 0.16	0.76 ± 0.06	0.53 ± 0.11
Mean	18530.84	12.19	133.24	26426.57	6717.04	216.64	1143.48	8568.46	55.89
GCV (%)	19.34	6.82	0.01	10.04	14.79	27.85	11.14	18.64	22.43
PVC (%)	21.84	18.84	26.93	15.73	19.81	50.32	18.87	21.38	30.82
ECV (%)	11.88	23.92	31.34	14.18	3.67	7.06	6.48	4.03	7.78
GA	6534.93	0.62	0	3486.75	1529.25	68.78	154.91	2869.25	18.8
GAM (%)	35.27	5.09	0	13.19	22.77	31.75	13.55	33.49	33.63

σ_G^2, genotypic variance; σ_Y^2, year variance; $\sigma_{G\times Y}^2$ genotype × year variance; σ_e^2, residual variance; σ_P^2, phenotypic variance; H^2, broad-sense heritability; GCV, genotypic coefficient of variation; PCV, phenotypic coefficient of variation; ECV, residual coefficient of variation; GA, genetic advance; GAM, genetic advance over mean.

4.4 Discussion

4.4.1 Nutritional value of leaves of *Gynandropsis gynandra*

Leaves of *G. gynandra* are highly nutritious and rich in potassium, calcium, sodium, phosphorus, magnesium, iron, manganese, zinc and copper. This aligns with previous reports on the species leaf nutritional value and potential in improving human nutrition (Omondi et al. 2017; Thovhogi et al. 2021; Jiménez-Aguilar and Grusak 2015; Moyo et al. 2018). The concentrations of iron, zinc, calcium, magnesium, manganese, phosphorus, copper, potassium, and sodium were comparable with those reported by Moyo et al. (2018), Omondi et al. (2017) and Thovhogi et al. (2021), but with some differences. For instance, the highest iron content in the present study (430.72 mg kg^{-1}) was comparable to that reported by Thovhogi et al. (2021) (431.3 mg kg^{-1}) but significantly lower than that of Omondi et al. (2017) (5892 mg kg^{-1}), with the latter pointing out potential contamination. The difference observed might also be associated with the genotype, environment and agricultural practices. Moyo et al. (2018) showed the superiority in mineral contents of *G. gynandra* over *Beta vulgaris* L. (Swiss chard) and *Brassica oleracea var. capitata* (cabbage), two world leading-consumed vegetables, with *G. gynandra* having 3.3- and 5.5-fold phosphorus, 1.4- and 1.8-fold potassium, 2.7- and 10.4-fold calcium, 4- and 2-fold iron and 1.2- and 2.1- fold zinc content more than *B. vulgaris* and *B. oleracea var. capitata*, respectively. The calcium, magnesium, and potassium concentrations in the leaves of *G. gynandra* were higher than those reported in *Amaranthus* species (Shukla et al. 2006; Sarker and Oba 2019).

Given the high mineral content, regular consumption of spider plant will be strategic in addressing micronutrient deficiencies and providing better human nutrition because of the diverse essential biological, physiological, and metabolic functions of minerals in the human body. There are multiple roles of iron in the human body, which include: (i) serving as an oxygen carrier through red blood cell hemoglobin from the lungs to tissues, (ii acting as an electron transporter within cells, and (iii) representing an essential component of enzyme machinery and DNA synthesis (Conrad and Umbreit 2000; FAO and WHO 2004). Iron also plays a crucial role in the immune system, as its deficiency affects the body's response to vaccines (Drakesmith et al. 2021). Iron represents one of the most deficient micronutrients in the diet of many populations, especially in Asia and Africa, and is responsible for diseases, including anemia, which mostly affects children, and pregnant and reproductive stage women in marginal regions of the world (Drakesmith et al. 2021). The recommended dietary allowance

(RDA) for iron is between 7 and 18 mg/day, depending on age group (Institute of Medicine 2006). With a serving size of 100 g fresh weight (FW), leaves of *G. gynandra* could provide 7.88 to 20.27% of the recommended dietary allowance (RDA) depending on the age group, with the lowest being for women between 19 and 50 years old (see Appendix 4.4). This agreed with previous reports on the species (Jiménez-Aguilar and Grusak 2015; van Jaarsveld et al. 2014) and some African green leafy vegetables (van Jaarsveld et al. 2014; Ejoh et al. 2021). Therefore, meeting the daily recommended intake of vegetables (300 g) (Willett et al. 2019) using spider plant will contribute up to 50% of the RDA of iron. More importantly, a consumption of 300 g of leaves of genotypes in cluster 3 would provide 80% of RDA for adults, except premenopausal women. Spider plant would, therefore, contribute to alleviating iron deficiency.

Zinc is the second essential micromineral, and because of its ability to bind to several enzymes and transcription factors, it is involved in several cellular functions and biochemical pathways, including DNA replication and damage repair, gene expression regulation, cell cycle progression, response to oxidative stress, apoptosis, homeostasis, immune responses, aging, and protein and collagen synthesis (Chasapis et al. 2020). Zinc deficiency remains an important health challenge in many low- and middle-income countries, including Sub-Saharan Africa (SSA), with a high prevalence in children, and pregnant and reproductive stage women, with consequences associated with adverse increased child morbidity and mortality, maternal health and pregnancy, and impaired childhood growth (Gupta et al. 2020). The consumption of 100 g fresh leaves of *G. gynandra* may provide between 5 and 7% of the RDA to adolescents and adults, respectively. This observation agreed with previous reports on the low contribution of the species and some other leafy vegetables to the diet for zinc (Ejoh et al. 2021; van Jaarsveld et al. 2014; Gowele et al. 2019; Jiménez-Aguilar and Grusak 2015). However, spider plant could be a good source of zinc (19.84% of the RDA) for infants between 1 and 3 years old (see Appendix 4.4).

Calcium confers rigidity to the skeleton (main cation of bone mineral), intervenes in most metabolic processes and is the second messenger of signals between the intracellular machinery and the plasma membrane (Power et al. 1999; Bronner and Pansu 1999). Magnesium, mostly in muscles and soft tissues but low in extracellular fluid, acts as a cofactor of more than 300 enzymes involved in many physiological and biological processes, such as RNA, DNA and protein synthesis, energy metabolism, electrical maintenance of the cell membranes and nervous tissues, and bone metabolism/remodeling (Glasdam et al. 2016; FAO and WHO 2004).

Depending on age group, the RDAs for calcium and magnesium were 500-1300 mg/day and 80-430 mg/day, respectively (53). While consuming 100 g of fresh leaves of spider plant provides 15% of RDA on average for adolescents, the same serving provides approximately 39% and 20% for infants (1-3 years old) and adults under 50 years old, respectively. In addition, *G. gynandra* leaves (100 g FW) could provide more than 50% of the RDA of magnesium for infants and children, with approximately 89% of the RDA to infants (1-3 years old) (see Appendix 4.4). Spider plant can be used to supplement calcium and magnesium for infants and children.

Manganese is a necessary nutrient for the human body, as it is crucial for the antioxidant system, development and metabolism (Avila et al. 2013). This mineral is required for several metabolic functions involved in the activation of certain metalloenzymes, nervous system function, reproductive hormone function, energy metabolism (metabolism of carbohydrates, fats, and proteins), immunological system function, and antioxidant enzymes protecting cells from free radical damage (Avila et al. 2013; Institute of Medicine 2006). It is also an essential component in blood clotting, cellular energy regulation, and bone and connective tissue growth and is a cofactor for various enzymes (those participating in neurotransmitter synthesis and metabolism) (Avila et al. 2013). More importantly, manganese is reported to be involved in the metabolism of brain glutamate to glutamine and stellate process production in astrocytes (Avila et al. 2013). Manganese deficiency was associated with generalized growth impairment, birth defects, reduced fertility, impaired bone formation, altered metabolism of lipids, proteins and carbohydrates, and several diseases (e.g., Down's syndrome, epilepsy, Perthest disease, osteoporosis Mseleni disease) (Avila et al. 2013). Irrespective of the age group, a serving size of 100 g of fresh leaves of spider plant could significantly supply the daily requirements of manganese (> 100%, see Appendix 4.4). Spider plant is, therefore, a prime source of manganese.

Potassium constitutes the major intracellular cation in the human body and refers to an electrolyte due to its role as an electrical charge messenger that activates various nerve and cell functions (Sobotka et al. 2008). It is essential for the maintenance of normal levels of fluid inside cells. Potassium is also involved in building proteins and muscle, maintaining normal body growth, and controlling the electrical activity of the heart and the acid-base balance. Potassium also helps muscles contract and supports normal blood pressure. It is a healthy heart mineral and is related to muscle contraction (Martínez-Ballesta et al. 2010). Sodium represents the major extracellular cation and is vital in regulating transmembrane gradients, fluid balance

(maintaining normal fluid levels outside of cells), and blood pressure (Thomas and Bishop 2013). Abnormal levels of potassium and sodium may lead to various pathological disorders, including hypernatremia (a high concentration of sodium, leading to edema, thirst, and lessened urine production), hyponatremia (a low concentration of sodium, often characterized by muscle spasms, headache, confusion, vomiting, seizures and nausea), hyperkalemia (a high concentration of potassium, associated with nausea, decreased urine production, irritability and cardiac arrest), hypokalemia (potassium deficiency, associated with fatigue, impaired glucose metabolism leading to elevated blood sugar, central nervous system changes, muscle weakness, decreased heart rate, slow reflexes, bone fragility, dry skin or acne, gastrointestinal disorders (ileus, nausea, vomiting, abdominal distention), renal effects (polyuria), and even death) (Pohl et al. 2013; Thomas and Bishop 2013; Ganong William 2005). Cardiac arrhythmia may result from a sudden loss of potassium (Pohl et al. 2013). The maintenance of the flux of these two electrolytes (membrane potential and osmotic equilibrium in cells) is assured by the Na+K+-ATPase pump, with the opposite movement of the two elements against the concentration gradients. The pump plays a crucial role in stabilizing the resting cell membrane potential, regulating cell volume, and cell signal transduction (Pivovarov et al. 2019). The sustained concentration gradient by the pump is crucial for many physiological processes, such as maintenance of filtering waste products in the nephrons (kidneys), production of the neuronal action potential, and sperm motility (Pirahanchi et al. 2021). Ma et al. (70) showed that higher sodium and lower potassium intakes were associated with a higher cardiovascular risk. Therefore, increasing potassium intake and reducing sodium intake is required, with a call for attention to the diet's Na:K ratio (Baer et al. 2021). The RDAs of sodium and potassium were 1000-1500 mg/day and 2000-3400 mg/day, respectively (National Academies of Sciences Engineering and Medicine 2019). A serving size of 100 g fresh leaves of *G. gynandra* may provide 0.81-1.22% of the RDA for sodium and 8.28-14.08% of the RDA for potassium according to the age group (see Appendix 4.4). Interestingly, the positive correlation between sodium and potassium associated with the low sodium and high potassium content with a high K/Na ratio (K/Na = 23) of spider plant leaves is an important outcome and shows the potential of the species in addressing cardiovascular risk, blood pressure, maintaining electrolyte balance and muscular function. This agrees with previous reports on the richness of green leafy vegetables as a source of potassium (Ejoh et al. 2021).

Phosphorus, available as phosphate, is the most abundant anion in the human body and the main component of nucleic acids (DNA and RNA) (Sobotka et al. 2008). This element is

essential for many metabolic processes, particularly those involved in maintaining acid-base balance (Chongtham et al. 2021). Phosphate is required to produce ATP, GTP and CP (energetic molecules) and regulate various enzymes through phosphorylation/ dephosphorylation reactions (Sobotka et al. 2008). Phosphorus is involved in teeth and bone formation and several body metabolic actions, including heart muscle contraction, cell growth and kidney functioning (Renkema et al. 2008; Martínez-Ballesta et al. 2010). Deficiency and excess phosphorus are called hyperphosphatemia and hypophosphatemia, respectively. Phosphorus deficiency is unusual, but when it occurs, it is associated with painful bones, skin sensitivity, numbness, fatigue, anxiety, changes in body weight, irregular breathing and growth retardation (Martínez-Ballesta et al. 2010; Renkema et al. 2008). Given that the RDAs of adolescents and adults are 1250 and 700 mg/day, respectively, consumption of 100 g of fresh leaves of spider plant could provide 7.30% and 13.05% of RDAs for adolescents and adults, respectively (see Appendix 4.4).

Copper functions as a vital constituent of many metalloenzymes (monoamine oxidase, ferroxidases, diamine oxidase, dopamine b-monooxygenase), which act as oxidases in molecular oxygen reduction (Institute of Medicine 2006). As such, copper participates in many biological systems, including immune function, neuropeptide synthesis, antioxidant defence, and iron metabolism (Bhattacharya et al. 2016). Copper deficiency is linked to diseases such as osteoporosis, hemosiderosis, abnormal bone formation with skeletal fragility, rheumatoid arthritis, hypochromic anaemia, neutropenia, hair and skin hypopigmentation, lowered immunity, joint pain, vascular aberrations and kinky hair (Watts 1989; Bhattacharya et al. 2016). The RDA of copper varies from 0.34 to 0.9 mg/day (Institute of Medicine 2006). Therefore, the consumption of 100 g of fresh leaves of spider plant may provide between 14.42% and 38.19% of the daily requirement of copper. Specifically, a serving of recommended intake of vegetables (300 g) (56) using spider plant may contribute up to more than 40% of the RDA for adolescents and adults (see Appendix 4.4). Spider plant, would, therefore, contribute to alleviating copper deficiency.

Given the above role of each mineral in human health, the positive correlations observed between calcium and magnesium, magnesium and manganese, calcium and manganese, copper and phosphorus, iron and zinc, zinc and manganese, iron and manganese, sodium and potassium, potassium and phosphorus, phosphorus and zinc are of great importance in maintaining the proper functioning of the human body. The positive association between iron, zinc and manganese will reinforce the immune and antioxidant systems (Cannas et al. 2020).

The association between calcium, magnesium and manganese is of importance in strengthening the bones, teeth, and nervous system (Quintaes and Diez-Garcia 2015). More importantly, these positive associations show the potential contribution of spider plant leaves in maintaining blood pressure, preventing cardiovascular disease, improving enzyme machinery, energy metabolism, fluid-electrolyte balance, regulating cell volume, and cell signal transduction. In addition, the leaves could contribute to improving the anti-inflammatory system, muscle contraction and relaxation, reproductive system, nucleic acid and protein synthesis, gene expression regulation, cell cycle progression, apoptosis and homeostasis.

Therefore, introducing the spider plant into the human diet will provide key essential minerals to overcome hidden hunger, as the species is also a rich source of vitamins and important phytochemicals (Sogbohossou et al. 2020; Sogbohossou et al. 2019; Omondi et al. 2017; Chataika et al. 2021; Moyo et al. 2018; Moyo and Aremu 2021). Based on the nutritional value of *Gynandropsis gynandra* leaves, the species can be used in biofortification programs, including medical supplementation and product fortification. Fortification refers to the process of commonly consumed food enrichment with nutrients (minerals and minerals) during processing through different methods to increase their nutritional value (Buturi et al. 2021; Olson et al. 2021). Efforts are still needed to popularize the species within and across countries/continents. To this end, genotypes in cluster 3 are potential candidates for species promotion. Several factors can affect leaf nutritional values, including soils, agronomic practices (fertilization, harvest time), developmental stages, cooking methods, and postharvest techniques. As nutritional value is genotype-specific, more investigations are needed to assess the effects of these factors on the nutritional values of the species (Moyo et al. 2018; Achigan-Dako et al. 2021; Sogbohossou et al. 2019). Other key components include the bioavailability of nutrients and the effects of the different cooking processes on bioavailability.

4.4.2 Genetic variation of leaf ionome in *Gynandropsis gynandra*

Spider plant exhibits a significantly wide range of variations in the leaf ionome, representing an important resource for breeding programs. This confirms previous reports of significant variation in leaf mineral concentrations among genotypes of *Gynandropsis gynandra* (Omondi et al. 2017; Thovhogi et al. 2021). The difference between these previous studies and the present study is the large collection used and the origin of the genotypes being from Asia and different regions of Africa, giving a unicity of the present study, as most previous studies used genotypes from East and Southern Africa (Omondi et al. 2017; Thovhogi et al. 2021; Jiménez-

Aguilar and Grusak 2015). Genotypes from this African region were found in the present study to cluster together and share the same genotypic background, as evidenced by Sogbohossou (2019). The observed variability was driven by the diverse provenance of the genotypes used as reported origin dependence in morphology (Wu et al. 2018; Sogbohossou et al. 2019), vitamin contents (Sogbohossou et al. 2019), secondary metabolite concentrations (Sogbohossou et al. 2020), seed mineral composition, seed morphology and germination (Blalogoe et al. 2020), antioxidant activity (Chataika et al. 2021), and photosynthesis traits (Reeves et al. 2018) in the species. The local adaptation in the species might further explain this.

4.4.3 Differentiation of genotypes and evidence of local adaptation

The present study demonstrated three groups in *G. gynandra* based on the leaf ionome, including two major groups, the first being the East/Southern African group and the second being the Asian/West African group. The East/Southern African group is characterized by high phosphorus, copper and zinc contents, while the Asian/West African group had higher calcium, magnesium and manganese contents. This grouping was similar to that obtained by Reeves et al. (2018) based on DNA sequencing and phylogenetic reconstruction and photosynthesis traits of nine accessions from Asia, East, Southern and West Africa. The authors found East/Southern African genotypes with lower core gene transcripts encoding C4 enzymes and water use efficiency, higher stomatal conductance and transpiration, higher vein and stomatal density, and small bundle sheath cell and area sizes compared to Asia/West African genotypes. Furthermore, this difference might be associated with the role of minerals (phosphorus, copper, zinc, calcium, magnesium and manganese) in photosynthesis and many other physiological, biochemical and metabolic processes in plants (Maathuis 2009; Hänsch and Mendel 2009). Another reason could be the induced changes by the environmental factors of the genotype origin with results of specific ion accumulation over time as local adaptation strategies (Huang and Salt 2016). This signal of geographical association, particularly local adaptation, with the ionome has also been reported for the leaves of accessions of *Arabidopsis halleri* from different European ecological regions (Stein et al. 2017) and for fruits in Indian accessions of *Artocarpus heterophyllus* Lam. (Debbarma et al. 2021). The observed variation offers an opportunity to investigate genes associated with ionome accumulation in the species as strategies for environmental adaptation, as reported in *Arabidopsis thaliana* (Campos et al. 2021). In-depth studies are required to understand the species' ability to absorb nutrients from soils and to what extent soil affects the leaf ionome in the species. The local adaptation might further be

explained by the genotype × year variance greater than the genotypic variance, which might translate the phenotypic plasticity in the species, as QTLs by environmental interactions were observed to control ionome divergence in rice (Tan et al. 2020), maize (Asaro et al. 2016), and switchgrass (Zhang et al. 2021b). However, evaluation under different environmental conditions is required. Because this study focused on leaves, future studies should include different plant parts, including stems, flowers, pods, and seeds.

4.4.4 Breeding and biofortification avenues for ionome-dense cultivars in *Gynandropsis gynandra*

Given the considerable variability observed, the present study offers several rooms for breeding nutrient-dense cultivars to tackle hidden hunger. The genotypes in cluster 3 are candidates for release and use in programs tackling micronutrient iron and zinc deficiencies. However, the biomass potential of the present germplasm used in this study should be assessed to identify morphological traits associated with mineral contents, as done by Sogbohossou et al. (2019), between morphology and vitamin concentrations in the species. Understanding the gene action controlling the leaf ionome will play a key role in designing appropriate breeding strategies for improved cultivar development. In addition, genes controlling the leaf ionome should be deciphered using a large natural collection and advanced populations, such as multiparent advanced generation intercross (MAGIC), recombinant inbred lines (RILs) and nested association mapping (NAM). These populations could be developed using the genotypes from clusters 1 and 2. The development of MAGIC will also help in exploiting the different nutritional potential of the three identified clusters in order to combine all mineral traits in elite cultivars. Several methods could be used, including genome-wide association studies (GWAS) and QTL mapping, which will be easier with ongoing efforts to release the genome of *G. gynandra* (Hendre et al. 2019; Sogbohossou 2019). The positive correlation between iron and zinc offers the possibility for simultaneous selection, as low heritability was observed for iron but moderate for zinc. This correlation contrasted with that observed by Omondi et al. (2017) and Thovhogi et al. (2021), who found no association between iron and zinc. This might be associated with the germplasm used, and these authors used genotypes from the same geographical region, East/Southern Africa. However, a positive correlation between calcium and magnesium was also reported Thovhogi et al. (2021), offering the possibility for simultaneous selection. The high genotype × year variance observed in the present study shows the roles of both genotype and environment in the leaf ionome in *G. gynandra*. Further investigations should be conducted to estimate the extent of these components' influence on

the leaf ionome in the species through multi-environmental trials. On the other hand, the nutritional value of the leaves can be enhanced through biofortification as a complementary strategy and incorporated into the breeding strategy. Consequently, agronomic mineral biofortification (Buturi et al. 2021) through the cultivation of *G. gynandra* in intensive agro-systems with the supply of nutrients through foliar fertilization, fertigation, soilless cultivation and organic fertilization will be key. This will particularly contribute to increasing the levels of zinc and iron in spider plant leaves, as a positive association was observed between the two elements.

4.5 Conclusion

The present study broadened the current knowledge on the nutritional value of *G. gynandra*, particularly its richness in minerals such as potassium, calcium, phosphorus, magnesium, iron, manganese, zinc, and copper. The species' genetic variability in the leaf ionome was revealed and provided a strong basis for developing more ion-dense cultivars for improved nutrition. This variability displayed some signals of local adaptation to the origin of the genotype, with genotypes from Asia clustered together with West African genotypes, on the one hand, and those from East Africa clustered together with Southern African genotypes, on the other hand, representing two significant gene pools for breeding higher nutritious cultivars. The Asian/West African group is rich in calcium, magnesium, and manganese, while the East/Southern African group had higher zinc, copper, and phosphorus contents. The two groups shared similar contents of iron, potassium and sodium. Additionally, genotypes combining the characteristics of these two groups were identified in a different cluster and are a prime resource for large-scale promotion in programs/projects tackling micronutrient deficiencies. Leaves of *G. gynandra* can be used as a supplement and in food fortification. Further investigations are required to assess micronutrient bioavailability, shelf-life and postharvest conditions, and cooking technique effects on the nutritional values of the species. Understanding the response to different growing conditions on leaf quality is required. Deciphering genes controlling each mineral and the extent of genotype-by-environment interaction effects on the leaf ionome are needed to boost more nutritious cultivars. Additionally, the potential association between morphological and leaf mineral contents should be assessed.

References

Achigan-Dako EG, Sogbohossou DEO, Houdegbe CA, Salaou MA, Sohindji FS, Blalogoe J, Chataika BY, Zohoungbogbo HF, Adje CAO, Fassinou Hotegni NV, Francisco R, Abukutsa-Onyango MO, Schranz ME (2021) Ten years of *Gynandropsis gynandra* research for improvement of nutrient-rich leaf consumption: lessons learnt and way forwards. Annu Rev Plant Biol 4 (3):767-812. 10.1002/9781119312994.apr0774

Asaro A, Ziegler G, Ziyomo C, Hoekenga OA, Dilkes BP, Baxter I (2016) The interaction of genotype and environment determines variation in the maize kernel ionome. G3 6 (12):4175-4183. 10.1534/g3.116.034827

Avila DS, Puntel RL, Aschner M (2013) Manganese in health and disease. Met Ions Life Sci 13:199-227. 10.1007/978-94-007-7500-8_7

Baer DJ, Althouse A, Hermann M, Johnson J, Maki KC, Marklund M, Vogt L, Wesson D, Stallings VA (2021) Targeting the dietary Na:K ratio—considerations for design of an intervention study to impact blood pressure. Adv Nutr:nmab099. 10.1093/advances/nmab099

Baxter I (2009) Ionomics: studying the social network of mineral nutrients. Curr Opin Plant Biol 12 (3):381-386. 10.1016/j.pbi.2009.05.002

Baxter IR, Vitek O, Lahner B, Muthukumar B, Borghi M, Morrissey J, Guerinot ML, Salt DE (2008) The leaf ionome as a multivariable system to detect a plant's physiological status. Proc Natl Acad Sci USA 105 (33):12081. 10.1073/pnas.0804175105

Baxter IR, Ziegler G, Lahner B, Mickelbart MV, Foley R, Danku J, Armstrong P, Salt DE, Hoekenga OA (2014) Single-kernel ionomic profiles are highly heritable indicators of genetic and environmental influences on elemental accumulation in maize grain (*Zea mays*). PLOS ONE 9 (1):e87628. 10.1371/journal.pone.0087628

Bernal-Vasquez A-M, Utz H-F, Piepho H-P (2016) Outlier detection methods for generalized lattices: a case study on the transition from ANOVA to REML. Theor Appl Genet 129 (4):787-804. 10.1007/s00122-016-2666-6

Bhattacharya PT, Misra SR, Hussain M (2016) Nutritional aspects of essential trace elements in oral health and disease: an extensive review. Scientifica 2016:5464373. 10.1155/2016/5464373

Blalogoe JS, Odindo AO, Sogbohossou EOD, Sibiya J, Achigan-Dako EG (2020) Origin-dependence of variation in seed morphology, mineral composition and germination percentage in *Gynandropsis gynandra* (L.) Briq. accessions from Africa and Asia. BMC Plant Biol 20 (1):168. 10.1186/s12870-020-02364-w

Bronner F, Pansu D (1999) Nutritional aspects of calcium absorption. J Nutr 129 (1):9-12. 10.1093/jn/129.1.9

Burton GW, DeVane EH (1953) Estimating heritability in tall fescue (*Festuca Arundinacea*) from replicated clonal material. Agron J 45 (10):478-481. 10.2134/agronj1953.00021962004500100005x

Butler D, Cullis B, Gilmour A, Gogel B, Thompson R (2017) ASReml-R Reference Manual Version 4. . VSN International Ltd Hemel Hempstead, HP1 1ES. UK

Buturi CV, Mauro RP, Fogliano V, Leonardi C, Giuffrida F (2021) Mineral biofortification of vegetables as a tool to improve human diet. Foods 10 (2):223. 10.3390/foods10020223

Campos ACAL, van Dijk WFA, Ramakrishna P, Giles T, Korte P, Douglas A, Smith P, Salt DE (2021) 1,135 ionomes reveal the global pattern of leaf and seed mineral nutrient and trace element diversity in *Arabidopsis thaliana*. Plant J 106 (2):536-554. 10.1111/tpj.15177

Cannas D, Loi E, Serra M, Firinu D, Valera P, Zavattari P (2020) Relevance of essential trace elements in nutrition and drinking water for human health and autoimmune disease risk. Nutrients 12 (7). 10.3390/nu12072074

Chasapis CT, Ntoupa P-SA, Spiliopoulou CA, Stefanidou ME (2020) Recent aspects of the effects of zinc on human health. Arch Toxicol 94 (5):1443-1460. 10.1007/s00204-020-02702-9

Chataika BY, Akundabweni LS-M, Houdegbe AC, Achigan-Dako EG, Sibiya J, Masamba K (2021) Dietary phytochemical screening of spider plant (*Gynandropsis gynandra* (L.) Briq.) accessions from Africa and Asia to identify genotypes for use in nutraceutical breeding. Front Sustain Food Syst 5 (344). 10.3389/fsufs.2021.733909

Chongtham N, Bisht MS, Santosh O, Bajwa HK, Indira A (2021) Mineral elements in bamboo shoots and potential role in food fortification. J Food Compost Anal 95:103662. 10.1016/j.jfca.2020.103662

Conrad ME, Umbreit JN (2000) Iron absorption and transport—an update. Am J Hematol 64 (4):287-298. 10.1002/1096-8652(200008)64:4<287::AID-AJH9>3.0.CO;2-L

Coulibali Z, Cambouris AN, Parent S-É (2020) Cultivar-specific nutritional status of potato (*Solanum tuberosum* L.) crops. PLOS ONE 15 (3):e0230458. 10.1371/journal.pone.0230458

Debbarma N, Manivannan S, Muddarsu VR, Umadevi P, Upadhyay S (2021) Ionome signatures discriminates the geographical origin of jackfruits (*Artocarpus heterophyllus* Lam.). Food Chem 339:127896. 10.1016/j.foodchem.2020.127896

Dinno A (2017) Package 'dunn. test': Dunn's test of multiple comparisons using rank sums. R package version 1.3.5. edn.,

Drakesmith H, Pasricha S-R, Cabantchik I, Hershko C, Weiss G, Girelli D, Stoffel N, Muckenthaler MU, Nemeth E, Camaschella C, Klenerman P, Zimmermann MB (2021) Vaccine efficacy and iron deficiency: an intertwined pair? Lancet Haematol 8 (9):e666-e669. 10.1016/S2352-3026(21)00201-5

Ejoh SI, Wireko-Manu FD, Page D, Mgc Renard C (2021) Traditional green leafy vegetables as underutilised sources of micronutrients in a rural farming community in south-west Nigeria II: consumption pattern and potential contribution to micronutrient requirements. S Afr J Clin Nutr 34 (2):46-51. 10.1080/16070658.2019.1652964

FAO, IFAD, UNICEF, WFP, WHO (2019) The state of food security and sutrition in the world 2019. Safeguarding against economic slowdowns and downturns. FAO, Rome

FAO, WHO (2004) Vitamin and mineral requirements in human nutrition. 2nd edn. World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO), Geneva

Ganong William F (2005) Review of medical Physiology. The McGraw-Hill Companies, Inc, New York, New York: Lange

Glasdam S-M, Glasdam S, Peters GH (2016) The importance of magnesium in the human body: a systematic literature review. Adv Clin Chem 73:169-193. 10.1016/bs.acc.2015.10.002

Gowele VF, Kinabo J, Jumbe T, Kirschmann C, Frank J, Stuetz W (2019) Provitamin A carotenoids, tocopherols, ascorbic acid and minerals in indigenous leafy vegetables from Tanzania. Foods 8 (1):35. 10.3390/foods8010035

Grubben G, Klaver W, Nono-Womdim R, Everaarts A, Fondio L, Nugteren JA, Corrado M (2014) Vegetables to combat the hidden hunger in Africa. Chron Hortic 54:24-32

Gupta S, Brazier AKM, Lowe NM (2020) Zinc deficiency in low- and middle-income countries: prevalence and approaches for mitigation. J Hum Nutr Diet 33 (5):624-643. 10.1111/jhn.12791

Hallauer AR, Carena MJ, Miranda Filho Jd (2010) Quantitative genetics in maize breeding, vol 6. Springer Science & Business Media, London

Hänsch R, Mendel RR (2009) Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). Curr Opin Plant Biol 12 (3):259-266. 10.1016/j.pbi.2009.05.006

Harrell Jr FE, Dupont C (2021) Hmisc: Harrell Miscellaneous. R package version 4.6-0 edn.,

Hendre PS, Muthemba S, Kariba R, Muchugi A, Fu Y, Chang Y, Song B, Liu H, Liu M, Liao X, Sahu SK, Wang S, Li L, Lu H, Peng S, Cheng S, Xu X, Yang H, Wang J, Liu X, Simons A, Shapiro H-Y, Mumm RH, Van Deynze A, Jamnadass R (2019) African Orphan Crops Consortium (AOCC): status of developing genomic resources for African orphan crops. Planta 250 (3):989-1003. 10.1007/s00425-019-03156-9

Hogan JA, Valverde-Barrantes OJ, Tang W, Ding Q, Xu H, Baraloto C (2021) Evidence of elemental homeostasis in fine root and leaf tissues of saplings across a fertility gradient in tropical montane forest in Hainan, China. Plant Soil 460 (1):625-646. 10.1007/s11104-020-04802-y

Huang X-Y, Salt DE (2016) Plant ionomics: from elemental profiling to environmental adaptation. Mol Plant 9 (6):787-797. 10.1016/j.molp.2016.05.003

Institute of Medicine (2006) Dietary reference intakes: the essential guide to nutrient requirements. The National Academies Press, Washington, DC. 10.17226/11537

Jiménez-Aguilar DM, Grusak MA (2015) Evaluation of minerals, phytochemical compounds and antioxidant activity of Mexican, Central American, and African green leafy vegetables. Plant Foods Hum Nutr 70 (4):357-364. 10.1007/s11130-015-0512-7

Jones J, J. B. (2001) Laboratory guide for conducting soil tests and plant analysis. CRC press, Boca Raton, Florida, USA

Kassambara A, Mundt F (2020) Factoextra: extract and visualize the results of multivariate data analyses. R package version 1.0.7 edn.,

Kirkby E (2012) Introduction, definition and classification of nutrients. In: Marschner P (ed) Marschner's Mineral Nutrition of Higher Plants (Third Edition). Academic Press, San Diego, pp 3-5. doi:10.1016/B978-0-12-384905-2.00001-7

Lê S, Josse J, Husson F (2008) FactoMineR: an R package for multivariate analysis. J Stat Softw 25 (1):1-18. 10.18637/jss.v025.i01

Maathuis FJM (2009) Physiological functions of mineral macronutrients. Curr Opin Plant Biol 12 (3):250-258. 10.1016/j.pbi.2009.04.003

Martínez-Ballesta MC, Dominguez-Perles R, Moreno DA, Muries B, Alcaraz-López C, Bastías E, García-Viguera C, Carvajal M (2010) Minerals in plant food: effect of agricultural practices and role in human health. A review. Agron Sustain Dev 30 (2):295-309. 10.1051/agro/2009022

Moyo M, Amoo SO, Aremu A, Gruz J, Šubrtová M, Jarošová M, Tarkowski P, Dolezal K (2018) Determination of mineral constituents, phytochemicals and antioxidant qualities of *Cleome gynandra*, compared to *Brassica oleracea* and *Beta vulgaris*. Front Chem 5:128. 10.3389/fchem.2017.00128

Moyo M, Aremu AO (2021) Nutritional, phytochemical and diverse health-promoting qualities of *Cleome gynandra*. Crit Rev Food Sci Nutr:1-18. 10.1080/10408398.2020.1867055

National Academies of Sciences Engineering and Medicine (2019) Dietary Reference Intakes for Sodium and Potassium. The National Academies Press, Washington, DC. 10.17226/25353

Neugart S, Baldermann S, Ngwene B, Wesonga J, Schreiner M (2017) Indigenous leafy vegetables of Eastern Africa—A source of extraordinary secondary plant metabolites. Food Res Int 100:411-422. 10.1016/j.foodres.2017.02.014

Neugebauer K, El-Serehy HA, George TS, McNicol JW, Moraes MF, Sorreano MCM, White PJ (2020) The influence of phylogeny and ecology on root, shoot and plant ionomes of 14 native Brazilian species. Physiol Plant 168 (4):790-802. 10.1111/ppl.13018

Nicolas O, Charles MT, Jenni S, Toussaint V, Parent S-É, Beaulieu C (2019) The ionomics of lettuce infected by *Xanthomonas campestris* pv. *vitians*. Front Plant Sci 10 (351). 10.3389/fpls.2019.00351

Olson R, Gavin-Smith B, Ferraboschi C, Kraemer K (2021) Food fortification: the advantages, disadvantages and lessons from sight and life programs. Nutrients 13 (4):1118. 10.3390/nu13041118

Omondi EO, Engels C, Nambafu G, Schreiner M, Neugart S, Abukutsa-Onyango M, Winkelmann T (2017) Nutritional compound analysis and morphological characterization of spider plant (*Cleome gynandra*)-an African indigenous leafy vegetable. Food Res Int 100:284-295. 10.1016/j.foodres.2017.06.050

Pilon-Smits EAH, Quinn CF, Tapken W, Malagoli M, Schiavon M (2009) Physiological functions of beneficial elements. Curr Opin Plant Biol 12 (3):267-274. 10.1016/j.pbi.2009.04.009

Pirahanchi Y, Jessu R, Aeddula N (2021) Physiology, sodium potassium pump. Treasure Island (FL): StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK537088/. Accessed 10 December 2021

Pivovarov AS, Calahorro F, Walker RJ (2019) Na+/K+-pump and neurotransmitter membrane receptors. Invert Neurosci 19 (1):1. 10.1007/s10158-018-0221-7

Pohl HR, Wheeler JS, Murray HE (2013) Sodium and potassium in health and disease. In: Sigel A, Sigel H, Sigel RKO (eds) Interrelations between essential metal ions and human diseases. Springer Netherlands, Dordrecht, pp 29-47. doi:10.1007/978-94-007-7500-8_2

Power ML, Heaney RP, Kalkwarf HJ, Pitkin RM, Repke JT, Tsang RC, Schulkin J (1999) The role of calcium in health and disease. Am J Obstet Gynecol 181 (6):1560-1569. 10.1016/S0002-9378(99)70404-7

Quintaes KD, Diez-Garcia RW (2015) The importance of minerals in the human diet. In: Guardia Mdl, Garrigues S (eds) Handbook of Mineral Elements in Food. Wiley Online Books. John Wiley & Sons, Ltd, West Sussex, UK, pp 1-21. doi:https://doi.org/10.1002/9781118654316.ch1

R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Reeves G, Singh P, Rossberg TA, Sogbohossou EOD, Schranz ME, Hibberd JM (2018) Natural variation within a species for traits underpinning C4 photosynthesis. Plant Physiol 177 (2):504. 10.1104/pp.18.00168

Renkema KY, Alexander RT, Bindels RJ, Hoenderop JG (2008) Calcium and phosphate homeostasis: concerted interplay of new regulators. Ann Med 40 (2):82-91. 10.1080/07853890701689645

Revelle W (2019) psych: procedures for psychological, psychometric, and personality research. R package version 1.9.12 edn. Northwestern University, Evanston, Illinois

Salt DE, Baxter I, Lahner B (2008) Ionomics and the study of the plant ionome. Annu Rev Plant Biol 59 (1):709-733. 10.1146/annurev.arplant.59.032607.092942

Sarker U, Oba S (2019) Protein, dietary fiber, minerals, antioxidant pigments and phytochemicals, and antioxidant activity in selected red morph *Amaranthus* leafy vegetable. PLOS ONE 14 (12):e0222517. 10.1371/journal.pone.0222517

Schönfeldt HC, Pretorius B (2011) The nutrient content of five traditional South African dark green leafy vegetables - A preliminary study. J Food Compos Anal 24:1141-1146. 10.1016/j.jfca.2011.04.004

Shukla S, Bhargava A, Chatterjee A, Srivastava J, Singh N, Singh SP (2006) Mineral profile and variability in vegetable amaranth (*Amaranthus tricolor*). Plant Foods Hum Nutr 61 (1):21-26. 10.1007/s11130-006-0004-x

Singh RK, Chaudhary BD (1985) Biometrical methods in quantitative genetic analysis. Third edition edn. Kalyani Publishers, New Delhi, India

Singh S, Parihar P, Singh R, Singh VP, Prasad SM (2016) Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. Front Plant Sci 6 (1143). 10.3389/fpls.2015.01143

Sobotka L, Allison S, Stanga Z (2008) Basics in clinical nutrition: water and electrolytes in health and disease. e-SPEN Eur E J Clin Nutr Metab 3 (6):e259-e266. 10.1016/j.eclnm.2008.06.004

Sogbohossou DEO (2019) Orphan no more : Ethnobotany and genetic analysis of leaf yield and secondary metabolites content in *Gynandropsis gynandra* (Cleomaceae). PhD Thesis, Wageningen University, Wageningen

Sogbohossou EOD, Achigan-Dako EG, Mumm R, de Vos RCH, Schranz ME (2020) Natural variation in specialised metabolites production in the leafy vegetable spider plant (*Gynandropsis gynandra* L. (Briq.)) in Africa and Asia. Phytochemistry 178:112468. 10.1016/j.phytochem.2020.112468

Sogbohossou EOD, Kortekaas D, Achigan-Dako EG, Maundu P, Stoilova T, Van Deynze A, de Vos RCH, Schranz ME (2019) Association between vitamin content, plant morphology and geographical origin in a worldwide collection of the orphan crop *Gynandropsis gynandra* (Cleomaceae). Planta 250:933–947. 10.1007/s00425-019-03142-1

Stein RJ, Höreth S, de Melo JRF, Syllwasschy L, Lee G, Garbin ML, Clemens S, Krämer U (2017) Relationships between soil and leaf mineral composition are element-specific, environment-dependent and geographically structured in the emerging model Arabidopsis halleri. New Phytol 213 (3):1274-1286

Tan Y, Zhou J, Wang J, Sun L (2020) The genetic architecture for phenotypic plasticity of the rice grain ionome. Front Plant Sci 11 (12). 10.3389/fpls.2020.00012

Thomas B, Bishop J (2013) Manual of dietetic practice. Wiley, Oxford, UK

Thovhogi F, McHau GR, Gwata ET, Ntushelo N (2021) Evaluation of leaf mineral, flavonoid, and total phenolic content in spider plant germplasm. Molecules 26 (12):3600. 10.3390/molecules26123600

Tulchinsky TH (2010) Micronutrient Deficiency Conditions: Global Health Issues. Public Health Rev 32 (1):243-255. 10.1007/BF03391600

van Jaarsveld P, Faber M, van Heerden I, Wenhold F, van Rensburg WJ, van Averbeke W (2014) Nutrient content of eight African leafy vegetables and their potential contribution to dietary reference intakes. J Food Compos Anal 33:77-84. 10.1016/j.jfca.2013.11.003

Watanabe T, Azuma T (2021) Ionomic variation in leaves of 819 plant species growing in the botanical garden of Hokkaido University, Japan. J Plant Res 134 (2):291-304. 10.1007/s10265-021-01254-y

Watanabe T, Maejima E, Yoshimura T, Urayama M, Yamauchi A, Owadano M, Okada R, Osaki M, Kanayama Y, Shinano T (2016) The ionomic study of vegetable crops. PLOS ONE 11 (8):e0160273. 10.1371/journal.pone.0160273

Watanabe T, Urayama M, Shinano T, Okada R, Osaki M (2015) Application of ionomics to plant and soil in fields under long-term fertilizer trials. SpringerPlus 4 (1):781. 10.1186/s40064-015-1562-x

Watts DL (1989) The nutritional relationships of copper. J Orthomol Med 4 (2):99-108

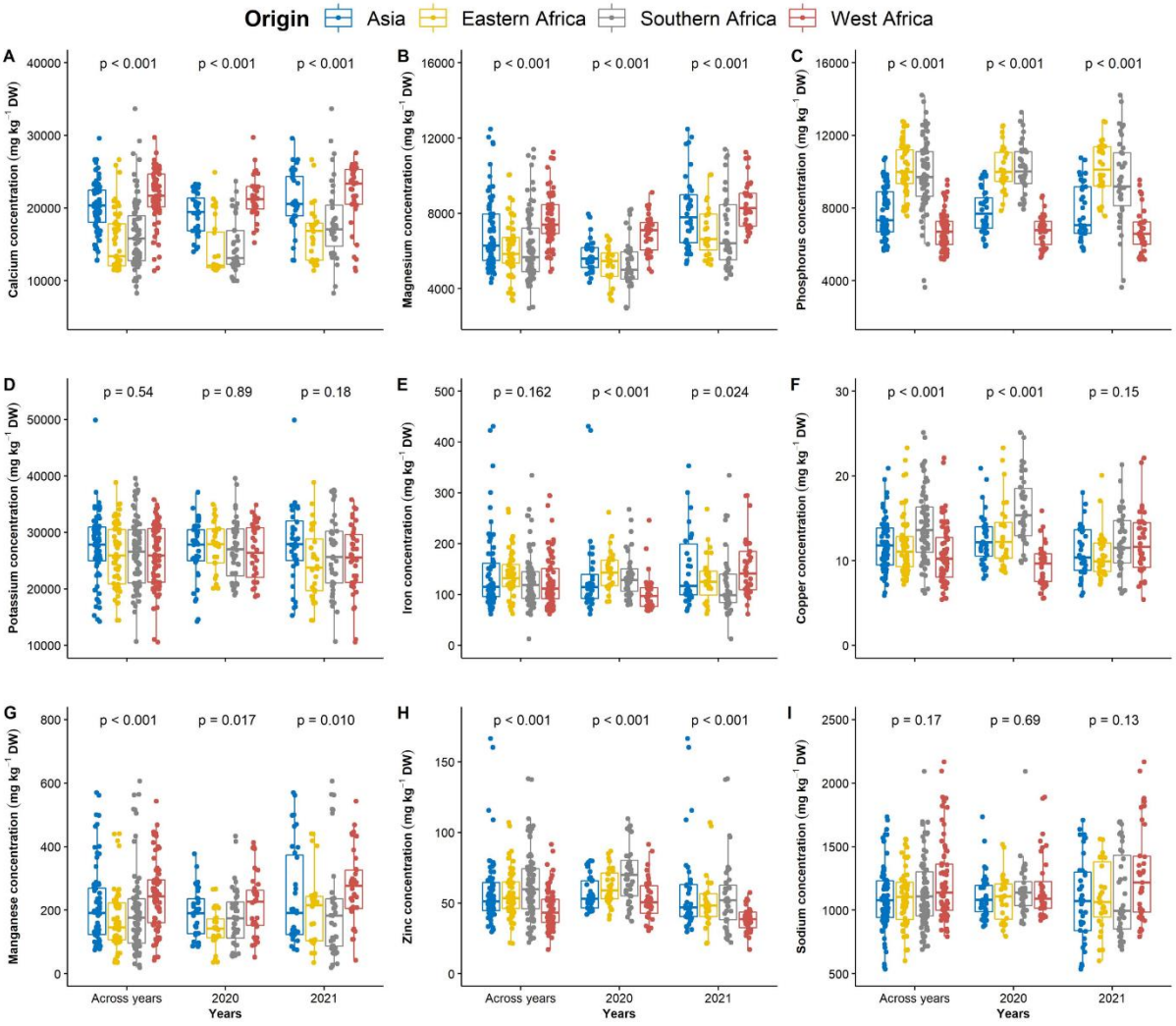
White PJ, Broadley MR, Thompson JA, McNicol JW, Crawley MJ, Poulton PR, Johnston AE (2012) Testing the distinctness of shoot ionomes of angiosperm families using the Rothamsted Park Grass Continuous Hay Experiment. New Phytol 196 (1):101-109. 10.1111/j.1469-8137.2012.04228.x

Willett W, Rockström J, Loken B, Springmann M, Lang T, Vermeulen S, Garnett T, Tilman D, DeClerck F, Wood A, Jonell M, Clark M, Gordon LJ, Fanzo J, Hawkes C, Zurayk R, Rivera JA, De Vries W, Majele Sibanda L, Afshin A, Chaudhary A, Herrero M, Agustina R, Branca F, Lartey A, Fan S, Crona B, Fox E, Bignet V, Troell M, Lindahl T, Singh S, Cornell SE, Srinath Reddy K, Narain S, Nishtar S, Murray CJL (2019) Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. Lancet 393 (10170):447-492. 10.1016/S0140-6736(18)31788-4

Wu T-h, Solberg SO, Yndgaard F, Chou Y-Y (2018) Morphological patterns in a world collection of *Cleome gynandra*. Genet Resour Crop Evol 65 (1):271-283. 10.1007/s10722-017-0529-x

Zhang C, Hiradate S, Kusumoto Y, Morita S, Koyanagi TF, Chu Q, Watanabe T (2021a) Ionomic responses of local plant species to natural edaphic mineral variations. Front Plant Sci 12 (530). 10.3389/fpls.2021.614613

Zhang L, MacQueen A, Bonnette J, Fritschi FB, Lowry DB, Juenger TE (2021b) QTL × environment interactions underlie ionome divergence in switchgrass. G3 11 (7):jkab144. 10.1093/g3journal/jkab144



Appendix 4.1. Boxplots showing variation in nine leaf ionome elements among regions of origin of a population of 70 advanced lines of G. gynandra evaluated in 2020 and 2021. (A) Calcium content; (B) Magnesium content; (C) Phosphorus content; (D) Potassium content; (E) Iron content; (F) Copper content; (G) Manganese content; (H) Zinc content; (I) Sodium content. Asia (n = 18), East Africa (n = 14), Southern Africa (n = 20), and West Africa (n = 18).

Appendix 4.2. Correlation between leaf ionome elements and the three first principal components.

Minerals	PC1 (27.27%)	PC2 (22.62%)	PC3 (14.96%)
Ca	**0.810*** **	-0.196	0.201
Cu	-0.400	0.322	0.281
Fe	0.316	**0.691*** **	-0.241
K	-0.027	0.278	**0.761*** **
Mg	**0.833*** **	0.070	0.068
Mn	0.569	**0.608*** **	-0.112
Na	0.150	0.096	**0.733*** **
P	**-0.703*** **	0.523	0.073
Zn	0.031	**0.824*** **	-0.172

Values in bold indicated significant correlation at $p < 0.001$ (***).

Appendix 4.3. Estimates of genetic parameters for the leaf ionome in 70 advanced lines of *G. gynandra* evaluated in 2020 and 2021.

Genetic parameters	Years	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
σ_G^2	2020	17676314.89 ± 3229288.98	8.72 ± 2.31	1700.07 ± 422.82	16669272.20 ± 3643837.70	1525784.27 ± 265165.07	7661.03 ± 1307.43	37023.09 ± 6922.75	3583141.40 ± 616539.21	266.52 ± 46.73
	2021	17238123.68 ± 3605998.72	2.65 ± 1.50	1721.47 ± 528.83	27107523.48 ± 6342627.57	3516612.67 ± 603905.04	32615.07 ± 5590.23	110735.06 ± 19204.59	4635441.66 ± 803749.80	589.96 ± 102.36
σ_e^2	2020	2501539.18 ± 422837.30	8.18 ± 1.41	1354.65 ± 231.79	8605527.44 ± 1454599.63	62791.85 ± 10613.76	36.77 ± 6.26	6977.85 ± 1187.99	76014.09 ± 12941.50	15.64 ± 2.64
	2021	7212900.16 ± 1235587.72	8.75 ± 1.57	2124.61 ± 374.27	17949556.28 ± 3074055.04	58527.93 ± 10111.75	426.63 ± 73.69	4001.03 ± 691.04	161962.71 ± 28201.33	22.09 ± 3.76
σ_P^2	2020	18927084.48 ± 3440707.63	12.81 ± 3.01	2377.40 ± 538.72	20972035.92 ± 4371137.51	1557180.19 ± 270471.95	7679.41 ± 1310.56	40512.02 ± 7516.75	3621148.45 ± 623009.96	274.34 ± 48.05
	2021	20844573.76 ± 4223792.58	7.03 ± 2.29	2783.77 ± 715.97	36082301.62 ± 7879655.10	3545876.63 ± 608960.91	32828.39 ± 5627.08	112735.58 ± 19550.11	4716423.02 ± 817850.47	601.01 ± 104.24
H^2	2020	0.93	0.68	0.72	0.79	0.98	0.99	0.91	0.99	0.97
	2021	0.83	0.38	0.62	0.75	0.99	0.99	0.98	0.98	0.98
Mean (mg kg^{-1} dry weight)	2020	17553.63	12.81	128.38	26859.04	5848.99	183.4	1139.84	8662.81	60.66
	2021	19528.01	11.56	138.69	25917.1	7584.79	249.91	1147	8471.8	51.12
GCV (%)	2020	23.95	23.05	32.12	15.2	21.12	47.72	16.88	21.85	26.91
	2021	21.26	14.08	29.92	20.09	24.72	72.26	29.01	25.41	47.51
PCV (%)	2020	24.78	27.94	37.98	17.05	21.33	47.78	17.66	21.97	27.3
	2021	23.38	22.94	38.04	23.18	24.83	72.5	29.27	25.63	47.96
ECV (%)	2020	9.01	22.33	28.67	10.92	4.28	3.31	7.33	3.18	6.52
	2021	13.75	25.59	33.23	16.35	3.19	8.26	5.51	4.75	9.19
GA	2020	8334.74	5.01	72.32	7452.72	2519.2	178.72	377.31	3880.84	33.1
	2021	7806.24	2.08	67.39	9280.59	3840.29	369.51	677.83	4384.29	49.49
GAM (%)	2020	47.48	39.14	56.33	27.75	43.07	97.45	33.1	44.8	54.56
	2021	39.97	17.95	48.59	35.81	50.63	147.86	59.1	51.75	96.82

σ_G^2, genotypic variance; σ_e^2, residual variance; σ_P^2, phenotypic variance; H^2, broad-sense heritability; GCV, genotypic coefficient of variation; PCV, phenotypic coefficient of variation; ECV, residual coefficient of variation; GA, genetic advance; GAM, genetic advance over mean.

Appendix 4.4. Contribution of 100 g fresh leaves od spider plant to daily recommended nutrient intake for all age groups

Minerals	Infant and Children		Adolescents				Adults			
	1-3 years	4-8 years	Males (9-13 years)	Males (14-18 years)	Females (9-13 years)	Females (14-18 years)	Males (19–50 years)	Males (> 50 years)	Females (19–50 years)	Females (> 50 years)
Calcium										
RDA	500	800	1300	1300	1300	1300	1000	1200	1000	1200
Concentration mg/100 g FW	197.82	197.82	197.82	197.82	197.82	197.82	197.82	197.82	197.82	197.82
Contribution (%) to RDA	39.56	24.73	15.22	15.22	15.22	15.22	19.78	16.48	19.78	16.48
Copper										
RDA	0.34	0.44	0.7	0.89	0.7	0.89	0.9	0.9	0.9	0.9
Concentration mg/100 g FW	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Contribution (%) to RDA	38.19	29.51	18.55	14.59	18.55	14.59	14.43	14.43	14.43	14.43
Iron										
RDA	7	10	8	11	8	11	8	8	18	8
Concentration mg/100 g FW	1.42	1.42	1.42	1.42	1.42	1.42	1.42	1.42	1.42	1.42
Contribution (%) to RDA	20.28	14.2	17.74	12.9	17.74	12.9	17.74	17.74	7.89	17.74
Potassium										
RDA	2000	2300	2500	3000	2300	2300	3400	3400	2600	2600
Concentration mg/100 g FW	281.62	281.62	281.62	281.62	281.62	281.62	281.62	281.62	281.62	281.62
Contribution (%) to RDA	14.08	12.24	11.26	9.39	12.24	12.24	8.28	8.28	10.83	10.83
Magnesium										
RDA	80	130	240	410	240	360	420	420	320	320
Concentration mg/100 g FW	71.7	71.7	71.7	71.7	71.7	71.7	71.7	71.7	71.7	71.7
Contribution (%) to RDA	89.63	55.15	29.88	17.49	29.88	19.92	17.07	17.07	22.41	22.41
Manganese										
RDA	1.2	1.5	1.9	2.2	1.6	1.6	2.3	2.3	1.8	1.8
Concentration mg/100 g FW	2.32	2.32	2.32	2.32	2.32	2.32	2.32	2.32	2.32	2.32
Contribution (%) to RDA	193.55	154.84	122.24	105.57	145.17	145.17	100.98	100.98	129.04	129.04

Appendix 4.4. Contribution of 100 g fresh leaves od spider plant to daily recommended nutrient intake for all age groups

Minerals	**Infant and Children**		**Adolescents**				**Adults**			
	1-3 years	**4-8 years**	**Males (9-13 years)**	**Males (14-18 years)**	**Females (9-13 years)**	**Females (14-18 years)**	**Males (19–50 years)**	**Males (> 50 years)**	**Females (19–50 years)**	**Females (> 50 years)**
Sodium										
RDA	1000	1200	1500	1500	1500	1500	1500	1300	1500	1300
Concentration mg/100 g FW	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2
Contribution (%) to RDA	1.22	1.02	0.81	0.81	0.81	0.81	0.81	0.94	0.81	0.94
Phosphorus										
RDA	460	500	1250	1250	1250	1250	700	700	700	700
Concentration mg/100 g FW	91.32	91.32	91.32	91.32	91.32	91.32	91.32	91.32	91.32	91.32
Contribution (%) to RDA	19.85	18.26	7.31	7.31	7.31	7.31	13.05	13.05	13.05	13.05
Zinc										
RDA	3	5	8	11	8	9	11	11	8	8
Concentration mg/100 g FW	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Contribution (%) to RDA	19.86	11.92	7.45	5.42	7.45	6.62	5.42	5.42	7.45	7.45

FW: fresh weight; RDA: recommended dietary allowance

CHAPTER 5

Combining ability and heterosis analysis for mineral content in the leafy vegetable *Gynandropsis gynandra* (L.) Briq.

Abstract

Gynandropsis gynandra (spider plant) is a leafy vegetable rich in micronutrients, including minerals, vitamins, and secondary metabolites, making it a promising crop for combating hidden hunger and promoting human health. However, knowledge of the inheritance of mineral content is limited, which hinders the development of improved cultivars for wider cultivation. Therefore, 222 F_1 hybrids involving 32 parental accessions were generated from a North Carolina mating design II. The F_1s were evaluated for gene action, combining ability effects and heterosis of mineral content. Significant differences ($P < 0.001$) were observed among and between hybrids and parents for the levels of all mineral contents. Significant general and specific combining ability effects together with variance components analysis revealed that both additive and nonadditive gene action controlled mineral content with a predominance of nonadditive gene action. Mid- and best-parent heterosis ranged from -84.98 and 404.79% for minerals. Parents with good general combining ability were identified, as well as crosses with high specific combining ability and heterosis. There were significant and moderate to strong correlations between mean hybrid performance, specific combining ability effects and heterosis levels and low to moderate correlations between general combining ability and mean parents' performance. Our study thus revealed that *G. gynandra* improvement would be possible through hybridization to exploit heterosis and that the crop could be used as a model to study the genetic mechanism underlying heterosis in orphan leafy vegetables.

Keywords: *Cleome gynandra*; Gene action; Hybrid breeding; Leafy vegetable; Micronutrient.

5.1 Introduction

The nutritive value of vegetables, particularly orphan or underutilized vegetables, is high, and they are an important source of micronutrients. The increasing interest in orphan leafy vegetables is particularly due to their distinct richness in minerals, vitamins, phytochemicals and antioxidants (Nyadanu and Lowor 2015; Moyo et al. 2018; Orech et al. 2007) and good adaptation to local conditions, representing a reliable resource to adapt to the changing climate. Ongoing actions to promote orphan crops include the development of genomic tools to speed up new cultivar development to meet consumers' preferences (Jamnadass et al. 2020), as well as raising population awareness of their potential value. In the case of leafy vegetables, the main breeding objective is to develop high leaf yielding cultivars (Sogbohossou et al. 2018) to optimize resource use and availability. This objective might conflict or align with the primary potential of leafy vegetables, which is their high micronutrient content. Marles (2017) provided evidence of a decline in mineral content in crops, including vegetables. The decrease in mineral content is referred to as the "dilution effect", which is an increase in yield in modern cultivars without a subsequent increase in mineral content or higher mineral nutrient concentrations in old cultivars than in improved cultivars. The decline was observed to be more pronounced in vegetables than in other commodity groups (Davis 2009). Therefore, assessing the nutritional value of the breeding material throughout the breeding process is important for the conservation of the primary benefits of leafy vegetables while increasing their productivity.

Gynandropsis gynandra (L.) Briq. (syn. *Cleome gynandra* L.), commonly known as spider plant, is interesting because of its high vitamin, mineral and secondary metabolite contents (Sogbohossou et al. 2019; Omondi et al. 2017b; Neugart et al. 2017; Moyo et al. 2018; Schönfeldt and Pretorius 2011; Gowele et al. 2019; Sogbohossou et al. 2020). The major vitamins reported in the species include vitamins C, A, and E, and minerals include iron, zinc, calcium, copper, potassium, magnesium, manganese, phosphorus and sodium (Omondi et al. 2017b; Sogbohossou et al. 2019). The species also contains several secondary metabolites, such as flavonoids, terpenoids, tannins, glucosinolates and several phenolic compounds essential for human heath (Neugart et al. 2017; Moyo et al. 2018; Sogbohossou 2019; Omondi et al. 2017b; Chataika et al. 2021). All these nutritional and health-promoting compounds exhibit a wide range of variation in the species, providing a strong basis for crop improvement (Sogbohossou 2019; Omondi et al. 2017b; Sogbohossou et al. 2019; Sogbohossou et al. 2020). Information on the inheritance, combining ability and heterosis level for nutritional elements

is crucial to designing breeding schemes, selecting superior parents and heterotic crosses, and deciding on the type of cultivars to be developed. Little is also known about the gene action controlling leaf yield and its related traits. To this end, different mating designs, including diallel, North Carolina Design II and line by tester designs, are used.

In this study, we generated knowledge on the genetic mechanism controlling mineral content in *G. gynandra* with a good perspective for hybrid cultivars development. The present study aimed to investigate the mineral profile of experimental hybrids of spider plant to select high-yielding and nutritious hybrids for cultivar development. Specifically, the study (i) assessed the mineral content and yield potential of two populations of F_1 *G. gynandra* hybrids and their parental lines; (ii) determined the gene action controlling the inheritance of mineral content in *G. gynandra* and the level of heterosis; (iii) evaluated the combining ability effects for mineral content of the parental lines of *G. gynandra*; and (iv) determined the extent of association between mean performance, heterosis and combining ability for mineral content in *G. gynandra*.

5.2 Materials and methods

5.2.1 Plant material

Thirty-two advanced lines derived from 32 accessions originating from various countries of Asia and Africa (Table 5.1) self-pollinated for three and four generations were used in this study. The lines were separated into two groups, 16 and 23 lines used as females and males, respectively, and crossed in a North Carolina design II during two summer seasons (season 1, from October 2018 to February 2019 and season 2: from October 2019 to March 2020) in a greenhouse at the Controlled Environment Facility (29°46′ S, 30°58′ E) of the University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa. During the first crossing season, only 28 advanced lines (13 females and 15 males) were used to generate the first populations of hybrids (Pop1), comprising 135 F_1 families (Table 5.2). Thirty advanced lines (13 females and 17 females) were used to generate the second populations of hybrids (Pop2) with 209 F_1 families (Table 5.2) during the second season. The 30 advanced lines used in the second crossing season included 26 lines used in the first crossing season. Across the two crossing seasons, a total of 222 unique single crosses were generated for evaluation. In addition, each line was self-pollinated during each crossing season. Crosses were performed following the protocol developed by Zohoungbogbo et al. (2018a).

Table 5.1. List of advanced lines of *G. gynandra* used as parents to generate the hybrids used in this study and their origin.

Lines	Genebank of the original accession	Country of origin	Continent	Parent	Generation of selfing in 2019	Generation of selfing in 2020
P18	KENRIK	Kenya	Africa	Female	S3	S4
P12	GBioS	Benin	Africa	Female	S3	S4
P14	GBioS	Benin	Africa	Female	S3	S4
P09	GBioS	Benin	Africa	Female	S3	S4
P24	WorldVeg	Rwanda	Africa	Female	S3	-
P23	WorldVeg	Thailand	Asia	Female	S3	S4
P05	WorldVeg	Laos	Asia	Female	S3	S4
P10	WorldVeg	Thailand	Asia	Female	S3	S4
P06	WorldVeg	Malaysia	Asia	Female	S3	S4
P25	WorldVeg	Malaysia	Asia	Female	S3	S4
P20	WorldVeg	Malaysia	Asia	Female	S3	S4
P17	WorldVeg	Laos	Asia	Female	S3	S4
P04	AVRDC	Malawi	Africa	Female	S3	S4
P03	KENRIK	Kenya	Africa	Male	S3	S4
P11	KENRIK	Kenya	Africa	Male	S3	S4
P07	KENRIK	Kenya	Africa	Male	S3	S4
P22	GBioS	Togo	Africa	Male	S3	S4
P27	GBioS	Togo	Africa	Male	S3	S4
P08	WorldVeg	Laos	Asia	Male	S3	S4
P02	WorldVeg	Laos	Asia	Male	S3	S4
P16	WorldVeg	Kenya	Africa	Male	S3	S4
P13	WorldVeg	Zambia	Africa	Male	S3	S4
P21	WorldVeg	South Africa	Africa	Male	S3	-
P01	WorldVeg	Uganda	Africa	Male	S3	S4
P19	WorldVeg	Uganda	Africa	Male	S3	S4
P15	WorldVeg	Uganda	Africa	Male	S3	S4
P26	WorldVeg	Kenya	Africa	Male	S3	S4
P28	WorldVeg	Zambia	Africa	Male	S3	S4
P29	GBioS	Benin	Africa	Male	-	S4
P30	WorldVeg	Malaysia	Asia	Male	-	S4
P31	WorldVeg	Malawi	Asia	Male	-	S4
P32	WorldVeg	South Africa	Africa	Female	-	S4

GBioS: Laboratory of Genetics, Horticulture and Seed Science, WorldVeg: World Vegetable Center, KENRIK: Kenya Resource Center for Indigenous Knowledge

Table 5.2. Representation of the North Carolina Design II implemented to generate the F1 hybrids used in the present study. A single cross hybrid is represented by x.

Males	**Females**												
Pop 1													
	P04	**P05**	**P06**	**P09**	**P10**	**P12**	**P14**	**P17**	**P18**	**P20**	**P23**	**P24**	**P25**
P01		x	x	x	x	x	x		x	x	x		
P02	x		x	x		x	x	x		x	x		x
P03	x	x	x	x	x		x		x		x		x
P07	x	x	x		x	x	x	x		x	x		
P08			x	x		x	x	x		x	x		x
P11	x	x	x	x		x	x	x	x	x	x		x
P13	x	x	x	x	x	x	x	x	x	x	x		x
P15	x		x	x	x	x	x	x		x	x		x
P16	x	x	x	x	x	x		x	x	x	x		
P19	x	x			x			x	x	x		x	
P21	x	x	x	x	x		x	x		x	x		
P22		x	x	x	x	x	x			x	x		x
P26	x		x				x		x	x	x	x	x
P27		x		x		x	x	x	x	x	x		x
P28			x		x				x		x	x	x
Pop 2													
	P04	**P05**	**P06**	**P09**	**P10**	**P12**	**P14**	**P17**	**P18**	**P20**	**P23**	**P25**	**P32**
P01	x	x	x	x	x	x	x	x	x	x	x	x	x
P02	x	x	x	x	x	x	x	x	x	x	x	x	x
P03	x	x	x	x	x	x	x	x	x	x	x	x	x
P07	x	x	x	x	x	x	x	x	x	x	x	x	x
P08	x	x	x	x	x	x	x	x		x	x	x	x
P11	x	x	x	x	x	x	x	x	x	x	x	x	x
P13	x	x	x	x	x	x	x	x	x	x	x	x	x
P15	x	x	x	x	x	x	x	x	x	x		x	x
P16	x	x	x	x	x	x	x	x	x	x	x	x	x
P19	x	x	x	x	x	x	x	x	x	x	x	x	x
P22	x	x	x	x	x	x	x	x	x	x	x	x	
P26	x	x	x	x	x	x	x	x	x	x	x	x	x
P27	x	x		x	x	x	x	x		x	x	x	x
P28	x	x	x		x	x	x	x	x	x	x	x	x
P29	x	x	x	x	x	x	x	x		x	x	x	
P30		x	x	x	x	x	x	x		x	x	x	x
P31		x	x	x	x		x	x	x	x	x	x	x

5.2.2 Experimental design and growth conditions

Evaluation of population 1 (Pop1)

The 28 advanced lines and 135 F_1 hybrids were grown using an alpha design (15 incomplete blocks with 9 entries per incomplete block for hybrids and 7 incomplete blocks with 4 entries per incomplete block for parents) with two replications in a greenhouse at the Controlled Environment Facility of the University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa. The parents were blocked separately from the hybrids, and the experiment was carried out from March 2019 to June 2019. Seeds of all genotypes were pretreated by heating at 40 °C for three days to improve germination before sowing in cell trays filled with growing media. Cell trays were established in the greenhouse, and germination was observed three days after planting. Seedlings were grown for four weeks, after which they were transplanted into a single row plot of 1 m length at a spacing of 20 cm between and within rows on raised beds measuring 1 m wide and 1 m high. The soil had 38.5% clay with a bulk density of 1.02 $g \cdot cm^{-3}$ and pH (KCl) of 5.58. The soil contained 78 mg kg^{-1} phosphorus, 133.5 mg kg^{-1} potassium, 2576 mg kg^{-1} calcium, 384.5 mg kg^{-1} magnesium, 21.9 mg kg^{-1} zinc, 11 mg kg^{-1} manganese, 5.5 mg kg^{-1} copper, 2.45% organic carbon and 0.19% nitrogen. Automated drip irrigation was used to water the plants, while weeds were controlled manually. Basal fertilizer composed of N:P:K (2:3:2) at a dose of 150 kg ha^{-1} was applied before transplanting, and limestone ammonium nitrate (28% N) was applied as topdressing two weeks after transplanting at a dose of 100 kg ha^{-1}. During the experiment, the average temperature was 28 °C day/20 °C night, while the average relative humidity was 78.5%.

Evaluation of population 2 (Pop2)

Pop2 (209 hybrids and their 30 parental lines) was evaluated in 2020 (September to December). The parental lines and hybrids were grown in adjacent trials using an alpha design (20 incomplete blocks with 10 entries per incomplete block for hybrids and 6 incomplete blocks with 5 entries per incomplete block for parents) with two replications in a greenhouse at the Controlled Environment Facility of the University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa. Seedlings were grown as described for pop1 and were transplanted into 10-liter pots with three plants per pot. Pots were filled with composted pine bark growing media. The growing media was characterized by 30.78% of carbon, 1.10% of nitrogen, 1.35% of calcium, 0.33% of magnesium, 0.25% of potassium, 0.34% of phosphorus, 469.99 mg kg^{-1} of sodium, 181.35 mg kg^{-1} of zinc, 42.41 mg kg^{-1} of copper, 1034.08 mg kg^{-1} of manganese,

13349.42 mg kg^{-1} of iron, and 6452.14 mg kg^{-1} of aluminium on dry matter basis. Fertilization and weed control were the same as those described for pop1. During the experiment, the average temperature and relative humidity were 28 °C day/20 °C night and 78.5%, respectively.

5.2.3 Mineral analysis

Four weeks after transplanting, young fresh leaves were randomly collected in paper bags from all the plants in each replicate and bulked to obtain at least 20 g per genotype. The collected leaves were immediately transported to the laboratory and washed before oven-drying at 65 °C for 72 h.

In 2019 (pop1), after cooling, dried leaves were ground using a mortar and pestle into powder and sieved using a 1 mm screen sieve. Three independent replicates of 0.5 g each of sieved powder were weighed in porcelain crucibles using an analytical balance (D&T, ES-E200A, max=200 g, d = 0.1 mg, China). Samples were then ashed in a muffle furnace at 550 °C for 2 hours. The obtained ashes were digested using 10 ml of double acid composed of nitric acid (HNO_3, 65%, Merck, Germany) and hydrochloric acid (HCl, 32%, Merck, Germany) mixed in a ratio of 1:3 (Jones 2001). The resultant mixtures were placed on a hot plate at 250 °C for 30 min and later cooled for 1 hour. Digestates were filtered using Whatman paper Grade 1 (Qualitative Filter Paper Standard Grade, circle, 125 mm, Merck, Germany) into a 100 ml volumetric flask and made up to the mark using deionized water. The resultant solutions were analysed using a fast-sequential atomic absorption spectrometer (Varian AA280FS, Varian Inc., Mulgrave, Victoria, Australia) for Ca, Cu, Fe, Zn, K, Mn, Mg and Na. Flame atomic absorbance spectroscopy was used for all elements except potassium, for which flame atomic emission spectroscopy was employed. The wavelengths used were 324.8 nm for Cu, 248.3 nm for Fe, 279.5 nm for Mn, 766.5 nm for K, 213.9 nm for Zn, 422.7 nm for Ca, 285.2 nm for Mg and 589.0 nm for Na. Multielement standard solution IV (23 elements) (1000 mg l^{-1} in HNO_3 Suprapur® 6.5%) was purchased from Merck, KGaA, Darmstadt, Germany and used for calibration. The phosphorus was analysed in the digested solution according to the 4500-P E ascorbic acid method (APHA et al. 1999) at 670 nm using an Alpha UV–VIS spectrophotometer (Spectronic Unicam, Berlin, Germany) (Kalra 1997).

In 2020 (pop2), due to the failure of the atomic absorption spectrometer, the dried plant materials were sent to the Plant Laboratory of the CEDARA (KZN Department of Agriculture and Environmental Affairs) for analysis of leaf elemental composition (Ca, Cu, Fe, K, Mn, Mg, Na, P and Zn). The analysis was performed based on Hunter (1975), and an inductively coupled

plasma-optical emission spectrometer (ICP–OES) (Agilent 5800 VDV, Agilent Technologies Australia (M) Pty Ltd. Inc., Mulgrave, Australia) was used. All mineral contents were reported in mg kg^{-1} on a dry weight basis (mg kg^{-1} DW) for Cu, Fe, Mn and Zn and in g kg^{-1} on a dry weight basis (g kg^{-1} DW) for Ca, K, Mg and P.

5.2.4 Statistical analysis

The software R version 4.1.1 (R Core Team 2021) was used to perform all statistical analyses. Before proceeding to the analyses, the quality of the data was assessed, mainly for outlier detection by the Bonferroni–Holm test based on studentized residuals at the level of significance of 5%, as recommended by Bernal-Vasquez et al. (2016). Data normality was assessed using the Shapiro–Wilk test. Descriptive statistics (minimum, maximum, mean, coefficient of variation, standard deviation) were used to characterise the parents and hybrids using the function *describe* from the R package *psych* (Revelle 2019). The significance of differences between the means of parents and hybrids was tested using the *t test* or *the Wilcoxon test* when necessary. Variance components for each mineral were estimated per population. Each hybrid and parental dataset were analysed separately for overall genotypic variance components and adjusted means (BLUEs) using the following statistical model 1:

$$y_{ik} = \mu + R_k + G_i + \varepsilon_{ik} \quad (1)$$

where y_{ik} is the phenotypic observation of the i^{th} genotype (hybrid or parental line) in the k^{th} replicate, μ is the overall mean, R_k is the random effect of the k^{th} replicate, G_i is the random effect of the i^{th} genotype, and ε_{ik} is the random residual. Standard broad-sense heritability was calculated according to Hallauer et al. (2010) as follows:

$$H^2 = \sigma_G^2/(\sigma_G^2 + \sigma_e^2/r) \quad (2)$$

where σ_G^2 is the total genotypic variance, σ_e^2 is the residual variance, and r is the number of replications.

In addition, statistical model 2 was used to determine the variance components of the specific combining ability (SCA) and general combining ability (GCA) using the hybrids data only:

$$Y_{fmk} = \mu + \alpha_f + \beta_m + \gamma_{fm} + r_k + \varepsilon_{fmk} \quad (3)$$

where $y_{fmk}$is the phenotypic observation of the hybrid between the f^{th} female and m^{th} male in the k^{th} replicate; μ is the overall mean; α_f is the random GCA effect of the f^{th} female; β_m is the

random GCA effect of the m[th] male; γ_{fm} is the random SCA effect of the cross between the f^{th} female and the m^{th} male; r_k is the random effect of the k^{th} replicate; and ε_{fmk} is the random residual. All linear mixed-effects models were fitted using the restricted maximum likelihood (REML) implemented in the "ASReml-R" package version 4.1.0.160 (Butler et al. 2017). The likelihood ratio test (Self and Liang 1987) was used to test the significance of the variance components using the function *lrt* implemented in the ASREML-R package. The additive genetic variance (σ_A^2), dominance genetic variance (σ_D^2), total phenotypic variance (σ_P^2), and narrow-sense (h^2) heritability estimates in each hybrid population were determined according to Hallauer et al. (2010) and Isik et al. (2017) as follows:

$$\sigma_A^2 = 2(\sigma_{GCA-M}^2 + \sigma_{GCA-F}^2) \quad (4)$$

$$\sigma_D^2 = 4\sigma_{SCA-F\times M}^2 \quad (5)$$

$$\sigma_P^2 = \sigma_G^2 + \sigma_e^2/r \quad (6)$$

$$h^2 = \sigma_A^2/\sigma_P^2 \quad (7)$$

where σ_A^2 is the additive genetic variance, σ_{GCA-M}^2 is the male GCA variance, $\sigma_{GCA-F}^2$is the female GCA variance, σ_D^2 is the dominance genetic variance, σ_P^2 is the total phenotypic variance, $\sigma_{SCA-F\times M}^2$ is the SCA variance, σ_e^2 is the residual variance, and *r* is the number of replications.

The average degree of dominance was computed as D = $(2\sigma_D^2/\sigma_A^2)^{0.5}$ (Dhillon 1990). The phenotypic best linear unbiased predictors (BLUPs) associated with the general combining ability effect of each female (GCA_f) and male (GCA_m) parent and the specific combining ability effect of each cross (SCA_{fm}) were derived from model 2 according to Isik et al. (2017). BLUPs associated with the combining ability effects were used due to the incomplete factorial mating design and interest in family represented by each parental line. In addition, BLUPs were used because they have good predictive accuracy due to their high correlation with the true values and have been recommended for phenotypic selection in plant breeding (Piepho et al. 2008; Molenaar et al. 2018; Kleinknecht et al. 2013). The significance of GCA and SCA effects was evaluated using a two-tailed t test (Dabholkar 1999) at the probability levels of 0.05, 0.01, and 0.001. The maternal effect was tested using the F test (Kearsey and Pooni 1996). The importance of dominance and additive gene effects was assessed through the predictability ratio of Baker (1978) using the following formula:

$$Predictability\ ratio = (\sigma^2_{GCA-M} + \sigma^2_{GCA-F})/(\sigma^2_{GCA-M} + \sigma^2_{GCA-F} + \sigma^2_{SCA-F\times M}) \quad (8)$$

where σ^2_{GCA-M} is the male GCA variance, σ^2_{GCA-F} is the female GCA variance, and $\sigma^2_{SCA-F\times M}$ is the SCA variance.

For heterosis analysis, the adjusted means of each parent and hybrid generated from model 1 were used to estimate the heterosis level. Mid-parent heterosis (MPH) and better-parent heterosis (BPH) were computed for each hybrid as follows:

$$MPH\ (\%) = [(F_1 - MP)/MP] \times 100 \quad (10)$$

$$BPH\ (\%) = [(F_1 - BP)/BP] \times 100 \quad (11)$$

where F_1 is the adjusted mean value of the hybrid, MP is the mid-parent adjusted mean value computed as the average adjusted mean values between the two parents of the hybrid, and BP is the adjusted mean value of the best parent. For Pop1, only 120 hybrids (out of 135 hybrids) were used because two parents (P27 and P28) had low germination and the number of plants per replicate was not enough. Therefore, hybrids with one of those two parents were removed. In contrast, all 209 hybrids of Pop2 were used in the heterosis analysis.

The genetic advance (GA) for each trait was computed as GA = i x H^2 x σ_P, where σ_P was the phenotypic standard deviation, H^2 was the broad-sense heritability, and i was the standardized selection differential at a selection intensity of 5% (i = 2.06) (Singh and Chaudhary 1985). Genetic advance over mean (GAM) was further computed as GAM = (GA/μ) x 100, where μ is the overall mean of the trait. Spearman correlation coefficients between combining ability, heterosis and mean performance of the parents and hybrids for all traits and their level of significance were performed using the function *corr* from the R package *Hmisc* (Harrell Jr and Dupont 2021).

5.3 Results

5.3.1 Performance of parents and hybrids

Two populations were used in the present study, and their descriptive statistics for nine minerals for both parents and hybrids are summarized in Table 5.3. A significant difference was observed among parents ($P < 0.01$) for all minerals in all the populations (Table 5.4). Similarly, hybrids were significantly different ($P < 0.001$) for all mineral contents. Traits with high variability and low variability were population specific. For pop 1, the highest variability

for the parents was observed for magnesium, with a coefficient of variation (CV) of 39.61%, while the lowest was for manganese (CV = 17.14%). High coefficients of variation (CVs) for hybrids were observed for zinc (48.30%), magnesium (42.20%) and manganese (41.11%) in population pop1. The lowest CV was found for potassium (12.59%). Potassium and iron showed the lowest and highest coefficients of variation, respectively, for both parents and hybrids in pop2. For both parents and hybrids, the importance of mineral element content in the leaves was in the order of calcium > potassium > phosphorus > magnesium > sodium > iron > manganese >zinc > copper across the two populations. The iron content for the parents ranged between 120.79 and 275.13 mg kg^{-1} dry weight (DW), with an average of 187.59 mg kg^{-1} DW, while the zinc content was between 29.75 and 74.34 mg kg^{-1} DW, with an average of 52.27 mg kg^{-1} DW.

Significant differences ($P < 0.01$) were observed between the parents and hybrids for all microminerals (Fe, Zn, Cu, Mn) and Na and P (Figure 5.1) in both pop1 and pop2. Hybrids performed better than their parents for Zn, P and Mn, with increases of 17.51%-45.89%, 17.53-22.24%, and 64.72-78.44%, respectively. In contrast, the mean Na content of the parents was higher than that of the hybrids across the two populations. While the mean of hybrids was lower than that of parents for Fe in pop1, higher mean hybrid values were observed in pop2. The inverse was noticed for Cu.

5.3.2 Variance components and heritability estimates

Estimates of genetic variance components, heritability estimates, degree of dominance, Baker's (Baker 1978) predictability ratio, and genetic advance are summarized for macroelements and microelements in Table 5.4 . For all minerals, the genotypic variance was higher than the environmental variance in both parents and hybrids.

Table 5.3. Descriptive statistics of nine minerals investigated in two populations of *Gynandropsis gynandra*. Pop 1 includes 135 hybrids and 26 parents, and Pop 2 includes 209 hybrids and 30 parents.

Minerals	Population	Mean		Minimum		Maximum		Standard deviation		Coefficient of variation (%)	
		Parents	Hybrids	Parents	Hybrids	Parents	Hybrids	Parents	Hybrids	Parents	Hybrids
Macroelements											
Ca (g kg^{-1})	Pop 1	30.26	29.03	13.07	13.81	53.21	50.40	8.81	7.54	29.13	25.96
	Pop 2	18.39	17.56	11.42	10.34	29.72	27.35	4.29	3.15	23.33	17.96
K (g kg^{-1})	Pop 1	23.79	24.25	12.26	16.12	35.06	36.32	4.18	3.09	17.57	12.74
	Pop 2	27.14	43.85	19.75	24.97	34.96	63.73	3.97	5.51	14.65	12.58
Mg (g kg^{-1})	Pop 1	3.67	3.73	1.53	1.34	9.38	8.69	1.45	1.58	39.54	42.38
	Pop 2	6.20	5.97	3.70	3.88	9.11	9.63	1.23	0.91	19.95	15.28
Na (g kg^{-1})	Pop 1	1.02	0.87	0.53	0.33	2.58	2.39	0.37	0.33	36.31	38.06
	Pop 2	1.17	0.95	0.83	0.40	1.88	3.46	0.23	0.34	19.75	36.66
P (g kg^{-1})	Pop 1	7.65	8.99	4.83	3.82	13.44	16.22	2.15	2.28	28.15	25.43
	Pop 2	8.54	10.44	5.53	6.22	12.17	16.30	1.81	1.63	21.20	15.69
Microelements											
Cu (mg kg^{-1})	Pop 1	9.32	10.83	3.03	1.83	20.54	30.77	3.05	3.88	32.76	35.89
	Pop 2	12.44	8.58	7.06	1.72	25.10	27.03	3.68	2.40	29.57	27.98
Fe (mg kg^{-1})	Pop 1	187.59	163.60	111.55	41.70	303.47	526.08	51.84	64.51	27.63	39.43
	Pop 2	136.75	145.36	67.76	67.60	430.72	847.09	67.84	68.73	49.61	47.28
Mn (mg kg^{-1})	Pop 1	26.89	44.30	14.40	14.91	45.45	115.92	5.44	18.45	20.22	41.65
	Pop 2	191.50	341.73.9	43.74	143.82	433.07	597.43	87.77	83.60	45.83	24.46
Zn (mg kg^{-1})	Pop 1	52.26	76.25	28.32	25.43	78.16	252.54	11.69	36.93	22.37	48.44
	Pop 2	60.11	70.68	37.16	34.59	104.31	163.59	15.59	16.08	25.93	22.57

Table 5.4. Estimates of genetic variance components, heritability estimates, degree of dominance, predictability ratio, and genetic advance for the mineral elements in two populations of *Gynandropsis gynandra*. Pop 1 includes 135 hybrids and 26 parents, and Pop 2 includes 209 hybrids and 30 parents.

Source variation	Ca		K		Mg		Na		P	
Populations	Pop1	Pop2	Pop1	Pop2	Pop1	Pop2	Pop1	Pop2	Pop1	Pop2
Parents										
σ_G^2	76.19 ± 21.87 ***	17.13 ± 4.71 ***	16.52 ± 4.79 ***	9.24 ± 3.43 ***	2.09 ± 0.60 ***	1.45 ± 0.40 ***	0.12 ± 0.04 ***	0.05 ± 0.01 ***	4.62 ± 1.32 ***	3.21 ± 0.86 ***
σ_e^2	3.40 ± 0.68	1.57 ± 0.41	1.26 ± 0.25	6.75 ± 1.74	0.07 ± 0.01	0.10 ± 0.03	0.01 ± 0.00	0.00 ± 0.00	0.13 ± 0.03	0.13 ± 0.03
H^2	0.98 ± 0.02	0.96 ± 0.03	0.96 ± 0.02	0.73 ± 0.42	0.98 ± 0.01	0.97 ± 0.01	0.94 ± 0.02	0.97 ± 0.01	0.99 ± 0.00	0.98 ± 0.01
Hybrids										
σ_G^2	57.11 ± 12.69 ***	9.11 ± 1.31 ***	9.33 ± 1.25 ***	30.35 ± 4.43 ***	2.48 ± 0.40 ***	0.73 ± 0.09 ***	0.14 ± 0.03 ***	0.11 ± 0.01 ***	5.42 ± 1.16 ***	1.79 ± 0.29 ***
$\sigma_{GCA-Females}^2$	26.72 ± 11.98 ***	1.26 ± 0.67 ***	0.70 ± 0.61ns	6.77 ± 3.26 ***	0.28 ± 0.18 **	0.05 ± 0.03 **	0.07 ± 0.03 ***	0.01 ± 0.01ns	2.20 ± 1.02 ***	0.38 ± 0.19 ***
$\sigma_{GCA-Males}^2$	6.07 ± 3.37 *	2.32 ± 0.99 ***	0.95 ± 0.70 *	5.42 ± 2.48 ***	0.65 ± 0.31 ***	0.16 ± 0.07 ***	0.02 ± 0.01 ***	0.01 ± 0.01 *	1.13 ± 0.52 ***	0.33 ± 0.16 ***
$\sigma_{SCA-F\times M}^2$	24.32 ± 3.33 ***	5.53 ± 0.63 ***	7.69 ± 1.06 ***	18.17 ± 1.99 ***	1.55 ± 0.21 ***	0.53 ± 0.06 ***	0.05 ± 0.01 ***	0.10 ± 0.01 ***	2.09 ± 0.30 ***	1.08 ± 0.16 ***
σ_e^2	0.69 ± 0.06	0.89 ± 0.09	0.41 ± 0.04	1.42 ± 0.14	0.05 ± 0.00	0.07 ± 0.01	0.00 ± 0.00	0.01 ± 0.00	0.23 ± 0.02	0.80 ± 0.08
σ_A^2	65.58 ± 24.83	7.15 ± 2.39	3.30 ± 1.90	24.36 ± 8.21	1.86 ± 0.72	0.41 ± 0.16	0.17 ± 0.07	0.03 ± 0.02	6.65 ± 2.28	1.42 ± 0.50
σ_D^2	97.28 ± 13.32	22.12 ± 2.52	30.74 ± 4.25	72.67 ± 7.97	6.19 ± 0.85	2.11 ± 0.24	0.21 ± 0.03	0.39 ± 0.04	8.37 ± 1.18	4.33 ± 0.65
H^2	0.99 ± 0.00	0.95 ± 0.01	0.99 ± 0.00	0.98 ± 0.00	0.99 ± 0.00	0.95 ± 0.01	0.99 ± 0.00	0.97 ± 0.00	0.99 ± 0.00	0.82 ± 0.03
h^2	0.40 ± 0.10	0.24 ± 0.07	0.10 ± 0.05	0.25 ± 0.07	0.23 ± 0.08	0.16 ± 0.06	0.44 ± 0.10	0.07 ± 0.04	0.44 ± 0.09	0.23 ± 0.07
$h_{Females}^2$	0.52 ± 0.12	0.18 ± 0.08	0.08 ± 0.07	0.27 ± 0.10	0.15 ± 0.09	0.09 ± 0.06	0.56 ± 0.12	0.06 ± 0.05	0.51 ± 0.12	0.24 ± 0.10
h_{Males}^2	0.20 ± 0.09	0.29 ± 0.09	0.11 ± 0.08	0.23 ± 0.08	0.29 ± 0.11	0.22 ± 0.08	0.24 ± 0.10	0.08 ± 0.05	0.35 ± 0.11	0.22 ± 0.09
Maternal effect	0.008	0.618562	0.613379	0.260587	0.708195	0.844001	0.046693	0.543681	0.090222	0.278584
Degree of dominance	1.22 ± 0.25	1.76 ± 0.32	3.05 ± 0.95	1.73 ± 0.31	1.82 ± 0.39	2.27 ± 0.47	1.12 ± 0.23	3.67 ± 1.06	1.12 ± 0.21	1.75 ± 0.35
Predictability ratio	0.57 ± 0.10	0.39 ± 0.09	0.18 ± 0.09	0.40 ± 0.09	0.38 ± 0.10	0.28 ± 0.08	0.62 ± 0.10	0.13 ± 0.07	0.61 ± 0.09	0.40 ± 0.09
Genetic advance (GA) at i = 5%	15.54 ± 1.73	6.07 ± 0.46	6.25 ± 0.42	11.22 ± 0.84	3.23 ± 0.26	1.72 ± 0.12	0.76 ± 0.09	0.68 ± 0.04	4.76 ± 0.52	2.49 ± 0.24
GAM (%)	53.52 ± 5.97	34.55 ± 2.60	25.76 ± 1.74	25.58 ± 1.91	86.62 ± 6.97	28.81 ± 1.94	86.97 ± 10.45	71.59 ± 3.92	52.95 ± 5.74	23.87 ± 2.30

σ^2_A: additive genetic variance, σ^2_D: dominance genetic variance, $\sigma^2_{GCA\text{-}F}$: female general combining ability variance, $\sigma^2_{GCA\text{-}M}$: male general combining ability variance, σ^2_{SCA}: specific combining ability variance, σ^2_E: residual variance, σ^2_G: genotypic variance, H^2: broad-sense heritability, h^2: narrow-sense heritability, GA: genetic advance, GAM: genetic advance over mean. ***, **, *: significantly different from zero at the 0.001, 0.01, and 0.05 probability levels, respectively. ns: not significantly different from zero at the 0.05 level of probability.

Table 5.4. Estimates of genetic variance components, heritability estimates, degree of dominance, predictability ratio, and genetic advance for the mineral elements in two populations of *Gynandropsis gynandra*. Pop 1 includes 135 hybrids and 26 parents, and Pop 2 includes 209 hybrids and 30 parents.

Source variation	Cu		Fe		Mn		Zn	
Populations	Pop1	Pop2	Pop1	Pop2	Pop1	Pop2	Pop1	Pop2
Parents								
σ_G^2	8.30 ± 2.47 ***	6.27 ± 2.69 **	2039.95 ± 643.34 ***	3216.16 ± 1050.55 ***	17.01 ± 6.07 ***	7780.91 ± 2050.64 ***	129.38 ± 37.61 ***	234.91 ± 63.29 ***
σ_e^2	1.25 ± 0.25	6.77 ± 1.78	686.57 ± 137.31	1441.82 ± 372.28	12.69 ± 2.54	55.33 ± 14.53	10.70 ± 2.10	12.02 ± 3.16
H^2	0.93 ± 0.04	0.65 ± 0.50	0.86 ± 0.20	0.82 ± 0.60	0.73 ± 0.56	0.99 ± 0.05	0.96 ± 0.09	0.98 ± 0.08
Hybrids								
σ_G^2	13.22 ± 2.94 ***	4.76 ± 0.52 ***	3571.37 ± 667.31 ***	4560.76 ± 472.06 ***	332.28 ± 46.12 ***	6886.85 ± 737.93 ***	1372.31 ± 280.00 ***	250.08 ± 30.08 ***
$\sigma_{GCA-Females}^2$	5.76 ± 2.79 ***	0.17 ± 0.20ns	1140.94 ± 595.64 ***	112.57 ± 158.74ns	49.05 ± 32.27 **	139.00 ± 213.61ns	564.08 ± 262.90 ***	34.77 ± 19.24 ***
$\sigma_{GCA-Males}^2$	0.14 ± 0.41ns	0.08 ± 0.18ns	15.49 ± 124.07ns	291.02 ± 232.31ns	0.00 ± NAns	752.37 ± 449.25 **	44.18 ± 51.64ns	27.18 ± 15.39 **
$\sigma_{SCA-F\times M}^2$	7.33 ± 1.14 ***	4.51 ± 0.53 ***	2414.93 ± 364.76 ***	4157.17 ± 449.40 ***	283.23 ± 36.92 ***	5995.48 ± 642.78 ***	764.05 ± 104.90 ***	188.13 ± 20.48 ***
σ_e^2	3.28 ± 0.28	0.93 ± 0.09	804.10 ± 69.72	203.28 ± 19.93	15.42 ± 1.33	181.76 ± 17.87	21.74 ± 1.88	12.21 ± 1.20
σ_A^2	11.79 ± 5.66	0.49 ± 0.54	2312.87 ± 1210.06	807.18 ± 566.18	98.10 ± 64.55	1782.74 ± 998.85	1216.52 ± 537.21	123.89 ± 49.17
σ_D^2	29.31 ± 4.58	18.04 ± 2.11	9659.73 ± 1459.03	16628.67 ± 1797.61	1132.92 ± 147.68	23981.91 ± 2571.12	3056.19 ± 419.60	752.51 ± 81.91
H^2	0.92 ± 0.02	0.91 ± 0.01	0.93 ± 0.01	0.98 ± 0.00	0.98 ± 0.00	0.99 ± 0.00	0.99 ± 0.00	0.98 ± 0.00
h^2	0.28 ± 0.10	0.03 ± 0.03	0.19 ± 0.09	0.05 ± 0.03	0.08 ± 0.05	0.07 ± 0.04	0.28 ± 0.10	0.14 ± 0.05
$h_{Females}^2$	0.43 ± 0.13	0.04 ± 0.04	0.31 ± 0.12	0.03 ± 0.04	0.15 ± 0.09	0.02 ± 0.03	0.42 ± 0.12	0.15 ± 0.07
h_{Males}^2	0.02 ± 0.05	0.02 ± 0.04	0.01 ± 0.05	0.07 ± 0.05	0.00 ± 0.00	0.11 ± 0.06	0.05 ± 0.06	0.13 ± 0.06
Maternal effect	0.001161	0.302414	0.002711	0.647376	0.015312	0.849677	0.002027	0.234184
Degree of dominance	1.58 ± 0.41	6.05 ± 3.42	2.04 ± 0.57	4.54 ± 1.67	3.40 ± 1.16	3.67 ± 1.08	1.59 ± 0.38	2.46 ± 0.52
Predictability ratio	0.45 ± 0.13	0.05 ± 0.06	0.32 ± 0.12	0.09 ± 0.06	0.15 ± 0.09	0.13 ± 0.07	0.44 ± 0.12	0.25 ± 0.08
Genetic advance (GA) at i = 5%	7.20 ± 0.86	4.29 ± 0.26	118.73 ± 11.88	137.59 ± 7.28	37.26 ± 2.63	169.84 ± 9.22	76.11 ± 7.81	32.19 ± 1.98
GAM (%)	66.44 ± 7.97	49.93 ± 3.01	72.57 ± 7.26	94.66 ± 5.01	84.10 ± 5.93	49.70 ± 2.70	99.81 ± 10.24	45.53 ± 2.81

σ^2_A: additive genetic variance, σ^2_D: dominance genetic variance, $\sigma^2_{GCA\text{-}F}$: female general combining ability variance, $\sigma^2_{GCA\text{-}M}$: male general combining ability variance, σ^2_{SCA}: specific combining ability variance, σ^2_E: residual variance, σ^2_G: genotypic variance, H^2: broad-sense heritability, h^2: narrow-sense heritability, GA: genetic advance, GAM: genetic advance over mean. ***, **, *: significantly different from zero at the 0.001, 0.01, and 0.05 probability levels, respectively. ns: not significantly different from zero at the 0.05 level of probability

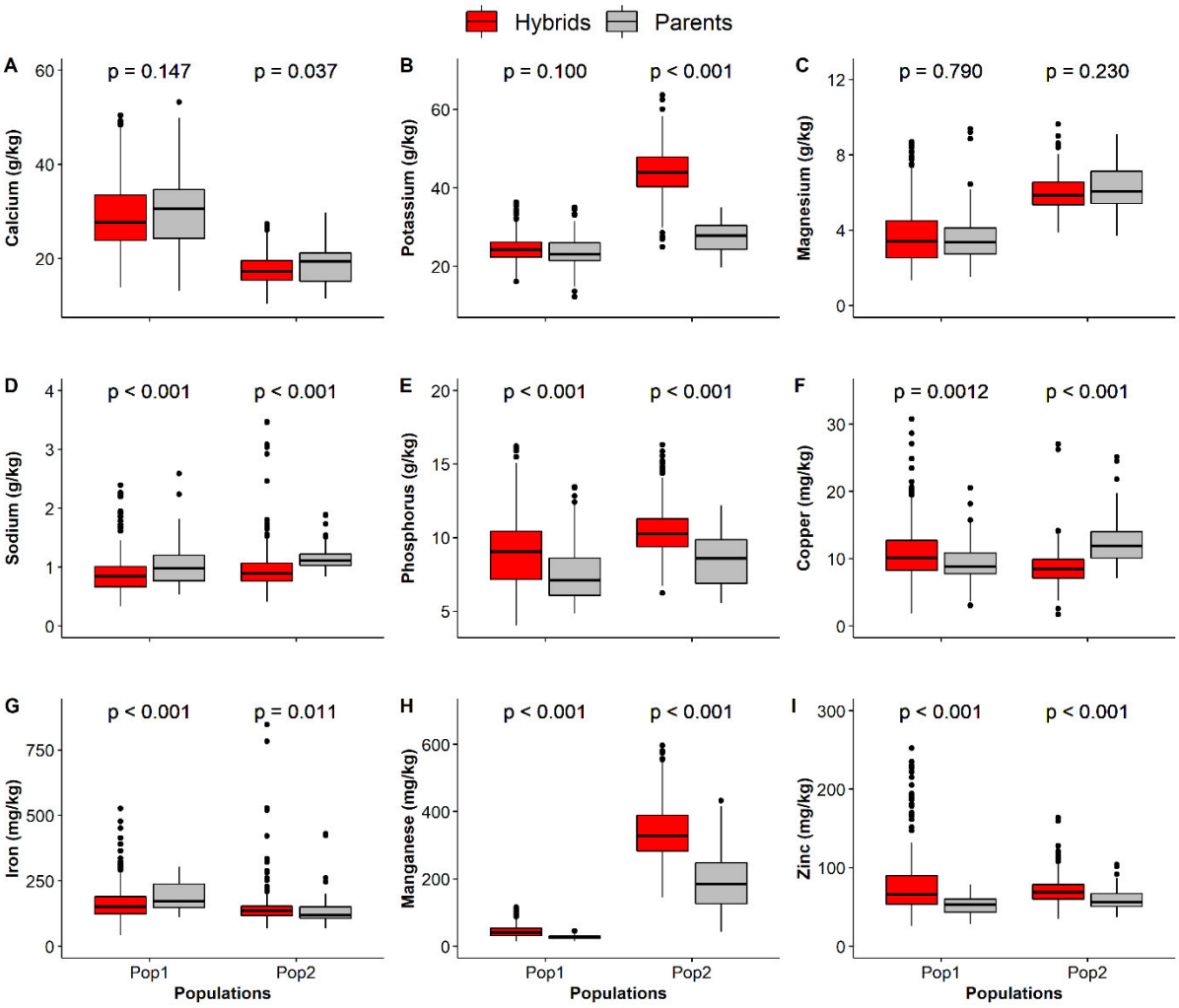


Figure 5.1. Comparison of hybrids and parents' performance in the two populations of *Gynandropsis gynandra*. Pop 1 includes 135 hybrids and 26 parents. Pop 2 includes 209 hybrids and 30 parents.

The partitioning of genotypic variance in hybrids showed that general combining ability (GCA) variance for females ($\sigma^2_{GCA\text{-}F}$) was significantly different from zero for Ca, Mg, P and Zn across the two populations, for Cu, Fe, Mn and Na in Pop1, and K in Pop2. The GCA variance for males ($\sigma^2_{GCA\text{-}M}$) was significantly different from zero for Ca, Na, P, Mg and K in both populations and for Mn and Zn in Pop2. While estimates of female GCA variance for Zn were larger than the male ones, male GCA variances were greater than female GCA variances for Mg across the two populations. For all mineral contents, the specific combining ability (SCA) variance ($\sigma^2_{SCA\text{-}FxM}$) was significantly different from zero. The estimate of $\sigma^2_{SCA\text{-}FxM}$ was greater than the average females and males GCA variance for all minerals across populations.

This was further confirmed by the additive variance (σ^2_A), which was low for all mineral elements. The degree of dominance was greater than unity for all mineral contents, showing their dominant nature. Overall, the predictability ratio was lower than 0.5 for most mineral contents and in both populations, with few exceptions. While traits displayed a predictability ratio less than 0.5 for all minerals in pop2, it was greater than 0.5 for all macroelements (Na (0.61), Ca (0.57), P (0.61)) except K in pop1.

Broad-sense heritability (H^2) estimates were high for all minerals (Table 5.4) in both hybrids and parents, ranging between 0.65 and 0.99 for parents and between 0.82 and 0.99 for hybrids. In contrast, narrow-sense heritability (h^2) estimates were low to moderate for mineral elements. Low h^2 values were observed for all microelements (0.03 to 0.28) across the two populations. While low h^2 values were obtained for all microelements in Pop2, moderate h^2 values were observed for Na (0.44), P (0.44), and Ca (0.40) in Pop 2. Furthermore, differential h^2 values were observed between males and females and were population specific. Genetic gains (> 20% of the current mean of the hybrid population) at 5% selection intensity were significant for all minerals (Table 5.4). The lowest and highest genetic gain estimates were 25.56% (Pop1) and 99.81% (Pop1) for P and Zn, respectively.

5.3.3 General combining ability effects of parents

Estimates of the general combining ability (GCA) effects of male and female parents are presented in Figure 5.2. Female parents had significant GCA effects for all minerals, while no significant GCA effects were observed for male parents for Zn and Cu in the two populations of study. Specifically, significant male GCA effects were observed for Mn and Fe in pop2 but not in pop1. Some female and male parents displayed multiple significant and positive GCA effects. Good general female combiners included parent P14 for Mg and Ca; parent P24 for Fe, P, Na and Cu; parent P04 for P, Na and Mn; parent P18 for Fe, Cu and Mn and K; and P09 for Ca, K and Mg. Males with multiple positive GCA effects comprised parent P31 for Fe and Mn; parent P29 for Ca, K and Na; parent P21 for Na, P and Zn; parents P22 and P27 and P03 for Mg and Ca; and parents P13 and P15 for Fe.

5.3.4 Specific combining ability effects of hybrids

A wide range of specific combining ability (SCA) effects from negative to positive was observed for all mineral contents (Appendix 5.1). The hybrids P20xP11, P09xP27, P17xP19 (Pop1), P14xP31, P09xP13, P32xP02, and P14xP29 (Pop2) exhibited highly significant SCA

effects for both Mg and Ca. The crosses P24xP28, P12xP11, P09xP11, and P09xP03 (pop1) and P25xP28, P04xP07, P23xP11, and P10xP03 exhibited desirable SCA effects for Cu, while hybrids P04xP21, P24xP19, P20xP11 (pop1), P17xP07, P12xP13, and P14xP01 (pop2) were the best for Na. For Fe, the highest and most significant SCA effects were observed in the hybrids P18xP16, P17xP11, P04xP13 (pop 1), P32xP31, P20xP19, and P32xP15 (Pop 2). The hybrids P20xP08, P04xP26, P09xP16, P12xP11 (pop 1), P18xP28, P10xP11 and P25xP22 (pop2) had high and significant positive SCA effects for potassium. For Mn, hybrids P18xP13, P04xP26, P10xP07 (pop1), P10xP29, P18xP26 and P18xP01 (pop2) showed high positive SCA effects. The crosses with highly significant and positive SCA effects for phosphorus were P23xP11, P14xP03, P05xP16 (pop 1), P17xP26, P18xP07, and P17xP16 (pop2). For Zn, crosses with high and significant SCA effects included P20xP21 P20xP01, P06xP03, and P20xP02 for pop1 and P20xP19, P18xP01 and P25xP02 for pop2.

5.3.5 Heterosis

The distributions of mid- and best-parent heterosis (MPH and BPH, respectively) for mineral elements are shown in Figure 5.3. A similar distribution pattern was observed for most traits in both study populations. The heterosis ranged between -80.38% and 292.11% for minerals and between -84.98 and 404.79% for Pop1 and Pop2 when mid- and best-parent heterosis were pooled. The species displayed both negative and positive heterosis. For Ca, the top three hybrids for BPH were P14xP21, P12xP15, P05xP16 (pop1), P18xP01, P05xP01 and P10xP11 (Pop2), while for MPH, they were P14xP03, P18xP19 and P12xP08 (pop1), P18xP01, P18xP28, and P06xP29 (pop2). Heterosis for magnesium ranged between -56.88% and 138.44% for MPH and -67.84% and 128.57% for BPH. The hybrids P20xP15, P20xP11 P06xP16, and P06xP15 (Pop1), P20xP19, P18xP01, P05xP11 and P05xP08 (pop2) for both MPH and BPH. For potassium, the best MPH was recorded in P04xP26, P09xP16, and P12xP11 (Pop1) and in P20xP19, P18xP01, P05xP28, and P05xP08 (pop2), while the best BPH was observed in crosses P04xP26, P09xP16, P12xP11, P14xP22 (pop1), P18xP28, P10xP29, P10xP11, and P09xP03 (pop2). The best combinations for Na were P04xP19, P04xP26, P24xP19, P24xP26 (pop1), P17xP07, P12xP13 and P14xP01 (pop2) for MPH and P04xP19, P04xP26, P04xP02 (pop1), P17xP07, P12xP13, and P14xP01 (pop2) for BPH. For phosphorus, the best cross combinations were P09xP01 (pop1), P05xP22, P12xP22, and P12xP27 (pop2) for both MPH and BHP.

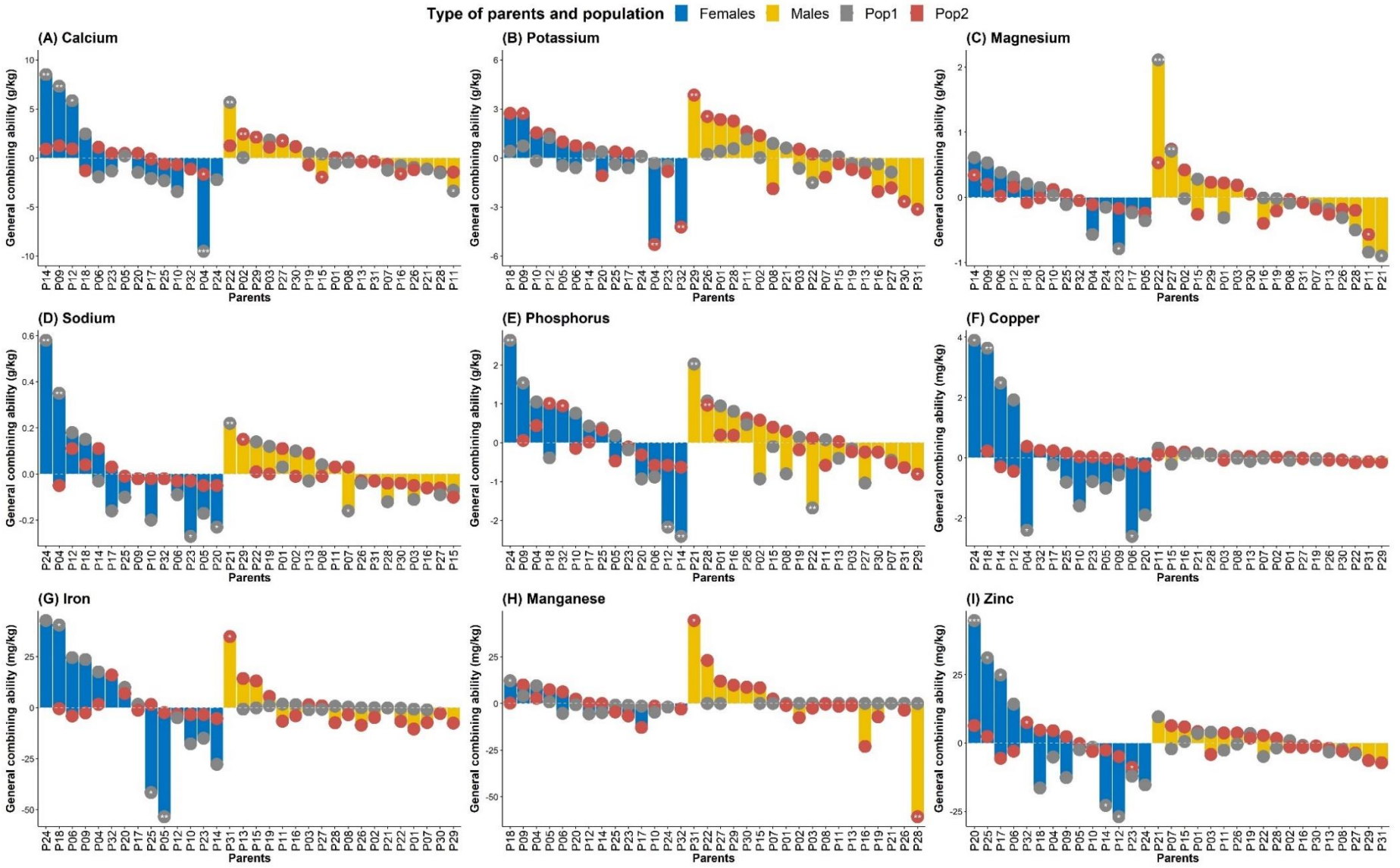
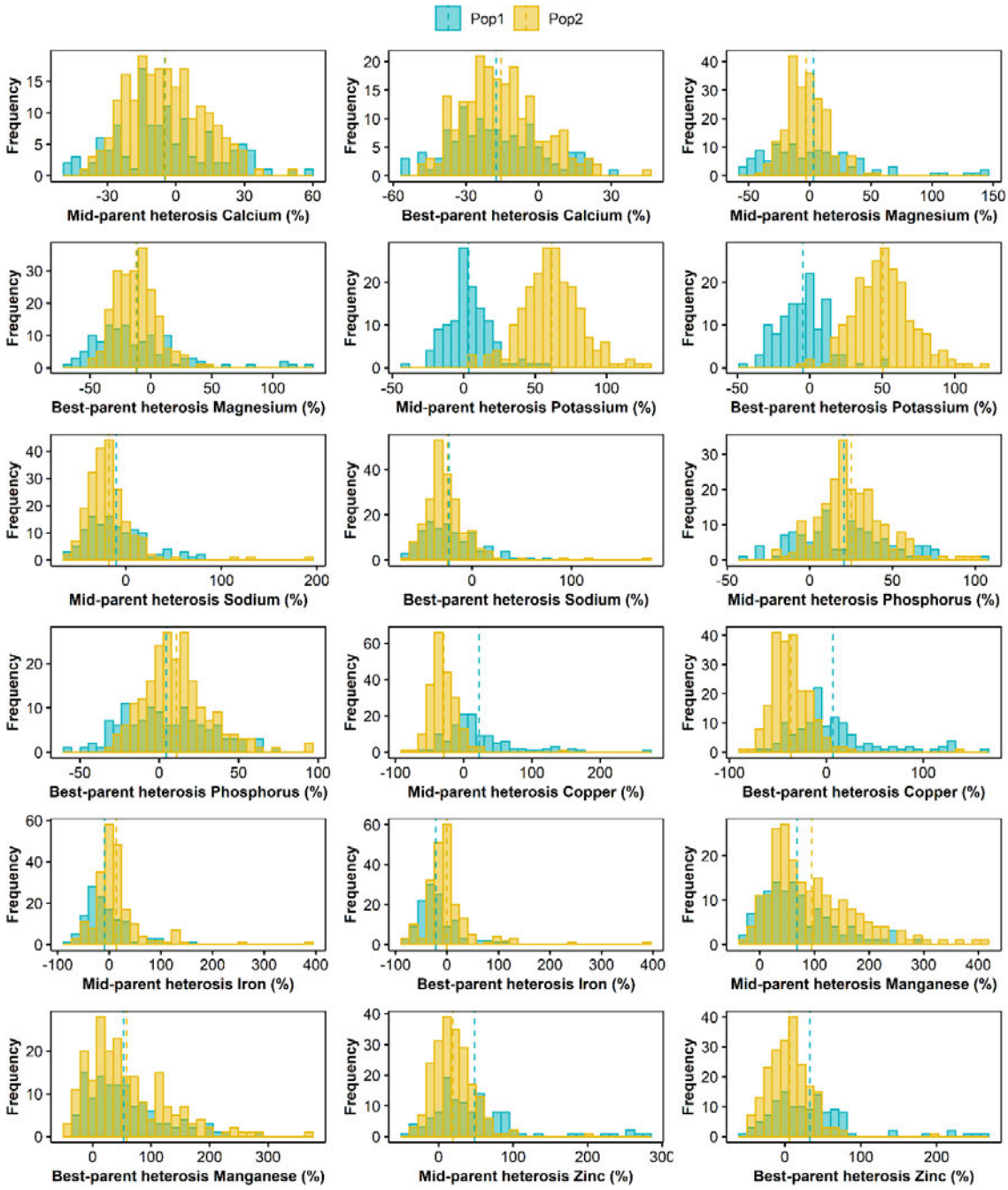


Figure 5.2. Estimates of general combining ability effects of male and female parents for mineral elements in two experimental hybrid populations of *Gynandropsis gynandra*. ***, **, * refer to estimate of general combining ability effect significantly different from zero at $P < 0.001$, 0.01 and 0.05, respectively.

Hybrids with high MPH and BHP were P14xP22, P18xP26, P18xP13, P18xP16 (pop1), P25xP28, P04xP07, P10xP03, P10xP29 and P04xP16 (pop2) for copper. For iron, the crosses P18xP16, P04xP13, P17xP11 and P20xP19 (pop1), P25xP28, P04xP07, P10xP03, and P10xP29 (pop2) displayed high MPH, while hybrids P14xP03, P18xP16, P04xP13, P17xP11 (pop1), P32xP31, P20xP19, P12xP27, and P17xP03 (pop2) were observed with high BPH. The cross combinations P14xP08, P18xP26, P04xP26, P18xP13 and P18xP11 (pop1) P32xP31, P20xP19, P12xP27, and P17xP03 (pop2) were among the highest for both MPHs, while P14xP08, P18xP26, P04xP26, P18xP13 (pop1), P20xP19, P10xP27, P25xP03, and P05xP31 (pop2) were the best for BPH for manganese. For zinc, the hybrids P06xP03, P20xP01, P20xP02, P20xP21 and P20xP19 (pop1) and P20xP19, P20xP31, P12xP07, and P09xP07 (pop 2) displayed high MPH and BPH.

5.3.6 Correlation between combining ability, heterosis and mean performance of the parents and hybrids

The associations between combining ability, heterosis and mean genotypic values of the parents and hybrids are summarized in Table 5.5. The *per se* performance of the parents exhibited a significantly positive correlation with their GCA effects for zinc, sodium, phosphorus and calcium across the two populations. The F_1 *per se* performance was significantly and positively correlated with SCA and the sum of GCA effects of the hybrids' parents for all minerals (Table 5.5). The r(SCA, F_1) was higher than the r(F_1, GCA) for Cu, Fe, K, and Mg. The correlation coefficients between SCA and F_1 ranged from moderate to strong. In addition, heterosis (both MPH and BPH) showed a highly significant and positive association with SCA and F_1 hybrid performance for all minerals, ranging from moderate to strong and trait specific. The correlation between the sum of GCA effects of hybrid parents and heterosis was population specific. For population 1, the sum of GCA effects of hybrid parents had a positive correlation with MPH and BPH for all minerals, except r(GCA, MPH) for K, which was not significant. In contrast, no weak correlations were observed between the sum of the GCA hybrid parents and the heterosis for population 2 (Table 5.5).



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127 Figure 5.3. Distribution of mid- and best-parent heterosis for nine mineral elements in two
128 populations of *Gynandropsis gynandra*.

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Table 5.5. Spearman correlation coefficients between general combining ability effects and parent *per se* performance r(*per se*, GCA), among mid- (MPH) and best- (BPH) parent heterosis, hybrid performance (F_1) and specific combining ability (SCA) and sum of general combining abilities of hybrids' parents (GCA) for nine minerals in *Gynandropsis gynandra*.

Traits	Population	r(*per se*, GCA)	r(F_1, SCA)	r(F_1, MPH)	r(F_1, BPH)	r(SCA, MPH)	r(SCA, BPH)	r(F_1, GCA)	r(GCA, MPH)	r(GCA, BPH)
Ca	Pop1	0.61***	0.69***	0.65***	0.61***	0.65***	0.63***	0.73***	0.32***	0.29**
	Pop2	0.72***	0.80***	0.58***	0.67***	0.75***	0.72***	0.67***	0.06	0.23***
Cu	Pop1	-0.16ns	0.75***	0.77***	0.74***	0.53***	0.54***	0.64***	0.54***	0.48***
	Pop2	0.39*	0.99***	0.70***	0.62***	0.73***	0.64***	0.41***	0.07	0.12
Fe	Pop1	-0.26ns	0.77***	0.89***	0.82***	0.62***	0.58***	0.67***	0.71***	0.63***
	Pop2	0.36*	0.90***	0.63***	0.62***	0.70***	0.71***	0.34***	-0.03	-0.07
K	Pop1	0.3ns	0.95***	0.58***	0.56***	0.62***	0.58***	0.59***	0.15	0.20*
	Pop2	0.42*	0.81***	0.71***	0.74***	0.71***	0.69***	0.66***	0.32***	0.40***
Mg	Pop1	0.25ns	0.83***	0.81***	0.71***	0.83***	0.77***	0.66***	0.36***	0.26**
	Pop2	0.54**	0.88***	0.57***	0.53***	0.70***	0.63***	0.58***	0.01	0.07
Mn	Pop1	0.47*	0.95***	0.94***	0.93***	0.95***	0.93***	0.40***	0.26**	0.29**
	Pop2	0.18ns	0.96***	0.52***	0.51***	0.54***	0.51***	0.41***	0.14*	0.20**
Na	Pop1	0.65***	0.72***	0.79***	0.72***	0.68***	0.59***	0.75***	0.49***	0.46***
	Pop2	0.48**	0.93***	0.84***	0.73***	0.86***	0.77***	0.48***	0.22**	0.11
P	Pop1	0.44*	0.71***	0.74***	0.58***	0.67***	0.57***	0.81***	0.49***	0.36***
	Pop2	0.46*	0.87***	0.55***	0.50***	0.66***	0.55***	0.61***	0.08	0.14
Zn	Pop1	0.54**	0.68***	0.88***	0.85***	0.68***	0.65***	0.76***	0.63***	0.61***
	Pop2	0.72***	0.85***	0.61***	0.53***	0.73***	0.62***	0.63***	0.08	0.09

Ca, K, Mg and Na (g kg^{-1}), Cu, Fe, Mn and Zn (mg kg^{-1}), ***, **, * = significantly different from zero at the 0.001, 0.01, and 0.05 probability levels, respectively. ns = not significantly different from zero at the 0.05 level of probability.

5.4 Discussion

5.4.1 Variability and hybrid performance in minerals

In the present study, we observed highly significant variation among parents and F_1 hybrids for mineral contents. The variation could be associated with the diversity in the origin of accessions used in the present study, in line with reports by Sogbohossou et al. (2019), Blalogoe et al. (2020) and Reeves et al. (2018). Moreover, as observed in this study, Omondi et al. (2017b) reported significant variation in mineral content between genotypes composed of farmers' cultivars, advanced lines and germplasm accessions from Eastern and Southern Africa. Similar observations for agronomic traits have also been reported for worldwide accessions (Wu et al. 2018; Sogbohossou et al. 2019), accessions from South Africa and Kenya (Kangai Munene et al. 2018), Ghana (Kwarteng et al. 2018), and Burkina-Faso (Kiebre et al. 2017). This phenotypic variation in *G. gynandra* has been reported to be associated with the genetic makeup of the accessions (Sogbohossou 2019; Omondi et al. 2017a).

Overall, hybrids outperformed their parents for several mineral contents, such as zinc, potassium, phosphorus and manganese, indicating hybrid vigor. Similar results were reported for mineral elements in *Brassica oleracea* var. *capitata* (Singh et al. 2009), in *Brassica rapa* (Xie et al. 2018), in *Brassica oleracea* var. *botrytis* L. (Ram et al. 2018). In addition, some hybrids were better or worse than their specific parents for various traits, showing that the inheritance of the traits is controlled by different mechanisms. The average performance of several hybrids for minerals was higher than that of the parents, as previously reported (Omondi et al. 2017b; Jiménez-Aguilar and Grusak 2015; Glew et al. 2009; Schönfeldt and Pretorius 2011; Jinazali et al. 2017). For instance, the average zinc content of 60 mg kg^{-1} dry weight (DW) with a maximum of 80 mg kg^{-1} DW reported by Omondi et al. (2017b) was lower than that of the present study, which was 76.26 mg kg^{-1} DW with a maximum of 252.54 mg kg^{-1} DW. The average Ca content in hybrids was twofold higher than that reported by Jiménez-Aguilar and Grusak (2015). However, we observed lower values in iron than in Omondi et al. (2017b) and Gowele et al. (2019). This might be due to the genotypes, leaf sampling stage, cultivation practices and growth conditions. For instance, Jinazali et al. (2017) observed significant variation in mineral content between genotypes collected from different agroecological areas of Malawi, while Mamboleo et al. (2018) and Makokha et al. (2019) reported the effect of harvesting stage and growing locations on the mineral content in *Gynandropsis gynandra*. More investigations are thus required to assess the phenotypic

plasticity and stability of the newly developed hybrids to identify the best genotypes under different agroecological and agronomic practices conditions.

5.4.2 Gene action, heritability and combining abilities

Knowledge of gene action and combining ability effects of traits is important for any crop breeding. Gene action is particularly crucial in the choice of breeding method (Priyadarshan 2019). Genetic variance components analysis showed that all traits were controlled by both additive and nonadditive genes. Nonadditive gene effects were predominant for most leaf mineral elements (Fe, Zn, Cu, Mg, K and Mn) in the species. This finding was supported by (1) the specific combining ability effects and dominance variances that were greater than the general combining ability effects and additive variances, respectively; (2) the degree of dominance greater than unity; and (3) the predictability ratio below 0.5 for most of the traits. Consequently, the selection for nutritious cultivars in *Gynandropsis gynandra* should focus on hybrid cultivars development for better exploitation of the dominance gene action through recurrent selection, especially reciprocal recurrent selection (Priyadarshan 2019). Similar findings were obtained by Xie et al. (2018) for Ca, Fe, Mg, and Zn in *Brassica rapa* (Chinese cabbage), Singh et al. (2012) for Fe, Zn, Cu, Mn, K and Ca in *Brassica oleracea* var. *capitata* L. (cabbage), where nonadditive gene action was predominant. Assessing epistasis was not part of the objectives of this study. Further studies using appropriate mating designs, such as North Carolina Design III and triple test cross should be implemented to estimate its contribution to mineral content in the species.

Heritability is a key parameter in breeding, particularly in the prediction of the response to selection. Broad-sense heritability (H^2) is a measure of the proportion of the total phenotypic variation attributable to the variance of genetic values (Visscher et al. 2008). Broad-sense heritability estimates were high (> 0.60) for all mineral contents, showing that phenotypic variation observed among genotypes is mostly due to genotypic variation. We, therefore, hypothesize that phenotypes can accurately predict genotypes, but this should be confirmed with multienvironmental trials. Similar results were found with respect to mineral content in *Amaranthus tricolor* L. (Shukla et al. 2006). Furthermore, narrow-sense heritability (h^2) is a measure of the proportion of the total phenotypic variation attributable to additive genetic variance. A high h^2 means that the phenotypic variation is mostly due to additive genetic effects. Thus, the higher the h^2 value is, the better the response to selection. We observed low to moderate narrow-sense heritability for all mineral contents (< 50%), which agreed with the

preponderance of dominance genes in the inheritance minerals content in the species. Xie et al. (2018) also reported low and moderate h^2 for mineral content in *Brassica rapa*, while Karmakar et al. (2013) reported low h^2 for mineral and antioxidant content in ridge gourd (*Luffa acutangula* Roxb.). However, there is a need to evaluate these parents and progenies in several environments to estimate the level of environmental influence and genotype-by-environment interactions in phenotypic variation.

The expected genetic gain, another important metric for breeding for quantitative traits, estimates the quantity of increase in performance between the selected and base populations and is key along with heritability in any breeding program (Xu et al. 2020). Our expected genetic gain at a selection intensity of 5% for all the traits ranged between 23.87% and 99.81%. Genetic gain over 20% was observed for all minerals, showing that significant improvement would be made through selection.

General combining ability is important for parental selection, and specific combining ability is important for best cross selection to exploit heterosis. Although nonadditive gene effects were predominant, high and significant general combining ability effects were observed for some female and/or male parents for mineral contents and were mainly due to additive and additive x additive gene effects (Dey et al. 2014). The parents with high good GCA effects are excellent founders for the development of improved populations and could be exploited through several generations of hybridization. In the present study, none of the male and female parents simultaneously showed significant GCA effects in the desirable direction for all mineral contents. This result concurs with previous findings with respect to minerals in cabbage head (Singh et al. 2012) and minerals, vitamins and antioxidants in cauliflower (Ram et al. 2018; Dey et al. 2014). However, some female (P14, P24, P04, P18, P09) and male (P31, P29, P21, P22, P27, P03, P13 P15) parents are good for their multiple positive and significant GCA effects. These parents are excellent and valuable candidates and resources for developing improved populations for research and breeding purposes.

Specific combining ability effects result from nonadditive gene effects, comprising dominance and epistasis (Sprague and Tatum 1942). In this study, none of the crosses displayed high and significant SCA effects for all minerals. This finding is similar to the results of Parkash et al. (2017) for antioxidant compounds in *Brassica oleracea* var. *capitata* but contrasts with those of Singh et al. (2012) and Xie et al. (2018). The latter researchers were able to identify at least one cross with significant and positive SCA effects for all the investigated minerals in cabbage

head and nonheading Chinese cabbage. However, depending on the targeted mineral, hybrids with the highest and most significant SCA in the desirable direction involved (i) both parents with good and significant GCA effects (e.g., P09xP27 for Ca, P32xP31 for Fe); (ii) one good and one poor combiner (e.g., P32xP02 for Ca, P18xP16 for Fe); and (iii) both parents with medium or bad GCA effects (e.g., P10xP11 for Ca, P12xP01 for Mn). This finding shows that depending on the trait, the observed SCA effect might result from (i) the cumulative effects of additive genes (good x good parents); (ii) the interaction between additive and nonadditive genes (good x poor general combiners or vice versa); and (iii) the over manifestation of the interaction between nonadditive genes, especially complementary epistatic effects (Xie et al. 2018; Dey et al. 2014; Singh et al. 2012; Singh et al. 2019; Sprague and Tatum 1942). We also observed that some crosses involving both parents with good GCA displayed significant and negative SCA effects. This might be the result of the absence of or weak interaction between the desirable alleles. Therefore, crosses from good general combiners might not always display desirable SCA effects. Based on the above, breeding strategies for high-quality leaves in *Gynandropsis gynandra* should consider both GCA and SCA in the selection of superior parents and crosses. Heterosis breeding and recurrent selection along with multiple crossing programs can be implemented, and types of cultivars might include hybrids, synthetics, composites and population improvements. Strategies implemented for allogamous species can be used for the species. Therefore, breeding strategies should focus on (i) selecting parents with good general combing ability, followed by (ii) selection based on specific combining ability. Reciprocal recurrent selection would be the best selection method to successfully exploit both additive and nonadditive gene action in the species.

5.4.3 Heterosis

Heterosis or hybrid vigor refers to the outperformance of F_1 progenies over their parents and has significantly contributed to increased crop productivity. Here, we report for the first time this phenomenon in *Gynandropsis gynandra*. The level of heterosis over the mid and best parents was large and variable between traits. This agrees with earlier reports on the existence of heterosis for mineral content, vitamins and antioxidants in vegetable crops such as amaranth, tomato, cabbage, eggplant, cauliflower and okra (Dey et al. 2014; Singh et al. 2009; Xie et al. 2018; Yadav et al. 2013). Specifically, the level of heterosis observed in the present study was higher than that reported for minerals in non-heading Chinese cabbage (Xie et al. 2018) and *Brassica oleracea* var. *capitata* (Singh et al. 2009). The result was comparable to the level of heterosis for vitamins and antioxidant pigments in cauliflower (Dey et al. 2014) and cabbage

(Parkash et al. 2017) and some bioactive properties in interspecific crosses between cultivated and wild relatives of eggplant (Kaushik et al. 2017). The wide range of heterosis could be explained by the previous observations on the reproductive biology of the species, revealing that the species in predominately outcrossing (Zohoungbogbo et al. 2018b; Raju and Rani 2016; Omondi et al. 2017a).

Both negative and positive mid- and best parent heterosis was observed in the species for all mineral content. This might be because several mechanisms are underlying heterosis expression in *Gynandropsis gynandra*. Three main models have been widely used to explain the heterosis in crops and include dominance, overdominance and epistasis (Fujimoto et al. 2018; Hochholdinger and Baldauf 2018; Liu et al. 2020; Bar-Zvi et al. 2017). Moreover, hybrids exhibiting a high level of heterosis are a combination of parents with either both good, both poor, average x good, good x poor, average x average or average x poor general combining abilities. The results showed that all three models or their combination could explain heterosis in *G. gynandra,* as most research has highlighted that a single model rarely occurs in plants (Fujimoto et al. 2018; Zhou et al. 2012). The present observation of the existence of heterosis in *G. gynandra* adds to previous reports (Sogbohossou et al. 2018; Sohindji et al. 2020) that the species could be used as a model for heterosis studies. A good exploitation of heterosis in *G. gynandra* requires the identification of heterotic patterns and groups in the species. To this end, the observed genetic differentiation between accessions based on geographical origin (Blalogoe et al. 2020; Sogbohossou et al. 2019) is key, and further investigation to assess the cross-compatibility between them and even within each region to avoid possible incompatibility between accessions is important. Additionally, the identification of common testers will help to speed up the exploitation of heterosis in the species.

5.4.4 Predicting hybrid performance

Both additive and nonadditive gene action are controlling minerals with a predominance of nonadditive genes in *Gynandropsis gynandra*. More importantly, we observed a positive association between F_1 performance and SCA, the sum of both parents GCA effects and heterosis, showing that the prediction of F_1 performance in *G. gynandra* should be based on models involving both GCA and SCA. More importantly, the correlations between F_1 performance and SCA and heterosis were strong, indicating that non-additive effects are the major contributor to heterosis in species for minerals content, confirming the observed gene action controlling minerals content. This is in agreement with the previous reports on iron and

zinc content in rice (Anusha et al. 2021) and agronomic traits in maize (Yu et al. 2020). There was no or weak correlation between GCA and the line per se performance, indicating that the performance of the given line could not predict its ability to transfer desirable traits in resultant crosses. The absence or weak association between the heterosis and the GCA of parental lines showed that the use of parental line performance could not be effective in determining the heterosis level of the crosses. A similar observation was reported by Yu et al. (2020) in maize for agronomic traits. In contrast, we observed a positive and significant association between the sum of GCA of parental line with hybrid performance, showing that in absence of SCA, the sum of GCA could be used as a predictor for the hybrid performance only. This was also reported in CMS rice (Gramaje et al. 2020), in maize (Yu et al. 2020). In contrast, models based on dominance were found to better predict heterosis in cucumber compared with models based on general combining ability. In our case as the correlation between SCA and hybrid performance was higher than that of the sum of GCA and Hybrid performance, models based on SCA could be more effective in hybrid performance prediction. To this end, a similar approach implemented by Liu et al. (2022) in cucumber using SNP-BLUP and GBLUP models based on dominance and SCA components could be implemented in heterosis prediction and hybrid performance. Additionally, genomic selection methods such as the multi-traits random forest (RF), the multi-traits genomic best linear unbiased predictor (GBLUP), and the multi-traits partial least squares (PLS) could be investigated. This opens the room for investigating genomic selection methods and machine learning techniques to predict hybrid performance and heterosis in the species.

5.5 Conclusion

The present study has generated critical and novel genetic knowledge on the gene action governing the inheritance of mineral content in *G. gynandra*. We observed significant variation among parents and hybrids for minerals. Genetic variance components analysis revealed significant effects of both general and specific combining ability, indicating the action of both additive and nonadditive genes with the predominance of nonadditive gene action in the inheritance of minerals in the species. The degrees of dominance observed were dominance and overdominance depending on the traits and populations. Our results also revealed the presence of both negative and positive mid- and best-parent heterosis for leaf mineral content in the species. High broad-sense and low to moderate narrow-sense heritability estimates were observed for all minerals. Significant genetic gain was observed for all mineral contents at a

selection pressure of 5%. The best crosses resulted from different parental combinations, ranging from good to poor combiners, suggesting that selection should be based on both general and specific combining ability effects. Heterosis breeding and reciprocal recurrent selection would be ideal breeding strategies to develop mineral-dense cultivars for better nutrition. Several cultivars can be developed, including hybrids and open-pollinated and synthetic varieties. We therefore suggest the use of *G. gynandra* as a model crop for investigating the mechanism underlying heterosis in plants. Further research on heterotic groups and patterns as well as tester identification to fully exploit heterosis in the species is needed. Overall, parents with good combining ability (P14, P24, P04, P18, P31, P29, P21, P22, P27) and crosses expressing promising hybrid vigor (P04xP26, P18xP16, P20xP19, P18xP01, P25xP28) were identified and represent resources for breeding and research purposes.

References

APHA, AWWA, WEF (1999) 4500-P Phosporus. In: Clesceri LS, Greenberg AE, Eaton AD (eds) Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC, pp 4-146-144-162

Baker RJ (1978) Issues in diallel analysis. Crop Sci 18 (4):533-536. https://doi.org/10.2135/cropsci1978.0011183X001800040001x

Bar-Zvi D, Lupo O, Levy AA, Barkai N (2017) Hybrid vigor: The best of both parents, or a genomic clash? Curr Opin Syst Biol 6:22-27. https://doi.org/10.1016/j.coisb.2017.08.004

Bernal-Vasquez A-M, Utz H-F, Piepho H-P (2016) Outlier detection methods for generalized lattices: a case study on the transition from ANOVA to REML. Theor Appl Genet 129 (4):787-804. 10.1007/s00122-016-2666-6

Blalogoe JS, Odindo AO, Sogbohossou EOD, Sibiya J, Achigan-Dako EG (2020) Origin-dependence of variation in seed morphology, mineral composition and germination percentage in *Gynandropsis gynandra* (L.) Briq. accessions from Africa and Asia. BMC Plant Biol 20 (1):168. 10.1186/s12870-020-02364-w

Butler D, Cullis B, Gilmour A, Gogel B, Thompson R (2017) ASReml-R Reference Manual Version 4. . VSN International Ltd Hemel Hempstead, HP1 1ES. UK

Chataika BY, Akundabweni LS-M, Houdegbe AC, Achigan-Dako EG, Sibiya J, Masamba K (2021) Dietary phytochemical screening of spider plant (*Gynandropsis gynandra* (L.) Briq.) accessions from Africa and Asia to identify genotypes for use in nutraceutical breeding. Front Sustain Food Syst 5 (344). 10.3389/fsufs.2021.733909

Dabholkar A (1999) Elements Of Bio Metrical Genetics (revised And Enlarged Edition). Concept publishing company, New Delhi, India

Davis DR (2009) Declining fruit and vegetable nutrient composition: What is the evidence? HortScience 44 (1):15-19. https://doi.org/10.21273/HORTSCI.44.1.15

Dey SS, Singh N, Bhatia R, Parkash C, Chandel C (2014) Genetic combining ability and heterosis for important vitamins and antioxidant pigments in cauliflower (*Brassica oleracea* var. *botrytis* L.). Euphytica 195 (2):169-181. https://doi.org/10.1007/s10681-013-0981-4

Dhillon BS (1990) Estimation of Degree of Dominance and Gene Frequency in Random Mating Populations. Biom J 32 (4):503-512. https://doi.org/10.1002/bimj.4710320414

Fujimoto R, Uezono K, Ishikura S, Osabe K, Peacock WJ, Dennis ES (2018) Recent research on the mechanism of heterosis is important for crop and vegetable breeding systems. Breed Sci 68 (2):145-158. https://doi.org/10.1270/jsbbs.17155

Glew RS, Amoako-Atta B, Ankar-Brewoo G, Presley J, Chuang L-T, Millson M, Smith BR, Glew RH (2009) Non-cultivated plant foods in West Africa: Nutritional analysis of the leaves of three indigenous leafy vegetables in Ghana. Food 3 (1):39-42

Gowele VF, Kinabo J, Jumbe T, Kirschmann C, Frank J, Stuetz W (2019) Provitamin A carotenoids, tocopherols, ascorbic acid and minerals in indigenous leafy vegetables from Tanzania. Foods 8 (1):35. 10.3390/foods8010035

Hallauer AR, Carena MJ, Miranda Filho Jd (2010) Quantitative genetics in maize breeding, vol 6. Springer Science & Business Media, London

Harrell Jr FE, Dupont C (2021) Hmisc: Harrell Miscellaneous. R package version 4.6-0 edn.,

Hochholdinger F, Baldauf JA (2018) Heterosis in plants. Curr Biol 28 (18):R1089-R1092. https://doi.org/10.1016/j.cub.2018.06.041

Hunter A (1975) New techniques and equipment for routine soil/plant analytical procedures. In: Borremiza E, Alvarado A (eds) Soil management in Tropical America. N.C. State University, Raleigh, NC,

Isik F, Holland J, Maltecca C (2017) Genetic data analysis for plant and animal breeding, vol 400. Springer. https://doi.org/10.1007/978-3-319-55177-7

Jamnadass R, Mumm RH, Hale I, Hendre P, Muchugi A, Dawson IK, Powell W, Graudal L, Yana-Shapiro H, Simons AJ, Van Deynze A (2020) Enhancing African orphan crops with genomics. Nat Genet 52 (4):356-360. https://doi.org/10.1038/s41588-020-0601-x

Jiménez-Aguilar DM, Grusak MA (2015) Evaluation of minerals, phytochemical compounds and antioxidant activity of Mexican, Central American, and African green leafy vegetables. Plant Foods Hum Nutr 70 (4):357-364. 10.1007/s11130-015-0512-7

Jinazali H, Mtimuni B, Chilembwe E (2017) Nutrient composition of cats whiskers (*Cleome gynandra* L.) from different agro ecological zones in Malawi. Afr J Food Sci 11 (1):24-29. https://doi.org/10.5897/AJFS2016.1478

Jones J, J. B. (2001) Laboratory guide for conducting soil tests and plant analysis. CRC press, Boca Raton, Florida, USA

Kalra Y (1997) Handbook of Reference Methods for Plant Analysis. CRC Press, Boca Raton, Florida, USA

Kangai Munene A, Nzuve F, Ambuko J, Odeny D (2018) Heritability Analysis and Phenotypic Characterization of Spider Plant (*Cleome gynandra* L.) for Yield. Adv Agric 2018:11. 10.1155/2018/8568424

Karmakar P, Munshi AD, Behera TK, Kumar R, Sureja AK, Kaur C, Singh BK (2013) Quantification and Inheritance of Antioxidant Properties and Mineral Content in Ridge Gourd (*Luffa acutangula*). Agric Res 2 (3):222-228. https://doi.org/10.1007/s40003-013-0070-x

Kaushik P, Gramazio P, Vilanova S, Raigón MD, Prohens J, Plazas M (2017) Phenolics content, fruit flesh colour and browning in cultivated eggplant, wild relatives and interspecific hybrids and implications for fruit quality breeding. Food Res Int 102:392-401. https://doi.org/10.1016/j.foodres.2017.09.028

Kearsey MJ, Pooni HS (1996) The Genetical Analysis of Quantitative Traits. Chapman and Hall, London

Kiebre Z, Bationa Kando P, Sawadogo N, Kiebre M, Sawadogo B, Traore RE, Sawadogo M, Zongo J-D (2017) Évaluation de la diversité agromorphologique d'une collection de *Cleome gynandra* L. du Burkina Faso. J Appl Biosci 118:11768-11780

Kleinknecht K, Möhring J, Singh K, Zaidi P, Atlin G, Piepho H (2013) Comparison of the performance of best linear unbiased estimation and best linear unbiased prediction of genotype effects from zoned Indian maize data. Crop Sci 53 (4):1384-1391. 10.2135/cropsci2013.02.0073

Kwarteng AO, Abogoom J, Adu Amoah R, Nyadanu D, Nyam CK, Ghunney T, Awuah E, Ziyaaba JZ, Ogunsanya JO, Orhin EE, Asiedu DD (2018) Phenomic characterization of twenty-four accessions of spider plant (*Cleome gynandra* L.) the Upper East region of Ghana. Sci Hortic 235:124-131. 10.1016/j.scienta.2018.02.046

Liu J, Li M, Zhang Q, Wei X, Huang X (2020) Exploring the molecular basis of heterosis for plant breeding. J Integr Plant Biol 62:287-298. https://doi.org/10.1111/jipb.12804

Makokha G, Owuor OP, Ongeri DMK (2019) Assessment of Levels of Nutrients in Selected ALVs at Different Harvesting Stages and Locations of Production in Western Kenya. Int J Biochem Res Rev 25 (3):1-16. https://doi.org/10.9734/ijbcrr/2019/v25i330076

Mamboleo TF, Msuya JM, Mwanri AW (2018) Vitamin C, iron and zinc levels of selected African green leafy vegetables at different stages of maturity. Afr J Biotechnol 17 (17):567-573. https://doi.org/10.5897/AJB2017.16346

Marles RJ (2017) Mineral nutrient composition of vegetables, fruits and grains: The context of reports of apparent historical declines. J Food Compos Anal 56:93-103. https://doi.org/10.1016/j.jfca.2016.11.012

Molenaar H, Boehm R, Piepho H-P (2018) Phenotypic selection in ornamental breeding: it's better to have the BLUPs than to have the BLUEs. Front Plant Sci 9:1511. 10.3389/fpls.2018.01511

Moyo M, Amoo SO, Aremu A, Gruz J, Šubrtová M, Jarošová M, Tarkowski P, Dolezal K (2018) Determination of mineral constituents, phytochemicals and antioxidant qualities of *Cleome gynandra*, compared to *Brassica oleracea* and *Beta vulgaris*. Front Chem 5:128. 10.3389/fchem.2017.00128

Neugart S, Baldermann S, Ngwene B, Wesonga J, Schreiner M (2017) Indigenous leafy vegetables of Eastern Africa—A source of extraordinary secondary plant metabolites. Food Res Int 100:411-422. 10.1016/j.foodres.2017.02.014

Nyadanu D, Lowor ST (2015) Promoting competitiveness of neglected and underutilized crop species: comparative analysis of nutritional composition of indigenous and exotic leafy

and fruit vegetables in Ghana. Genet Resour Crop Evol 62 (1):131-140. https://doi.org/10.1007/s10722-014-0162-x

Omondi EO, Debener T, Linde M, Abukutsa-Onyango M, Dinssa FF, Winkelmann T (2017a) Mating biology, nuclear DNA content and genetic diversity in spider plant (*Cleome gynandra*) germplasm from various African countries. Plant Breed 136:578-589. 10.1111/pbr.12485

Omondi EO, Engels C, Nambafu G, Schreiner M, Neugart S, Abukutsa-Onyango M, Winkelmann T (2017b) Nutritional compound analysis and morphological characterization of spider plant (*Cleome gynandra*)-an African indigenous leafy vegetable. Food Res Int 100:284-295. 10.1016/j.foodres.2017.06.050

Orech FO, Christensen DL, Larsen T, Friis H, Aagaard-Hansen J, Estambale BA (2007) Mineral content of traditional leafy vegetables from western Kenya. Int J Food Sci Nutr 58 (8):595-602. https://doi.org/10.1080/09637480701350288

Parkash C, Kumar S, Thakur N, Kumar S, Dey SS, Bhatia R, Dhiman MR, Sharma VK, Kumar R (2017) Genetic analysis of important antioxidant compounds in cabbage (*Brassiaca oleracea* var. *capitata* L.). J Crop Improv 31 (3):418-437. https://doi.org/10.1080/15427528.2017.1310165

Piepho HP, Möhring J, Melchinger AE, Büchse A (2008) BLUP for phenotypic selection in plant breeding and variety testing. Euphytica 161 (1):209-228. 10.1007/s10681-007-9449-8

Priyadarshan PM (2019) PLANT BREEDING: Classical to Modern. Springer Nature, Singapore. https://doi.org/10.1007/978-981-13-7095-3

R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Raju AS, Rani DS (2016) Reproductive ecology of *Cleome gynandra* and *Cleome viscosa* (Capparaceae). Phytol Balcan 22 (1):15-28

Ram H, Dey SS, Gopala Krishnan S, Kar A, Bhardwaj R, Arun Kumar MB, Kalia P, Sureja AK (2018) Heterosis and Combining Ability for Mineral Nutrients in Snowball Cauliflower (*Brassica oleracea* var. *botrytis* L.) Using Ogura Cytoplasmic Male Sterile Lines. Proc Natl Acad Sci, India, Sect B Biol Sci 88 (4):1367-1376. https://doi.org/10.1007/s40011-017-0874-8

Reeves G, Singh P, Rossberg TA, Sogbohossou EOD, Schranz ME, Hibberd JM (2018) Natural variation within a species for traits underpinning C4 photosynthesis. Plant Physiol 177 (2):504. 10.1104/pp.18.00168

Revelle W (2019) psych: procedures for psychological, psychometric, and personality research. R package version 1.9.12 edn. Northwestern University, Evanston, Illinois

Schönfeldt HC, Pretorius B (2011) The nutrient content of five traditional South African dark green leafy vegetables - A preliminary study. J Food Compos Anal 24:1141-1146. 10.1016/j.jfca.2011.04.004

Self SG, Liang K-Y (1987) Asymptotic Properties of Maximum Likelihood Estimators and Likelihood Ratio Tests under Nonstandard Conditions. J Am Stat Assoc 82 (398):605-610. 10.1080/01621459.1987.10478472

Shukla S, Bhargava A, Chatterjee A, Srivastava J, Singh N, Singh SP (2006) Mineral profile and variability in vegetable amaranth (*Amaranthus tricolor*). Plant Foods Hum Nutr 61 (1):21-26. 10.1007/s11130-006-0004-x

Singh BK, Sharma SR, Singh B (2009) Heterosis for mineral elements in single cross-hybrids of cabbage (*Brassica oleracea* var. *capitata* L.). Sci Hortic 122 (1):32-36. https://doi.org/10.1016/j.scienta.2009.04.007

Singh BK, Sharma SR, Singh B (2012) Genetic combining ability for concentration of mineral elements in cabbage head (*Brassica oleracea* var. *capitata* L.). Euphytica 184 (2):265-273. https://doi.org/10.1007/s10681-011-0598-4

Singh RK, Chaudhary BD (1985) Biometrical methods in quantitative genetic analysis. Third edition edn. Kalyani Publishers, New Delhi, India

Singh S, Dey SS, Bhatia R, Kumar R, Sharma K, Behera TK (2019) Heterosis and combining ability in cytoplasmic male sterile and doubled haploid based *Brassica oleracea* progenies and prediction of heterosis using microsatellites. PLoS ONE 14 (8):e0210772. https://doi.org/10.1371/journal.pone.0210772

Sogbohossou DEO (2019) Orphan no more : Ethnobotany and genetic analysis of leaf yield and secondary metabolites content in *Gynandropsis gynandra* (Cleomaceae). PhD Thesis, Wageningen University, Wageningen

Sogbohossou EOD, Achigan-Dako EG, Maundu P, Solberg S, Deguenon EMS, Mumm RH, Hale I, Van Deynze A, Schranz ME (2018) A roadmap for breeding orphan leafy

vegetable species: a case study of *Gynandropsis gynandra* (Cleomaceae). Hortic Res 5 (1):2. 10.1038/s41438-017-0001-2

Sogbohossou EOD, Achigan-Dako EG, Mumm R, de Vos RCH, Schranz ME (2020) Natural variation in specialised metabolites production in the leafy vegetable spider plant (*Gynandropsis gynandra* L. (Briq.)) in Africa and Asia. Phytochemistry 178:112468. 10.1016/j.phytochem.2020.112468

Sogbohossou EOD, Kortekaas D, Achigan-Dako EG, Maundu P, Stoilova T, Van Deynze A, de Vos RCH, Schranz ME (2019) Association between vitamin content, plant morphology and geographical origin in a worldwide collection of the orphan crop *Gynandropsis gynandra* (Cleomaceae). Planta 250:933–947. 10.1007/s00425-019-03142-1

Sohindji FS, Sogbohossou DEO, Zohoungbogbo HPF, Houdegbe CA, Achigan-Dako EG (2020) Understanding Molecular Mechanisms of Seed Dormancy for Improved Germination in Traditional Leafy Vegetables: An Overview. Agronomy 10:57. https://doi.org/10.3390/agronomy10010057

Sprague GF, Tatum LA (1942) General vs. Specific Combining Ability in Single Crosses of Corn 1. Agron J 34 (10):923-932. https://doi.org/10.2134/agronj1942.00021962003400100008x

Visscher PM, Hill WG, Wray NR (2008) Heritability in the genomics era — concepts and misconceptions. Nat Rev Genet 9 (4):255-266. 10.1038/nrg2322

Wu T-h, Solberg SO, Yndgaard F, Chou Y-Y (2018) Morphological patterns in a world collection of *Cleome gynandra*. Genet Resour Crop Evol 65 (1):271-283. 10.1007/s10722-017-0529-x

Xie F, Zha J, Tang H, Xu Y, Liu X, Wan Z (2018) Combining ability and heterosis analysis for mineral elements by using cytoplasmic male-sterile systems in non-heading Chinese cabbage (*Brassica rapa*). Crop Pasture Sci 69 (3):296-302. https://doi.org/10.1071/CP17357

Xu Y, Liu X, Fu J, Wang H, Wang J, Huang C, Prasanna BM, Olsen MS, Wang G, Zhang A (2020) Enhancing Genetic Gain through Genomic Selection: From Livestock to Plants. Plant Commun 1 (1):100005. https://doi.org/10.1016/j.xplc.2019.100005

Yadav SK, Singh BK, Baranwal DK, Solankey SS (2013) Genetic study of heterosis for yield and quality components in tomato (*Solanum lycopersicum*). Afr J Agric Res 8 (44):5585-5591

Zhou G, Chen Y, Yao W, Zhang C, Xie W, Hua J, Xing Y, Xiao J, Zhang Q (2012) Genetic composition of yield heterosis in an elite rice hybrid. Proc Natl Acad Sci USA 109 (39):15847. https://doi.org/10.1073/pnas.1214141109

Zohoungbogbo HPF, Blalogoe JS, Sogbohossou EDO, Houdegbe CA, Achigan-Dako EG (2018a) Tips for successful crosses in Cleome. Laboratory of Genetics Horticulture and Seed Science, Cotonou, Benin. doi:10.13140/RG.2.2.11977.90723

Zohoungbogbo HPF, Houdegbe CA, Sogbohossou DEO, Tossou MG, Maundu P, Schranz EM, Van Deynze A, Zoundjihekpon J, Achigan-Dako EG (2018b) Andromonoecy in *Gynandropsis gynandra* (L.) Briq. (Cleomaceae) and effects on fruit and seed production. Genet Resour Crop Evol 65 (8):2231–2239. https://doi.org/10.1007/s10722-018-0687-5

Appendix 5.1. Specific combining ability (SCA) of crosses for nine minerals in two populations of *Gynandropsis gynandra*. All minerals (mg kg^{-1} dry weight). Values in bold and italic represent the top 10 and bottom 10 crosses, respectively.

Crosses	Population	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
P20xP21	Pop1	0 5	2 37	1 95	1 78	-0 75	-5 88	0 01	1 37*	**109.04*** **
P20xP01	Pop1	1 28	0 8	7 88	0 45	-0 36	22 95***	-0 04	0 48	**99.95*** **
P06xP03	Pop1	*-6.26***	-1 47	-40 71*	-1 38	-0 87	3 58	*-0.32****	*-3.27****	**91.52*** **
P20xP02	Pop1	4 14*	1 03	**76.74*** **	-1 65	1 82***	6 16	0 15	0 6	**82.27*** **
P20xP19	Pop2	**4.24*** **	1 02	**357.04*** **	**6.68*** **	**2.41*** **	0	0 04	1 04	**80.11*** **
P20xP19	Pop1	*-10.36****	-0 64	**94.19*** **	*-3.61****	*-2.45****	-17 50***	*-0.48****	-1 31	**66.63*** **
P20xP15	Pop1	3 63	-1 59	-7 98	-0 86	1 90***	18 81***	0 16	-0 67	**61.01*** **
P20xP26	Pop1	-4 11*	-1 01	-18 08	*-4.18****	-0 41	-9 80*	-0 17	-0 2	**47.78*** **
P25xP03	Pop1	1 57	**3.06***	13 43	-2 75**	0 11	*-20.51****	-0 18	**2.96*** **	**43.13*** **
P18xP01	Pop2	2 13*	1 06	-29 64	-1 55	**1.80*** **	**202.86*** **	-0 02	0 73	**42.56*** **
P25xP02	Pop2	-1 54	-0 23	25 19	-1 35	0 34	-63 36**	0 14	1 37*	**37.14*** **
P04xP26	Pop2	-0 12	-0 68	-0 98	0 16	0 14	-21 91	-0 24*	-0 57	**36.74*** **
P09xP07	Pop2	2 65**	0 46	18 44	-3 56*	-0 23	**160.79*** **	-0 30**	-0 13	**32.95*** **
P09xP21	Pop1	0 06	1 25	-2 29	1	-0 09	-5 83	0 07	**2.93*** **	**31.42****
P32xP13	Pop2	0 01	2 86***	-16 47	4 67**	-0 38	40 38	-0 03	1 24*	**30.72*** **
P14xP28	Pop2	-2 56*	-1 37	4 93	5 01**	0 5	-16 07	0 1	0 63	**30.57*** **
P04xP15	Pop2	1 27	1 83*	18 52	4 97**	**1.64*** **	-10 93	0 1	**1.47***	**29.70*** **
P18xP11	Pop2	-1 38	-1 07	-7 28	-2 97	-0 89**	-84 06***	-0 26**	*-1.72***	**27.47*** **
P05xP01	Pop2	2 91**	-0 62	-45 93*	-0 41	0 53	-26 65	-0 19	0 81	**27.26*** **
P12xP07	Pop2	*-4.58****	-0 95	-30 87	4 66**	-0 5	-83 93***	*-0.35****	0 04	23 52***
P10xP29	Pop2	-0 68	**3.32*** **	-6 34	**8.44*** **	0 34	**208.48*** **	0 21*	0 14	21 22***
P17xP21	Pop1	-1 1	0 7	-7 77	1 35	-0 34	-3 32	-0 02	1 1	**20.81***
P32xP08	Pop2	**4.18*** **	-0 27	-18 29	-4 37**	**1.35*** **	118 42***	0 06	**1.50***	20 59***
P04xP13	Pop1	-1 62	1 27	**103.94*** **	-1 6	-0 72	-12 55*	*-0.35****	0 81	19 98*
P10xP15	Pop2	-0 78	**3.51*** **	-7 1	-5 29**	-0 29	12 28	-0 19	0 67	18 90***
P20xP31	Pop2	0 46	-0 7	**137.54*** **	0 73	0 59*	**184.51*** **	-0 13	-0 39	18 46***
P25xP11	Pop1	2 43	2 59*	18 2	1 37	-0 15	9 44	0 13	**2.16*** **	18 42
P25xP28	Pop2	-0 31	**16.09*** **	56 32**	-3 35*	-0 37	-59 88**	-0 29**	-0 18	18 18***
P14xP26	Pop2	0 65	1 13	-2 13	-4 57**	-0 62*	-17 55	*-0.44****	**1.57***	18 11***
P04xP16	Pop1	-3 91	-0 52	-14 4	-1 98*	-1 23*	9 79*	-0 24*	-1 40*	17 61
P23xP02	Pop1	-5 23**	2 09	-25 83	**7.02*** **	-0 58	13 72**	-0 01	-0 61	16 97
P17xP07	Pop2	0 92	0 38	24 66	1 09	0 44	-25 59	**2.16*** **	-0 26	16 48***
P18xP16	Pop1	-2 44	**3.91****	**249.22*** **	**2.69****	0 05	-4 83	0 17	0 97	16 43
P32xP16	Pop2	-1 17	2 71***	4 6	-4 35**	-0 71*	-66 54**	-0 20*	0 22	15 66**
P32xP27	Pop2	-0 9	0 58	-17 16	-0 85	-0 13	*-116.46****	-0 03	-0 86	15 20**
P17xP22	Pop2	-0 08	0 61	2 23	4 39**	-0 57*	-31 78	*-0.31***	0 98	14 30**
P09xP26	Pop2	1 61	0 03	17 73	1 35	-0 32	-57 16**	-0 15	1 16	14 03**
P25xP08	Pop1	-4 56*	1 27	-27 35	-2 45*	-0 97	-15 58**	0 02	-0 38	14 02
P25xP27	Pop1	*-6.10***	0 55	7 5	-0 74	*-1.54***	4 63	-0 14	-0 04	13 46
P17xP11	Pop2	-2 68**	1 14	4 18	-2 66	0	-49 40*	-0 13	0 45	13 41**
P10xP21	Pop1	-1 42	*-3.09**	-29	1 25	-0 59	-17 13***	-0 15	-0 41	13
P06xP01	Pop1	0 89	1 69	-2 52	-0 99	-0 14	12 78**	-0 06	-0 49	12 81
P04xP21	Pop1	-4	1 9	50 88*	0 33	-0 19	**30.50*** **	**0.80*** **	-0 12	12 72
P04xP11	Pop2	0 2	2 36**	34 65	-4 39**	0 72*	108 96***	-0 04	0 54	11 93*
P05xP26	Pop2	-1 57	1 08	-10 01	1 59	-0 68*	70 37**	-0 27**	0 91	11 85*
P09xP02	Pop2	4 07***	0 4	19 23	0 94	0 80**	-15 2	-0 05	1 17	11 40*
P10xP19	Pop1	0 59	0 67	**91.12*** **	1 13	-0 52	-12 44*	0 26*	1 12	11 37
P05xP22	Pop2	-2 32*	-0 69	14 25	**7.40*** **	*-0.97****	91 59***	-0 05	**1.75****	11 18*
P23xP11	Pop2	-0 54	**4.27*** **	0 88	-1 1	-0 14	88 98***	0 07	0 17	10 87*
P12xP22	Pop2	2 22*	-1 14	31 54	-3 60*	0 34	-19	-0 17	1 18	10 80*
P05xP30	Pop2	3 64***	-0 23	10 16	3 30*	-0 01	17 39	-0 02	0 89	10 75*
P23xP28	Pop1	2 68	0 86	-13 71	1 91	-0 23	5 55	0 20*	1 83**	10 59

Crosses	Population	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
P25xP22	Pop1	2 93	-0 64	1 8	-2 33*	1 57**	15 84**	0 01	-1 11	10 5
P14xP13	Pop1	-2 14	0 15	-16 91	1 90*	-0 71	-10 00*	0 03	-0 56	10 14
P05xP27	Pop2	1 69	-1 14	-12 33	0 82	0 26	143 29***	-0 05	-0 99	9 96*
P05xP15	Pop2	*-3.33****	1 58*	12 56	*-7.63****	-0 87**	*-127.07****	*-0.38****	0 65	9 90*
P18xP07	Pop2	-1 99*	2 45**	17 82	1 73	*-1.33****	-55 31*	-0 13	**1.99****	9 86*
P04xP19	Pop2	-2 84**	-0 96	-29 69	*-7.60****	-0 70*	-71 31**	-0 25*	-0 3	9 73*
P12xP27	Pop1	5 11*	**3.52****	-0 38	**3.05****	1 00*	-8 67	0 29**	1 26	9 41
P10xP28	Pop1	-2 21	*-3.04**	28 58	-1 43	-0 38	-17 48***	-0 03	-1 16	9 32
P14xP11	Pop1	-5 12**	2 59*	16 91	-0 95	-0 64	-10 72*	-0 03	-0 63	9 27
P10xP16	Pop2	0 44	-0 01	8 29	3 26*	0 43	87 63***	-0 06	0 31	9 14
P23xP11	Pop1	4 60*	2 41*	44 72*	1	0 5	-6 07	0 12	**3.31*****	8 74
P12xP13	Pop2	-1 37	-0 24	31 02	0 46	0 07	-49 47*	**2.06*****	-0 07	8 57
P18xP26	Pop1	2 59	**5.99*****	-9 64	**2.95****	0 72	**45.65*****	-0 08	0 77	8 55
P09xP11	Pop2	-1 44	-0 47	-13 41	5 53***	-0 32	-13 41	0 06	0 1	8 31
P23xP08	Pop1	-2 33	0 39	-8 09	**3.62*****	-0 74	-15 73***	-0 20*	-1 47*	8 26
P05xP11	Pop1	-2 36	-2 12	*-62.79***	1 05	-0 85	*-20.32****	-0 08	-0 71	8 25
P09xP16	Pop1	-1 33	1 82	59 09**	**8.53*****	**2.36*****	6 42	**0.36*****	*-2.91****	7 68
P09xP01	Pop2	-0 61	-0 06	-16 8	-0 97	-0 51	-32 52	0 05	-0 25	7 49
P04xP03	Pop1	*-5.65***	-2 88*	-35 34	1 21	-1 25*	3 67	-0 06	1 1	7 48
P25xP15	Pop2	1 56	-1 02	-18 32	-0 65	0 63*	23 2	-0 14	-0 67	7 34
P09xP03	Pop2	2 29*	0 93	19 19	5 10**	**1.35*****	111 19***	0 06	0 39	7 19
P32xP31	Pop2	*-5.01****	-1 75*	**604.01*****	0 23	*-1.42****	-19 22	-0 22*	-0 71	7 08
P14xP21	Pop1	5 65**	0 82	-15 1	0 19	0 77	-5 41	0 03	-0 84	6 85
P24xP28	Pop1	-2 2	**10.88*****	38 52	1 24	-0 64	-10 07	*-0.59****	**2.19***	6 69
P10xP02	Pop2	-1 8	0 34	-16 63	-2 49	-0 22	57 16**	-0 04	0 2	6 64
P17xP26	Pop2	-0 18	-0 31	2 76	3 21*	-0 29	*-107.75****	-0 29**	**2.09*****	6 6
P06xP07	Pop1	3 16	0 22	-32 77	1 19	0 8	-6 14	0 01	1 66**	6 5
P10xP03	Pop2	*-5.44****	**3.57*****	-14 07	-4 40**	*-1.27****	9 87	-0 24*	1 18	6 38
P17xP03	Pop2	2 68**	-0 08	**138.03*****	6 25***	-0 1	17 08	-0 15	-0 29	6 36
P12xP08	Pop2	2 47*	-0 2	15 86	6 33***	1 04***	**149.66*****	-0 09	**1.41***	6 31
P17xP11	Pop1	-0 45	-2 95*	**198.24*****	0 74	-0 3	**25.10*****	0 13	1 15	6 2
P05xP07	Pop1	-2 23	-0 92	-37 9	-1 83	-1 05*	-7 78	-0 17	*-2.45****	6 04
P06xP02	Pop2	**4.67*****	2 62***	14 86	-4 24**	0 85**	102 16***	-0 23*	0 29	6 04
P18xP16	Pop2	-1 06	-0 18	-31 37	1 52	-0 18	-64 76**	0 11	-0 77	5 74
P25xP15	Pop1	-1 4	-1 48	*-50.83**	1 01	-0 43	4 02	0 09	0 91	5 7
P09xP08	Pop1	1 21	-2 35	20 65	0 06	0 47	-1 61	-0 19*	0 89	5 64
P18xP19	Pop1	6 21**	0 59	-25 72	0 88	0 82	23 29***	0 06	1 02	5 59
P17xP28	Pop2	2 99**	1 86*	16 05	-4 91**	0 33	-17 46	-0 31**	-0 73	5 58
P05xP11	Pop2	1 07	2 32**	5 93	-2 15	0 35	-46 95*	-0 13	0 25	5 57
P23xP03	Pop2	**5.19*****	-1 04	-49 45**	4 98**	0 74**	17 51	0 09	0 18	5 52
P18xP13	Pop2	-0 18	**2.95*****	**158.13*****	-2 31	0 91**	-33 44	0 30**	1 26*	5 51
P17xP16	Pop1	-4 65*	0 21	-11 09	0 11	*-1.56***	**28.00*****	-0 14	**1.99****	5 42
P25xP29	Pop2	-2 81**	-0 15	-18 74	-3 22	-0 70*	23 19	-0 1	0 06	5 33
P14xP08	Pop1	2 53	2 59*	-14 41	1 64	0 71	**36.53*****	0 26**	0 53	5 29
P18xP22	Pop2	-3 12**	2 07**	-20 03	-5 83***	-0 24	-66 61**	0 04	0 02	5 27
P10xP01	Pop2	-1 04	1 28	13 53	-0 63	-0 23	-81 44***	-0 22*	0 47	5 1
P25xP22	Pop2	3 68***	-2 31**	-33 7	**9.38*****	0 29	-40 21	0 16	1	4 96
P23xP16	Pop2	-1 25	2 75***	8 6	*-7.17****	-0 48	-8 15	*-0.43****	0 68	4 96
P06xP08	Pop1	-1 14	0 46	**96.52*****	2 05*	-0 14	1 44	0 19*	1 37*	4 89
P09xP30	Pop2	-0 87	1 44	-1 2	0 05	-0 45	47 04*	0 02	0 35	4 89
P06xP19	Pop2	1 74	0 1	-14 91	2 31	0 28	-24 14	0 35***	-0 39	4 65
P17xP13	Pop1	1 6	0 39	-31 87	-2 24*	0 11	0 23	-0 04	-0 84	4 62
P25xP27	Pop2	-0 97	-1 19	43 54*	-1 87	0 61*	41 39	-0 18	-0 49	4 44
P12xP11	Pop1	*-12.28****	**8.06*****	61 16**	**7.50*****	-0 83	-16 03**	0 24*	-0 27	4 32
P09xP19	Pop2	-3 19**	0 94	18 92	-5 40**	-0 79**	-6 79	*-0.34****	-0 87	3 82
P20xP22	Pop2	0 9	-0 37	4 89	-5 91***	-0 42	15 53	-0 03	-0 71	3 79

Crosses	Population	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
P23xP15	Pop1	-3 08	-1 75	41 34*	-1 33	-1 07*	11 74**	-0 12	0 64	3 7
P06xP22	Pop2	-0 85	0 6	-13 43	0 01	0 73*	119 50***	0 1	-0 37	3 68
P12xP01	Pop2	-0 9	1 77*	-25 83	-1 58	0 36	95 67***	-0 13	0 4	3 66
P23xP07	Pop2	0 21	-0 94	-13 88	-0 07	0 18	-53 40*	-0 02	-0 09	3 63
P14xP15	Pop2	1 42	-0 27	-7 09	-0 07	0 04	-46 58*	-0 12	-0 01	3 38
P06xP22	Pop1	-0 24	-2 44*	25 22	0 9	0 66	-12 26**	**0.36*** **	0 32	3 37
P25xP03	Pop2	-0 29	-0 71	-18 3	1 4	-0 41	-2 97	-0 08	0 84	3 3
P06xP01	Pop2	0 46	-0 26	-25 84	3 28*	-0 2	-14 35	-0 11	-0 21	3 29
P04xP16	Pop2	-2 49*	**3.07****	57 83**	*-8.38****	*-1.33****	-10 64	*-0.37****	-0 84	3 16
P32xP19	Pop2	-1 12	0 89	-27 04	-1 87	-0 33	-46 34*	-0 1	1 24*	3 08
P14xP30	Pop2	1 73	-1 49	-39 97*	-2 8	0 68*	**151.37*****	-0 27**	-0 22	3 06
P06xP16	Pop2	-0 48	1 87*	4 73	1 12	-0 47	-19 35	-0 11	-0 23	2 88
P06xP30	Pop2	-0 55	-0 89	0 08	-5 56***	-0 3	-18 51	-0 02	0 34	2 62
P12xP30	Pop2	2 62*	-1	-29 64	3 49*	-0 56	3 98	-0 02	-0 6	2 5
P09xP11	Pop1	*-6.00***	**7.53*****	**73.91*****	1 91*	-1 42**	*-22.27****	-0 15	-0 9	2 49
P14xP11	Pop2	-2 21*	**3.22*****	-2 87	-1 94	*-1.26****	41 85	0 26**	-0 44	2 49
P12xP15	Pop1	5 72**	1 09	4 61	1 63	1 47**	5 95	0 18	-0 3	2 43
P04xP28	Pop2	*-3.37****	-0 49	24 29	*-7.44****	-0 92**	75 38***	-0 13	0 23	2 28
P10xP16	Pop1	-0 08	1	18 62	-2 39*	0 55	-10 91*	-0 02	-0 22	2 22
P12xP07	Pop1	*-11.67****	-1 89	-15 89	1 6	*-1.62***	1 3	0 03	-0 38	1 95
P10xP01	Pop1	-5 10*	0 73	-3 43	0 09	-1 38**	*-19.65****	-0 06	1 50*	1 94
P06xP15	Pop2	-0 58	1 2	-7 53	3 66*	-0 44	125 29***	-0 07	0 38	1 91
P25xP31	Pop2	3 62***	-0 7	*-61.99****	-2 94	0 84**	-78 02***	-0 17	0 69	1 77
P25xP26	Pop2	-0 39	0 43	-8 83	-1 51	0 47	-13 11	0 07	0 72	1 75
P17xP13	Pop2	-1 14	0 91	-32 02	0 42	0 08	102 44***	-0 04	-0 8	1 72
P32xP26	Pop2	-1 99*	0 6	7 83	-2 87	0 02	-95 82***	**0.38*****	-0 49	1 72
P25xP02	Pop1	-0 84	-0 51	-12 61	1 99*	-0 79	9 83*	0 05	-0 25	1 64
P18xP28	Pop2	0 39	-1 56*	-30 93	**13.78*****	-0 16	-16 27	0 02	0 62	1 42
P17xP15	Pop2	-1 54	**3.32*****	-34 36	6 26***	0 02	73 32**	0 11	1 18	1 38
P17xP02	Pop1	-1 17	1 55	-5 95	-0 05	-0 06	-7 36	0 01	-0 19	1 26
P18xP13	Pop1	0 53	1 68	40 39	0 75	-0 06	**56.58*****	0 24*	0 95	1 15
P17xP19	Pop1	**10.11*****	1 15	*-53.11**	-2 63**	**2.68*****	-2 36	0 04	-0 16	1 02
P06xP08	Pop2	0 04	-0 94	-2 14	-6 59***	0 14	-39 46	0 06	-0 71	0 98
P17xP15	Pop1	2 4	0 89	-0 63	-1 85	-0 44	*-19.89****	-0 17	-0 64	0 79
P04xP07	Pop2	2 04*	**4.69*****	-29 47	0 69	0 97***	22 78	0 02	0 16	0 69
P14xP31	Pop2	**6.76*****	-2 39**	*-67.34****	-1 93	0 71*	-79 47***	**0.38*****	-1 07	0 66
P05xP27	Pop1	-4 13*	0 28	-29 61	-2 83**	*-1.60***	-11 25*	-0 17	-1 05	0 56
P06xP13	Pop1	3 26	-0 94	-23 51	*-4.48****	2 07***	-7 55	-0 08	-1	0 55
P23xP01	Pop2	2 03*	-1 82*	-15 06	3 20*	0 79**	-10 23	0 01	-0 79	0 42
P05xP16	Pop1	**8.95*****	0 91	20 02	-1 1	0 6	-15 20**	0 13	**3.10*****	0 4
P04xP07	Pop1	0 63	0 29	25 41	-0 28	-0 34	-8 82	-0 03	0 2	0 34
P05xP03	Pop1	2 15	0 24	-4 15	0 57	0 01	**26.26*****	0 20*	**2.29*****	0 28
P20xP03	Pop2	1 61	1 11	-22 14	-1 11	0 59*	65 96**	0 07	0 69	0 22
P17xP07	Pop1	0 77	-1 46	-35 78	0 22	0 33	14 55**	-0 17	0 09	0 21
P14xP01	Pop1	4 16*	*-4.25****	-21 72	-2 17*	1 21*	-7 48	-0 05	*-1.69***	0 13
P20xP30	Pop2	-1 45	-1 53*	*-57.37***	-4 39**	-0 33	-35 42	-0 22*	-0 94	0 11
P14xP08	Pop2	-1 38	1 61*	-19 58	4 21*	-0 59*	121 42***	-0 15	-0 12	-0 03
P32xP15	Pop2	1 24	0 71	**240.61*****	2 27	0 07	-62 60**	-0 18	-0 12	-0 28
P12xP02	Pop2	2 32*	-0 63	-7 04	2 3	1 11***	-66 89**	0 06	-0 77	-0 31
P23xP28	Pop2	-3 19**	-1 08	-14 91	-2 95	-0 29	-80 69***	-0 1	0 82	-0 58
P23xP08	Pop2	1 76	2 51**	0 45	-0 14	-0 90**	*-158.25****	-0 16	**1.92****	-0 71
P14xP16	Pop2	-1 64	0 38	-19 27	0 68	-0 01	-57 61**	0 20*	0 91	-0 76
P05xP22	Pop1	6 60**	-0 38	-25 98	*-3.34****	1 02*	13 82**	0 24*	-1 62*	-0 8
P25xP11	Pop2	1 42	-0 18	-42 54*	2 83	-0 11	-29 95	0	-0 19	-0 87
P17xP01	Pop2	-1 05	2 05**	4 79	-0 22	0 12	*-110.84****	0 02	0 5	-0 95
P14xP07	Pop1	-2 97	**2.93***	-6 19	-0 27	0 29	-0 91	0 01	-0 08	-1

Crosses	Population	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
P17xP27	Pop2	3 15**	0 25	-4 08	-1 17	0 18	-52 35*	-0 15	0 99	-1 04
P06xP28	Pop1	2 59	-0 16	**100.32*** **	-1 52	0 72	5 7	0 18	1 36*	-1 06
P06xP29	Pop2	**4.75*** **	-1 85*	-21 19	-1	-0 07	-43 56	0 11	-1 16	-1 07
P14xP27	Pop2	-0 49	-1 56*	-1 72	3 69*	0 59*	-51 37*	-0 05	0 5	-1 1
P23xP27	Pop1	3 12	-0 51	-20 09	-2 59**	0 2	11 14*	-0 05	0 16	-1 21
P06xP13	Pop2	-0 21	0 27	-11 71	-0 44	-0 84**	15 18	*-0.38****	0 75	-1 22
P20xP29	Pop2	-1 58	-2 26**	-32 66	2 73	*-0.98****	-81 58***	0 31**	*-2.11****	-1 25
P12xP13	Pop1	2 28	-1 49	-12 81	0 52	0 68	9 29	-0 09	-0 83	-1 35
P10xP07	Pop2	0 95	0 09	-10 83	-3 38*	0 77**	38 48	-0 17	-0 67	-1 4
P05xP29	Pop2	1 23	0 3	12 19	2 14	-0 17	-74 47**	0 21*	0 76	-1 53
P05xP13	Pop1	-5 27**	-2 49	*-50.22**	1 98*	-1 40**	-9 71*	0	0 62	-1 63
P12xP26	Pop2	1 11	0 88	-15 85	-1 95	1 23***	62 40**	0 14	0 07	-1 64
P06xP07	Pop2	-1 64	-0 9	-4 73	-3 39*	-0 42	-40 91	-0 18	-1 16	-1 72
P23xP26	Pop1	-1 15	-1 05	27 84	-3 31***	-0 78	-8 23	-0 16	0 16	-1 81
P04xP02	Pop2	-1 97*	0 22	7 23	4 00*	-0 86**	-4 83	-0 11	0 69	-1 9
P09xP13	Pop2	**6.20*** **	0 17	-32 16	-2 46	**1.80*** **	76 50***	-0 01	0 2	-1 95
P23xP13	Pop2	-1 88	0 78	-15 4	0 29	-0 45	92 51***	-0 26**	0 44	-1 97
P12xP03	Pop2	0 01	*-3.11****	*-49.81***	-6 46***	-0 61*	-4 37	0 03	*-1.65***	-2 01
P20xP07	Pop2	-0 54	0 39	-3 77	-4 17*	0 01	27 6	-0 16	0 24	-2 02
P23xP02	Pop2	-1 13	1 60*	42 80*	-2 7	-0 57*	23 7	0 12	0 14	-2 04
P25xP16	Pop2	1 19	*-2.95****	-29 05	**6.54*** **	0 60*	-53 32*	0 34***	0 65	-2 1
P10xP22	Pop2	0 07	0 06	-21 96	0 95	0 34	2 83	-0 05	0 28	-2 13
P20xP16	Pop2	1 8	1 46	-21 07	2 64	0 35	-28 34	-0 05	0 47	-2 22
P10xP22	Pop1	2 78	0	-33 63	-2 28*	2 02***	-18 17***	0 07	0 12	-2 25
P20xP28	Pop2	1 43	-0 72	-6 61	-4 14*	-0 28	-27 24	-0 07	0 76	-2 26
P23xP27	Pop2	-2 90**	1 16	-1 33	-3 06	-0 26	-46 27*	-0 13	-0 66	-2 34
P04xP11	Pop1	0 56	1 24	-15 69	*-3.80****	0 02	-9 92*	*-0.46****	-0 9	-2 59
P05xP19	Pop1	*-6.14***	0 11	43 97*	-1 36	-0 47	13 56**	*-0.37****	-0 95	-2 6
P10xP11	Pop2	2 27*	-1 49*	-23 49	**9.53*** **	0 62*	-38 38	0 13	0 11	-2 62
P09xP22	Pop1	-0 84	*-4.44****	-1 91	0 13	*-1.93****	-13 83**	*-0.26***	0 61	-2 66
P04xP08	Pop2	-0 5	-0 73	-23 4	2 2	-0 23	27 21	-0 12	-0 73	-2 81
P14xP19	Pop2	0 59	1 43	-29 88	-1 55	0 32	8 4	-0 13	0 2	-2 83
P17xP08	Pop1	-3 98	0 37	-22 66	-2 16*	-0 59	*-21.96****	0 07	-1 12	-3 21
P06xP03	Pop2	0 16	-0 74	-3 01	5 29**	0 28	0 58	0 01	0 53	-3 25
P17xP27	Pop1	*-5.42***	-1 15	-25 84	-0 21	-1 12*	*-21.50****	0 17	-0 97	-3 38
P20xP26	Pop2	-0 89	-1 24	-22 65	5 63***	-0 2	117 51***	-0 05	0 13	-3 6
P04xP26	Pop1	6 57**	1 14	-49 10*	**8.96*** **	0 99	**56.07*** **	0 26*	0 18	-3 65
P12xP19	Pop2	-0 26	-0 81	-24 12	6 07***	0 23	-31 25	-0 12	0 56	-3 85
P32xP30	Pop2	-3 02**	0 05	4 6	0 94	-0 06	72 51**	0 34***	0 51	-3 89
P10xP15	Pop1	-3 54	-2 4	-38 61	2 19*	-1 30**	-3 3	-0 04	-0 01	-3 95
P20xP27	Pop2	1 93	-0 3	-16 73	-3 16	0 33	41 25	-0 20*	-0 6	-4 16
P17xP31	Pop2	-2 62**	-0 49	*-55.62***	1 64	-0 58*	33 54	-0 15	0 39	-4 18
P23xP01	Pop1	0	0 41	-26 13	-0 36	0 28	4 11	**0.36*** **	-0 12	-4 21
P32xP07	Pop2	-1 09	0 17	6 06	1 33	0 11	-18 32	-0 16	0 56	-4 22
P14xP26	Pop1	-3 94	-1 42	-3 2	0 49	-0 15	-1 7	-0 18	-0 24	-4 38
P12xP16	Pop1	0 94	-1	-25 55	2 17*	-0 5	-15 04**	-0 09	0 65	-4 46
P14xP03	Pop2	0 91	-1 89*	**113.40*** **	*-9.45****	-0 07	-101 45***	-0 1	-0 89	-4 63
P09xP15	Pop2	0 95	2 84***	-7 73	2 82	0 55	95 53***	0 13	-0 17	-4 65
P06xP11	Pop2	0 74	-0 37	-13 82	3 80*	0 02	0 82	0 01	-0 11	-4 67
P06xP16	Pop1	5 23**	-0 95	*-50.57**	*-6.13****	1 77***	*-19.82****	*-0.26***	*-1.95***	-4 72
P18xP15	Pop2	-0 91	-2 60***	*-71.79****	-0 29	-0 84**	-105 78***	-0 13	*-1.90***	-4 89
P14xP03	Pop1	**7.24*** **	0 19	0	0 36	0 58	10 31*	0 07	**3.29*** **	-4 92
P10xP26	Pop2	-2 09*	-0 58	-24 13	0 65	-0 55	-100 70***	-0 09	-0 83	-4 94
P23xP13	Pop1	0 66	1 53	-16 48	-1 33	-0 33	-10 03*	0 26**	0 06	-4 95
P12xP29	Pop2	1 75	-1 53*	-17	-3 87*	-0 11	-2 37	0	-0 35	-4 97
P05xP28	Pop2	-0 57	0 27	8 49	0 24	0 49	-12 19	0 26**	-0 19	-5 01

Crosses	Population	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
P09xP16	Pop2	-0 6	1 77*	18 37	2 45	-0 07	60 54**	0 06	0 98	-5 09
P32xP02	Pop2	**5.45*** **	-0 34	*-57.11***	-1 38	**1.29*** **	91 32***	0 18	-1 09	-5 15
P10xP08	Pop2	-1 7	1 56*	-17 5	-1 85	-0 53	-42 83	-0 05	-0 72	-5 18
P04xP27	Pop2	0 08	2 10**	17 2	0 94	-0 82**	6 26	-0 12	-0 07	-5 57
P09xP01	Pop1	-4 35*	-2 18	-34 72	1 27	-0 86	5 92	**0.30****	**3.08*****	-5 75
P05xP01	Pop1	5 86**	0 43	41 47*	1 28	**2.24*****	14 80**	0 05	1 2	-5 8
P23xP29	Pop2	0 29	0 17	-6 61	2 86	-0 51	-53 73*	0 04	0 75	-5 81
P10xP13	Pop1	-2 32	0 17	-10 92	1 24	-0 99*	1 92	0 02	1 85**	-5 84
P05xP13	Pop2	1 82	0 01	-14 7	1 57	-0 22	-96 66***	-0 29**	-1 03	-5 87
P06xP26	Pop2	*-3.43****	-1 65*	11 93	1 02	-0 07	-1 79	0 05	0 21	-6
P17xP30	Pop2	0 08	1 44	13 19	0	-0 55	*-111.01****	*-0.43****	-0 41	-6
P23xP07	Pop1	1 43	-1 11	28 63	-1 21	-0 06	-1 24	-0 12	-0 28	-6 17
P20xP08	Pop2	-1 07	-0 43	-4 69	-0 92	-0 2	-63 53**	0 01	-0 01	-6 32
P23xP30	Pop2	-0 52	-1 51*	0 4	-0 93	0 17	-26 74	-0 09	-0 16	-6 34
P18xP27	Pop1	-0 06	-0 18	-21 62	*-3.94****	-0 29	-13 79**	-0 15	-0 81	-6 42
P23xP16	Pop1	-2 13	-0 17	-21 59	-3 20***	-0 77	-11 47*	0 01	0 08	-6 49
P25xP08	Pop2	*-4.54****	-0 1	16 58	-3 01	-0 82**	-25 96	0 14	-0 43	-6 54
P09xP08	Pop2	1 1	-0 19	-14 85	3 90*	0 3	23 38	0 23*	-0 36	-6 64
P10xP30	Pop2	-0 88	-0 41	55 28**	-3 31*	0 48	-54 60*	0 33**	0 1	-6 7
P05xP07	Pop2	2 37*	-1 27	-30 18	**7.03*****	0 34	110 10***	0 15	-1 21*	-6 71
P09xP22	Pop2	-0 39	*-3.35****	-46 67*	-6 56***	0 08	-37 58	0 03	*-1.44**	-6 71
P18xP31	Pop2	-0 7	2 44**	-7 05	-0 51	0 34	82 28***	0 36***	**1.95****	-6 72
P20xP13	Pop2	-2 02*	-0 81	-21 25	-1 95	-0 19	-54 57*	-0 11	0 52	-6 73
P32xP01	Pop2	0 21	-0 81	-41 21*	-4 52**	-0 68*	23 9	0	-0 41	-6 76
P23xP19	Pop2	1 71	0 24	-21 64	-1 05	0 03	-42	0 09	-0 3	-7 18
P04xP13	Pop2	-2 30*	0 7	-6 14	*-10.90****	-0 71*	-62 25**	-0 16	-0 33	-7 19
P14xP22	Pop2	1 44	-2 39**	-45 85*	5 80***	**2.05*****	127 05***	**0.39*****	-0 5	-7 29
P12xP02	Pop1	**7.50*****	1 45	33 98	1 69	0 59	5 77	-0 15	0 43	-7 39
P10xP28	Pop2	1 78	-2 34**	-23 34	1 49	0 24	-77 54***	0 27**	-0 01	-7 44
P25xP13	Pop1	5 11*	-2 12	-43 93*	-1 1	1 19*	-3 79	0 14	-0 52	-7 5
P09xP27	Pop1	**10.43*****	-0 13	-16 97	-1 86	**3.55*****	-7 72	-0 14	0 91	-7 51
P12xP28	Pop2	-2 21*	-0 73	-40 19*	0 7	-0 88**	-65 49**	-0 02	-0 69	-7 56
P18xP28	Pop1	-2 75	-2 23	*-58.88***	2 08*	-0 24	-19 39***	0 02	-0 01	-7 7
P14xP07	Pop2	1 69	-0 96	-26 41	-2 48	0	-38 61	*-0.33****	-0 09	-7 78
P12xP22	Pop1	3 19	-1 08	25 29	-0 62	0 05	-2 14	0 06	0 36	-7 83
P05xP21	Pop1	-3 24	2 65*	-7 94	0 39	-0 47	0 62	0 04	-0 26	-8 01
P05xP02	Pop2	-1 22	0 96	-5 56	2 15	-0 37	-13 42	0 03	1 28*	-8 12
P25xP30	Pop2	1 98	-0 39	3 41	0 25	1 11***	22 92	-0 08	-0 66	-8 42
P25xP26	Pop1	3 01	-1 17	-14 45	-1 58	0 83	-0 36	-0 05	-1 17	-8 43
P18xP11	Pop1	-1 06	-1 02	-28 61	-1 28	0 38	**37.16*****	-0 11	*-1.62**	-8 44
P09xP13	Pop1	-4 73*	-2 81*	-0 57	0 47	-0 79	17 35***	-0 17	-0 53	-8 63
P12xP27	Pop2	0 67	0 55	28 43	-2	-0 01	-103 98***	-0 1	**1.59***	-8 74
P10xP07	Pop1	**9.21*****	1 54	-41 93*	0 05	**2.21*****	**49.59*****	-0 05	0 9	-8 76
P23xP22	Pop2	-1 12	-1 28	55 30**	-1 51	0 26	33 59	0 12	-0 19	-8 85
P20xP01	Pop2	-1 32	-2 37**	-18 64	3 47*	*-0.93***	-12 37	-0 13	-0 84	-8 89
P20xP02	Pop2	-1 41	0 99	-12 35	-2 36	0 03	56 75**	0 07	-0 39	-8 92
P14xP22	Pop1	6 23**	**5.57*****	-6 05	2 08*	0 98*	-17 10***	0 15	0 44	-8 93
P14xP27	Pop1	**8.70*****	-2 94*	-6 63	-1 16	**2.93*****	-16 08***	0 09	0 22	-9 23
P23xP31	Pop2	-0 95	0 82	-43 59*	1 73	-0 49	4 54	0 15	-0 62	-9 5
P20xP15	Pop2	-1 36	0 52	9 27	-0 2	-0 78**	-35 37	-0 03	0 41	-9 53*
P06xP21	Pop1	0 01	0 79	-5 23	-0 28	-0 24	9 25*	0 17	0 86	-9 65
P04xP22	Pop2	2 52*	-1 81*	-23 06	-3 72*	-0 1	-10 82	-0 09	*-1.63***	-9 68*
P32xP11	Pop2	-0 92	2 25**	7 64	-4 31**	-0 11	79 64***	0 07	-0 31	-9 71*
P05xP08	Pop2	-0 19	-1	19 13	-1 87	0 62*	0 56	-0 05	-0 21	-9 73*
P18xP02	Pop2	0 85	0 25	16 23	4 09*	-0 35	-64 54**	-0 14	0 72	-10 00*
P17xP08	Pop2	-0 21	0 57	-0 66	-4 20*	-0 29	*-113.59****	0 06	-0 56	-10 10*

Crosses	Population	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
P14xP29	Pop2	**4.98*** **	*-2.94***	1 08	-1 09	**2.11*** **	103 76***	0 23*	-1 02	-10 12*
P25xP19	Pop2	-1 27	0 29	-2 13	-1 49	-0 03	121 44***	0 26**	0 5	-10 13*
P24xP19	Pop1	2 04	*-3.58**	-19 67	1 96	0 24	-1 47	**0.58*** **	-0 24	-10 16
P14xP13	Pop2	-0 73	-0 18	-29 71	2 91	-0 08	-13 4	0 11	0 06	-10 24*
P05xP16	Pop2	-2 95**	0 85	-30 56	*-6.67***	-0 82**	69 61**	-0 13	-0 51	-10 29*
P23xP21	Pop1	-0 99	0 71	-5 98	-1 11	-0 24	-6 51	-0 22*	-0 88	-10 38
P09xP02	Pop1	-1 94	-2 55*	-13 66	-1 68	-0 75	24 53***	-0 19*	-1 50*	-10 55
P04xP03	Pop2	-2 92**	1 62*	2 84	2 92	-0 15	-57 69**	-0 1	0 96	-10 95*
P04xP19	Pop1	-0 36	-1 4	2 84	0 93	-0 36	-2	0 30**	0 79	-11 22
P12xP08	Pop1	**8.73*** **	*-4.55***	-18 68	*-5.71***	**2.13*** **	-1 78	-0 06	-1 19	-11 24
P09xP15	Pop1	**8.59*** **	*-3.28***	-18 79	-2 27*	**2.38*** **	12 60**	**0.32*** **	-0 12	-11 33
P32xP03	Pop2	-2 39*	-0 54	-41 84*	0 39	0 33	2 23	-0 13	0 11	-11 47*
P10xP27	Pop2	2 39*	-0 92	-19 3	0 27	**1.63*** **	**180.55*** **	0 26*	0 29	-11 49*
P06xP28	Pop2	0 73	-1 97*	-33 48	5 82***	0 54	-55 22*	0 05	0 6	-11 67*
P25xP13	Pop2	-0 54	-2 41**	**141.01*** **	2 15	-0 66*	30 07	-0 03	*-1.40**	-11 73*
P32xP28	Pop2	1 52	-0 83	*-65.96***	3 32*	0 12	*-130.79***	-0 27**	1 33*	-11 85*
P04xP29	Pop2	0 96	-0 61	10 36	**7.99*** **	0 21	86 52***	0 23*	1 14	-12 11*
P20xP11	Pop2	1 31	-2 01**	-13 18	3 53*	-0 1	-74 99***	-0 18	0 84	-12 12*
P25xP01	Pop2	-1 05	1 36	27 83	1 26	-0 37	-67 59**	-0 07	0 19	-12 16*
P06xP31	Pop2	-0 78	-1 60*	-29 83	-3 07	0 14	**160.11*** **	-0 13	-0 42	-12 27*
P12xP15	Pop2	-2 64**	-0 23	**59.34** **	*-7.04***	-0 62*	125 46***	-0 23*	-0 6	-12 35*
P06xP02	Pop1	-1 83	2 46*	6 25	2 24*	-0 39	-4 62	0 19*	1 54*	-12 55
P14xP01	Pop2	*-3.85***	1 29	22 19	2 13	-0 89**	-18 72	**1.48*** **	-0 52	-12 56**
P05xP03	Pop2	-0 27	-2 10**	-49 50**	-0 72	-0 57*	24 01	0 15	*-2.19***	-12 61**
P10xP13	Pop2	1 45	*-2.88***	52 99**	2 59	-0 22	-56 34**	-0 13	-0 75	-12 66**
P18xP03	Pop2	1 11	-1 71*	-5 17	-2 43	0 49	-101 62***	-0 17	-0 58	-12 66*
P04xP15	Pop1	-1 81	-0 7	41 75*	-0 66	-0 62	2 02	*-0.37***	0 51	-12 73
P10xP19	Pop2	0 57	-2 32**	-46 72*	1 61	-0 34	-92 87***	-0 15	-0 55	-12 89**
P14xP15	Pop1	*-11.17***	-2 19	13 74	2 27*	*-2.14***	-10 62*	-0 25**	-1 18	-13 06
P23xP22	Pop1	-2 72	*-2.99**	-27 67	*-3.67***	*-1.61***	-8 18	-0 21*	-1 58*	-13 69
P05xP19	Pop2	0 3	*-2.85***	-49 78**	3 31*	-0 08	15 06	0 04	-1 27*	-13 70**
P05xP31	Pop2	-0 58	2 21**	**76.24*** **	*-7.47***	-0 33	**169.28*** **	-0 1	-1 07	-13 79**
P09xP31	Pop2	-2 52*	*-3.80***	-29 82	4 37**	-0 17	14 05	-0 14	-0 23	-14 82**
P14xP02	Pop2	*-3.32***	-1 54*	-46 75*	3 07	0 12	*-117.08***	0 14	-0 79	-14 93**
P18xP03	Pop1	-0 17	*-3.40***	*-50.83**	-1 35	0 21	*-29.29***	0 1	-1 25	-15 54
P23xP03	Pop1	3 93*	-1 83	-8 72	-0 52	1 13*	15 46***	-0 06	-1 47*	-15 7
P18xP01	Pop1	-0 61	-0 69	-8 67	1 89	-0 45	*-25.33***	-0 12	-0 39	-15 91
P12xP16	Pop2	4 10***	-0 6	-17 73	**6.72*** **	1 28***	-10 56	0 19	-0 6	-16 02**
P17xP16	Pop2	0 22	0 13	-10 3	-5 23**	0 05	-81 59***	-0 22*	-0 64	-16 18***
P17xP29	Pop2	-0 62	-1 78*	7 68	-1 54	0 75*	-36 59	**0.51*** **	-0 6	-16 32***
P10xP31	Pop2	1 46	-1 98**	-24 22	-3 31*	0 1	*-117.90***	-0 16	-0 62	-16 41***
P18xP19	Pop2	-0 94	0 57	20 05	1 71	-0 54	110 32***	0 24*	0 88	*-16.76***
P24xP26	Pop1	-1 86	-2 34	**71.47** **	-2 09	-0 42	0 55	**0.46*** **	0 57	-17 21
P09xP29	Pop2	*-3.24***	-1 65*	-37 18*	-0 52	-0 07	-51 80*	0 01	-0 24	*-17.38***
P09xP03	Pop1	5 55**	**6.40*** **	-14 76	0 6	0	7 87	0 05	-0 98	-17 85
P25xP07	Pop2	-2 47*	-1 94*	-21 71	-3 41*	*-0.97***	-25 18	-0 16	-1 04	*-19.01***
P10xP03	Pop1	-1 04	2 38	-18 38	-1 87	0 55	20 57***	-0 14	*-2.95***	-19 13
P04xP01	Pop2	2 15*	-1 42	1 04	4 36**	0 97***	43 63*	**0.56*** **	0 54	*-19.40***
P17xP02	Pop2	0 81	*-3.35***	-49 67**	2 54	-0 73*	-46 71*	-0 24*	-0 91	*-19.77***
P09xP27	Pop2	-0 6	-1 09	-4 49	0 25	0 11	53 21*	0 03	-0 48	*-20.11***
P14xP02	Pop1	-1 43	-0 88	0 82	-2 66**	-0 46	4 85	-0 15	-1 57*	-21 04*
P17xP19	Pop2	-1 22	-0 61	*-71.55***	-5 09**	*-1.19***	2 36	0 01	-1 33*	*-21.23***
P18xP26	Pop2	0 09	1 22	-26 35	0 4	0 18	**205.93*** **	**0.47*** **	-0 32	*-21.38***
P06xP11	Pop1	-4 74*	-0 37	-5 8	0 89	*-1.59***	-13 88**	-0 19*	*-2.72***	-22 19*
P12xP01	Pop1	-4 21*	-1 68	*-62.05***	1 88	-1 28*	-10 93*	*-0.27***	*-1.80***	-22 30*
P06xP15	Pop1	2 25	-0 19	-1 09	0 37	0 92	-4 22	-0 03	0 67	-22 62*

Crosses	Population	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
P12xP11	Pop2	-1 31	*-4.02***	-31 39	-0 35	-0 71*	5 48	**0.44*** **	*-1.58**	*-24.35****
P06xP26	Pop1	-4 94*	-2 4	-14 2	0 57	*-1.51***	5 03	-0 22*	0 79	*-27.50***
P23xP26	Pop2	**4.33*** **	*-5.79****	*-52.38***	5 42***	0 13	-69 37**	0 04	*-2.59****	*-27.87****
P04xP02	Pop1	0 93	*-3.41***	*-73.13****	*-6.75****	0 56	-14 14**	**0.43*** **	-0 18	*-34.84****
P20xP07	Pop1	-3 27	-0 84	*-57.62***	1 64	-0 84	6 92	-0 04	-0 5	*-35.28****
P20xP16	Pop1	-3 71	0 17	0 2	-1 67	-1 28**	-11 35*	-0 11	1 19	*-48.03****
P25xP28	Pop1	-4 25	-2 58*	20 15	2 35*	-0 41	-9 02	-0 15	*-2.20***	*-48.70****
P20xP08	Pop1	-2 11	0 51	13 33	**10.14*** **	-1 07*	-18 78***	0 05	-0 11	*-60.64****
P20xP13	Pop1	1 32	-1 91	-46 49*	1 03	0 52	-14 28**	-0 03	-0 75	*-61.81****
P20xP22	Pop1	4 76*	-0 52	1 56	-3 10**	**2.27*** **	4 37	0 02	-0 65	*-62.61****
P20xP27	Pop1	-4 37*	-0 09	-11 22	**3.31*** **	-1 43**	5 29	-0 18*	-1 60*	*-67.45****
P20xP11	Pop1	**10.93*** **	-0 68	-33 4	0 84	**2.89*** **	9 27*	**0.49*** **	1 26*	*-70.16****

CHAPTER 6

Genetic analysis of biomass and related traits provides insights into hybrid breeding in the leafy vegetable *Gynandropsis gynandra* (L.) Briq

Abstract

Knowledge of quantitative genetic parameters associated with the inheritance of target traits is important for selecting appropriate breeding methods for cultivar development, but is still limited for biomass and related traits in *Gynandropsis gynandra*. Therefore, 331 F_1 hybrids generated from a North Carolina mating design II and their 39 parental lines were evaluated for gene action, combining ability effects and heterosis of biomass yield and related traits. The evaluation was done across seven environments between 2019 and 2021 using an alpha lattice design with two replicates per environment. Significant differences ($P < 0.001$) were observed among and between hybrids and parents for all agronomic traits. Overall, hybrids performed better than their parents for stem diameter (21.12%), plant height (34.45%), primary branch length (37.63%), leaf traits (17.50-50.91%), total (80.58%) and edible (54.70%) fresh biomass. Significant general and specific combining ability (GCA and SCA) effects together with variance component analysis revealed that both additive and nonadditive gene action controlled biomass and related traits in the species with the predominance of additive gene action. The environment significantly interacted with genotype, GCA and SCA. Mid- and best-parent heterosis varied between -51.89% and 192.10%. Specifically, only positive heterosis effects were observed for leaf area and total fresh biomass, with an average mid-parent heterosis greater than 50%. Parents with good GCA and crosses with high SCA and heterosis were identified. There were similar patterns of positive associations among plant height, stem diameter, leaf traits and plant biomass in both parents and hybrids. In contrast, there were significant changes from parents to hybrids in the association of harvest index and time to 50% flowering with biomass per plant and leaf traits on the one hand and between harvest index and dry matter content on the other hand. The study thus revealed that reciprocal recurrent selection would be a sound breeding strategy for *G. gynandra* improvement with the development of hybrid cultivars to exploit heterosis. These findings contribute to the potential of using *G. gynandra* to decipher the genetic mechanism underlying heterosis in orphan leafy vegetables.

Keywords: *Cleome gynandra*, combining ability, genetic gain, heterosis, variance components, heritability, leaf yield, breeding.

6.1 Introduction

Feeding the ever-growing population remains the main challenge of the world and country's agriculture agenda. Plant breeding has significantly contributed to this objective (Qaim 2020; Ortiz 2015; Bradshaw 2017; Woeste et al. 2010; Wallace et al. 2018; Altman et al. 2021) but still reported that world food production should be doubled by 2050 (Parry and Hawkesford 2010; Alexandratos and Bruinsma 2012; McKenzie and Williams 2015; Tomlinson 2013) as total food demand will increase by 56% (van Dijk et al. 2021). Achieving this objective is hampered by the changing climate associated with increasing undernourished people, poverty, and extreme disasters, including drought, heat, and floods (Myers et al. 2017; Lesk et al. 2016; Wiebe et al. 2015). These disasters were projected to significantly affect the major crops, such as wheat (Raimondo et al. 2021; Pequeno et al. 2021), maize (Hristov et al. 2020; Bassu et al. 2021; Tesfaye et al. 2015) and rice (Hussain et al. 2020; Van Oort and Zwart 2018), the primary source of human foods with yield and production reduction (Challinor et al. 2014; Wiebe et al. 2015; Zhao et al. 2017). The changing paradigms and adoption of new and sustainable approaches are thus required (Anderson et al. 2020; Myers et al. 2017; McKenzie and Williams 2015). Consequently, there is an increasing call for crop diversification to mitigate the plausible failure of the main crops in the future climate (Li and Siddique 2020; Lin 2011; Bioversity International 2017; FAO 2019; Ulian et al. 2020).

One option to improve agrobiodiversity includes bringing into large-scale cultivation old crops, referred to as orphan crops or neglected or underutilized crops, that local communities have conserved and have thrived over the harsh local conditions (Dawson et al. 2018; Jamnadass et al. 2020; Tian et al. 2021). Plant breeding and genomics will play a critical role in speeding up this process by extrapolating the gained knowledge from well-studied and known crops to orphan crops (Jamnadass et al. 2020; Dawson et al. 2019; Kamenya et al. 2021; Chiurugwi et al. 2019). Orphan crops have not only the potential to adapt to harsh climate conditions but are also an important source of nutrients and incomes, indispensable to combat food insecurity, malnutrition and hidden hunger (Padulosi et al. 2021; Mabhaudhi et al. 2019).

Orphan crops encompass diverse groups of plants, including African leafy vegetables (AVLs), which are rich in minerals and vitamins and are mostly deficient in staple crops (Yang and Keding 2009; Padulosi et al. 2021). In addition, ALVs contain several healthy compounds or phytonutrients, such as phenolics, carotenoids, flavonoids and glucosinolates, that are essential for human well-being (Neugart et al. 2017; Moyo et al. 2021), which supports their large use

by local communities in preventing and curing several diseases. Increasing the consumption of AVLs is thus crucial and will contribute to combating malnutrition and hidden hunger in the world, especially in sub-Saharan Africa, where approximately 264.2 million people are undernourished in 2020 (FAO et al. 2021). A prerequisite to achieve this is to assure the all-around year production and consumption of AVLs. Providing farmers with improved varieties and best agricultural practices is key in a context where few sustainable orphan leafy vegetable crop breeding programs are established.

Gynandropsis gynandra (L.) Briq. syn *Cleome gynandra* L., known as spider plant, is mostly used by local communities in Africa and Asia but is also found in Southern America and Oceania (Sogbohossou 2019; Feodorova et al. 2010; Vandebroek and Voeks 2018). The leaves of *G. gynandra* are a rich source of vitamins A, E and C, iron, zinc, calcium, potassium, magnesium and manganese (Moyo and Aremu 2021; Sogbohossou et al. 2019; Omondi et al. 2017b). The species is also an extraordinary source of secondary metabolites and antioxidants (Neugart et al. 2017; Sogbohossou et al. 2020; Moyo and Aremu 2021; Chataika et al. 2021). This potential of the species is endorsed with a huge within-species diversity offering a strong basis for a breeding program (Sogbohossou et al. 2019; Omondi et al. 2017b; Wu et al. 2018). The observed variation in the species is strongly correlated with the geographical origin of the plant material (Reeves et al. 2018; Blalogoe et al. 2020; Sogbohossou et al. 2020; Sogbohossou et al. 2019; Chataika et al. 2021), and the exploitation of this variability will be fundamental for the successful breeding of the species.

The reproductive biology of any species is an important driver in defining the appropriate breeding strategies. *G. gynandra* is both self- and cross-compatible but predominantly out-crossing (Raju and Rani 2016; Zohoungbogbo et al. 2018b; Omondi et al. 2017a), opening the door for developing inbred and hybrid cultivars. As out-crossing is predominant in the species (Zohoungbogbo et al. 2018b), heterosis might occur as reported for several out-crossing species and yet to be investigated. Developing hybrid cultivars might be a good approach in species breeding because hybrid cultivars offer the possibility to exploit heterosis, which is the target of several breeding programs for cross-pollinated as well as self-pollinated crops for increased genetic gain (Gupta et al. 2019; Cowling et al. 2020; Labroo et al. 2021; Longin et al. 2013). This will help increase the productivity of the species and ensure the availability of the vegetable for large-scale consumption.

However, the production of the species is still hindered by its early flowering, erratic germination and low leaf yield (Onyango et al. 2013; Sogbohossou et al. 2018) due to the lack of improved cultivars. Information on the inheritance, combining ability and heterosis for agronomic traits is crucial to designing breeding schemes, selecting superior parents and heterotic crosses, and deciding on the type of cultivars to be developed. Unfortunately, little is known about the combining ability, heterosis level and gene action controlling biomass and its related traits in *G. gynandra*. To this end, different mating designs, including diallel, North Carolina Design II and line by tester designs, are used.

The objective of the present study was to investigate the genetics of the inheritance of biomass and related traits in *G. gynandra* to inform breeding programs on the type of cultivars to be developed. Specifically, we aimed to (i) evaluate the agronomic performance of experimental F_1 *G. gynandra* hybrids and their parental lines, (ii) investigate the correlations among biomass and related traits, (iii) estimate variance components and heritability of biomass and related traits in *G. gynandra*, (iv) determine the combining ability effects for biomass and related traits of selected parental lines of *G. gynandra* and (v) identify the type of gene action and the level of heterosis for biomass and related traits in *G. gynandra*.

6.2 Materials and methods

6.2.1 Germplasm and crossing blocks

Thirty-nine lines derived from accessions originating from various countries of Asia and Africa (Table 6.1) were used in this study. The parental lines were separated into two groups, 16 and 23 lines used as females and males, respectively, and crossed in a North Carolina design II during two cropping seasons: 2018/2019 (from October 2018 to February 2019) and 2019/2020 (from October 2019 to March 2020) in a greenhouse at the Controlled Environment Facility (29°46′ S, 30°58′ E) of the University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa. During the first crossing season, only 28 parental lines (13 females and 15 males) were used to generate the first set of hybrids (Set 1), comprising 135 F_1 families (Appendix), whereas 37 parental lines (15 females and 22 females) were used to generate the second set of hybrids (Set 2) of 319 F_1 families (Appendix 6.1) during the second crossing season. The 37 parental lines used in the second crossing season included 26 parental lines used in the first crossing season. Across the two crossing seasons, a total of 331 unique single crosses were generated

for evaluation. In addition, each parental accession was self-pollinated during each crossing season. Crosses were performed according to Zohoungbogbo et al. (2018a).

Table 6.1. List of advanced lines of *G. gynandra* used as parents to generate the hybrids used in this study and their origin.

Lines	Genebank of the original accession	Country of origin	Continent	Parent	Generation of selfing in 2019	Generation of selfing in 2020
F1	KENRIK	Kenya	Africa	Female	S3	S4
F10	WorldVeg	Thailand	Asia	Female	S3	S4
F11	WorldVeg	Laos	Asia	Female	S3	S4
F12	WorldVeg	Malaysia	Asia	Female	S3	S4
F13	WorldVeg	Malaysia	Asia	Female	S3	S4
F14	WorldVeg	Malaysia	Asia	Female	S3	S4
F15	WorldVeg	Malawi	Africa	Female	S3	S4
F16	WorldVeg	South Africa	Africa	Female	-	S4
F2	GBioS	Benin	Africa	Female	S3	S4
F3	GBioS	Benin	Africa	Female	S3	S4
F4	GBioS	Benin	Africa	Female	-	S4
F5	GBioS	Benin	Africa	Female	S3	S4
F6	GBioS	Togo	Africa	Female	-	S4
F7	WorldVeg	Rwanda	Africa	Female	S3	-
F8	WorldVeg	Thailand	Asia	Female	S3	S4
F9	WorldVeg	Laos	Asia	Female	S3	S4
M1	KENRIK	Kenya	Africa	Male	S3	S4
M10	WorldVeg	Kenya	Africa	Male	S3	S4
M11	WorldVeg	Zambia	Africa	Male	S3	S4
M12	WorldVeg	South Africa	Africa	Male	S3	-
M13	WorldVeg	Malaysia	Asia	Male	-	S4
M14	WorldVeg	Laos	Asia	Male	S3	S4
M15	WorldVeg	Uganda	Africa	Male	S3	S4
M16	WorldVeg	Uganda	Africa	Male	S3	S4
M17	WorldVeg	Uganda	Africa	Male	-	S4
M18	WorldVeg	Uganda	Africa	Male	S3	S4
M19	WorldVeg	Malawi	Africa	Male	-	S4
M2	KENRIK	Kenya	Africa	Male	S3	S4
M20	WorldVeg	Kenya	Africa	Male	-	S4
M21	WorldVeg	Kenya	Africa	Male	S3	S4
M22	WorldVeg	Zambia	Africa	Male	S3	S4
M23	WorldVeg	Malaysia	Asia	Male	-	S4
M3	KENRIK	Kenya	Africa	Male	S3	S4
M4	GBioS	Benin	Africa	Male	-	S4
M5	GBioS	Togo	Africa	Male	-	S4
M6	GBioS	Togo	Africa	Male	S3	S4
M7	GBioS	Togo	Africa	Male	S3	S4

Lines	Genebank of the original accession	Country of origin	Continent	Parent	Generation of selfing in 2019	Generation of selfing in 2020
M8	GBioS	Ghana	Africa	Male	-	S4
M9	WorldVeg	Laos	Asia	Male	S3	S4

GBioS: Laboratory of Genetics, Horticulture and Seed Science, WorldVeg: World Vegetable Center, KENRIK: Kenya Resource Center for Indigenous Knowledge

6.2.2 Planting sites, experimental design and crop management

Evaluation of Set 1

Set 1 of 135 F_1 progenies along with their 28 parents were subjected to evaluation in three contrasting environments, including two field experiments and one greenhouse experiment in 2019. The first field experiment was established at the Ukulinga Research Farm (29°40′ S, 30°24′ E) of the University of KwaZulu-Natal in South Africa from March to June 2019, while the second one was implemented in the Abomey-Calavi's experimental site (6°25′ N, 2°20′ E) of the Laboratory of Genetics, Horticulture and Seed Science (GBioS) of the Faculty of Agronomic Sciences (FSA), University of Abomey-Calavi (UAC) in Republic of Benin (West Africa) from May to August 2019. The greenhouse experiment was carried out from March to June 2019 under the Controlled Environment Facility (29°46′ S, 30°58′ E) of the University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa.

In each environment, the 135 hybrids were planted in a 15 x 9 alpha design with two replications, whereas the 28 parents were laid out in a 7 x 4 alpha design with two replications in blocks adjacent to the hybrid trial. At Abomey-Calavi and Ukulinga, the plot size was 0.8 m² (1 m x 0.8 m) and separated by 0.5 m, while the incomplete blocks were 1 m apart. In each plot, the spacing was 0.2 m between and within rows (0.2 m x 0.2 m), leading to four rows of five plants, for a total of 20 plants. In the greenhouse, the plot was a single row of 1 m of five plants, resulting from the inter- and intra-row spacing of 20 cm.

Abomey-Calavi's experimental site belongs to the Guinean area characterized by bimodal rainfall. The soil was classified as ferralitic soil with sandy loam texture and a pH (KCl) of 5.92. The clay, organic carbon and nitrogen (N) contents of the soil were 8.61%, 0.56% and 0.08%, respectively (Table 6.2). During the experiment, the average monthly temperature was 27.5°C, the average monthly relative humidity was 86.2%, the total rainfall was 476.4 mm and the total evaporation was 428.58 mm (Table 6.3). The soil was ploughed at a depth of 30 cm,

and beds were raised. Top dressing fertilizers composed of poultry manure (containing 2.66% N, 1.1% P and 2.06% K) at a dose of 20 t ha^{-1} and urea (46% N) at a dose of 100 kg/ha were applied one and two weeks after transplanting, respectively. Water was applied twice a day using a water can of 11 L. The organic pesticide biomass, an *Azadirachta indica* A. Juss-based product, was used to control the pests, mainly caterpillars. Manual weeding was performed to keep the plots clean.

Table 6.2. Physico-chemical properties of the soil and growing media used in Abomey-Calavi, Ukulinga and Greenhouse in 2019, 2020 and 2021.

Characteristics	Environments						
	AB2019	UK2019	GH2019	UK2020	GH2020	AB2021	AL2021
Organic carbon (%)	0.56	2.30	2.45	3.6	30.78	0.5	1.1
Nitrogen (%)	0.08	0.18	0.19	0.21	1.10	0.05	0.13
Clay (%)	8.61	39.00	38.50	45.00	-	17.00	14.00
pH (KCl)	5.92	4.32	5.59	4.00	6.0	5.39	5.79
Phophorus (mg/kg)	304.50	21.74	76.10	18.89	3405.85	29.00	5.00
Potassium (mg/kg)	37.145	227.05	130.24	155.56	2450.92	93.00	42.00
Calcium (mg/kg)	665.00	1552.66	2513.17	484.44	13507.64	440.00	651.00
Magnesium (mg/kg)	77.47	607.73	375.12	132.22	3286.30	107.00	275.00

UK2019: Ukulinga 2019, AB2019: Abomey-Calavi 2019, GH2019: Greenhouse 2019, UK2020: Ukulinga 2020, GH2020: Greenhouse 2020, AB2021: Abomey-Calavi 2021 and AL2021: Allada 2021.

The Ukulinga Research Farm belongs to the Coast Hinterland Thornveld (Camp 1999). The soil was the westleigh soil form, plinthic paleustalf (Soil Classification Working Group 1991), with a predominantly clay loam texture (Moodley et al. 2004). The soil contained 39% clay, 2.3% organic carbon and 0.18% nitrogen (N) with a pH (KCl) of 4.32 (Table 6.2). Basal

fertilizer composed of N:P:K (2:3:4) at 150 kg ha^{-1} was applied before transplanting, and top dressing of limestone ammonium nitrate (LAN) (28% N) was applied two weeks after transplanting at 100 kg ha^{-1}. Manual weeding was performed to keep the plots clean. The trial was irrigated using a sprinkler irrigation system twice a day for an average of 30 minutes each. The average monthly temperature was 18.4°C, the average monthly relative humidity was 70.75%, the total rainfall was 196 mm and the total evaporation was 307 mm (Table 6.3).

The greenhouse experiment was established on raised beds measuring 1 m wide and 1 m high. The soil had 38.5% clay and a pH (KCl) of 5.58. The soil contained 0.19% nitrogen and 2.45% organic carbon (Table 6.2). Automated drip irrigation was used while weeds were controlled manually. Basal fertilizer composed of N:P:K (2:3:2) at 150 kg ha^{-1} was applied before transplanting, and top dressing LAN (28% N) was applied two weeks after transplanting at 100 kg ha^{-1}. The average temperature was 28°C day/18°C night, and the average relative humidity was 78.5% during the experiment.

Evaluation of Set 2

Set 2 of 319 F_1 progenies along with their 37 parents was evaluated in four contrasting environments, including two fields, one pot field and one greenhouse pot experiment, from September 2020 to September 2021. The first field experiment was established at the Ukulinga Research Farm, University of KwaZulu-Natal in South Africa from October 2020 to January 2021. The second field experiment was implemented from June to September 2021 at Allada's multiplication site (6°36′6″ N, 2°30′6″ E) of the Seed Services Cooperative of the Laboratory of Genetics, Horticulture and Seed Science (GBioS) in the Republic of Benin. The pot-field experiment was established at Abomey-Calavi's experimental site of the Laboratory of Genetics, Horticulture and Seed Science (GBioS) in the Republic of Benin from May to August 2021. The greenhouse experiment was carried out from September 2020 to December 2020 under the Controlled Environment Facility, University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa.

In each environment, 310 hybrids were planted in a 31 x 10 alpha design with two replications, whereas the 37 parents were laid out in a 10 x 4 alpha design with two replications in blocks adjacent to the hybrid trial. At the Allada and Ukulinga sites, the plot size was 0.6 m^2 (1 m x 0.6 m) and separated by 0.5 m, while the incomplete blocks were 1 m apart. In each plot, the spacing was 0.2 m between and within rows (0.2 m x 0.2 m), leading to three rows of five plants, for a total of 15 plants. In the greenhouse, 10 litre pots filled with composted pine bark

growing media were used. The physico-chemical characteristics of the growing media are presented in Table 6.2.

Similar to Abomey-Calavi's experimental site, Allada's site belongs to the Guinean area characterized by bimodal rainfall. The soil was classified as ferralitic soil with sandy loam texture and a pH (KCl) of 5.79. The clay, organic carbon and nitrogen (N) contents of the soil were 14%, 1.1% and 0.13%, respectively (Table 6.2). During the experiment, the average monthly temperature was 25.7°C, the average monthly relative humidity was 90.0%, and the total rainfall was 455.36 mm (Table 6.3). The soil was manually ploughed at a depth of 30 cm and flattened. Top dressing fertilizer of urea (46% N) at 100 kg/ha was applied two weeks after transplanting. Water was applied twice a day using a water can of 11 L. Pesticides, including alpha-cyhalothrin and acetamiprid, were used to control the pests, mainly caterpillars and aphids. Manual weeding was performed to keep the plots clean.

Table 6.3. Weather conditions during the field experiments at Abomey-Calavi, Ukulinga, Allada in 2019, 2020 and 2021.

Locations	Months and years	Total rainfall (mm)	Total evaporation (mm)	Average solar radiation (MJ m^{-2} day^{-1})	Average temperature (°C)	Average relative humidity (%)
UKulinga						
	March 2019	77.26	100.60	16.22	21.62	78.33
	April 2019	100.33	67.33	11.71	18.75	79.30
	May 2019	17.50	74.17	12.55	18.58	66.67
	June 2019	0.51	65.10	11.28	16.22	53.85
Abomey-Calavi						
	April 2019	84.80	126.19	17.93	29.31	84.36
	May 2019	149.30	113.84	15.91	28.31	84.18
	June 2019	191.60	87.22	14.76	26.68	87.7
	July 2019	50.70	101.33	NA	25.62	88.5
UKulinga						
	October 2020	42.22	96.76	15.05	19.16	72.35
	November 2020	92.95	113.97	19.34	19.73	79.76
	December 2020	87.62	125.13	20.01	21.68	79.27
	January 2021	139.17	118.99	19.06	21.78	82.05
Abomey-Calavi						
	May 2021	145.03	137.98	21.88	26.75	83.24
	June 2021	124.3	121.58	18.40	26.97	84.00
	July 2021	85.90	117.93	18.05	27.00	83.34
	August 2021	125.1	107.57	16.61	26.58	85.47
Allada						
	June 2021	196.25	NA	12.72	26.19	88.71
	July 2021	98.644	NA	12.44	25.50	89.42
	August 2021	17.94	NA	10.49	25.60	90.08
	September 2021	142.526	NA	10.63	25.53	91.96

Source: IITA-Cotonou, Ukulinga wheather Station UKZN-PMB, NA: Not available.

The same Ukulinga Research Farm used in 2019 was used in 2020 but in different fields. The soil contained 45% clay, 3.6% organic carbon and 0.21% nitrogen (N) with a pH (KCl) of 4.0 (Supplement File 1). Basal fertilizer composed of N:P:K (2:3:4) at 150 kg ha^{-1} was applied before transplanting, and top dressing of calcium ammonium nitrate (27% N) was applied two weeks after transplanting at 100 kg ha^{-1}. Manual weeding was performed to keep the plots clean. The trial was irrigated using a sprinkler irrigation system twice a day for an average of 30 minutes each. The average monthly temperature was 20.58°C, the average monthly relative humidity was 78.35%, the total rainfall was 361.96 mm and the total evaporation was 454.85 mm (Table 6.3).

For the greenhouse experiment, fertilization and weed control were the same as those described for set 1. During the experiment, the average temperature and relative humidity were 28 °C day/20 °C night and 78.5%, respectively.

Across the two hybrid set evaluations, a total of seven environments were obtained and included Ukulinga 2019 (UK2019), Abomey-Calavi (AB2019), Greenhouse 2019 (GH2019), Ukulinga 2020 (UK2020), Greenhouse 2020 (GH2020), Abomey-Calavi 2021 (AB2021) and Allada 2021 (AL2021). For all experiments, seeds were preheated at 40°C for 3 days in an oven before planting in seedling trays filled with compost and/or sand. Seedlings were grown and transplanted four weeks after sowing. Seedlings trays were established in a glasshouse at the University of KwaZulu-Natal and under a shelter at the University of Abomey-Calavi.

6.2.3 Trait measurements

Fourteen agronomic traits, including time to 50% flowering (DFlow), stem diameter (StDiam), plant height (PHeight), number of primary branches (NPBr), primary branch length (PBrLeng), central leaflet length (CtlLeng), central leaflet width (CtlWid), leaf width (LWid), petiole length (PtilLeng), leaf area (LfArea), total fresh biomass per plant (FBiom), edible fresh biomass per plant (EDBiom), harvest index (HI) and dry matter content (DMC) were assessed four weeks after transplanting. The measurements were taken on five randomly selected plants in each plot for field experiments, all five plants per plot for the 2019 greenhouse experiment, and two plants per pot for pot experiments. The time to 50% flowering were recorded as the number of days from the sowing date to the day when 50% of the plants in each plot/pot flowered. The central leaflet length (cm), central leaf width (cm), leaf width (cm) and petiole

length (cm) were collected on a fully developed primary leaf randomly selected on each plant using a ruler. The selected leaf was scanned, and the resultant image was used to calculate leaf area using the R package "*LeafArea*" (Katabuchi 2015). Plant height (cm) was measured from the base to the top of the plant with a tape measure, while the stem diameter was measured using a digital Vernier calliper at the plant collar. Each plant was harvested by cutting at a height of 15 cm above the ground, and the resultant biomass was weighed to determine the total fresh biomass per plant (g plant^{-1}). The edible parts of the total biomass were separated and weighed to record the edible fresh biomass per plant. The ratio of edible biomass to total fresh biomass was computed and reported as the harvest index (HI). These measurements were taken on two plants out of the three plants per pot and on three to five plant per field plot. For dry matter content (DM), edible biomass of the plants per genotype in each replicate was bulked, and a sample of 20 g was taken and oven-dried at 65 °C for 72 h. DM (%) was computed as DM = (dry weight)/(fresh weight) x 100.

Statistical analysis

The software R version 4.1.1 (R Core Team 2021) was used to perform all statistical analyses. A single-stage analysis procedure was used to analyse the data as described by Damesa et al. (2017). This approach is efficient when error, block, and replicate variances are environment-specific (So and Edwards 2011). The quality of the data was assessed for outlier detection following Bernal-Vasquez et al. (2016) using the Bonferroni–Holm test based on studentized residuals at the significance level of 5%. The difference among hybrids was tested using Student's t test or the Wilcoxon signed-rank test, when necessary. Variance components across years were estimated by fitting a linear mixed-effect model using the restricted maximum likelihood (REML) implemented in the ASReml-R package version 4.1.0.160 (Butler et al. 2017) according to the following statistical model:

$$y_{ijkl} = \mu + E_j + R_k(E_j) + B_l[R_k(E_j)] + G_i + GE_{ij} + \varepsilon_{ijkl} \quad (1)$$

where y_{ijkl} is the phenotypic observation of the i^{th} genotype (parent or hybrid) in the l^{th} incomplete block within the k^{th} replicate at the j^{th} environment, μ is the overall mean, E_j is the random effect of the j^{th} environment, $R_k(E_j)$ is the random effect of the k^{th} replicate within the j^{th} environment, $B_l[R_k(E_j)]$ is the random effect of the l^{th} incomplete block within the k^{th} replicate at the j^{th} environment, G_i is the random effect of the i^{th} genotype (parent or hybrid), GE_{ij} is the random effect of the interaction between the i^{th} genotype (parent or hybrid) and the

j^{th} environment, and ε_{ijkl} is the random residual. Heterogeneous variances were assumed for residual, block and replicate effects in different environments. The likelihood ratio test (Self and Liang 1987) was used to test the significance of the variance components using the function *lrt.asreml* implemented in the ASREML-R package. In addition, the phenotypic best linear unbiased predictors (BLUPs) were estimated for parents and hybrids separately from model 1.

Furthermore, the genetic effect of hybrids was partitioned into the general combining ability effect of females (GCA_f), the general combining ability effect of males (GCA_m) and the specific combining ability effect of the cross between females and males (SCA_{fm}). Hence, using the hybrid data only, model 1 was rewritten as follows:

$$y_{fmjkl} = \mu + E_j + R_k(E_j) + B_l[R_k(E_j)] + GCA_f + GCA_m + SCA_{fm} + (GCA:E)_{fj} + (GCA:E)_{mj} + (SCA:E)_{fmj} + \varepsilon_{fmjkl} \quad (2)$$

in which y_{fmjkl} was the phenotypic observation of the hybrid between the f^{th} female and m^{th} male in the l^{th} incomplete block within the k^{th} replicate at the j^{th} environment, μ was the intercept, E_j was the random effect of the j^{th} environment, $R_k(E_j)$ was the random effect of the k^{th} replicate within the j^{th} environment, $B_l[R_k(E_j)]$ was the random effect of the l^{th} incomplete block within the k^{th} replicate at the j^{th} environment, GCA_f was the random GCA effect of the f^{th} female, GCA_m was the random GCA effect of the m^{th} male, SCA_{fm} was the random SCA effect of the cross between the f^{th} female and the m^{th} male, $(GCA:E)_{fj}$ was the random effect of the interaction between the f^{th} female and the j^{th} environment, $(GCA:E)_{mj}$ was the random interaction effect between the GCA of the m^{th} male and the j^{th} environment, $(SCA:E)_{fmj}$ was the random interaction effect between the SCA effect of the cross between the f^{th} female and m^{th} male and the j^{th} environment, and ε_{fmjkl} was the random residual. Heterogeneous variances were assumed for residual, block and replicate effects in different environments.

Variance components associated with the GCA effects of males and females, SCA effects and their interaction with the environment were estimated from model 2. The likelihood ratio test (Self and Liang 1987) was used to test the significance of the variance components, in which full and reduced (variance component of interest was removed) models were used using the function *lrt.asreml* implemented in the ASREML-R package. All linear mixed-effects models were fitted in ASReml-R package version 4.1.0.154 (Butler et al. 2017).

The additive genetic variance (σ_A^2), dominance genetic variance (σ_D^2) and total phenotypic variance (σ_P^2) in the hybrid population were estimated according to Holland et al. (2003) and Isik et al. (2017) as follows:

$$\sigma_A^2 = 2(\sigma_{GCAf}^2 + \sigma_{GCAm}^2)$$

$$\sigma_D^2 = 4\sigma_{SCA}^2$$

$$\sigma_P^2 = \sigma_A^2 + \sigma_D^2 + \frac{\sigma_{GCAf\times E}^2}{n} + \frac{\sigma_{GCAm\times E}^2}{n} + \frac{\sigma_{SCA\times E}^2}{n} + \frac{\sigma_e^2}{rn}$$

where σ_A^2 is the additive genetic variance, σ_{GCAm}^2 is the male GCA variance, σ_{GCAf}^2 is the female GCA variance, σ_D^2 is the dominance genetic variance, σ_P^2 is the total phenotypic variance, σ_{SCA}^2 is the SCA variance, $\sigma_{GCAf\times E}^2$ is the interaction between the female GCA and environment variance, $\sigma_{GCAm\times E}^2$is the interaction between the male GCA and environment variance, $\sigma_{SCA\times E}^2$ is the interaction between the SCA and environment variance, σ_e^2 is the residual variance, r is the number of replications and n is the number of environments.

Assuming the epistatic effects were negligible, broad- (H^2) and narrow-sense (h^2) heritability estimates in the hybrid population were determined following Hallauer et al. (2010) as follows:

$$H^2 = \frac{\sigma_A^2 + \sigma_D^2}{\sigma_P^2}$$

$$h^2 = \frac{\sigma_A^2}{\sigma_P^2}$$

where σ_A^2 is the additive genetic variance, σ_D^2 is the dominance genetic variance and σ_P^2 is the total phenotypic variance.

For parental lines, standard broad-sense heritability (Holland et al. 2003) was calculated as follows:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{G\times E}^2}{n} + \frac{\sigma_e^2}{rn}}$$

where σ_G^2 is the genotypic variance of parental lines, $\sigma_{G\times E}^2$ is the parental lines × environment interaction variance, σ_e^2 is the residual variance, r is the number of replications, and n is the number of environments.

The general combining ability effects of each female (GCA_f) and male (GCA_m) parents and the specific combining ability effect of each cross (SCA_{fm}) were predicted as the best linear unbiased prediction (BLUPs) from model 2 according to Isik et al. (2017). BLUPs associated with the combining ability effects were used due to the incomplete factorial mating design, unbalanced data across environments and interest in family represented by each parental accession. The advantages of BLUP over BLUE in plant breeding have been highlighted by Piepho et al. (2008). Methods based on BLUP predict genetic effects more accurately by maximizing the correlation between true genotypic and predicted values. The significance of GCA and SCA effects was assessed using a two-tailed t test (Dabholkar 1999) at the probability levels of 0.05, 0.01, and 0.001. The importance of dominance and additive gene effects was assessed through the predictability ratio of Baker (1978) using the following formula:

$$Predictability\ ratio = \frac{(\sigma^2_{GCAf} + \sigma^2_{GCAm})}{(\sigma^2_{GCAf} + \sigma^2_{GCAm} + \sigma^2_{SCA})}$$

where σ^2_{GCAf} is the female GCA variance, $\sigma^2_{GCAm}$is the male GCA variance, and σ^2_{SCA} is the SCA variance.

The average degree of dominance (D) (Dhillon 1990) was computed as:

$$D = \sqrt{\frac{2\sigma^2_D}{\sigma^2_A}}$$

where σ^2_A is the additive genetic variance and σ^2_D is the dominance genetic variance.

The genetic advance (GA) for each trait was computed as $GA = i \times H^2 \times \sigma_P$, where σ_P was the phenotypic standard deviation, H^2 was the broad-sense heritability, and i was the standardized selection differential at a selection intensity of 5% ($i = 2.06$) (Singh and Chaudhary 1985). Genetic advance over mean (GAM) was further computed as $GAM = \frac{GA}{\mu} \times 100$, where μ is the overall mean of the trait.

The phenotypic best linear unbiased predictors (BLUPs) generated from model 1 were referred to as the mean genotypic values and used in further analyses. BLUPs were used because they have good predictive accuracy over the best linear unbiased estimators (BLUEs) due to their high correlation with the true values and their ability to handle environmental effects and have been recommended for phenotypic selection in plant breeding (Piepho et al. 2008; Molenaar et

al. 2018; Kleinknecht et al. 2013). Spearman rank's correlation coefficients among all traits and their level of significance were calculated using the function *corr* from the R package *"Hmisc"* (Harrell Jr and Dupont 2021) and plotted using the *"metan"* R package (Olivoto and Lúcio 2020).

For heterosis analysis, BLUPs of hybrids and their corresponding parents across environments were used. To this end, mid-parent heterosis (MPH) and better-parent heterosis (BPH) were computed for each hybrid as follows:

$$MPH\ (\%) = \frac{F_1 - MP}{MP} \times 100$$

$$BPH\ (\%) = \frac{F_1 - BP}{BP} \times 100$$

where F_1 is the BLUP value of the hybrid, MP is the mid-parent BLUP value computed as the average BLUP value between the two parents of the hybrid, and BP is the BLUP value of the best parent.

6.3 Results

6.3.1 Performance of parents and hybrids

Significant differences ($P < 0.01$) were observed between the parents and hybrids for all agronomic traits and varied between environments (Figure 6.1). Across environments, hybrids performed better than their parents for stem diameter, plant height, primary branch length, central leaflet length, central leaflet width, leaf width, petiole length, leaf area, total fresh biomass, and edible fresh biomass, with increases of 21.12%, 34.45%, 37.63%, 19.87%, 18.99%, 17.50%, 25.05%, 50.91%, 80.58%, and 54.70%, respectively. In contrast, the mean of the parents was higher than that of hybrids for time to 50% flowering and harvest index. The decrease in hybrid performance compared to the parent performance was 15.39% and 9.59% for time to 50% flowering and harvest index, respectively. Overall, similar performance between hybrids and parents for the number of primary branches was observed.

6.3.2 Variance components and heritability estimates

Genotypic (σ_G^2) and genotype × environment interaction variances ($\sigma_{G\times E}^2$) were significant for all traits for both hybrids and parents (Table 6.4). The importance of σ_G^2 over $\sigma_{G\times E}^2$ varied from parents to hybrids and was trait specific. Overall, genotype × environment interaction variances were higher than genotypic variances (σ_G^2) for most traits in hybrids and parents except for petiole length and primary branch length (hybrids and parents), time to 50% flowering (hybrids only), stem diameter, number of primary branches and leaf width (parents only).

The partitioning of genotypic variance in hybrids revealed that general combining ability (GCA) variance for males (σ_{GCAm}^2) was significantly different from zero for all agronomic traits except dry matter content. The GCA variance for females (σ_{GCAf}^2) was significantly different from zero for most traits except for stem diameter, total and edible fresh biomass, dry matter content and harvest index. Similar to σ_{GCAf}^2, specific combining ability (SCA) variance (σ_{SCA}^2) was significantly different from zero for most traits except for total and edible fresh biomass, dry matter content, and harvest index. Additionally, male GCA × environment interaction variances ($\sigma_{GCAm\times E}^2$), female GCA × environment interaction variances ($\sigma_{GCAf\times E}^2$) and SCA × environment interaction variances ($\sigma_{SCA\times E}^2$) were significantly different from zero for all traits except dry matter content. Only the SCA × environment interaction variance ($\sigma_{SCA\times E}^2$) was significantly different from zero for dry matter content.

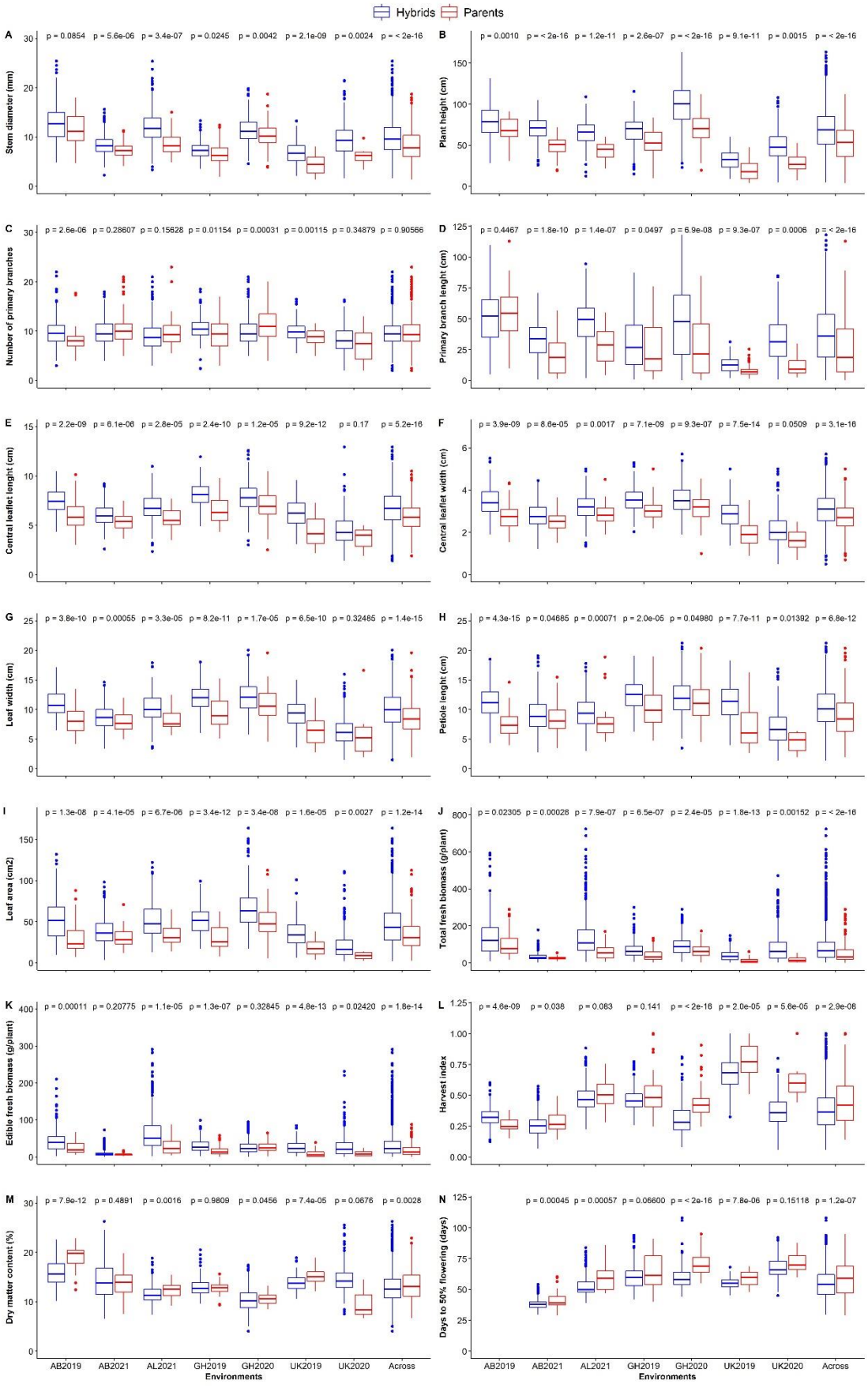

Figure 6.1. Comparison of hybrid and parent performance of *G. gynandra* across environments.

Estimates of male GCA variance were larger than the female ones for most traits except for time to 50% flowering, number of primary branches, primary branch length, petiole length and dry matter content. The latter traits had female GCA variances larger than the male ones. In addition, estimates of specific combining ability (SCA) variance (σ^2_{SCA}) were lower than the average female and male GCA variances for all agronomic traits except dry matter content. Similarly, the additive variance (σ^2_A) was greater than that of dominance (σ^2_D) for all agronomic traits except dry matter content.

The degree of dominance was greater than unity for dry matter content, showing only their dominant nature for dry matter content. In contrast, the degree of dominance was lower than unity for other traits, showing their partially dominant nature. The predictability ratio was greater than 0.5 for all agronomic traits, showing the preponderance of additive gene action for the investigated traits in the species. The broad-sense heritability (H^2) was moderate to high for all traits and ranged between 0.35 ± 0.11 (edible biomass per plant) and 0.91 ± 0.03 (petiole length) for parents and between 0.44 ± 0.18 (dry matter content) and 0.94 ± 0.01 (number of primary branches) (Table 6.4). Similarly, narrow-sense heritability (h^2) estimates were moderate to high for all agronomic traits, except dry matter content (Table 6.4). A low h^2 value was obtained for dry matter content (0.20 ± 0.12). High h^2 values (> 0.60) were observed for time to 50% flowering, petiole length, primary branch length, number of primary branches and leaf width. In the current hybrid population, genetic gains at 5% selection intensity were variable (Table 6.4). Estimates of genetic gains over the mean of the current hybrid population varied from low for dry matter content (5.09%) to high for leaf area (60.94%). Specifically, significant genetic gains (> 20%) were observed for most traits except dry matter content (5.09%) and harvest index (11.63%).

Table 6.4. Estimates of genetic variance components, heritability estimates, degree of dominance, predictability ratio, and genetic advance for the fourteen agronomic traits in 39 parents and 331 hybrids of *Gynandropsis gynandra* evaluated across seven environments from 2019 to 2021.

Source variation	StDiam	PHeight	PBrLeng	NPBr	CtLleng	CtLwid	Lwid
Parents							
σ_G^2	1.63 ± 0.53***	68.99 ± 23.03***	137.80 ± 42.55***	5.83 ± 1.65***	0.53 ± 0.19***	0.08 ± 0.03***	2.35 ± 0.73***
$\sigma_{G\times E}^2$	1.30 ± 0.35***	75.33 ± 15.37***	133.71 ± 22.38***	3.80 ± 0.62***	0.70 ± 0.13***	0.14 ± 0.03***	1.89 ± 0.38***
σ_e^2	3.17 ± 0.56	79.26 ± 20.21	74.11 ± 10.89	4.04 ± 1.83	0.54 ± 0.11	0.14 ± 0.03	4.26 ± 1.70
H^2	0.80 ± 0.06	0.81 ± 0.06	0.85 ± 0.04	0.88 ± 0.04	0.79 ± 0.07	0.73 ± 0.09	0.80 ± 0.06
Hybrids							
σ_G^2	0.83 ± 0.15***	63.98 ± 8.30***	2.60 ± 0.26***	47.94 ± 7.94***	0.33 ± 0.05***	0.09 ± 0.01***	1.38 ± 0.17***
σ_{GCAf}^2	0.05 ± 0.07	6.75 ± 4.55*	34.70 ± 16.49***	1.34 ± 0.55***	0.04 ± 0.02*	0.03 ± 0.01***	0.28 ± 0.13***
σ_{GCAm}^2	0.54 ± 0.21***	39.15 ± 13.56***	14.38 ± 6.35***	0.91 ± 0.31***	0.22 ± 0.08***	0.04 ± 0.01***	0.91 ± 0.31***
σ_{SCA}^2	0.16 ± 0.08*	21.01 ± 4.75***	5.90 ± 3.70	0.42 ± 0.08***	0.07 ± 0.02***	0.02 ± 0.01***	0.18 ± 0.06**
$\sigma_{G\times E}^2$	3.10 ± 0.21***	100.88 ± 6.92***	1.54 ± 0.12***	124.13 ± 9.27***	0.56 ± 0.05***	0.12 ± 0.01***	1.59 ± 0.12***
$\sigma_{GCAf\times E}^2$	0.50 ± 0.13***	14.38 ± 4.09**	42.02 ± 8.69***	0.48 ± 0.11***	0.09 ± 0.02***	0.01 ± 0.00***	0.22 ± 0.06***
$\sigma_{GCAm\times E}^2$	0.36 ± 0.10***	7.78 ± 3.11**	19.18 ± 4.89***	0.18 ± 0.06***	0.11 ± 0.03***	0.03 ± 0.01***	0.33 ± 0.08***
$\sigma_{SCA\times E}^2$	1.69 ± 0.17***	79.83 ± 6.59***	60.04 ± 7.73***	0.93 ± 0.11***	0.36 ± 0.04***	0.08 ± 0.01***	1.10 ± 0.11***
σ_e^2	3.36 ± 0.20	103.92 ± 4.92	159.80 ± 7.41	2.03 ± 0.09	0.74 ± 0.03	0.16 ± 0.01	1.94 ± 0.08
σ_A^2	1.19 ± 0.44	91.79 ± 28.55	98.16 ± 35.31	4.50 ± 1.25	0.50 ± 0.16	0.14 ± 0.04	2.38 ± 0.67
σ_D^2	0.63 ± 0.33	84.04 ± 18.99	23.61 ± 14.81	1.66 ± 0.32	0.29 ± 0.09	0.09 ± 0.02	0.71 ± 0.25
H^2	0.75 ± 0.06	0.89 ± 0.02	0.81 ± 0.05	0.94 ± 0.01	0.86 ± 0.03	0.89 ± 0.02	0.89 ± 0.02
h^2	0.49 ± 0.12	0.46 ± 0.09	0.65 ± 0.11	0.69 ± 0.07	0.54 ± 0.10	0.55 ± 0.09	0.69 ± 0.08
Degree of dominance	0.73 ± 0.24	0.96 ± 0.19	0.49 ± 0.18	0.61 ± 0.10	0.76 ± 0.18	0.79 ± 0.16	0.55 ± 0.13
Predictability ratio	0.79 ± 0.11	0.69 ± 0.08	0.89 ± 0.07	0.84 ± 0.05	0.78 ± 0.08	0.76 ± 0.07	0.87 ± 0.05
Genetic advance (GA) at i = 5%	2.41 ± 0.44	25.75 ± 2.74	20.45 ± 3.79	4.97 ± 0.55	1.70 ± 0.23	0.92 ± 0.10	3.42 ± 0.44
GAM	25.06 ± 4.63	40.12 ± 4.27	58.73 ± 10.87	51.50 ± 5.67	25.35 ± 3.38	30.32 ± 3.37	34.35 ± 4.41

StDiam: stem diameter (mm), PHeight: plant height (cm), PBrLeng: primary branch length (cm), NPBr: number of primary branches, CtLleng: central leaflet length (cm), CtLwid: central leaflet width (cm), Lwid: leaf width (cm), Ptillen: petiole length (cm), LfArea: leaf area (cm^2), FBiom: total fresh biomass per plant (g), EDBiom: edible fresh biomass per plant (g), HI: harvest index, DM: dry matter content (%), DFlow: time to 50% flowering (days), σ_e^2 = residual variance, σ_G^2 = genotypic variance, $\sigma_{G\times E}^2$ = genotype × environment variance, σ_{GCAm}^2 = male general combining ability variance, σ_{GCAf}^2 = female general combining ability variance, σ_{SCA}^2 = specific combining ability variance, $\sigma_{GCAf\times E}^2$ = female general combining ability × environment variance, $\sigma_{GCAm\times E}^2$ = male general combining ability × environment variance, $\sigma_{SCA\times E}^2$ = specific combining ability × environment variance, = genotypic variance, σ_A^2 = additive genetic variance, σ_D^2 = dominance genetic variance, H^2 = broad-sense heritability, h^2 = narrow-sense heritability, GA = Genetic advance; GAM = genetic advance over mean. ***, **, * = significantly different from zero at the 0.001, 0.01, and 0.05 probability levels, respectively. *ns* = not significantly different from zero at the 0.05 level of probability.

Table 6.4. Estimates of genetic variance components, heritability estimates, degree of dominance, predictability ratio, and genetic advance for the fourteen agronomic traits in 39 parents and 331 hybrids of *Gynandropsis gynandra* evaluated across seven environments from 2019 to 2021.

Source variation	Ptillen	LfArea	FBiom	EDBiom	HI	DM	DFAS
Parents							
σ_G^2	4.98 ± 1.40***	36.41 ± 19.64**	57.01 ± 26.69***	29.55 ± 12.82***	0.0018 ± 0.0009**	0.30 ± 0.15**	34.88 ± 11.85***
$\sigma_{G\times E}^2$	2.64 ± 0.51***	121.49 ± 22.73***	90.68 ± 29.22***	76.52 ± 13.28***	0.0051 ± 0.0013***	0.49 ± 0.19***	39.77 ± 8.04***
σ_e^2	1.88 ± 0.30	66.63 ± 10.62	1288.72 ± 188.38	94.90 ± 20.81	0.0112 ± 0.0028	3.66 ± 1.85	24.83 ± 7.20
H^2	0.91 ± 0.03	0.62 ± 0.14	0.35 ± 0.11	0.63 ± 0.11	0.53 ± 0.14	0.47 ± 0.17	0.82 ± 0.06
Hybrids							
σ_G^2	2.77 ± 0.29***	79.63 ± 10.94***	198.19 ± 41.78***	26.41 ± 5.88***	0.0004 ± 0.0002***	0.09 ± 0.05*	9.10 ± 1.06***
σ_{GCAf}^2	1.30 ± 0.53***	9.43 ± 5.69**	9.46 ± 28.03	1.34 ± 3.08	0.0002 ± 0.0001	0.03 ± 0.02	10.37 ± 4.46***
σ_{GCAm}^2	1.19 ± 0.40***	54.06 ± 19.24***	175.97 ± 72.90***	25.49 ± 10.70***	0.0003 ± 0.0001**	0.02 ± 0.02	3.87 ± 1.41***
σ_{SCA}^2	0.23 ± 0.08***	18.09 ± 5.63***	22.29 ± 21.21	4.58 ± 3.61	0.0000 ± 0.0000	0.03 ± 0.05	1.51 ± 0.37***
$\sigma_{G\times E}^2$	1.88 ± 0.16***	127.66 ± 9.93***	623.22 ± 56.56***	100.87 ± 8.83***	0.0031 ± 0.0003***	0.66 ± 0.10***	3.89 ± 0.77***
$\sigma_{GCAf\times E}^2$	0.44 ± 0.11***	14.80 ± 4.81***	226.45 ± 60.19***	20.40 ± 6.28***	0.0007 ± 0.0002***	0.02 ± 0.03	4.45 ± 1.06***
$\sigma_{GCAm\times E}^2$	0.37 ± 0.09***	23.88 ± 6.26***	184.04 ± 47.32***	29.00 ± 7.27***	0.0005 ± 0.0001***	0.03 ± 0.03	1.70 ± 0.49***
$\sigma_{SCA\times E}^2$	1.13 ± 0.14***	99.12 ± 8.75***	331.97 ± 38.91***	64.91 ± 6.84***	0.0017 ± 0.0002***	0.60 ± 0.10***	1.02 ± 0.48*
σ_e^2	2.72 ± 0.12	150.27 ± 9.06	4764.97 ± 258.06	569.79 ± 31.27	0.0061 ± 0.0003	3.05 ± 0.15	32.32 ± 1.50
σ_A^2	4.97 ± 1.33	126.98 ± 40.01	370.86 ± 155.64	53.66 ± 22.17	0.0008 ± 0.0003	0.11 ± 0.06	6.29 ± 4.22
σ_D^2	0.92 ± 0.31	72.35 ± 22.54	89.14 ± 84.83	18.30 ± 14.44	0.0001 ± 0.0004	0.14 ± 0.18	0.09 ± 0.11
H^2	0.93 ± 0.02	0.87 ± 0.03	0.51 ± 0.10	0.56 ± 0.09	0.54 ± 0.14	0.44 ± 0.18	0.90 ± 0.03
h^2	0.78 ± 0.06	0.55 ± 0.10	0.41 ± 0.11	0.42 ± 0.11	0.48 ± 0.15	0.20 ± 0.12	0.74 ± 0.07
Degree of dominance	0.43 ± 0.09	0.75 ± 0.17	0.49 ± 0.26	0.58 ± 0.27	0.36 ± 0.63	1.12 ± 0.86	0.46 ± 0.10
Predictability ratio	0.92 ± 0.03	0.78 ± 0.08	0.89 ± 0.10	0.85 ± 0.11	0.94 ± 0.20	0.62 ± 0.36	0.90 ± 0.04
Genetic advance (GA) at i = 5%	4.81 ± 0.60	27.09 ± 3.50	31.47 ± 8.94	13.05 ± 3.40	0.05 ± 0.02	0.68 ± 0.39	11.47 ± 1.73
GAM	46.11 ± 5.71	60.94 ± 7.87	37.83 ± 10.75	46.99 ± 12.24	11.63 ± 4.56	5.10 ± 2.93	20.37 ± 3.07

StDiam: stem diameter (mm), PHeight: plant height (cm), PBrLeng: primary branch length (cm), NPBr: number of primary branches, CtLleng: central leaflet length (cm), CtLwid: central leaflet width (cm), Lwid: leaf width (cm), Ptillen: petiole length (cm), LfArea: leaf area (cm^2), FBiom: total fresh biomass per plant (g), EDBiom: edible fresh biomass per plant (g), HI: harvest index, DM: dry matter content (%), DFlow: time to 50% flowering (days), σ_e^2 = residual variance, σ_G^2 = genotypic variance, $\sigma_{G\times E}^2$ = genotype × environment variance, σ_{GCAm}^2 = male general combining ability variance, σ_{GCAf}^2 = female general combining ability variance, σ_{SCA}^2 = specific combining ability variance, $\sigma_{GCAf\times E}^2$ = female general combining ability × environment variance, $\sigma_{GCAm\times E}^2$ = male general combining ability × environment variance, $\sigma_{SCA\times E}^2$ = specific combining ability × environment variance, = genotypic variance, σ_A^2 = additive genetic variance, σ_D^2 = dominance genetic variance, H^2 = broad-sense heritability, h^2 = narrow-sense heritability, GA = Genetic advance; GAM = genetic advance over mean. ***, **, * = significantly different from zero at the 0.001, 0.01, and 0.05 probability levels, respectively. *ns* = not significantly different from zero at the 0.05 level of probability.

6.3.3 General combining ability effects of parents

Estimates of the GCA effects of male and female parents are presented in Figures 6.2 and 6.3, respectively. Significant male parent GCA effects were observed for most agronomic traits except dry matter content (Figure 6.2). For female parental lines, significant GCA effects were observed for plant height, number of primary branches, primary branch length, central leaflet width, leaf width, petiole length and time to 50% flowering (Figure 6.3). Some female and male parents displayed multiple significant and positive GCA effects. Good general male combiners included parent M22 for most agronomic traits except dry matter content and primary branch length; parent M1 for most agronomic traits except number of primary branches, harvest index and time to 50% flowering; M16 for number of primary branches, central leaflet length, leaf width, leaf area, and time to 50% flowering; M10 for stem diameter, number of primary branches, petiole length, and biomass, M2 for time to 50% flowering and biomass, M18 for time to 50% flowering and leaf area, M20 for time to 50% flowering, petiole length and time to 50% flowering. Female parental lines such as F15, F11, F16, F1, F2 and F3 were good combiners. F15 displayed significant and positive general combining ability for number of primary branches, leaf width, petiole length and time to 50% flowering but also had positive GCA effects for stem diameter, leaf area and edible fresh biomass. F11 was a good combiner for the number of primary branches, leaf traits (leaf area, central leaflet width, central leaflet length), harvest index and dry matter content. F1 and F2 displayed positive GCA effects for plant height, primary branch length and biomass. F3 was a good combiner for biomass, dry matter content, primary branch length and plant height.

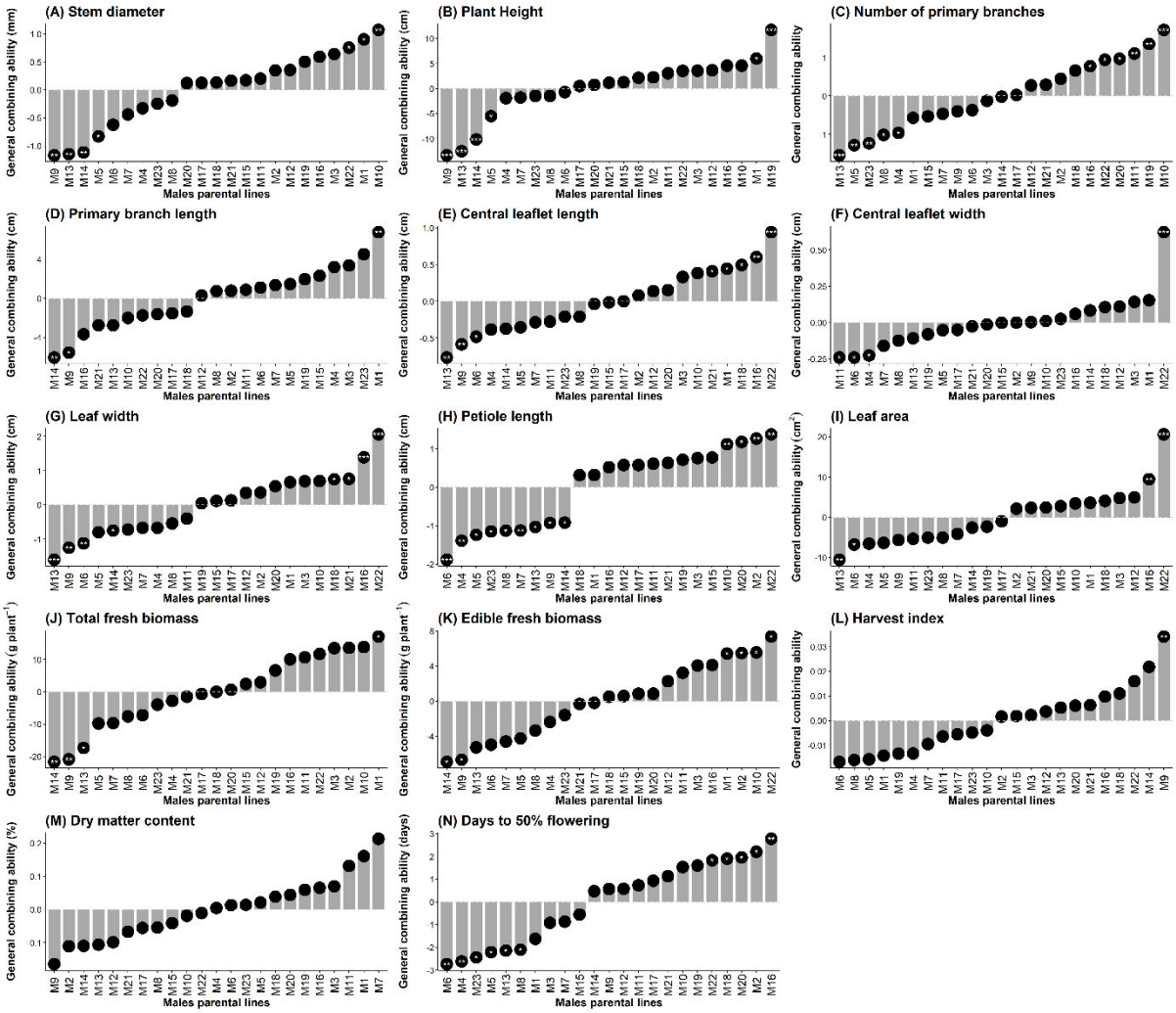

Figure 6.2. Estimates of general combining ability effects of male parental lines for fourteen yield- and yield-related traits in *Gynandropsis gynandra*. ***, **, * refer to estimates of the general combining ability effect significantly different from zero at P < 0.001, 0.01 and 0.05, respectively.

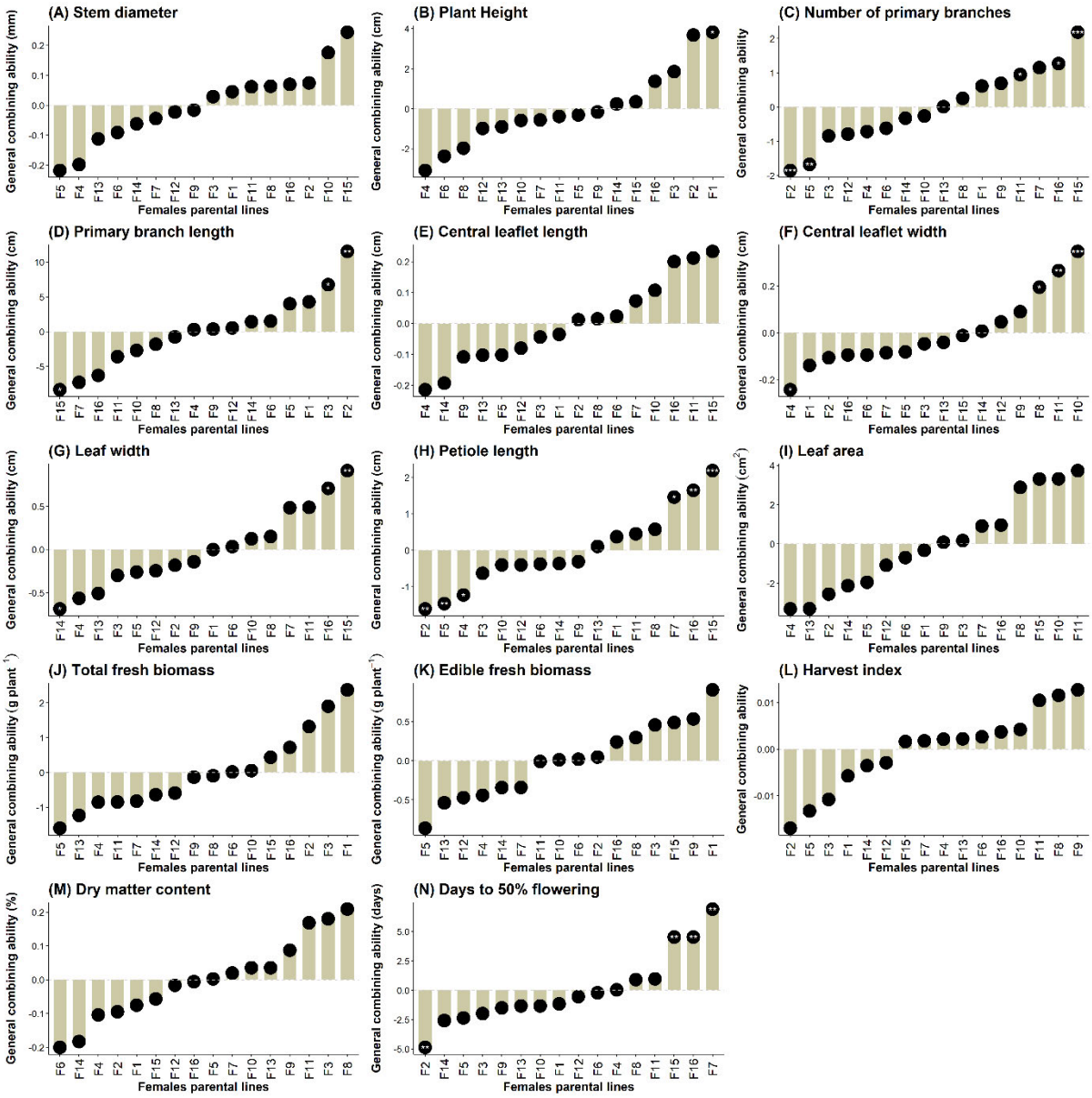

Figure 6.3. Estimates of general combining ability effects of female parental lines for fourteen yield- and yield-related traits in *Gynandropsis gynandra*. ***, **, * refer to estimates of the general combining ability effect significantly different from zero at P < 0.001, 0.01 and 0.05, respectively.

6.3.4 Specific combining ability effects of hybrids

A wide range of specific combining ability (SCA) effects from negative to positive was observed for all biomass and related traits. Regarding total and edible fresh biomass, the highest SCA effects were observed for the crosses F3xM1, F14xM1, F16xM2, F10xM16, and

F11xM11. Significant and positive SCA effects for time to 50% flowering were exhibited by crosses F6xM16, F16xM19, F3xM22, F3xM2 and F15xM7. Forty-eight percent of the hybrids exhibited positive SCA effects for stem diameter, with crosses F11xM10, F9xM3, F15xM14, F16xM2 and F6xM3 having the highest SCA effects. The hybrid F15xM14 displayed the highest and most significant SCA effect for plant height, followed by F10xM22, F11xM22, F3xM14 and F4xM9. Regarding the number of primary branches, hybrids F15xM10, F1xM10, F5xM14, F16xM19, F15xM21 and F1xM11 showed significant SCA effects in the desirable direction. High and positive SCA effects were observed for crosses F15xM14, F6xM11, F2xM2 and F13xM19 for primary branch length. Hybrids F10xM22, F15xM14, and F15xM18 displayed positive and significant SCA effects for central leaflet width. For leaf area, the hybrid F11xM22 displayed the highest and significant SCA effect, followed by F3xM1, F9xM3, F10xM17 and F10xM22. None of the crosses displayed significant and positive SCA effects for dry matter content, although crosses F8xM1, F13xM6, F3xM8, F3xM7 and F1xM20 exhibited positive and high SCA effects for dry matter content. High and positive SCA effects in the desirable direction were observed for the F10xM11, F13xM18 and F11xM14 hybrids for the harvest index.

6.3.5 Heterosis

The distributions of mid- and best-parent heterosis (MPH and BPH, respectively) for all fourteen agronomic traits are shown in Figure 6.4. The mid-parent heterosis ranged between -34.11% and 192.10%, while the best-parent heterosis varied from -51.89% to 176.32% for all agronomic traits. Both negative and positive heterosis were observed in the species (Figure 6.4). Only positive heterosis effects were observed for leaf area and total fresh biomass, with an average mid-parent heterosis greater than 50%. Although positive and negative heterosis effects were observed, average mid- and best-parent heterosis were negative for dry matter content, harvest index, time to 50% flowering and number of primary branches, with the latter having a positive average mid-parent heterosis. The top crosses with the highest mid- and best-parent heterosis were F10xM8, F9xM8, and F9xM3 for stem diameter; F10xM8, F10xM22, and F10xM17 for plant height; F9xM3, F11xM22, and F8xM3 for leaf area; F9xM3, F10xM16, and F10xM22 (MPH) for total fresh biomass; F10xM8 and F11xM11 edible fresh biomass (> 100%); F14xM10, F10xM11, F14xM11, and F1xM20 for dry matter content; and F3xM21, F3xM22, and F6xM16 (MPH) for time to 50% flowering time.

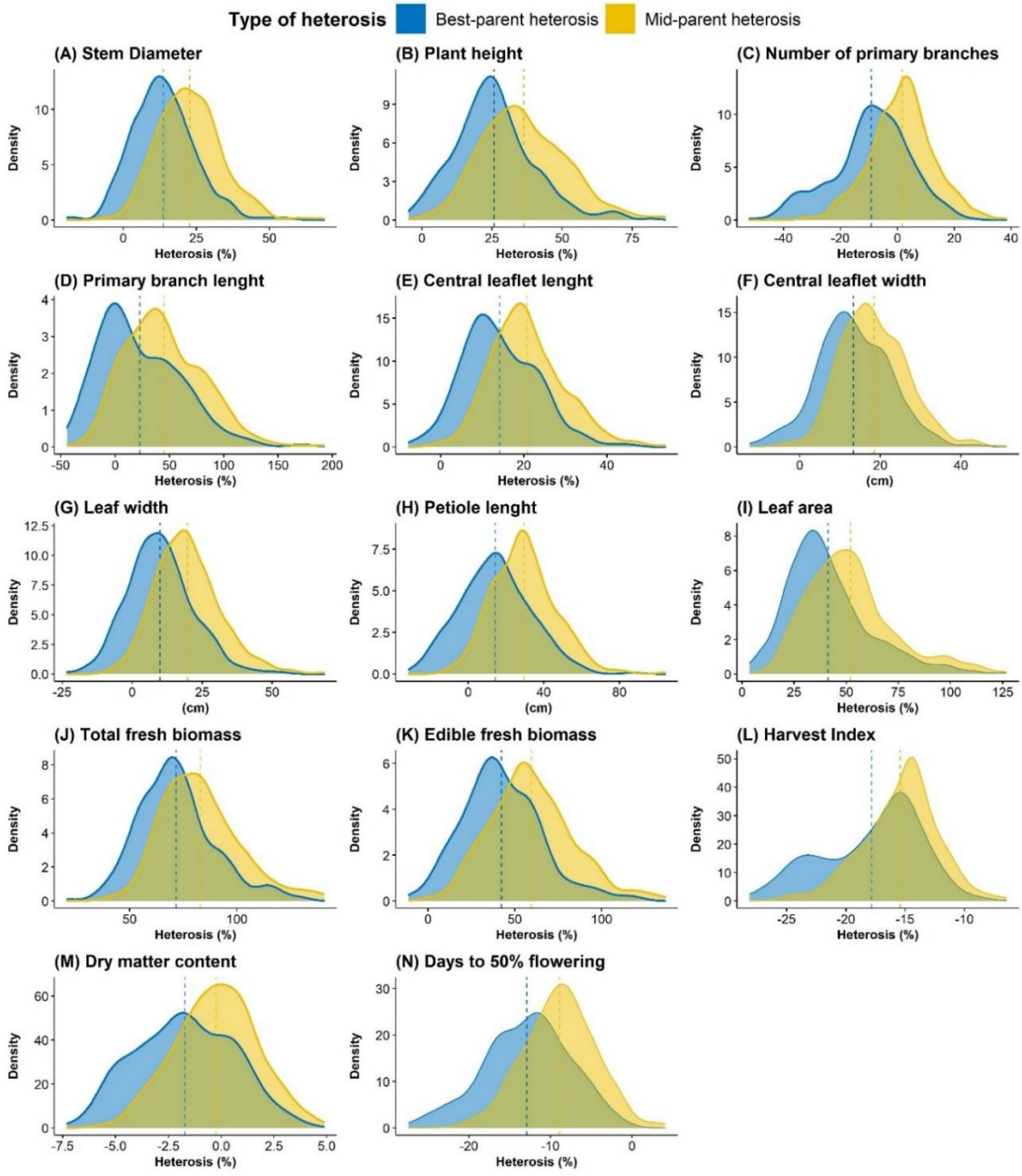

Figure 6.4. Distribution of mid- and best-parent heterosis for fourteen agronomic traits in *Gynandropsis gynandra*.

6.3.6 Association among agronomic traits

Significant phenotypic correlation coefficients were observed among the fourteen agronomic traits for both parents and hybrids (Figure 6.5). Overall, phenotypic correlations showed similar patterns for the hybrids and parents with slight differences. While the phenotypic correlation coefficients ranged from -0.74 to 0.94 for parental lines, the phenotypic correlation coefficients varied between -0.57 and 0.93 for the hybrids. A highly significant and positive correlation was

observed between edible and total fresh biomass per plant for parents ($r = 0.88$, $p < 0.001$) and hybrids ($r = 0.92$, $p < 0.001$) (Figure 6.5). Total and edible biomass per plant had moderate to strong and positive correlations with plant height, stem diameter and leaf-related traits (central leaflet length, leaf width, petiole length and leaf area) and primary branch length for both parents and hybrids. There were moderate to strong and positive correlations among leaf traits, with leaf area being moderately or strongly and positively correlated with central leaflet length, central leaflet width and leaf width for both populations. While time to 50% flowering had a strong and negative correlation with primary branch length for parents (-0.78 for parents, $p < 0.001$), a moderate correlation was observed for hybrids ($r = -0.58$, $p < 0.001$) (Figure 6.5). The time to 50% flowering had moderate and positive correlations with the number of primary branches, petiole length and harvest index for hybrids and parents (Figure 6.5). In addition, while time to 50% flowering had no correlation with leaf traits and biomass in parents, moderate and positive correlations were observed between time to 50% flowering and leaf traits, and weak and positive correlations were observed with total and edible fresh biomass for hybrids. A moderate and negative correlation between time to 50% flowering and dry matter content observed in parents changed to no correlation for hybrids (Figure 6.5). A similar observation was noticed between the dry matter content and harvest index. A strong and positive correlation was observed between stem diameter and plant height, which had a moderate to strong positive correlation with leaf traits (Figure 6.5).

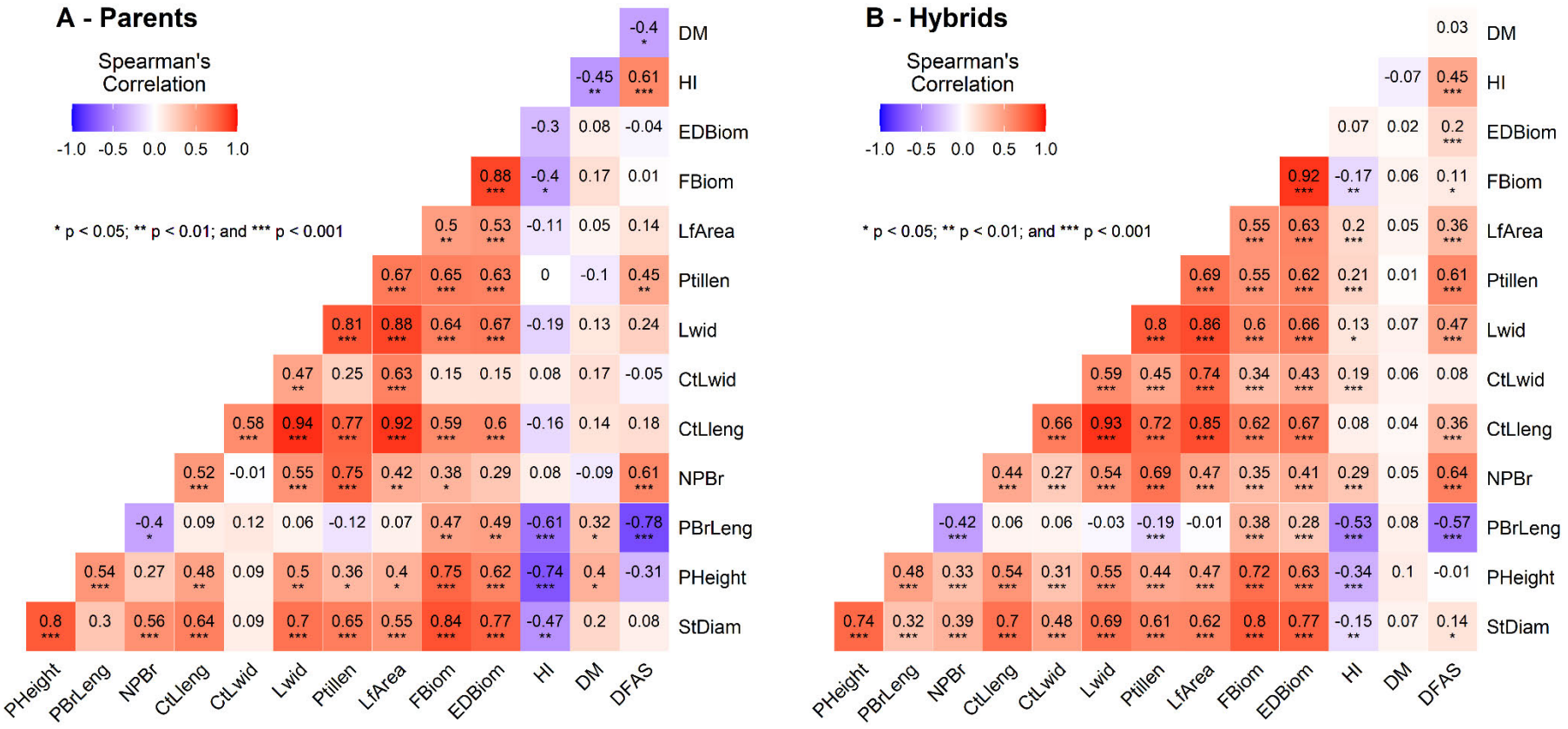

Figure 6.5. Plots of Spearman rank correlation coefficients for fourteen agronomic traits of 39 parental lines and 331 hybrids of *Gynandropsis gynandra*. StDiam: stem diameter (mm), PHeight: plant height (cm), PBrLeng: primary branch length (cm), NPBr: number of primary branches, CtLleng: central leaflet length (cm), CtLwid: central leaflet width (cm), Lwid: leaf width (cm), Ptillen: petiole length (cm), LfArea: leaf area (cm^2), FBiom: total fresh biomass per plant (g), EDBiom: edible fresh biomass per plant (g), HI: harvest index, DM: dry matter content (%), DFAS: time to 50% flowering (days)

6.4 Discussion

6.4.1 Performance of parents and F1 hybrids

Overall, hybrids outperformed their parents for several agronomic traits, including plant height, stem diameter, total and edible fresh biomass per plant, leaf traits (leaf width, central leaflet length and width, leaf area) and branching traits (number of primary branches and primary branch length), indicating hybrid vigor. Similar results were reported in *Amaranthus cruentus* L. for leaf length, leaf width, plant height (Kulakow and Jain 1987) and biomass yield (Lehmann et al. 1991) and in *Brassica oleracea* L. for head weight and leaf traits (Tanaka and Niikura 2006). In addition, some hybrids were better or worse than their specific parents for various traits, showing that the inheritance of the traits is controlled by different mechanisms. Several hybrid performances for total fresh biomass per plant were higher than those of the parents, as previously reported (Omondi et al. 2017b). For instance, the total fresh biomass per plant obtained by Omondi et al. (2017b) was between 25.7 g and 70.4 g, which was lower than that of hybrids in the present study, which ranged between 1.15 and 893.63 g. Significant variation in parent and hybrid performance among environments calls for investigation of the extent of environmental influence on genotype performance. Additionally, we investigated the phenotypic plasticity and stability of the newly developed hybrids to identify the best genotypes under different agroecological and agronomic practices conditions.

6.4.2 Gene action and combining ability estimates

General combining ability is important for parental selection, and specific combining ability is important for best cross selection to exploit heterosis. We observed a preponderance of additive gene effects for most agronomic traits, and high and significant general combining ability effects were observed for female and/or male parental lines. This is mainly due to additive and additive x additive gene effects (Dey et al. 2014). The parents with high GCA effects are excellent founders for the development of improved populations and could be exploited through several generations of hybridization. In the present study, none of the male and female parental lines simultaneously exhibited significant GCA effects in the desirable direction for all agronomic traits. This result concurs with previous findings with respect to agronomic traits in cauliflower (Ram et al. 2018; Dey et al. 2014; Singh et al. 2019) and yield and related traits in eggplant (*Solanum melongena* L.) (Kaushik et al. 2018). However, parental lines such as males M22, M1, M16, M10, M2, M18, M20 and females F15, F11, F16, F1, F2 and F3 are

good combiners for their multiple positive and significant GCA effects. These parents are excellent and valuable candidates and resources for developing improved populations for research and breeding purposes. However, evaluation of these selected parents for maternel effects is needed to fully exploit potential cytoplasmic heredity in the species.

Specific combining ability effects result from nonadditive gene effects, comprising dominance and epistasis (Sprague and Tatum 1942). In this study, none of the crosses displayed high and significant SCA effects for all yield and yield-related traits. This finding is similar to the results of Kaushik et al. (2018) for agronomic traits in eggplant. However, depending on the trait, hybrids with the highest and most significant SCA in the desirable direction involved (i) both parents with good and significant GCA effects (e.g., F3xM1 for biomass per plant, F15xM10 for number of primary branches, F11xM22 for leaf area, F8xM1 for dry matter content) and (ii) one good and one average combiner (e.g., F6xM16, for time to 50% flowering, F9xM3 for stem diameter), (iii) one bad and one average combiner (e.g., F15xM14, F10xM22 for plant height) and (iv) both parents with medium or bad GCA effects (e.g., F5xM7 for stem diameter). This finding shows that depending on the trait, the observed SCA effect might result from (i) the cumulative effects of additive genes (good x good parents) and (ii) the interaction between additive and nonadditive genes (good x poor general combiners or vice versa); and (iii) the over manifestation of the interaction between nonadditive genes, especially complementary epistatic effects (Xie et al. 2018; Dey et al. 2014; Singh et al. 2012; Singh et al. 2019; Sprague and Tatum 1942).

Based on the above, breeding strategies for high-yielding and high-quality leaves in *G. gynandra* should consider both GCA and SCA in the selection of superior parents and crosses. Heterosis breeding and recurrent selection along with multiple crossing programs can be implemented, and types of cultivars might include hybrids, synthetics, composites and population improvements. Strategies implemented for allogamous species can be used for the species. Therefore, breeding strategies should focus on (i) selecting parents with good general combing ability, followed by (ii) selection based on specific combining ability.

6.4.3 Heterosis

Heterosis or hybrid vigor refers to the outperformance of F_1 progenies over their parents and has significantly contributed to increased crop productivity. Here, we report for the first time this phenomenon for yield and yield-related traits in *Gynandropsis gynandra*. The level of heterosis over the mid and best parents was large and variable between traits. This agrees with

earlier reports on the existence of heterosis for agronomic traits in vegetable crops such as amaranth, tomato, cabbage, eggplant, cauliflower and okra (Dey et al. 2014; Singh et al. 2009; Singh et al. 2019; Mistry et al. 2018; Lehmann et al. 1991; Xie et al. 2018; Yadav et al. 2013; Wamm et al. 2010). Specifically, the level of heterosis observed in the present study was comparable to that reported for various morphological traits in other crops, including yield in *Amaranthus* species (Patel et al. 2013; Lehmann et al. 1991), eggplant (Kaushik et al. 2016; Mistry et al. 2018) and okra (Wamm et al. 2010). The wide range of heterosis could be explained by previous observations on the reproductive biology of the species, revealing that the species in predominately outcrossing (Zohoungbogbo et al. 2018b; Raju and Rani 2016; Omondi et al. 2017a).

Both negative and positive mid- and best-parent heterosis were observed in the species for some agronomic traits. This might be because several mechanisms underlie heterosis expression in *Gynandropsis gynandra*. Three main models have been widely used to explain heterosis in crops and include dominance, overdominance and epistasis (Fujimoto et al. 2018; Hochholdinger and Baldauf 2018; Liu et al. 2020; Bar-Zvi et al. 2017). Moreover, hybrids exhibiting a high level of heterosis are a combination of parents with either good, both poor, average x good, good x poor, average x average or average x poor general combining abilities. The results showed that all three models or their combination could explain heterosis in *G. gynandra,* as most research has highlighted that a single model rarely occurs in plants (Fujimoto et al. 2018; Zhou et al. 2012). The present observation of the existence of heterosis in *G. gynandra* adds to previous reports (Sogbohossou et al. 2018; Sohindji et al. 2020) that the species could be used as a model for heterosis studies. A good exploitation of heterosis in *G. gynandra* requires the identification of heterotic patterns and groups in the species. To this end, the observed genetic differentiation between accessions based on geographical origin (Blalogoe et al. 2020; Sogbohossou et al. 2019) is key, and further investigation to assess the cross-compatibility between them and even within each region to avoid possible incompatibility between accessions is important. Additionally, the identification of common testers will help to speed up the exploitation of heterosis in the species.

6.4.4 Association among traits

Information on associations among target traits is crucial for establishing breeding programs. Overall, we observed similar patterns of positive association between most agronomic traits in both populations, particularly among stem diameter, number of primary branches, plant height,

leaf traits and total and edible plant biomass. Similar findings were reported in accessions of *G. gynandra* (Kiebre et al. 2017; Sogbohossou et al. 2019). These might result from a possible linkage between loci controlling these traits and offer good opportunities for simultaneous and direct selection, as these traits are desired by farmers (Sogbohossou 2019; Kiebre et al. 2015). In contrast, we observed some significant changes in the association between some traits from parents to hybrids. Specifically, we observed changes of no association of flowering time with fresh biomass per plant (total and edible) on the one hand and with leaf traits on the other hand from parents to hybrids to low or moderate. These changes could be associated with probable independent segregation between loci controlling these traits, as the composition of the population changes from parents to hybrids. This needs to be investigated at the molecular level and using advanced generations to reveal the true association between these traits as well as genomic regions associated with these traits.

6.5 Conclusion

The present study, for the first time, generated critical and novel knowledge on the quantitative genetic parameters and gene action governing the inheritance of biomass and related traits in *G. gynandra*. We observed significant variation among parent and hybrid for biomass and related traits, with hybrids superior to their parents for biomass, plant architecture and leaf traits. Quantitative genetic parameters analysis revealed significant effects of both general and specific combining ability, indicating the importance of both additive and nonadditive genes in the inheritance of biomass and related traits, with a predominance of additive gene action. The degree of dominance observed was partial dominance for all traits except dry matter content, for which complete dominance was observed. Our results also revealed the presence of positive and negative mid- and best-parent heterosis but were trait specific. While only a positive level of heterosis was observed for leaf area and total fresh biomass, negative heterosis effects were observed for harvest index and time to 50% flowering. Consequently, hybrids with higher leaf yields and late flowering time could be developed. Moderate to high broad-sense and narrow-sense heritability estimates were observed for most agronomic traits except dry matter content, which had low narrow sense heritability. Significant genetic gains (> 20%) were observed for most traits except dry matter content (5.09%) and harvest index (11.63%) at the selection pressure of 5%. The best crosses resulted from different parental combinations, ranging from good to poor combiners. Heterosis breeding and reciprocal recurrent selection would be sound breeding strategies to develop high-yielding cultivars with hybrids and open-pollinated and

synthetic varieties as cultivars to be developed. The observed positive association among traits is good for simultaneous selection. Moreover, changes in the association between traits from parents to hybrids provide insights into potential independent segregation among these traits. We therefore suggest the use of *G. gynandra* as a model crop for investigating the mechanism underlying heterosis in plants. Further research on heterotic groups and patterns as well as tester identification to fully exploit heterosis in the species is needed. Furthermore, future investigations should aim to decipher the genomic basis of heterosis in the species to contribute to uncovering the mechanism underlying heterosis in plants.

References

Alexandratos N, Bruinsma J (2012) World agriculture towards 2030/2050: the 2012 revision. ESA Working Papers 12-03. doi:10.22004/ag.econ.288998

Altman A, Fan L, Foyer C, Cowling W, Mittler R, Qaim M, Weber AP, Reynolds M, Varshney RK, Fernie A (2021) Past and Future Milestones of Plant Breeding. Trends in Plant Science 26 (6):530-538. https://doi.org/10.1016/j.tplants.2021.03.013

Anderson R, Bayer PE, Edwards D (2020) Climate change and the need for agricultural adaptation. Current Opinion in Plant Biology 56:197-202. https://doi.org/10.1016/j.pbi.2019.12.006

Baker RJ (1978) Issues in diallel analysis. Crop Sci 18 (4):533-536. https://doi.org/10.2135/cropsci1978.0011183X001800040001x

Bar-Zvi D, Lupo O, Levy AA, Barkai N (2017) Hybrid vigor: The best of both parents, or a genomic clash? Curr Opin Syst Biol 6:22-27. https://doi.org/10.1016/j.coisb.2017.08.004

Bassu S, Fumagalli D, Toreti A, Ceglar A, Giunta F, Motzo R, Zajac Z, Niemeyer S (2021) Modelling potential maize yield with climate and crop conditions around flowering. Field Crops Research 271:108226. https://doi.org/10.1016/j.fcr.2021.108226

Bernal-Vasquez A-M, Utz H-F, Piepho H-P (2016) Outlier detection methods for generalized lattices: a case study on the transition from ANOVA to REML. Theor Appl Genet 129 (4):787-804. 10.1007/s00122-016-2666-6

Bioversity International (2017) Mainstreaming agrobiodiversity in sustainable food systems: Scientific foundations for an agrobiodiversity index. Bioversity International, Rome, Italy

Blalogoe JS, Odindo AO, Sogbohossou EOD, Sibiya J, Achigan-Dako EG (2020) Origin-dependence of variation in seed morphology, mineral composition and germination percentage in *Gynandropsis gynandra* (L.) Briq. accessions from Africa and Asia. BMC Plant Biol 20 (1):168. 10.1186/s12870-020-02364-w

Bradshaw JE (2017) Plant breeding: past, present and future. Euphytica 213 (3):60. 10.1007/s10681-016-1815-y

Butler D, Cullis B, Gilmour A, Gogel B, Thompson R (2017) ASReml-R Reference Manual Version 4. . VSN International Ltd Hemel Hempstead, HP1 1ES. UK

Camp K (1999) The Bioresource Groups of KwaZulu-Natal. Cedara report. KwaZulu-Natal Department of Agriculture,

Challinor AJ, Watson J, Lobell DB, Howden SM, Smith DR, Chhetri N (2014) A meta-analysis of crop yield under climate change and adaptation. Nature Climate Change 4:287. 10.1038/nclimate2153

Chataika BY, Akundabweni LS-M, Houdegbe AC, Achigan-Dako EG, Sibiya J, Masamba K (2021) Dietary phytochemical screening of spider plant (*Gynandropsis gynandra* (L.) Briq.) accessions from Africa and Asia to identify genotypes for use in nutraceutical breeding. Front Sustain Food Syst 5 (344). 10.3389/fsufs.2021.733909

Chiurugwi T, Kemp S, Powell W, Hickey LT (2019) Speed breeding orphan crops. Theoretical and Applied Genetics 132 (3):607-616. 10.1007/s00122-018-3202-7

Cowling WA, Gaynor RC, Antolín R, Gorjanc G, Edwards SM, Powell O, Hickey JM (2020) In silico simulation of future hybrid performance to evaluate heterotic pool formation in a self-pollinating crop. Scientific Reports 10 (1):4037. 10.1038/s41598-020-61031-0

Dabholkar A (1999) Elements Of Bio Metrical Genetics (revised And Enlarged Edition). Concept publishing company, New Delhi, India

Damesa TM, Möhring J, Worku M, Piepho HP (2017) One step at a time: Stage-wise analysis of a series of experiments. Agronomy Journal 109 (3):845-857

Dawson IK, Hendre P, Powell W, Sila D, McMullin S, Simons T, Revoredo-Giha C, Odeny DA, Barnes AP, Graudal L, Watson CA, Hoad S, Burnett F, Muchugi A, Roshetko JM, Hale IH, Van Deynze A, Mayes S, Kindt R, Prabhu R, Cheng S, Xu X, Guarino L, Jamnadass R (2018) Supporting human nutrition in Africa through the integration of

new and orphan crops into food systems: placing the work of the African Orphan Crops Consortium in context. ICRAF Working Paper No 276 Nairobi, World Agroforestry Centre http://dx.doi.org/10.5716/WP18003.PDF. http://dx.doi.org/10.5716/WP18003.PDF

Dawson IK, Powell W, Hendre P, Bančič J, Hickey JM, Kindt R, Hoad S, Hale I, Jamnadass R (2019) The role of genetics in mainstreaming the production of new and orphan crops to diversify food systems and support human nutrition. New Phytologist 224 (1):37-54. https://doi.org/10.1111/nph.15895

Dey SS, Singh N, Bhatia R, Parkash C, Chandel C (2014) Genetic combining ability and heterosis for important vitamins and antioxidant pigments in cauliflower (*Brassica oleracea* var. *botrytis* L.). Euphytica 195 (2):169-181. https://doi.org/10.1007/s10681-013-0981-4

Dhillon BS (1990) Estimation of Degree of Dominance and Gene Frequency in Random Mating Populations. Biom J 32 (4):503-512. https://doi.org/10.1002/bimj.4710320414

FAO (2019) The state of the world's biodiversity for food and agriculture. FAO Commission on Genetic Resources for Food and Agriculture Assessments, Rome.

FAO, IFAD, UNICEF, WFP, WHO (2021) The State of Food Security and Nutrition in the World 2021. Transforming food systems for food security, improved nutrition and affordable healthy diets for all. FAO, Rome. https://doi.org/10.4060/cb4474en

Feodorova TA, Voznesenskaya EV, Edwards GE, Roalson EH (2010) Biogeographic patterns of diversification and the origins of C4 in Cleome (Cleomaceae). Syst Bot 35 (4):811-826

Fujimoto R, Uezono K, Ishikura S, Osabe K, Peacock WJ, Dennis ES (2018) Recent research on the mechanism of heterosis is important for crop and vegetable breeding systems. Breed Sci 68 (2):145-158. https://doi.org/10.1270/jsbbs.17155

Gupta PK, Balyan HS, Gahlaut V, Saripalli G, Pal B, Basnet BR, Joshi AK (2019) Hybrid wheat: past, present and future. Theoretical and Applied Genetics 132 (9):2463-2483. 10.1007/s00122-019-03397-y

Hallauer AR, Carena MJ, Miranda Filho Jd (2010) Quantitative genetics in maize breeding, vol 6. Springer Science & Business Media, London

Harrell Jr FE, Dupont C (2021) Hmisc: Harrell Miscellaneous. R package version 4.6-0 edn.,

Hochholdinger F, Baldauf JA (2018) Heterosis in plants. Curr Biol 28 (18):R1089-R1092. https://doi.org/10.1016/j.cub.2018.06.041

Holland JB, Nyquist WE, Cervantes-Martínez CT (2003) Estimating and interpreting heritability for plant breeding: an update. Plant Breed Rev 22:9-112. 10.1002/9780470650202.ch2

Hristov J, Toreti A, Pérez I, Domínguez FD, Fellmann T, Elleby C, Ceglar A, Fumagalli D, Niemeyer S, Cerrani I (2020) Analysis of climate change impacts on EU agriculture by 2050, EUR 30078 EN, Publications Office of the European Union, Luxembourg, 2020, doi:10.2760/121115, JRC119632. Publications Office of the European Union, Luxembourg. Luxembourg. doi:10.2760/121115,JRC119632

Hussain S, Huang J, Huang J, Ahmad S, Nanda S, Anwar S, Shakoor A, Zhu C, Zhu L, Cao X, Jin Q, Zhang J (2020) Rice Production Under Climate Change: Adaptations and Mitigating Strategies. In: Fahad S, Hasanuzzaman M, Alam M et al. (eds) Environment, Climate, Plant and Vegetation Growth. Springer International Publishing, Cham, pp 659-686. doi:10.1007/978-3-030-49732-3_26

Isik F, Holland J, Maltecca C (2017) Genetic data analysis for plant and animal breeding, vol 400. Springer. https://doi.org/10.1007/978-3-319-55177-7

Jamnadass R, Mumm RH, Hale I, Hendre P, Muchugi A, Dawson IK, Powell W, Graudal L, Yana-Shapiro H, Simons AJ, Van Deynze A (2020) Enhancing African orphan crops with genomics. Nat Genet 52 (4):356-360. https://doi.org/10.1038/s41588-020-0601-x

Kamenya SN, Mikwa EO, Song B, Odeny DA (2021) Genetics and breeding for climate change in Orphan crops. Theoretical and Applied Genetics 134 (6):1787-1815. 10.1007/s00122-020-03755-1

Katabuchi M (2015) LeafArea: an R package for rapid digital image analysis of leaf area. Ecol Res 30 (6):1073-1077. 10.1007/s11284-015-1307-x

Kaushik P, Plazas M, Prohens J, Vilanova S, Gramazio P (2018) Diallel genetic analysis for multiple traits in eggplant and assessment of genetic distances for predicting hybrids performance. PLoS ONE 13 (6):e0199943. https://doi.org/10.1371/journal.pone.0199943

Kaushik P, Prohens J, Vilanova S, Gramazio P, Plazas M (2016) Phenotyping of Eggplant Wild Relatives and Interspecific Hybrids with Conventional and Phenomics Descriptors

Provides Insight for Their Potential Utilization in Breeding. Front Plant Sci 7:677. https://doi.org/10.3389/fpls.2016.00677

Kiebre Z, Bationo Kando P, Barro A, Sawadogo B, Kiebre M, Ouedraogo MH, Sawadogo M, Zongo JD (2017) Estimates of genetic parameters of spider plant (*Cleome gynandra* L.) of Burkina Faso. Int J Agric Policy Res 5 (9):138-144. 10.15739/IJAPR.17.016

Kiebre Z, Bationo Kando P, Sawadogo N, Sawadogo M, Zongo J-D (2015) Selection phenotypic interests for the cultivation of the plant *Cleome gynandra* L. in the vegetable garderns in Burkina-Faso. J Exp Biol Agric Sci 3 (3):288-297. 10.18006/2015.3(3).288.297

Kleinknecht K, Möhring J, Singh K, Zaidi P, Atlin G, Piepho H (2013) Comparison of the performance of best linear unbiased estimation and best linear unbiased prediction of genotype effects from zoned Indian maize data. Crop Sci 53 (4):1384-1391. 10.2135/cropsci2013.02.0073

Kulakow P, Jain S (1987) Genetics of grain amaranths : 4. Variation and early generation response to selection in *Amaranthus cruentus* L. Theor Appl Genet 74 (1):113-120. https://doi.org/10.1007/BF00290093

Labroo MR, Studer AJ, Rutkoski JE (2021) Heterosis and Hybrid Crop Breeding: A Multidisciplinary Review. Frontiers in Genetics 12 (234). 10.3389/fgene.2021.643761

Lehmann JW, Clark RL, Frey KJ (1991) Biomass Heterosis and Combining Ability in Interspecific and Intraspecific Matings of Grain Amaranths. Crop Sci 31 (5):1111-1116. https://doi.org/10.2135/cropsci1991.0011183X003100050004x

Lesk C, Rowhani P, Ramankutty N (2016) Influence of extreme weather disasters on global crop production. Nature 529 (7584):84-87. 10.1038/nature16467

Li X, Siddique KHM (2020) Future Smart Food: Harnessing the potential of neglected and underutilized species for Zero Hunger. Maternal & Child Nutrition 16 (S3):e13008. https://doi.org/10.1111/mcn.13008

Lin BB (2011) Resilience in agriculture through crop diversification: adaptive management for environmental change. BioScience 61 (3):183-193

Liu J, Li M, Zhang Q, Wei X, Huang X (2020) Exploring the molecular basis of heterosis for plant breeding. J Integr Plant Biol 62:287-298. https://doi.org/10.1111/jipb.12804

Longin CFH, Gowda M, Mühleisen J, Ebmeyer E, Kazman E, Schachschneider R, Schacht J, Kirchhoff M, Zhao Y, Reif JC (2013) Hybrid wheat: quantitative genetic parameters and consequences for the design of breeding programs. Theor Appl Genet 126 (11):2791-2801. 10.1007/s00122-013-2172-z

Mabhaudhi T, Chimonyo VGP, Hlahla S, Massawe F, Mayes S, Nhamo L, Modi AT (2019) Prospects of orphan crops in climate change. Planta 250 (3):695-708. 10.1007/s00425-019-03129-y

McKenzie FC, Williams J (2015) Sustainable food production: constraints, challenges and choices by 2050. Food Security 7 (2):221-233. 10.1007/s12571-015-0441-1

Mistry CR, Kathiria KB, Sabolu S, Kumar S (2018) Heterosis and inbreeding depression for fruit yield attributing traits in eggplant. Curr Plant Biol 16:27-31. https://doi.org/10.1016/j.cpb.2018.10.004

Molenaar H, Boehm R, Piepho H-P (2018) Phenotypic selection in ornamental breeding: it's better to have the BLUPs than to have the BLUEs. Front Plant Sci 9:1511. 10.3389/fpls.2018.01511

Moodley M, Johnston MA, Hughes JC, Titshall LW (2004) Effects of a water treatment residue, lime, gypsum, and polyacrylamide on the water retention and hydraulic conductivity of two contrasting soils under field conditions in KwaZulu-Natal, South Africa. Soil Research 42 (3):273-282

Moyo M, Aremu AO (2021) Nutritional, phytochemical and diverse health-promoting qualities of *Cleome gynandra*. Crit Rev Food Sci Nutr:1-18. 10.1080/10408398.2020.1867055

Moyo S, Serem J, Bester M, Mavumengwana V, Kayitesi E (2021) African green leafy vegetables health benefits beyond nutrition. Food Reviews International 37 (6):601-618

Myers SS, Smith MR, Guth S, Golden CD, Vaitla B, Mueller ND, Dangour AD, Huybers P (2017) Climate Change and Global Food Systems: Potential Impacts on Food Security and Undernutrition. Annual Review of Public Health 38 (1):259-277. 10.1146/annurev-publhealth-031816-044356

Neugart S, Baldermann S, Ngwene B, Wesonga J, Schreiner M (2017) Indigenous leafy vegetables of Eastern Africa—A source of extraordinary secondary plant metabolites. Food Res Int 100:411-422. 10.1016/j.foodres.2017.02.014

Olivoto T, Lúcio ADC (2020) metan: An R package for multi-environment trial analysis. Methods Ecol Evol 11 (6):783-789. 10.1111/2041-210X.13384

Omondi EO, Debener T, Linde M, Abukutsa-Onyango M, Dinssa FF, Winkelmann T (2017a) Mating biology, nuclear DNA content and genetic diversity in spider plant (*Cleome gynandra*) germplasm from various African countries. Plant Breed 136:578-589. 10.1111/pbr.12485

Omondi EO, Engels C, Nambafu G, Schreiner M, Neugart S, Abukutsa-Onyango M, Winkelmann T (2017b) Nutritional compound analysis and morphological characterization of spider plant (*Cleome gynandra*)-an African indigenous leafy vegetable. Food Res Int 100:284-295. 10.1016/j.foodres.2017.06.050

Onyango CM, Kunyanga CN, Ontita EG, Narla RD, Kimenju JW (2013) Current status on production and utilization of spider plant (*Cleome gynandra* L.) an underutilized leafy vegetable in Kenya. Genet Resour Crop Evol 60:2183-2189. 10.1007/s10722-013-0036-7

Ortiz RR (2015) Plant breeding in the omics era. Springer, Switzerland. https://doi.org/10.1007/978-3-319-20532-8

Padulosi S, King EIO, Hunter D, Swaminathan M (2021) Orphan Crops for Sustainable Food and Nutrition Security: Promoting Neglected and Underutilized Species. Routledge,

Parry MA, Hawkesford MJ (2010) Food security: increasing yield and improving resource use efficiency. Proceedings of the nutrition Society 69 (4):592-600

Patel KS, Patel PJ, Parihar A, Gami RA (2013) Genetics analysis of various quantitative traits in grain amaranth. Crop Improv 40 (2):163-167

Pequeno DNL, Hernández-Ochoa IM, Reynolds M, Sonder K, MoleroMilan A, Robertson RD, Lopes MS, Xiong W, Kropff M, Asseng S (2021) Climate impact and adaptation to heat and drought stress of regional and global wheat production. Environmental Research Letters 16 (5):054070. 10.1088/1748-9326/abd970

Piepho HP, Möhring J, Melchinger AE, Büchse A (2008) BLUP for phenotypic selection in plant breeding and variety testing. Euphytica 161 (1):209-228. 10.1007/s10681-007-9449-8

Qaim M (2020) Role of New Plant Breeding Technologies for Food Security and Sustainable Agricultural Development. Applied Economic Perspectives and Policy 42 (2):129-150. https://doi.org/10.1002/aepp.13044

R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Raimondo M, Nazzaro C, Marotta G, Caracciolo F (2021) Land degradation and climate change: Global impact on wheat yields. Land Degradation & Development 32 (1):387-398. https://doi.org/10.1002/ldr.3699

Raju AS, Rani DS (2016) Reproductive ecology of *Cleome gynandra* and *Cleome viscosa* (Capparaceae). Phytol Balcan 22 (1):15-28

Ram H, Dey SS, Gopala Krishnan S, Kar A, Bhardwaj R, Arun Kumar MB, Kalia P, Sureja AK (2018) Heterosis and Combining Ability for Mineral Nutrients in Snowball Cauliflower (*Brassica oleracea* var. *botrytis* L.) Using Ogura Cytoplasmic Male Sterile Lines. Proc Natl Acad Sci, India, Sect B Biol Sci 88 (4):1367-1376. https://doi.org/10.1007/s40011-017-0874-8

Reeves G, Singh P, Rossberg TA, Sogbohossou EOD, Schranz ME, Hibberd JM (2018) Natural variation within a species for traits underpinning C4 photosynthesis. Plant Physiol 177 (2):504. 10.1104/pp.18.00168

Self SG, Liang K-Y (1987) Asymptotic Properties of Maximum Likelihood Estimators and Likelihood Ratio Tests under Nonstandard Conditions. J Am Stat Assoc 82 (398):605-610. 10.1080/01621459.1987.10478472

Singh BK, Sharma SR, Singh B (2009) Heterosis for mineral elements in single cross-hybrids of cabbage (*Brassica oleracea* var. *capitata* L.). Sci Hortic 122 (1):32-36. https://doi.org/10.1016/j.scienta.2009.04.007

Singh BK, Sharma SR, Singh B (2012) Genetic combining ability for concentration of mineral elements in cabbage head (*Brassica oleracea* var. *capitata* L.). Euphytica 184 (2):265-273. https://doi.org/10.1007/s10681-011-0598-4

Singh RK, Chaudhary BD (1985) Biometrical methods in quantitative genetic analysis. Third edition edn. Kalyani Publishers, New Delhi, India

Singh S, Dey SS, Bhatia R, Kumar R, Sharma K, Behera TK (2019) Heterosis and combining ability in cytoplasmic male sterile and doubled haploid based *Brassica oleracea*

progenies and prediction of heterosis using microsatellites. PLoS ONE 14 (8):e0210772. https://doi.org/10.1371/journal.pone.0210772

So Y-S, Edwards J (2011) Predictive Ability Assessment of Linear Mixed Models in Multienvironment Trials in Corn. Crop Science 51 (2):542-552. https://doi.org/10.2135/cropsci2010.06.0338

Sogbohossou DEO (2019) Orphan no more : Ethnobotany and genetic analysis of leaf yield and secondary metabolites content in *Gynandropsis gynandra* (Cleomaceae). PhD Thesis, Wageningen University, Wageningen

Sogbohossou EOD, Achigan-Dako EG, Maundu P, Solberg S, Deguenon EMS, Mumm RH, Hale I, Van Deynze A, Schranz ME (2018) A roadmap for breeding orphan leafy vegetable species: a case study of *Gynandropsis gynandra* (Cleomaceae). Hortic Res 5 (1):2. 10.1038/s41438-017-0001-2

Sogbohossou EOD, Achigan-Dako EG, Mumm R, de Vos RCH, Schranz ME (2020) Natural variation in specialised metabolites production in the leafy vegetable spider plant (*Gynandropsis gynandra* L. (Briq.)) in Africa and Asia. Phytochemistry 178:112468. 10.1016/j.phytochem.2020.112468

Sogbohossou EOD, Kortekaas D, Achigan-Dako EG, Maundu P, Stoilova T, Van Deynze A, de Vos RCH, Schranz ME (2019) Association between vitamin content, plant morphology and geographical origin in a worldwide collection of the orphan crop *Gynandropsis gynandra* (Cleomaceae). Planta 250:933–947. 10.1007/s00425-019-03142-1

Sohindji FS, Sogbohossou DEO, Zohoungbogbo HPF, Houdegbe CA, Achigan-Dako EG (2020) Understanding Molecular Mechanisms of Seed Dormancy for Improved Germination in Traditional Leafy Vegetables: An Overview. Agronomy 10:57. https://doi.org/10.3390/agronomy10010057

Soil Classification Working Group (1991) Soil Classification: A Taxonomic System for South Africa. Department of Agriculture and Development, Pretoria, South Africa,

Sprague GF, Tatum LA (1942) General vs. Specific Combining Ability in Single Crosses of Corn 1. Agron J 34 (10):923-932. https://doi.org/10.2134/agronj1942.00021962003400100008x

Tanaka N, Niikura S (2006) Genetic analysis of the developmental characteristics related to the earliness of head formation in cabbage (*Brassica oleracea* L.). Breed Sci 56 (2):147-153. https://doi.org/10.1270/jsbbs.56.147

Tesfaye K, Gbegbelegbe S, Cairns JE, Shiferaw B, Prasanna BM, Sonder K, Boote K, Makumbi D, Robertson R (2015) Maize systems under climate change in sub-Saharan Africa. International Journal of Climate Change Strategies and Management 7 (3):247-271. 10.1108/IJCCSM-01-2014-0005

Tian Z, Wang J-W, Li J, Han B (2021) Designing future crops: challenges and strategies for sustainable agriculture. The Plant Journal 105 (5):1165-1178. https://doi.org/10.1111/tpj.15107

Tomlinson I (2013) Doubling food production to feed the 9 billion: a critical perspective on a key discourse of food security in the UK. Journal of rural studies 29:81-90

Ulian T, Diazgranados M, Pironon S, Padulosi S, Liu U, Davies L, Howes MJR, Borrell JS, Ondo I, Pérez-Escobar OA (2020) Unlocking plant resources to support food security and promote sustainable agriculture. Plants, People, Planet 2 (5):421-445

van Dijk M, Morley T, Rau ML, Saghai Y (2021) A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. Nature Food 2 (7):494-501. 10.1038/s43016-021-00322-9

Van Oort PA, Zwart SJ (2018) Impacts of climate change on rice production in Africa and causes of simulated yield changes. Global change biology 24 (3):1029-1045

Vandebroek I, Voeks R (2018) The Gradual Loss of African Indigenous Vegetables in Tropical America: A Review. Econ Bot 72 (4):543-571. 10.1007/s12231-019-09446-3

Wallace JG, Rodgers-Melnick E, Buckler ES (2018) On the Road to Breeding 4.0: Unraveling the Good, the Bad, and the Boring of Crop Quantitative Genomics. Annual Review of Genetics 52 (1):421-444. 10.1146/annurev-genet-120116-024846

Wamm DT, Kadams AM, Jonah PM (2010) Combining ability analysis and heterosis in a diallel cross of okra (*Abelmoschus esculentus* L. Moench). Afr J Agric Res 5 (16):2108-2115

Wiebe K, Lotze-Campen H, Sands R, Tabeau A, van der Mensbrugghe D, Biewald A, Bodirsky B, Islam S, Kavallari A, Mason-D'Croz D, Müller C, Popp A, Robertson R, Robinson S, van Meijl H, Willenbockel D (2015) Climate change impacts on agriculture in 2050

under a range of plausible socioeconomic and emissions scenarios. Environmental Research Letters 10 (8):085010. 10.1088/1748-9326/10/8/085010

Woeste KE, Blanche SB, Moldenhauer KA, Nelson CD (2010) Plant Breeding and Rural Development in the United States. Crop Science 50 (5):1625-1632. https://doi.org/10.2135/cropsci2009.10.0572

Wu T-h, Solberg SO, Yndgaard F, Chou Y-Y (2018) Morphological patterns in a world collection of *Cleome gynandra*. Genet Resour Crop Evol 65 (1):271-283. 10.1007/s10722-017-0529-x

Xie F, Zha J, Tang H, Xu Y, Liu X, Wan Z (2018) Combining ability and heterosis analysis for mineral elements by using cytoplasmic male-sterile systems in non-heading Chinese cabbage (*Brassica rapa*). Crop Pasture Sci 69 (3):296-302. https://doi.org/10.1071/CP17357

Yadav SK, Singh BK, Baranwal DK, Solankey SS (2013) Genetic study of heterosis for yield and quality components in tomato (*Solanum lycopersicum*). Afr J Agric Res 8 (44):5585-5591

Yang R-Y, Keding GB (2009) Nutritional contributions of important African indigenous vegetables. In: Shackleton CM, Pasquini MW, Drescher AW (eds) African Indigenous Vegetables in Urban Agriculture. Earthscan, UK and USA, pp 105-143

Zhao C, Liu B, Piao S, Wang X, Lobell DB, Huang Y, Huang M, Yao Y, Bassu S, Ciais P, Durand J-L, Elliott J, Ewert F, Janssens IA, Li T, Lin E, Liu Q, Martre P, Müller C, Peng S, Peñuelas J, Ruane AC, Wallach D, Wang T, Wu D, Liu Z, Zhu Y, Zhu Z, Asseng S (2017) Temperature increase reduces global yields of major crops in four independent estimates. Proceedings of the National Academy of Sciences 114 (35):9326. 10.1073/pnas.1701762114

Zhou G, Chen Y, Yao W, Zhang C, Xie W, Hua J, Xing Y, Xiao J, Zhang Q (2012) Genetic composition of yield heterosis in an elite rice hybrid. Proc Natl Acad Sci USA 109 (39):15847. https://doi.org/10.1073/pnas.1214141109

Zohoungbogbo HPF, Blalogoe JS, Sogbohossou EDO, Houdegbe CA, Achigan-Dako EG (2018a) Tips for successful crosses in Cleome. Laboratory of Genetics Horticulture and Seed Science, Cotonou, Benin. doi:10.13140/RG.2.2.11977.90723

Zohoungbogbo HPF, Houdegbe CA, Sogbohossou DEO, Tossou MG, Maundu P, Schranz EM, Van Deynze A, Zoundjihekpon J, Achigan-Dako EG (2018b) Andromonoecy in *Gynandropsis gynandra* (L.) Briq. (Cleomaceae) and effects on fruit and seed production. Genet Resour Crop Evol 65 (8):2231–2239. https://doi.org/10.1007/s10722-018-0687-5

Males	Females															
	F1	F10	F11	F12	F13	F14	F15	F16	F2	F3	F4	F5	F6	F7	F8	F9
M1	XY	XY	Y	XY	Y	XY	XY	Y	Y	XY	Y	XY	Y		XY	XY
M10	XY	XY	XY	Y	XY	XY	XY	Y	XY	Y	Y	Y	Y		XY	XY
M11	XY	XY	XY	XY	XY	XY	XY	Y	XY	XY	Y	XY	Y		XY	XY
M12		X	X		X	X	X			X					X	X
M13		Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y
M14	Y	Y	XY	XY	XY	XY	XY	Y	XY	XY	Y	XY	Y		XY	Y
M15	XY	XY	Y	Y	XY	XY	Y	Y	XY	XY	Y	XY	Y		XY	XY
M16	XY	XY	XY	Y	XY	Y	XY	Y	Y	XY	Y	Y	Y	X	Y	XY
M17	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y
M18	Y	Y	XY	XY	XY	XY	XY	Y	XY	XY	Y	XY	Y		XY	Y
M19	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y
M2	XY	Y	XY	XY	XY	XY	XY	Y	XY	XY	Y	XY	Y		XY	XY
M20	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y
M21	XY	Y	Y	XY	XY	XY	XY	Y	XY	Y	Y	Y	Y	X	XY	Y
M22	XY	XY	Y	XY	Y	XY	Y	Y	Y	Y	Y	Y	Y	X	XY	Y
M23	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y
M3	XY	XY	XY	Y	XY	XY	XY	Y	XY	XY	Y	Y	Y		XY	XY
M4		Y	Y	Y	Y	Y	Y		Y	Y	Y	Y	Y		Y	Y
M5		Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y
M6	Y	XY	XY	XY	XY	XY	Y		XY	XY	Y	XY	Y		XY	XY
M7	X	Y	XY	XY	XY		Y	Y	XY	XY	Y	XY	Y		XY	XY
M8		Y	Y	Y	Y	Y			Y	Y	Y	Y	Y		Y	Y
M9		Y	XY	XY	XY	XY	Y	Y	XY	XY	Y	XY	Y		XY	Y

Appendix 6.1. Representation of the North Carolina Design II implemented to generate the 331 unique F1 hybrids used in the present study. A single cross hybrid is represented by X or Y. X, Y and XY refer to single cross hybrids of set1, set2 and both sets, respectively.

CHAPTER 7

Genomic dissection of combining ability and heterosis of biomass yield and related traits in *Gynandropsis gynandra* (L.) Briq.

Abstract

Hybrid vigour or heterosis refers to the better performance of the first filial generation compared to their parents. This was recently reported in *Gynandropsis gynandra* at a significant level, adding the species to the list of potential plants to uncover the genetic mechanism controlling heterosis in plants, which is still unclear. The present study aimed to contribute to an understanding of the genetic basis of heterosis in plants with a focus on dissecting the genomic basis of the combining ability and heterosis level of biomass and related traits in the species using 266 hybrids developed from 38 lines of spider plant. The hybrids and parents were genotyped using diversity array technology sequencing (DArt-seq) technology for single nucleotide polymorphism (SNP) marker identification. We successfully identified 594 high-quality markers that differentiated the 38 lines into three groups linked with the geographical origin of the accession used to develop the lines. Using these 594 SNPs, we identified two markers linked to heterosis level for flowering time, a single marker for edible biomass, one marker for total fresh biomass and one marker for the number of primary branches. Specifically, the marker MABiomLa1 was a pleiotropic marker and was associated with heterosis level for biomass and leaf area. In contrast, no consistent markers were associated with general combining ability and this might be due to the low number of parents and the density of markers used. The identified markers represent an important resource for marker-assisted selection in the species for better exploitation of heterosis. This finding paves the way for further studies on heterosis in the African leafy vegetables.

Keywords: Hybrid vigour, marker-trait association, genetic improvement, marker-assisted selection.

7.1 Introduction

Gynandropsis gynandra (L.) Briq. (syn *Cleome gynandra* L.) is a promising leafy vegetable mostly used by local populations in Africa and Asia. However, its production by local farmers is constrained by poor germination, low yield, early flowering, seed availability, pest and diseases and environmental stresses (Sogbohossou et al. 2018; Kwarteng et al. 2018; Onyango et al. 2013; Abukutsa-Onyango 2007). Increasing the yield potential of the species is required for wider production and is achievable through the development of improved cultivars. This, in turn, requires an understanding of the genetics of the inheritance of target traits. Knowledge of the genetic inheritance of mineral content and biomass and related traits revealed that both additive and nonadditive gene action control these traits with a significant amount of heterosis (Chapter 6). Therefore, hybrid breeding is a promising approach to speed up the productivity of the species, with reciprocal recurrent selection being suggested to maximize hybrid performance and harness hybrid vigour.

Hybrid vigour, also known as heterosis, refers to the better performance of the first filial (F_1) generation compared to their parents (Darwin 1877; Shull 1908; Hochholdinger and Baldauf 2018). This phenomenon is highly researched by breeders to increase crop yield and has been successfully exploited in many crops, including self- and cross-pollinated species (Schnable and Springer 2013). In cross-pollinated crops, heterosis use is easier and contributes to increasing up to 100% of the given species potential. Heterosis has been reported for yield but also for multiple traits in several plant species (Labroo et al. 2021). For instance, heterosis is known for biomass in *Arabidopsis thaliana* (Yang et al. 2017), in vegetable crops (Pearson 1983; Wehner and heterosis 1999), in maize (Fritsche-Neto et al. 2021; Liu et al. 2020a), and in wheat (Longin et al. 2013; Schwarzwälder et al. 2022). However, little is known about this phenomenon in orphan crops and particularly in leafy vegetables. In addition, the molecular mechanism controlling heterosis is still unclear and requires further investigation (Fujimoto et al. 2018; Hochholdinger and Baldauf 2018; Blum 2013; Liu et al. 2020b).

To better exploit heterosis, combining ability has been the genetic factor used by plant breeders. Combining ability refers to the ability of a genotype to transfer its desirable traits to its crosses and includes general combining ability (GCA) and specific combining ability (SCA) (Sprague and Tatum 1942). The general combining ability of a genotype is its average performance in hybrid combinations, while the specific combining ability (SCA) refers to the deviation of a cross performance from the expected performance, which is the sum of average performance

(GCA) of the lines involved (Sprague and Tatum 1942). The GCA effects are attributed to additive gene action, and the SCA effects are associated with nonadditive gene action. General combining ability is important for parental selection, and SCA is important for best cross selection to exploit heterosis (Sprague and Tatum 1942).

Several approaches are available to dissect heterosis and combining ability in plants and include the use of mating designs and molecular tools, which are complementary. Quantitative trait locus (QTL) mapping and genome-wide association studies (GWAS) are common molecular approaches used to decipher the loci and genomic regions controlling heterosis in plants. Populations that have been successfully used for mapping and GWAS include F_2 populations, "immortalized F_2" generated by paired-cross recombinant inbred lines (RILs), cross between RIL populations and the backcross population (RILBC1), chromosome segment substitution lines (CSSLs), residual heterozygosity offspring (RH-F_2), diverse hybrid crosses or F1 lines and multiple-hybrid populations (MHPs) (Liu et al. 2020b). Populations of multiple hybrids have more allelic variation resulting in high mapping resolution due to the presence of more comprehensive heterotic loci compared with the other populations (Liu et al. 2020b). For instance, this population has been used in rice (Huang et al. 2015) and *Arabidopsis thaliana* (Yang et al. 2017) with GWAS. Moreover, Wang et al. (2017) highlighted the theoretical basis for using this type of population in GWAS and recommended the development of the population using various mating designs, including full diallel and North Carolina Design II (NCDII). Furthermore, NCDII was identified as the best training set for predicting hybrids taken from a full diallel design (Fritsche-Neto et al. 2021).

The GWAS is an efficient method to dissect several complex traits and has been implemented in several crops, including rice (Mogga et al. 2018), maize (Yang et al. 2014; Xiao et al. 2017), sorghum (Boyles et al. 2016) and other crops (Liu et al. 2017). It has become the most popular approach to identify genes controlling any trait and has been facilitated by the availability of reference genomes (Xiao et al. 2017). In addition, GWAS has the advantage of covering the entire genome of the species, including genes with minor effects that are mostly difficult to cover in biparental quantitative trait locus (QTL) mapping (Varshney et al. 2014; Burghardt et al. 2017). However, the quality of the GWAS depends on the efficiency of the genotyping and phenotyping systems used. For efficient GWAS, the use of super high density markers is required (Xu et al. 2017). This is resolved by the recent development of next-generation sequencing enabling the availability of several technologies for genotyping, including genotyping by sequencing (GBS), diversity array technology sequencing (DArT-seq),

sequence-based genotyping (SBG), and restriction fragment sequencing (REST-seq) (Rasheed et al. 2017). Among these techniques, GBS and DArT-seq are commonly used (Rasheed et al. 2017).

Diversity Arrays Technology (DArT) is a microarray-based DNA marker technique for genome-wide discovery and genotyping of genetic variation (Wittenberg et al. 2005). It is an inexpensive and high-throughput whole-genome genotyping technique that does not require sequence information (Wittenberg et al. 2005; Wenzl et al. 2004; Rasheed et al. 2017). The DArT-seq is a GBS platform that allows the identification of genome regions associated with active genes (through GWAS and QTL mapping), genomic selection and genetic diversity studies (Schouten et al. 2012; Liu et al. 2017). This technology has been applied for several crops, including "orphan crops" (Kilian et al. 2012), but has not yet been reported in African leafy vegetables. The present study is among the first to use DArT-seq in African leafy vegetables, especially in *Gynandropsis gynandra*.

The present study aims to elucidate the genetic basis of heterosis and combining ability for biomass yield and related traits in *Gynandropsis gynandra* using a panel of diverse hybrid crosses through genome-wide association studies (GWAS) towards the implementation of marker-assisted selection and genomic selection in the species.

7.2 Materials and Methods

7.2.1 Plant material

Thirty-eight parental lines derived from accessions originating from various countries in Asia and Africa (Table 7.1) were used in this study. The parental lines were separated into two groups, 15 and 23 lines used as females and males, respectively, and crossed in a NCDII during two cropping seasons: 2018/2019 (from October 2018 to February 2019) and 2019/2020 (from October 2019 to March 2020) in a greenhouse at the Controlled Environment Facility (29°46′ S, 30°58′ E) of the University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa. Across the two crossing seasons, a total of 266 unique single crosses were generated for evaluation. In addition, each parental accession was self-pollinated during each crossing season. Crosses were performed according to Zohoungbogbo et al. (2018).

Table 7.1. List of advanced lines of *G. gynandra* used as parents to generate the hybrids used in this study and their origin.

Lines	Genebank of the original accession	Country of origin	Continent	Parent
F1	KENRIK	Kenya	Africa	Female
F10	WorldVeg	Thailand	Asia	Female
F11	WorldVeg	Laos	Asia	Female
F12	WorldVeg	Malaysia	Asia	Female
F13	WorldVeg	Malaysia	Asia	Female
F14	WorldVeg	Malaysia	Asia	Female
F15	WorldVeg	Malawi	Africa	Female
F16	WorldVeg	South Africa	Africa	Female
F2	GBioS	Benin	Africa	Female
F3	GBioS	Benin	Africa	Female
F4	GBioS	Benin	Africa	Female
F5	GBioS	Benin	Africa	Female
F6	GBioS	Togo	Africa	Female
F8	WorldVeg	Thailand	Asia	Female
F9	WorldVeg	Laos	Asia	Female
M1	KENRIK	Kenya	Africa	Male
M10	WorldVeg	Kenya	Africa	Male
M11	WorldVeg	Zambia	Africa	Male
M12	WorldVeg	South Africa	Africa	Male
M13	WorldVeg	Malaysia	Asia	Male
M14	WorldVeg	Laos	Asia	Male
M15	WorldVeg	Uganda	Africa	Male
M16	WorldVeg	Uganda	Africa	Male
M17	WorldVeg	Uganda	Africa	Male
M18	WorldVeg	Uganda	Africa	Male
M19	WorldVeg	Malawi	Africa	Male
M2	KENRIK	Kenya	Africa	Male
M20	WorldVeg	Kenya	Africa	Male
M21	WorldVeg	Kenya	Africa	Male
M22	WorldVeg	Zambia	Africa	Male
M23	WorldVeg	Malaysia	Asia	Male
M3	KENRIK	Kenya	Africa	Male
M4	GBioS	Benin	Africa	Male
M5	GBioS	Togo	Africa	Male
M6	GBioS	Togo	Africa	Male
M7	GBioS	Togo	Africa	Male
M8	GBioS	Ghana	Africa	Male
M9	WorldVeg	Laos	Asia	Male

GBioS: Laboratory of Genetics, Horticulture and Seed Science, WorldVeg: World Vegetable Center, KENRIK: Kenya Resource Center for Indigenous Knowledge

7.2.2 Phenotyping

A total of 266 unique single crosses and their 38 parental lines were evaluated across seven environments from 2019 to 2021 in two locations in the Republic of Benin (Abomey-Calavi and Allada) and two locations in South Africa (Greenhouse and Ukulinga research farm). The location and year combination led to a total of seven environments, namely, Ukulinga 2019 (UK2019), Abomey-Calavi (AB2019), Greenhouse 2019 (GH2019), Ukulinga 2020 (UK2020), Greenhouse 2020 (GH2020), Abomey-Calavi 2021 (AB2021) and Allada 2021 (AL2021). The physico-chemical properties of the soil and growing media used are summarized in Table 7.2. The weather conditions during the field conditions are presented in Table 7.3.

Table 7.2. Physico-chemical properties of the soil and growing media used in Abomey-Calavi, Ukulinga research farm and Greenhouse in 2019, 2020 and 2021.

Characteristics	Environments						
	AB2019	UK2019	GH2019	UK2020	GH2020	AB2021	AL2021
Organic carbon (%)	0.56	2.30	2.45	3.6	30.78	0.5	1.1
Nitrogen (%)	0.08	0.18	0.19	0.21	1.10	0.05	0.13
Clay (%)	8.61	39.00	38.50	45.00	-	17.00	14.00
pH (KCl)	5.92	4.32	5.59	4.00	6.0	5.39	5.79
Phosphorus (mg/kg)	304.50	21.74	76.10	18.89	3405.85	29.00	5.00
Potassium (mg/kg)	37.145	227.05	130.24	155.56	2450.92	93.00	42.00
Calcium (mg/kg)	665.00	1552.66	2513.17	484.44	13507.64	440.00	651.00
Magnesium (mg/kg)	77.47	607.73	375.12	132.22	3286.30	107.00	275.00

UK2019: Ukulinga 2019, AB2019: Abomey-Calavi 2019, GH2019: Greenhouse 2019, UK2020: Ukulinga 2020, GH2020: Greenhouse 2020, AB2021: Abomey-Calavi 2021 and AL2021: Allada 2021.

For all experiments, seeds of hybrids and parental lines were preheated at 40°C for 3 days in an oven before planting in seedling trays filled with compost and/or sand. Seedlings were

established in a glasshouse at the University of KwaZulu-Natal and under a shelter at the University of Abomey-Calavi. Hybrids and their parental lines were evaluated using an alpha lattice design with two replicates per environment. Parental lines were planted in a separate trial adjacent to the hybrid trial.

Table 7.3. Weather conditions during the field experiments at Abomey-Calavi, Ukulinga research farm, Allada in 2019, 2020 and 2021.

Locations	Month and year	Total rainfall (mm)	Total evaporation (mm)	Average solar radiation (MJ m^{-2} day^{-1})	Average temperature (°C)	Average relative humidity (%)
Ukulinga						
	March 2019	77.26	100.60	16.22	21.62	78.33
	April 2019	100.33	67.33	11.71	18.75	79.30
	May 2019	17.50	74.17	12.55	18.58	66.67
	June 2019	0.51	65.10	11.28	16.22	53.85
Abomey-Calavi						
	April 2019	84.80	126.19	17.93	29.31	84.36
	May 2019	149.30	113.84	15.91	28.31	84.18
	June 2019	191.60	87.22	14.76	26.68	87.7
	July 2019	50.70	101.33	NA	25.62	88.5
Ukulinga						
	October 2020	42.22	96.76	15.05	19.16	72.35
	November 2020	92.95	113.97	19.34	19.73	79.76
	December 2020	87.62	125.13	20.01	21.68	79.27
	January 2021	139.17	118.99	19.06	21.78	82.05
Abomey-Calavi						
	May 2021	145.03	137.98	21.88	26.75	83.24
	June 2021	124.3	121.58	18.40	26.97	84.00
	July 2021	85.90	117.93	18.05	27.00	83.34
	August 2021	125.1	107.57	16.61	26.58	85.47
Allada						
	June 2021	196.25	NA	12.72	26.19	88.71
	July 2021	98.644	NA	12.44	25.50	89.42
	August 2021	17.94	NA	10.49	25.60	90.08
	September 2021	142.526	NA	10.63	25.53	91.96

Source: IITA-Cotonou, Ukulinga weather Station UKZN-PMB, NA: Not available.

Details on the experimental management and cropping practices in all environments are described in Chapter 6 of this thesis.

Data on six agronomic traits, including time to 50% flowering (DFlow), plant height (PHeight), number of primary branches (NPBr), leaf area (LfArea), total fresh biomass per plant (FBiom), and edible fresh biomass per plant (EDBiom) were collected four weeks after transplanting. The measurements were taken on five randomly selected plants in each plot for field experiments, all five plants per plot for the 2019 greenhouse experiment and two plants per pot for pot experiments. The time to 50% flowering were recorded as the number of days from the sowing date to the day when 50% of the plants in each plot/pot had flowered. A fully developed primary leaf was randomly selected on each plant and scanned using a Canon PIXMA G2411 scanner (Canon INC; Tokyo, Japan). The resultant image was used to calculate leaf area using the R package "*LeafArea*" (Katabuchi 2015). Plant height (cm) was measured from the base to the top of the plant with a tape measure. Each plant was harvested by cutting at a height of 15 cm above the ground, and the resultant biomass was weighed to determine the total fresh biomass per plant (g plant^{-1}). The edible parts of the total biomass were separated and weighed to record the edible fresh biomass per plant. These measurements were taken on two plants out of the three plants per pot and three to five plants per field plot.

7.2.3 Phenotypic data analysis

The software R version 4.1.1 (R Core Team 2021) was used to perform all statistical analyses. A single-stage analysis procedure was used to analyse the data as described by Damesa et al. (2017). This approach is efficient when error, block, and replicate variances are environment-specific (So and Edwards 2011). The quality of the data was assessed for outlier detection following Bernal-Vasquez et al. (2016) using the Bonferroni–Holm test based on studentized residuals at the significance level of 5%. The difference among hybrids was tested using Student's t test or the Wilcoxon signed-rank test, when necessary. Variance components across years were estimated by fitting a linear mixed-effect model using the restricted maximum likelihood (REML) implemented in the ASReml-R package version 4.1.0.160 (Butler et al. 2017) according to the following statistical model:

$$y_{ijkl} = \mu + E_j + R_k(E_j) + B_l[R_k(E_j)] + G_i + GE_{ij} + \varepsilon_{ijkl} \quad (1)$$

where y_{ijkl} is the phenotypic observation of the i^{th} genotype (parent or hybrid) in the l^{th} incomplete block within the k^{th} replicate at the j^{th} environment, μ is the overall mean, E_j is the

random effect of the j^{th} environment, $R_k(E_j)$ is the random effect of the k^{th} replicate within the j^{th} environment, $B_l[R_k(E_j)]$ is the random effect of the l^{th} incomplete block within the k^{th} replicate at the j^{th} environment, G_i is the random effect of the i^{th} genotype (parent or hybrid), GE_{ij} is the random effect of the interaction between the i^{th} genotype (parent or hybrid) and the j^{th} environment, and ε_{ijkl} is the random residual. Heterogeneous variances were assumed for residual, block and replicate effects in different environments. The likelihood ratio test (Self and Liang 1987) was used to test the significance of the variance components using the function *lrt.asreml* implemented in the ASREML-R package. In addition, the phenotypic best linear unbiased predictors (BLUPs) were estimated for parents and hybrids separately from model 1.

Furthermore, the genetic effect of hybrids was partitioned into the general combining ability effect of females (GCA_f), the general combining ability effect of males (GCA_m) and the specific combining ability effect of the cross between females and males (SCA_{fm}). Hence, using the hybrid data only, model 1 was rewritten as follows:

$$y_{fmjkl} = \mu + E_j + R_k(E_j) + B_l[R_k(E_j)] + GCA_f + GCA_m + SCA_{fm} + (GCA:E)_{fj} + (GCA:E)_{mj} + (SCA:E)_{fmj} + \varepsilon_{fmjkl} \quad (2)$$

in which y_{fmjkl} was the phenotypic observation of the hybrid between the f^{th} female and m^{th} male in the l^{th} incomplete block within the k^{th} replicate at the j^{th} environment, μ was the intercept, E_j was the random effect of the j^{th} environment, $R_k(E_j)$ was the random effect of the k^{th} replicate within the j^{th} environment, $B_l[R_k(E_j)]$ was the random effect of the l^{th} incomplete block within the k^{th} replicate at the j^{th} environment, GCA_f was the random GCA effect of the f^{th} female, GCA_m was the random GCA effect of the m^{th} male, SCA_{fm} was the random SCA effect of the cross between the f^{th} female and the m^{th} male, $(GCA:E)_{fj}$ was the random effect of the interaction between the f^{th} female and the j^{th} environment, $(GCA:E)_{mj}$ was the random interaction effect between the GCA of the m^{th} male and the j^{th} environment, $(SCA:E)_{fmj}$ was the random interaction effect between the SCA effect of the cross between the f^{th} female and the m^{th} male and the j^{th} environment, and ε_{fmjkl} was the random residual. Heterogeneous variances were assumed for residual, block and replicate effects in different environments.

Variance components associated with the GCA effects of males and females, SCA effects and their interaction with the environment were estimated from model 2. The likelihood ratio test (Self and Liang 1987) was used to test the significance of the variance components, in which

full and reduced (variance component of interest was removed) models were used by implementing the function *lrt.asreml* in the ASREML-R package. All linear mixed-effects models were fitted in ASReml-R package version 4.1.0.154 (Butler et al. 2017).

For parental lines and hybrids, standard broad-sense heritability (Holland et al. 2003) was calculated as follows:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{G\times E}^2}{n} + \frac{\sigma_e^2}{rn}}$$

where σ_G^2 is the genotypic variance (parental lines or hybrids), $\sigma_{G\times E}^2$ is the genotype × environment interaction variance, σ_e^2 is the residual variance, r is the number of replications, and n is the number of environments.

The general combining ability effects of each female (GCA_f) and male (GCA_m) parent and the specific combining ability effect of each cross (SCA_{fm}) were predicted as the best linear unbiased prediction (BLUPs) from model 2 according to Isik et al. (2017). BLUPs associated with the combining ability effects were used due to the incomplete factorial mating design, unbalanced data across environments and interest in family represented by each parental accession. The advantages of BLUP over BLUE in plant breeding have been highlighted by Piepho et al. (2008). Methods based on BLUP predict genetic effects more accurately by maximizing the correlation between true genotypic and predicted values.

For heterosis analysis, phenotypic best linear unbiased predictors (BLUPs) generated from model 1 of hybrids and their corresponding parents across environments were used. To this end, mid-parent heterosis (MPH) and better-parent heterosis (BPH) were computed for each hybrid as follows:

$$MPH\ (\%) = \frac{F_1 - MP}{MP} \times 100$$

$$BPH\ (\%) = \frac{F_1 - BP}{BP} \times 100$$

where F_1 is the BLUP value of the hybrid, MP is the mid-parent BLUP value computed as the average BLUP value between the two parents of the hybrid, and BP is the BLUP value of the best parent.

7.2.4 Genotyping

Dried seed samples (30-50 seeds) were collected per genotype (hybrid or parental line) and shipped to the SEQART AFRICA (formerly known as Integrated Genotyping Support and Service - IGSS) platform (https://ordering.seqart.net/cgi-bin/order/login.pl) located at the Biosciences East and Central Africa (BeCA) hub in Nairobi (http://hub.africabiosciences.org/activities/services) for genotyping based on DArT-seq GBS Technology (http://www.diversityarrays.com/) for SNP discovery. The genotyping yielded 37617 SNP markers, which were aligned to the draft reference genome (Sogbohossou 2019).

7.2.5 SNP filtering and quality

The dartR package (Gruber et al. 2018) was used to filter and assess the quality of the SNP markers (Gruber et al., 2018). Single nucleotide polymorphism (SNP) markers were filtered by removing markers with missing data > 15%, reproducibility rate < 95%, minor allele frequency (MAF) < 5%, unknown position on the reference genome, and deviation from Hardy-Weinberg equilibrium at a threshold of 5%. The resultant data were imputed using the Ensemble Method algorithm implemented in the "Optimal Imputation V. 1.0.5" plugin on the KDcompute pipeline (https://kdcompute.seqart.net/kdcompute/plugins). This method was used based on scoring the highest simple matching coefficient. The imputed marker data were refiltered using the same criteria as described above. This resulted in 3337 quality markers. Additionally, downstream filtering was performed by removing all markers with heterozygosity for any of the parental lines. Therefore, only 594 markers at the homozygosity level were used in the analysis.

7.2.6 Genetic diversity and analysis of molecular variance

Genetic diversity parameters, including the average observed heterozygosity (Ho), expected heterozygosity (He) and allele frequencies, were estimated using the dartR package for hybrid and parent genotypes (Gruber et al. 2018). The number of clusters/subpopulations obtained from the analysis with STRUCTURE was used in the analysis of molecular variance with the R package *poppr* (Kamvar et al. 2014). Furthermore, a pairwise Fst analysis was conducted to assess the genetic differentiation between clusters using the *pegas* R package (Paradis 2010).

7.2.7 Population structure

The population structure of parental lines was inferred using the Bayesian clustering method implemented in STRUCTURE v2.3.4 software (Pritchard et al. 2000). The settings used were a burn-in period of 10000 and Monte Carlo Markov chain (MCMC) iterations of 10000. Three independent runs with cluster values (K) ranging from 1 to 10 were performed to determine the number of subpopulations. The optimal K-value (number of clusters) was determined based on the Evanno method (Evanno et al. 2005) using the R *pophelper* package (Francis 2017). Analysis of molecular variance (AMOVA) will be used to summarize the divergence between identified clusters (Grzebelus et al. 2014).

7.2.8 Genome-wide association

Genome-wide association analysis was performed based on four models, Bayesian-information and linkage disequilibrium Iteratively Nested Keyway (BLINK), Fixed and random model Circulating Probability Unification (FarmCPU), Mixed Linear Model (MLM), and Multiple Locus Mixed Linear Model (MMLM) algorithms implemented in the R Genomic Association and Prediction Integrated Tool (Version 3) (GAPIT3) (Wang and Zhang 2021). For the association analysis, the best linear unbiased predictor (BLUP) phenotypic values of all 266 hybrids and 38 parents were computed from the across environments analysis and used as phenotypic data. In addition, the molecular data were converted into HapMap format using the "Conversion > DarT to HapMap" plugin on the KDcompute pipeline (https://kdcompute.seqart.net/kdcompute/plugins) for suitability for the analysis. It is important to mention that population structure was considered by setting PCA.total = 3 in the GAPIT3 package during the analysis. Furthermore, the kinship level between genotypes was included in the analysis using the arguments kinship.algorithm = "VanRaden", kinship.cluster = "average", kinship.group = "Mean". The additive relationship or kinship matrix using the molecular data was systematically generated according to VanRaden (2008) and incorporated into the analysis. The Bonferroni-corrected threshold (cut-off) for the p values will be $p = \alpha/n$ (n = number of markers and $\alpha = 0.05$) with a corresponding – log(p)-value (Yang et al. 2014). The cut-off point on the Manhattan plot was -log(p) = 4.28.

7.3 Results

7.3.1 Phenotypic variability of parents and hybrids and combining ability effects

Genotypic (σ_G^2) and genotype × environment interaction variances ($\sigma_{G\times E}^2$) were significant for all traits for both hybrids and parents (Table 7.4). The environmental variances (σ_E^2) were significant for all traits in the parental lines and for all traits in hybrids except the number of primary branches and edible biomass. The partitioning of genotypic variance in hybrids revealed that general combining ability (GCA) variance for males (σ_{GCAm}^2) was significantly different from zero for all agronomic traits. GCA variance for females (σ_{GCAf}^2) was significantly different from zero for most traits except for total and edible fresh biomass. Specific combining ability (SCA) variance (σ_{SCA}^2) was significantly different from zero for plant height, time to 50% flowering, the number of primary branches and leaf area. Additionally, male general combining ability × environment interaction variances ($\sigma_{GCAm\times E}^2$) and female general combining ability × environment interaction variances ($\sigma_{GCAf\times E}^2$) were significantly different from zero for all traits except plant height. Specific combining ability × environment interaction variances ($\sigma_{SCA\times E}^2$) were significantly different from zero for all agronomic traits, except time to 50% flowering. The broad-sense heritability (H^2) was moderate to high for all traits and ranged between 0.35 ± 0.11 (total fresh biomass per plant) and 0.88 ± 0.03 (number of primary branches) for parents and between 0.31 ± 0.06 (edible fresh biomass per plant) and 0.88 ± 0.01 (number of primary branches) (Table 7.4).

7.3.2 Heterosis

Figure 7.1 presents the distributions of mid- and best-parent heterosis (MPH and BPH, respectively) for all fourteen agronomic traits. The distribution showed that heterosis levels followed a normal distribution. The amount of heterosis ranged between -50.95% and 141.89% for all agronomic traits. Both negative and positive heterosis were observed in the species (Figure 7.1). Only positive heterosis effects were observed for leaf area and total fresh biomass, with an average mid-parent heterosis greater than 50%. Although positive and negative heterosis effects were observed, average mid- and best-parent heterosis were negative for time to 50% flowering and number of primary branches, with the latter having a positive average mid-parent heterosis.

Table 7.4. Estimates of genetic variance components, heritability estimates, degree of dominance, predictability ratio, and genetic advance for the fourteen agronomic traits in 38 parents and 266 hybrids of *Gynandropsis gynandra* evaluated across seven environments from 2019 to 2021.

Source variation	PHeight	NPBr	LfArea	FBiom	EDBiom	DFAS
Parents						
σ_G^2	71.31 ± 23.93***	6.03 ± 1.72***	39.48 ± 20.47**	56.37 ± 26.17***	31.05 ± 13.12***	34.60 ± 11.71***
σ_E^2	359.92 ± 212.85***	2.52 ± 1.82***	159.63 ± 98.14***	1024.15 ± 623.48***	90.71 ± 56.61***	119.15 ± 77.34***
$\sigma_{G\times E}^2$	75.65 ± 15.62***	3.83 ± 0.63***	116.92 ± 22.65***	83.55 ± 27.34***	72.68 ± 12.97***	37.29 ± 7.66***
σ_e^2	79.24 ± 20.38	4.03 ± 1.82	245.29 ± 22.65	1329.42 ± 197.48	97.83 ± 21.13	29.50 ± 8.34
H^2	0.81 ± 0.06	0.88 ± 0.03	0.65 ± 0.13	0.35 ± 0.11	0.64 ± 0.10	0.80 ± 0.06
Hybrids						
σ_G^2	58.52 ± 8.42***	2.67 ± 0.29***	70.52 ± 10.94***	204.17 ± 46.42***	26.25 ± 6.50***	8.67 ± 1.09***
σ_{GCAf}^2	8.01 ± 5.15*	1.44 ± 0.6***	9.04 ± 6.26*	28.08 ± 35.35ns	3.24 ± 3.76ns	6.52 ± 2.93***
σ_{GCAm}^2	33.26 ± 11.82***	0.84 ± 0.29***	44.29 ± 16.67***	146.71 ± 66.43***	22.63 ± 10.32***	3.87 ± 1.44***
σ_{SCA}^2	19.14 ± 5.04***	0.42 ± 0.09***	21.09 ± 6.15***	23.63 ± 23.27ns	4.54 ± 4.05ns	1.71 ± 0.43***
σ_E^2	415.29 ± 263.87*	0.05 ± 0.07ns	235.23 ± 137.97**	1891.09 ± 1196.78***	131.07 ± 86.81ns	91.71 ± 59.39**
$\sigma_{G\times E}^2$	95.34 ± 7.30***	1.58 ± 0.13***	122.51 ± 10.38***	628.78 ± 62.26***	103.62 ± 9.79***	3.12 ± 0.79***
$\sigma_{GCAm\times E}^2$	3.66 ± 2.83ns	0.54 ± 0.12***	4.43 ± 2.78***	502.50 ± 354.91***	39.98 ± 28.09***	4.17 ± 1.02***
$\sigma_{GCAf\times E}^2$	8.15 ± 5.03ns	0.21 ± 0.06***	24.75 ± 6.70***	194.58 ± 52.78***	32.16 ± 8.40***	1.74 ± 0.54***
$\sigma_{SCA\times E}^2$	79.47 ± 7.28***	0.9 ± 0.11***	87.92 ± 8.93***	317.73 ± 42.11***	67.23 ± 7.70***	0.78 ± 0.53ns
σ_e^2	102.91 ± 5.20	1.91 ± 0.09	145.38 ± 9.28	5010.39 ± 287.16	597.61 ± 36.55	36.02 ± 1.96
H^2	0.74 ± 0.03	0.88 ± 0.01	0.72 ± 0.04	0.31 ± 0.05	0.31 ± 0.06	0.71 ± 0.03

StDiam: stem diameter (mm), PHeight: plant height (cm), PBrLeng: primary branch length (cm), NPBr: number of primary branches, CtLleng: central leaflet length (cm), CtLwid: central leaflet width (cm), Lwid: leaf width (cm), Ptillen: petiole length (cm), LfArea: leaf area (cm^2), FBiom: total fresh biomass per plant (g), EDBiom: edible fresh biomass per plant (g), HI: harvest index, DM: dry matter content (%), DFlow: time to 50% flowering (days), σ_e^2 = residual variance, σ_G^2 = genotypic variance, σ_E^2 = environmental variance, $\sigma_{G\times E}^2$ = genotype × environment variance, σ_{GCAm}^2 = male general combining ability variance, σ_{GCAf}^2 = female general combining ability variance, σ_{SCA}^2 = specific combining ability variance, $\sigma_{GCAf\times E}^2$ = female general combining ability × environment variance, $\sigma_{GCAm\times E}^2$ = male general combining ability × environment variance, $\sigma_{SCA\times E}^2$ = specific combining ability × environment variance, H^2 = broad-sense heritability. ***, **, * = significantly different from zero at the 0.001, 0.01, and 0.05 probability levels, respectively. *ns* = not significantly different from zero at the 0.05 level of probability.

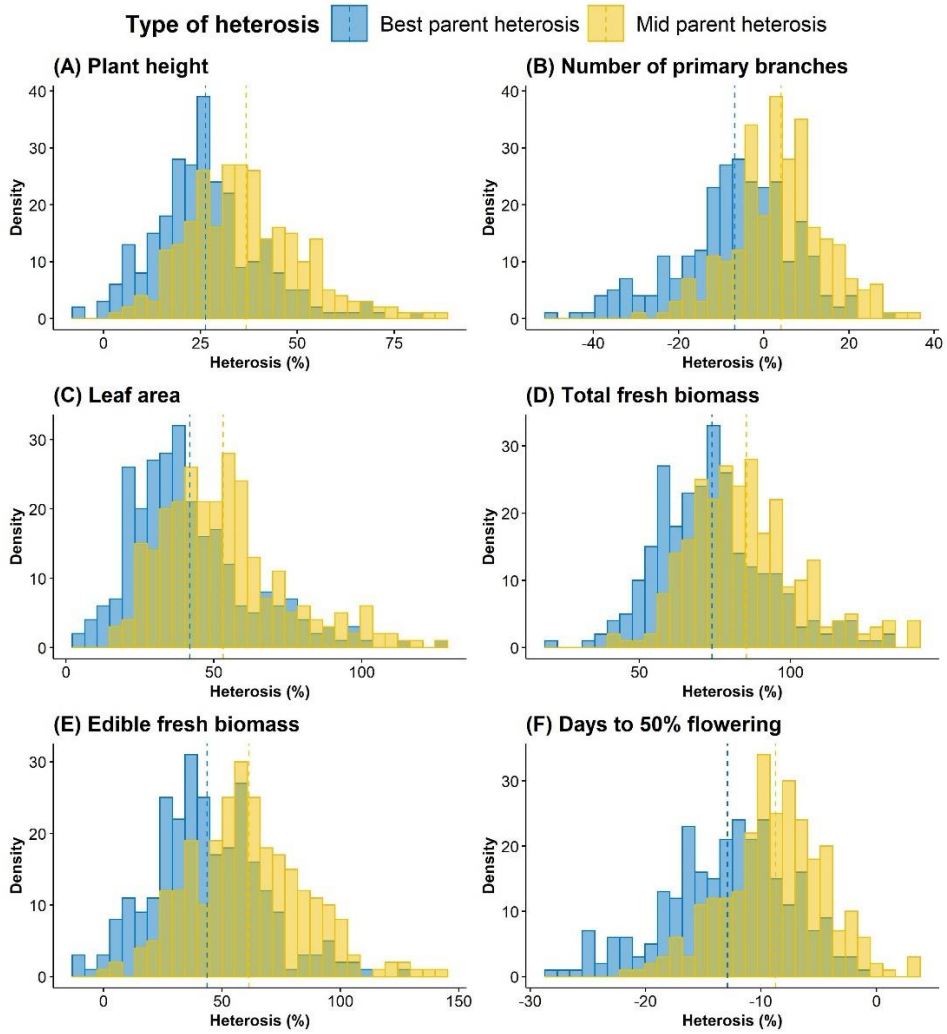

Figure 7.1. Distribution of mid- and best-parent heterosis for six agronomic traits in *Gynandropsis gynandra*.

7.3.3 General combining ability effects of parental lines

Estimates of the general combining ability (GCA) effects of parents are presented in Figure 7.2. Both significant and positive GCA effects were observed for all six agronomic traits but were trait specific. More importantly, the general combining ability effects were observed to follow a normal distribution.

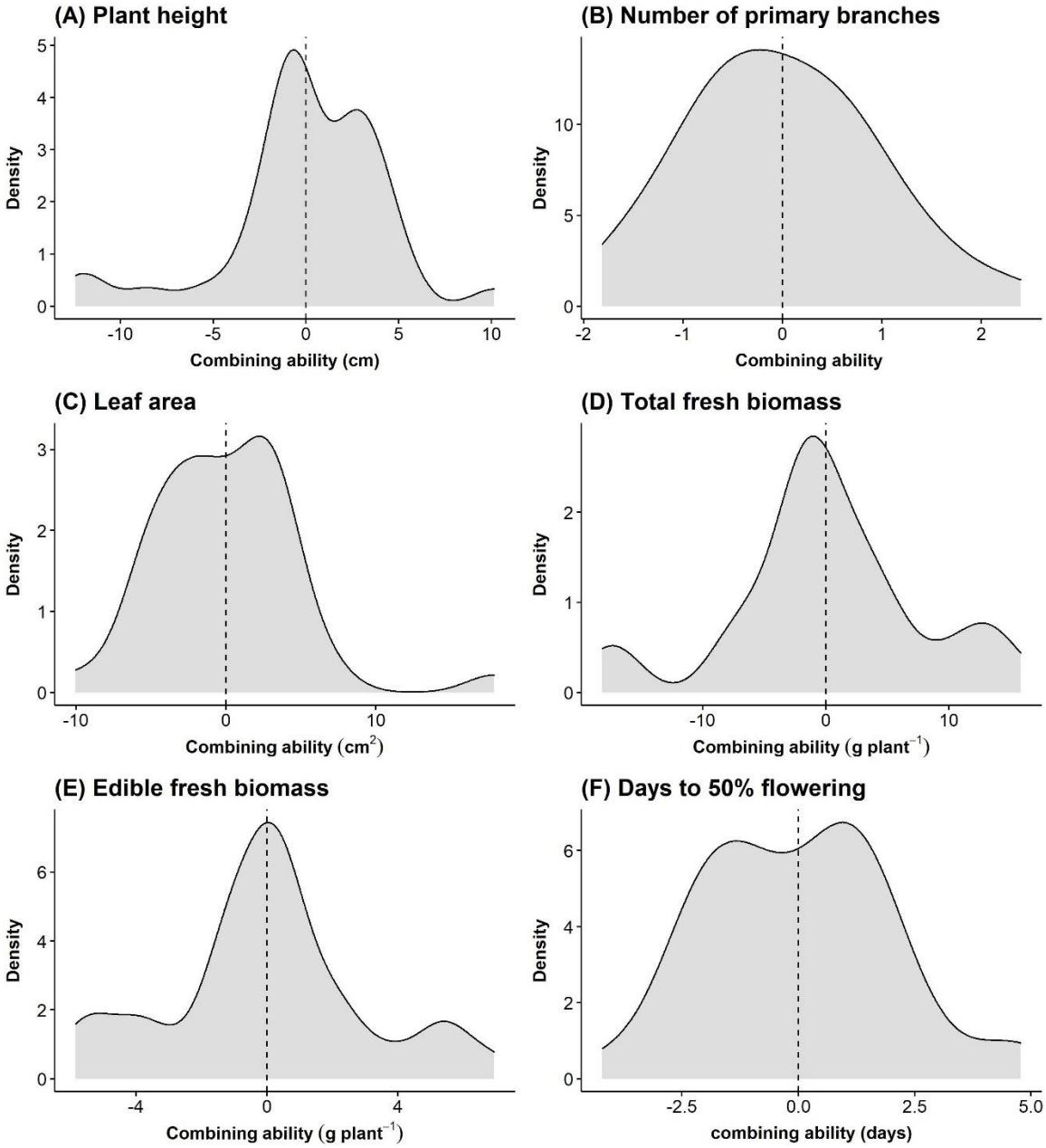

Figure 7.2. Distribution of general combining ability for six agronomic traits in 38 parental lines of *Gynandropsis gynandra*.

7.3.4 Characteristics of the SNP markers

The 594 SNP markers were distributed across the seventeen linkage groups of *Gynandropsis gynandra* (Figure 7.3). The density per linkage group was low and ranged from 13 in linkage group 3 to 69 in linkage group 4. The markers were characterized by adenosine (27.94%), guanine (23.23%), thymine (26.57%) and cytosine (22.26%). The transition and transversion mutation percentage rates revealed that transitions (57.74%) were higher than transversion

mutations (42.26%) with a transversions/transitions ratio of 1.36. As only homozygote markers were selected, the observed heterozygosity was 0.00 for parents and 0.36 in hybrids and lower than the expected heterozygosity of 0.01 for parents and 0.42 for hybrids.

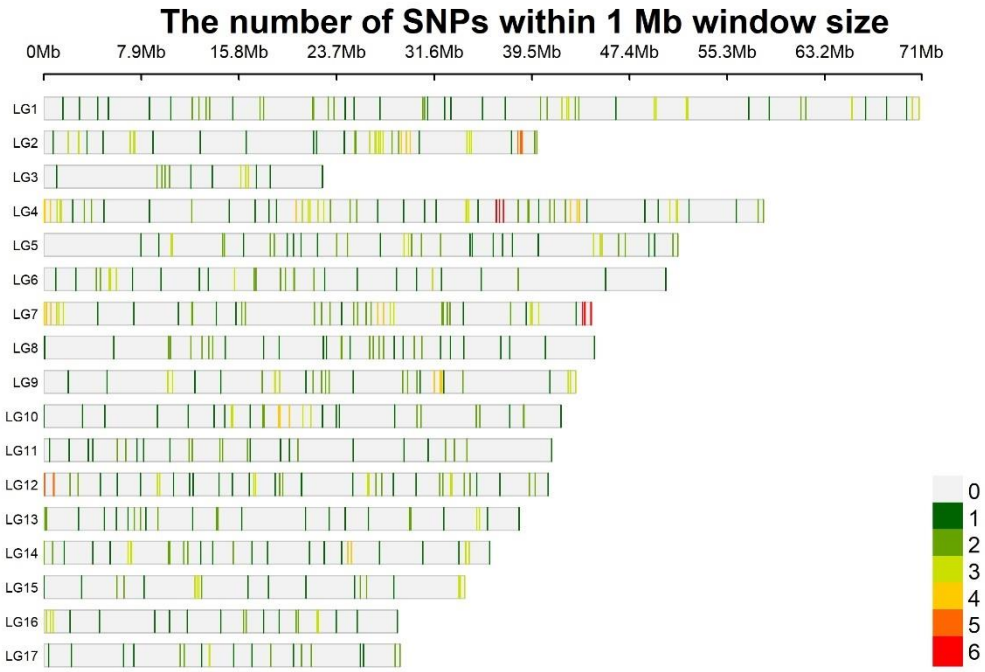

Figure 7.3. Distribution of the 594 SNP markers across the linkage groups.

7.3.5 Population structure and genetic differentiation of parental lines

The structuration of the parental lines was assessed using STRUCTURE software. Based on the Evanno method (Evanno et al., 2005), the optimal K-value (number of clusters) is 3, corresponding to the pick of the delta K values (Figure 7.4). Therefore, the parental lines in the present study were clustered into three groups. The three groups were associated with the geographical provenance of the original accession from which the lines were derived (Figure 7.5). Populations 1, 2 and 3 were Asia (blue), East and Southern Africa (red) and West Africa (light blue) (Figure 7.4). Furthermore, the molecular analysis of variance showed that 97.64% of the variation in the 38 parental lines was due to differences among populations, while only a few were attributable to differences among individuals within populations (Table 7.5). The pairwise Fst values showed a significant differentiation among the three populations (Table

7.6). Nei's genetic distance among the three groups varied from 0.55 to 1.30. A low value was observed between Asian and West African lines, showing the relatedness of the two clusters.

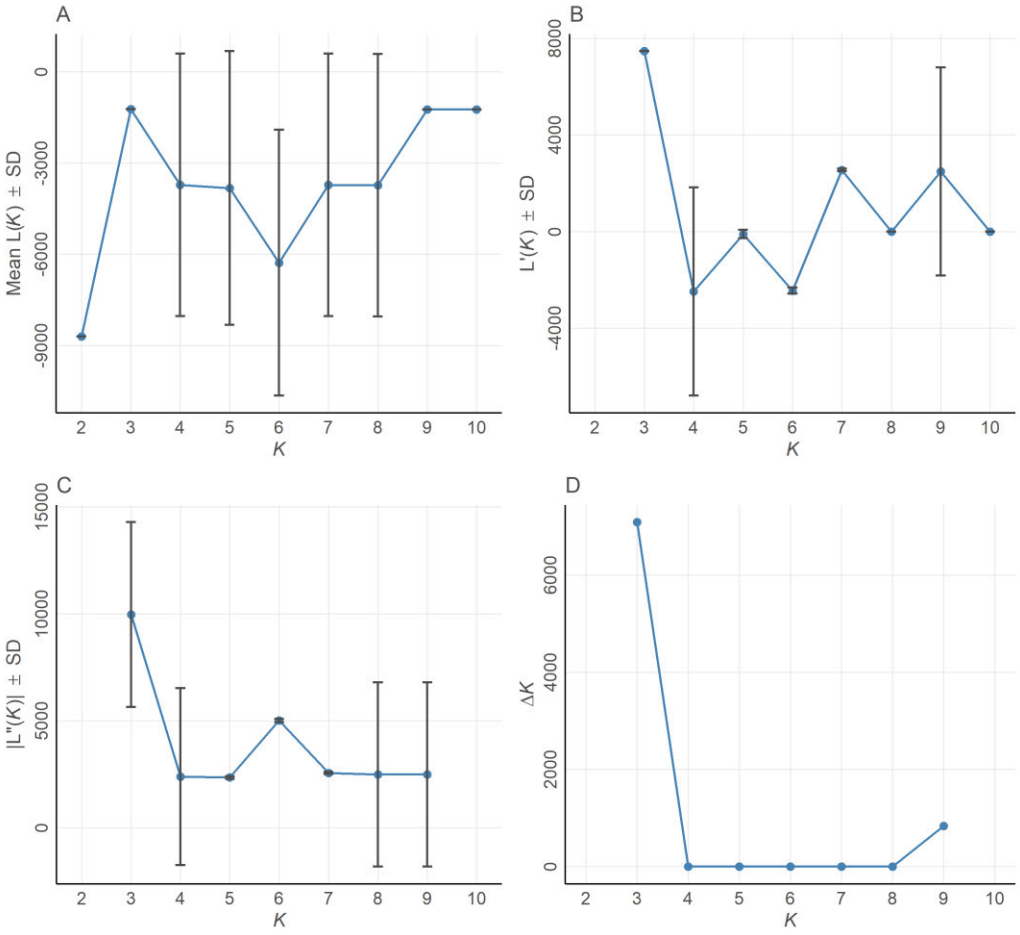

Figure 7.4. Output of Evanno analysis. (A) Estimated log probability of data of runs over increasing values of K, (B) first derivative, (C) second derivative and (D) Delta K over values of K.

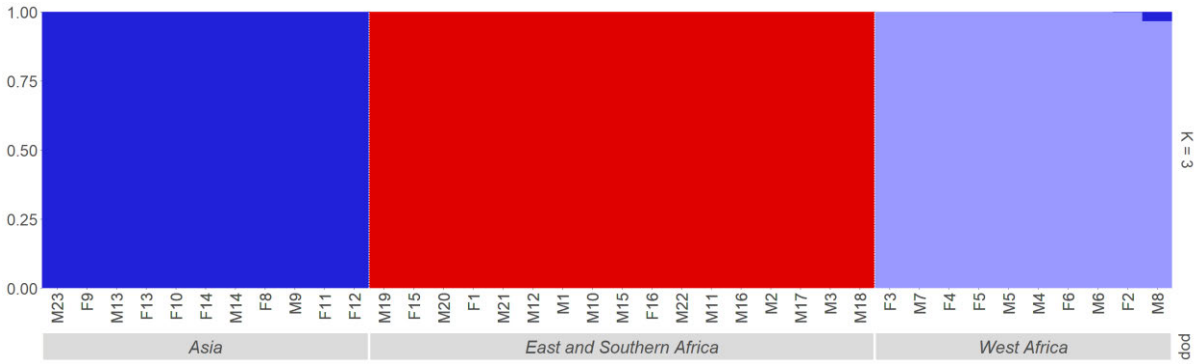

Figure 7.5. Population structure of 38 parental lines of *Gynandropsis gynandra* based on 594 SNP markers.

Table 7.5. Molecular analysis of variance among the clusters of *Gynandropsis gynandra*

Source of variation	Df	SS	MS	Variance	%	Pvalue
Between populations	2	9262.44	4631.22	188.05	97.64	**
Between samples within populations	35	317.34	9.06	4.53	2.35	ns
Within samples	38	0	0	0	0	ns
Total	75	9579.79	127.73	192.58	100	

Table 7.6. Pairwise Fst (below the diagonal) and Nei's genetic distance (above diagonal) analysis among clusters of *Gynandropsis gynandra*

Regions/Clusters	Asia	West Africa	East and Southern Africa
Asia	-	0.551	1.184
West Africa	0.977***	-	1.309
East and Southern Africa	0.977***	0.975***	-

7.3.6 Genome-wide association studies of heterosis

The genome-wide association analysis on mid- and best-parent heterosis identified several markers associated with the number of primary branches, time to 50% flowering, total and edible fresh biomass, and leaf area (Table 7.7). The four models yielded various markers associated with the level of heterosis in the species (Figures 7.6-7.14). FarmCPU and BLINK depicted more SNP markers than MMLM and MLM, which yielded similar results (Figures 7.6-7.14). For the sake of false SNP discovery, we only considered consistent markers across the four tested models. Consequently, two markers were associated with best parent heterosis (BPH) (Figure 7.6) and one for mid-parent heterosis (MPH) for flowering, a single marker associated with MPH for edible biomass (Figure 7.8), one marker for total fresh biomass, and one marker for both BPH and MPH for the number of primary branches (Figures 7.13-7.14).

7.3.7 Genome-wide association studies of general combining ability

No consistent markers associated with combining ability were observed for general combining ability across the four models used and might be due to the low number of parents and the density of markers used.

Table 7.7. Significant markers associated with heterosis level for time to 50% flowering, total and edible fresh biomass, leaf area and number of primary branches in *Gynandropsis gynandra*

Traits	**Code**	**SNP**	**Linkage group**	**Position**	**P value**	**maf**	**FDR Adjusted P values**	**Effect**
BPH_DFAS	MAFlow1	100414092|F|0-57:G>A-57:G>A	LG 14	750156	4E-06	0.27	0.001759	-4.88
BPH_DFAS	MAFlow1	100135756|F|0-6:G>A-6:G>A	LG 15	12554118	5.92E-06	0.06	0.001759	-5.04
MPH_DFAS	MAFlow1	100135756|F|0-6:G>A-6:G>A	LG 15	12554118	3.80E-05	0.06	0.022571	-3.42
MPH_EDBiom	MABiomLa1	100449774|F|0-52:G>A-52:G>A	LG 13	35901810	6.16E-05	0.07	0.036604	-17.88
MPH_FBiom	MABiom2	100548217|F|0-11:G>T-11:G>T	LG 1	12581138	7.76E-05	0.08	0.046072	13.76
BPH_LfArea	MABiomLa1	100449774|F|0-52:G>A-52:G>A	LG 13	35901810	5.62E-06	0.07	0.003338	-13.22
MPH_NPBr	MANBrch1	100245623|F|0-56:C>T-56:C>T	LG 4	36613451	1.57E-06	0.05	0.000933	13.23
BPH_NPBr	MANBrch1	100245623|F|0-56:C>T-56:C>T	LG 4	36613451	1.74E-05	0.05	0.010365	14.04

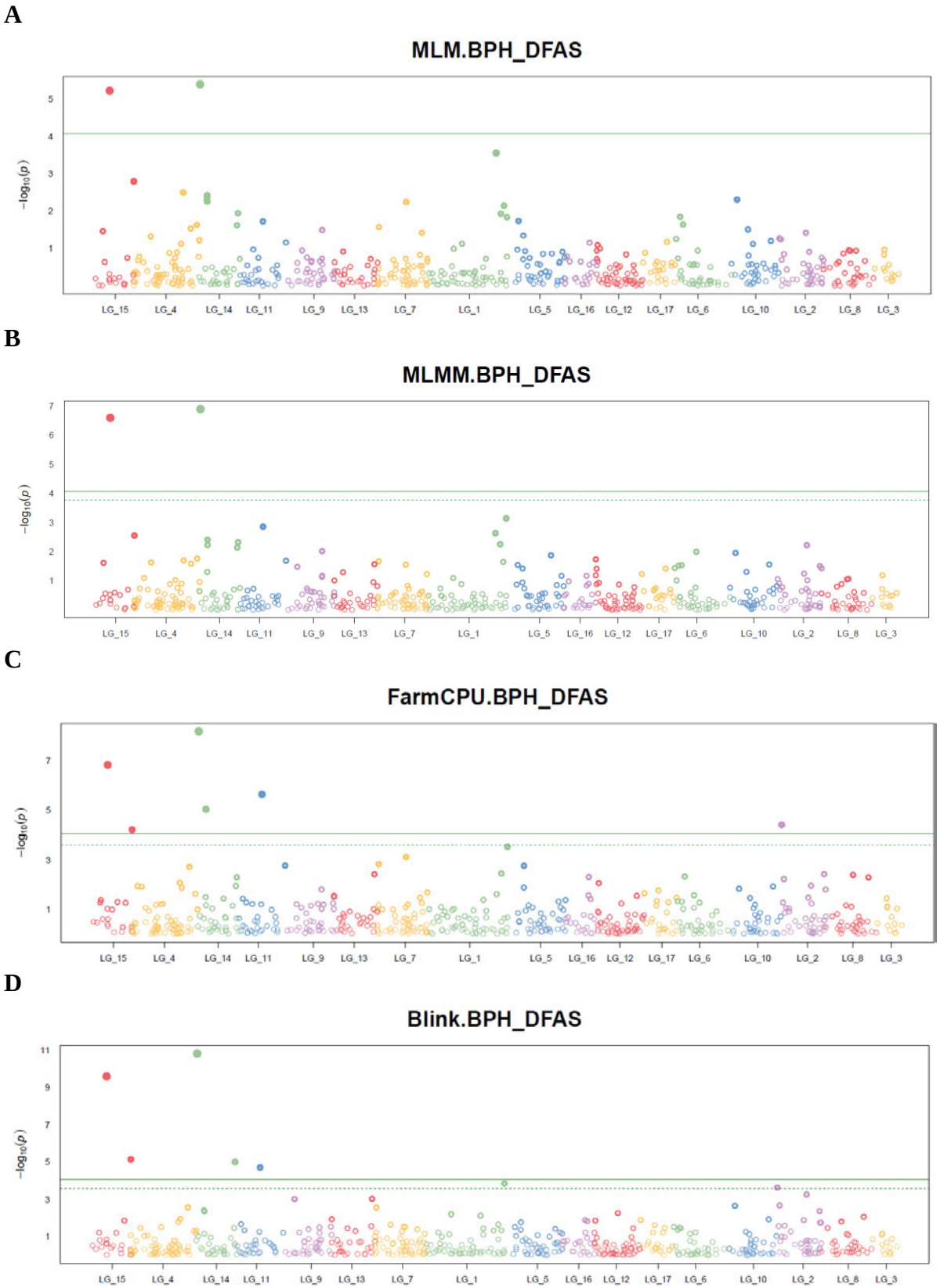

Figure 7.6. Genome association analysis of best-parent heterosis for flowering time in 266 hybrids of *Gynandropsis gynandra* using (A) MLN, (B) MMLM, (C) FarmCPU and (D) BLINK algorithm of GAPIT.

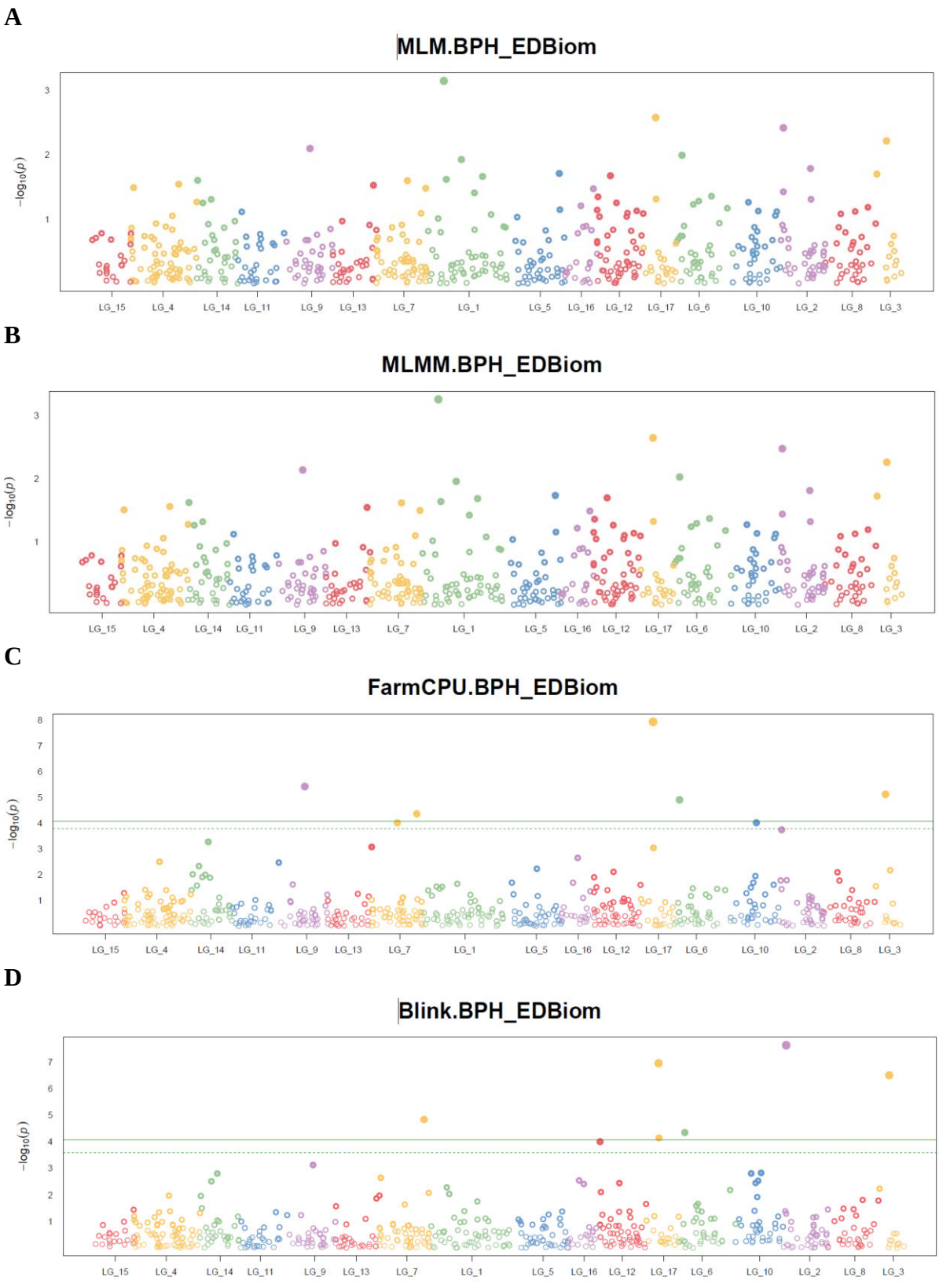

Figure 7.7. Genome association analysis of best-parent heterosis for edible fresh biomass in 266 hybrids of *Gynandropsis gynandra* using (A) MLN, (B) MMLM, (C) FarmCPU and (D) BLINK algorithm of GAPIT.

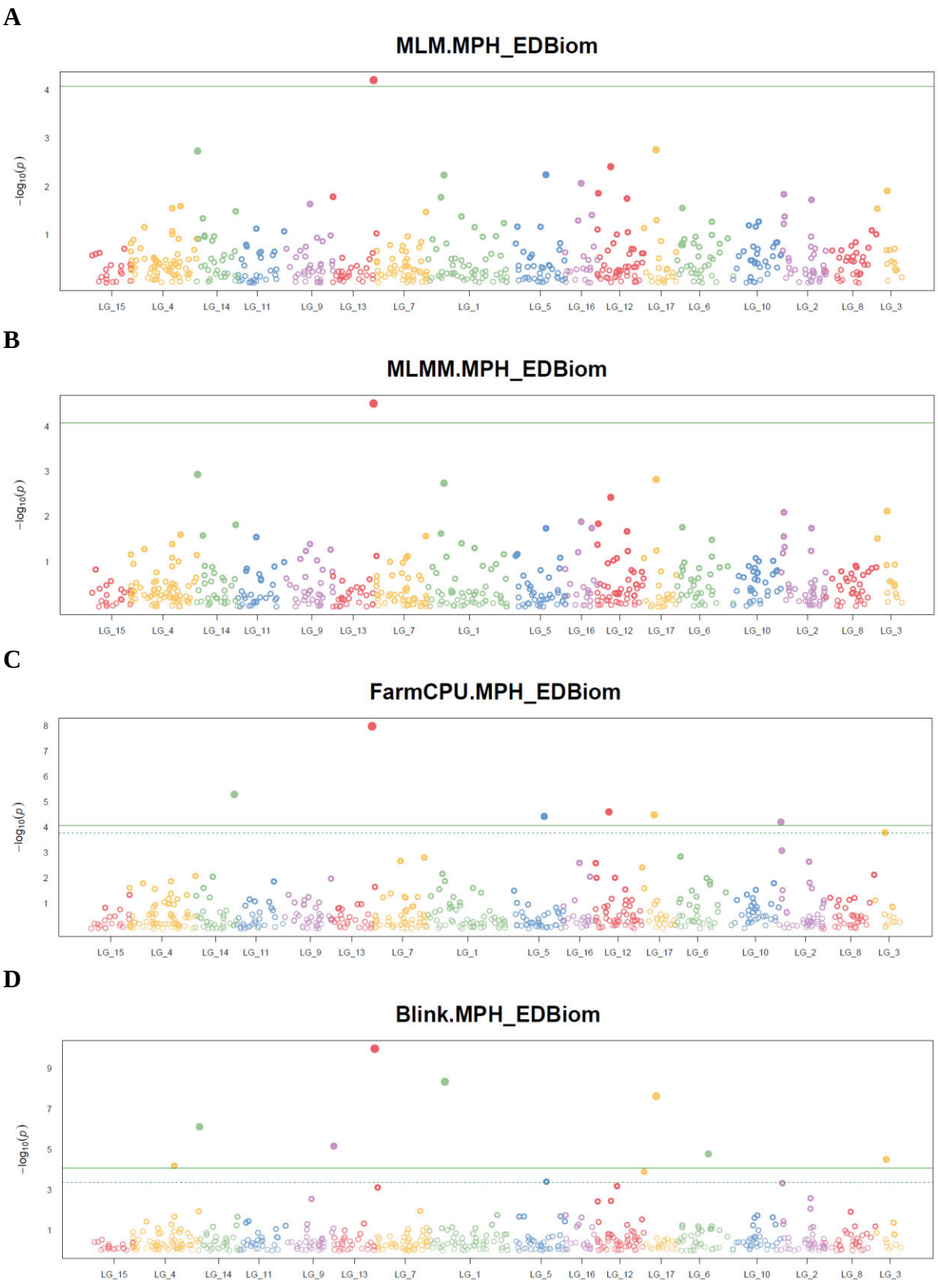

Figure 7.8. Genome association analysis of mid-parent heterosis for edible fresh biomass in 266 hybrids of *Gynandropsis gynandra* using (A) MLN, (B) MMLM, (C) FarmCPU and (D) BLINK algorithm of GAPIT.

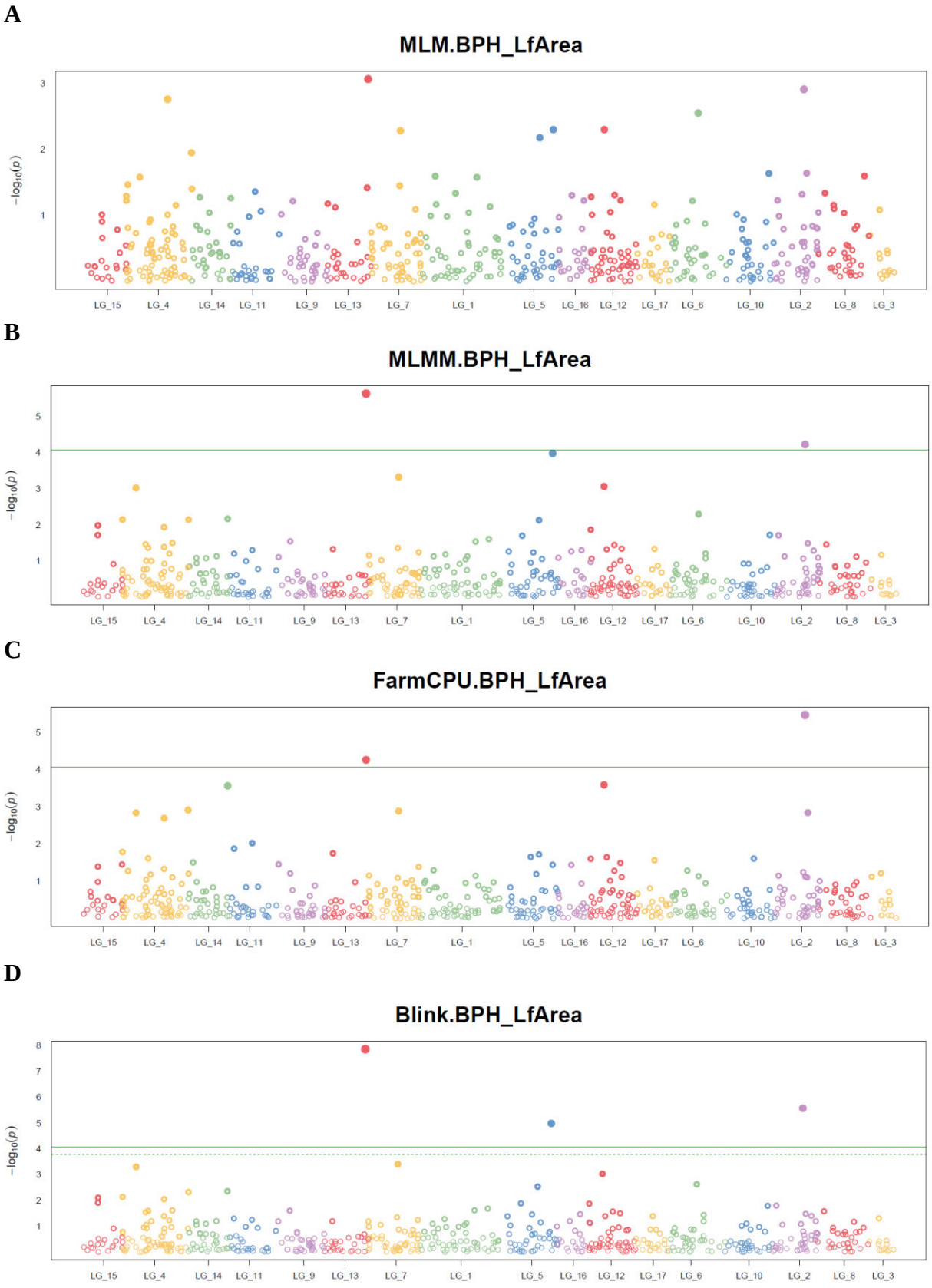

Figure 7.9. Genome association analysis of best-parent heterosis for leaf area in 266 hybrids of *Gynandropsis gynandra* using (A) MLN, (B) MMLM, (C) FarmCPU and (D) BLINK algorithm of GAPIT.

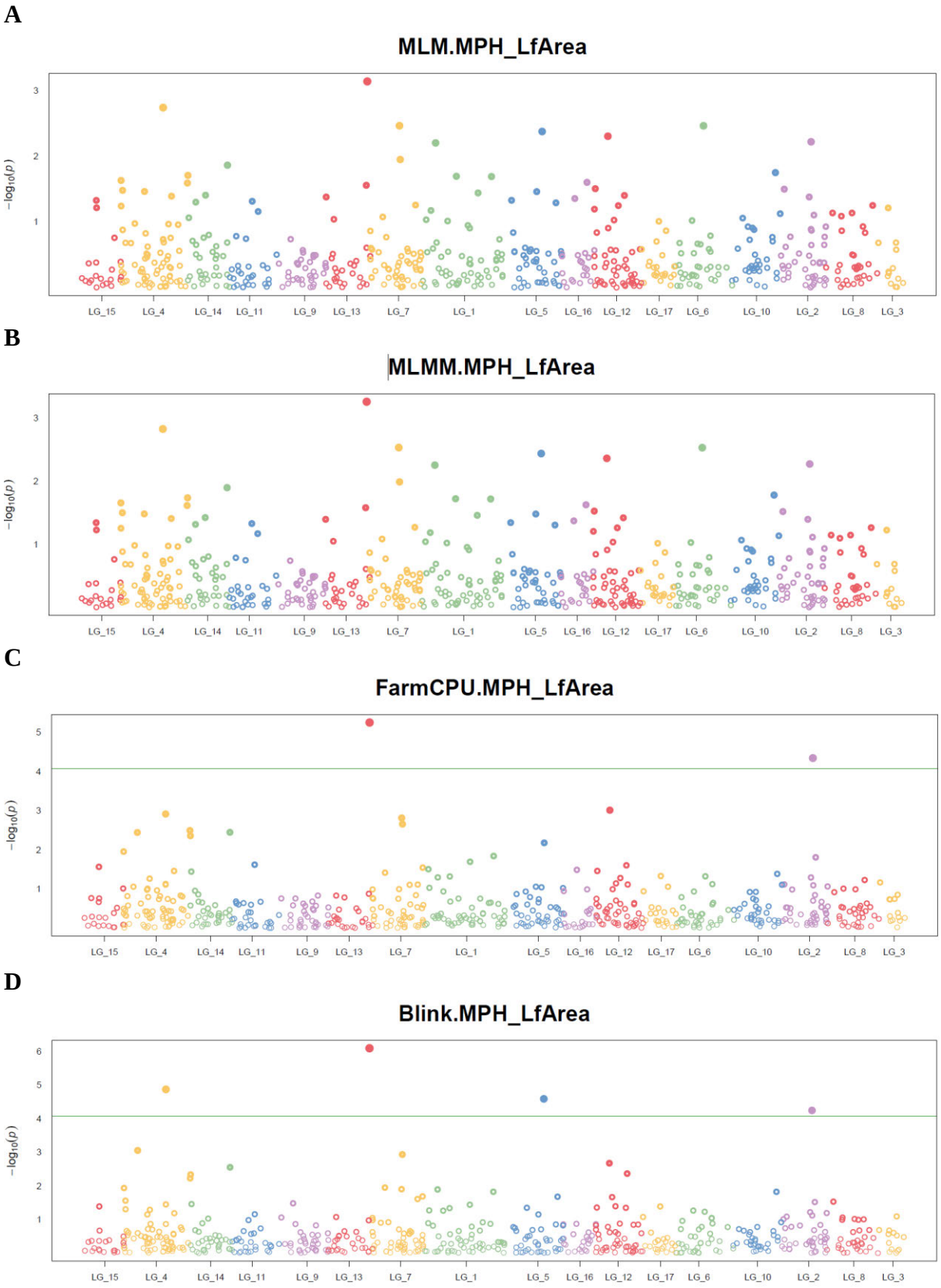

Figure 7.10. Genome association analysis of mid-parent heterosis for leaf area in 266 hybrids of *Gynandropsis gynandra* using (A) MLN, (B) MMLM, (C) FarmCPU and (D) BLINK algorithm of GAPIT.

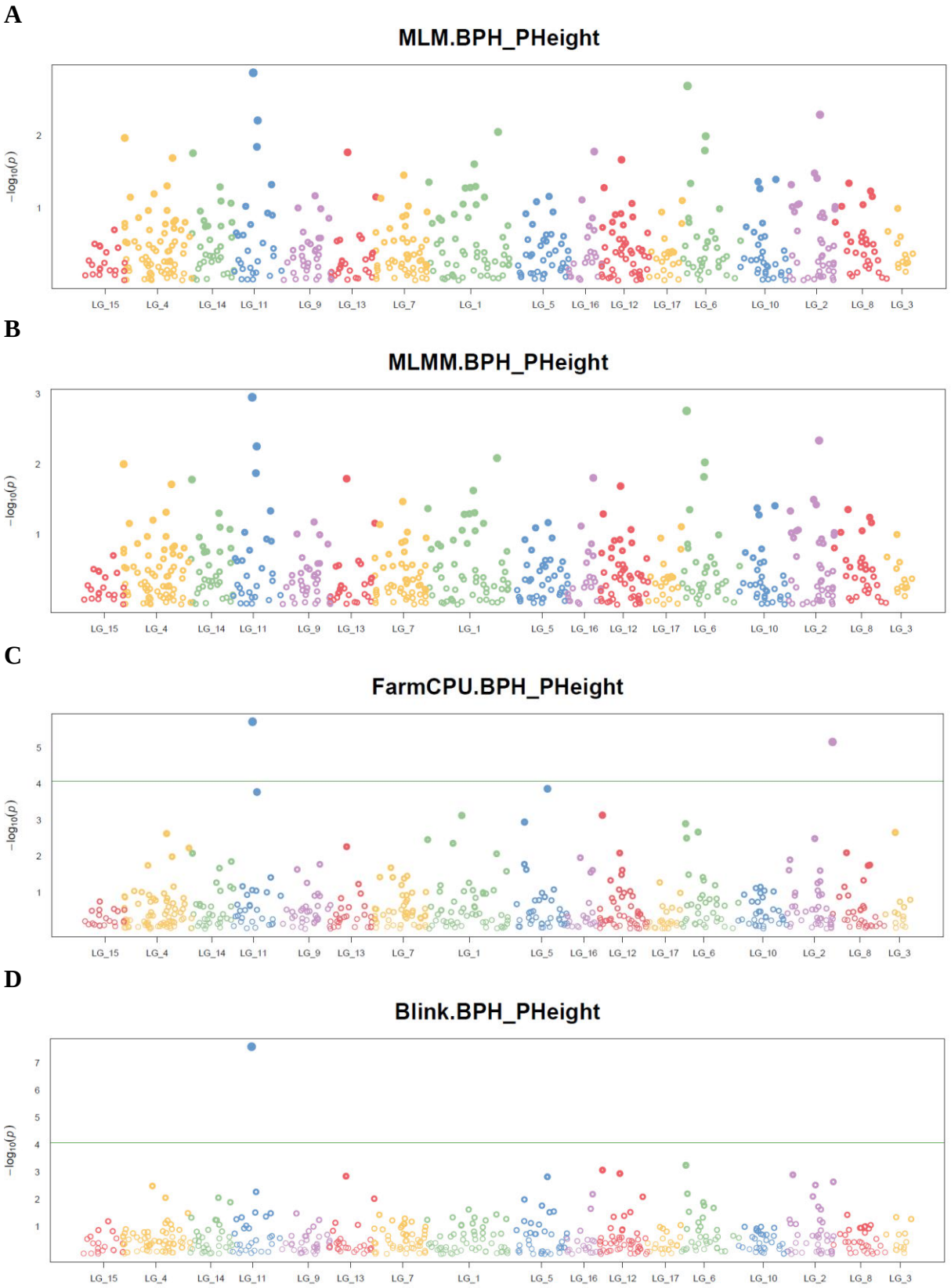

Figure 7.11. Genome association analysis of best-parent heterosis for plant height in 266 hybrids of *Gynandropsis gynandra* using (A) MLN, (B) MMLM, (C) FarmCPU and (D) BLINK algorithm of GAPIT.

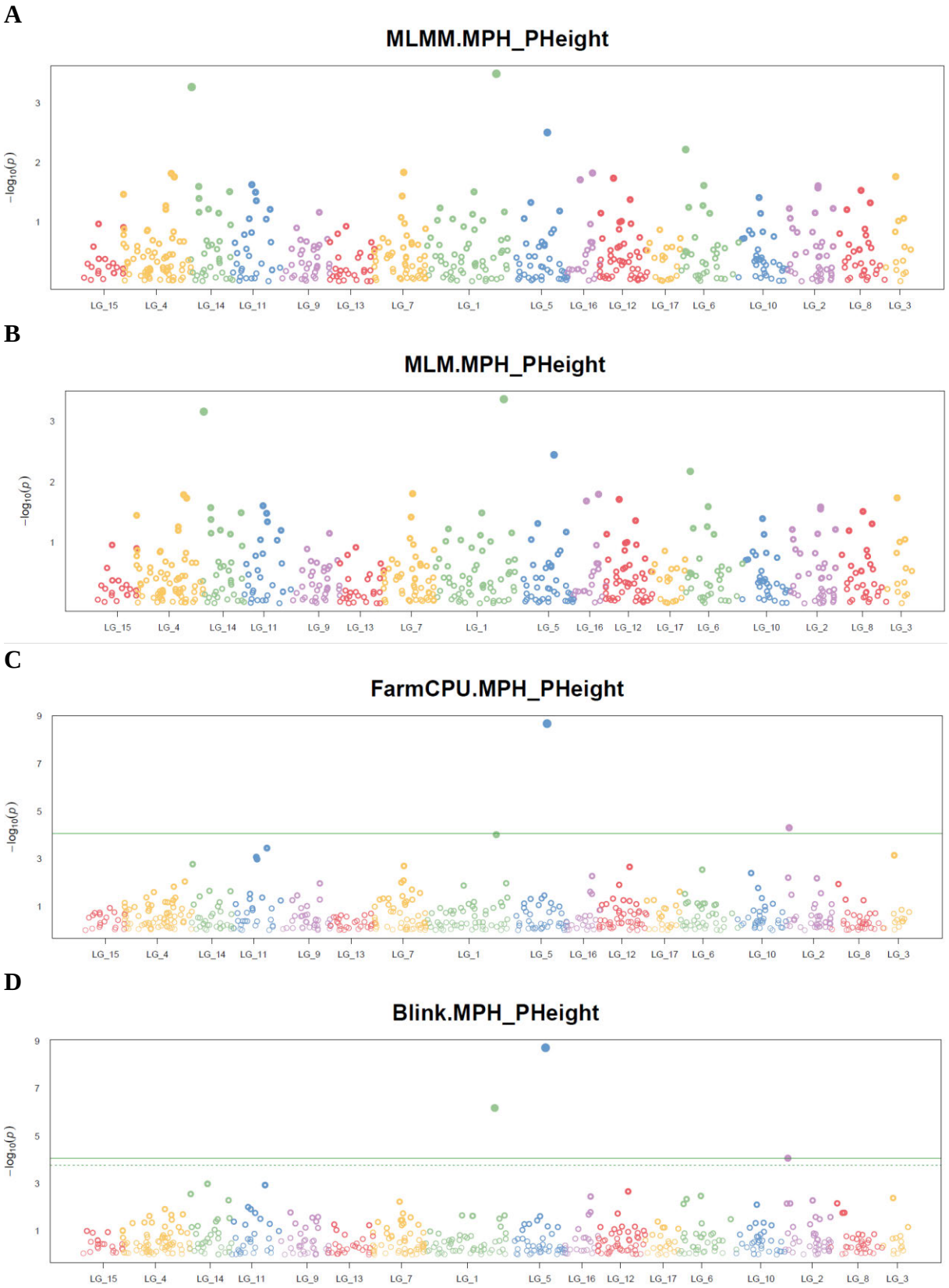

Figure 7.12. Genome association analysis of mid-parent heterosis for plant height in 266 hybrids of *Gynandropsis gynandra* using (A) MLN, (B) MMLM, (C) FarmCPU and (D) BLINK algorithm of GAPIT.

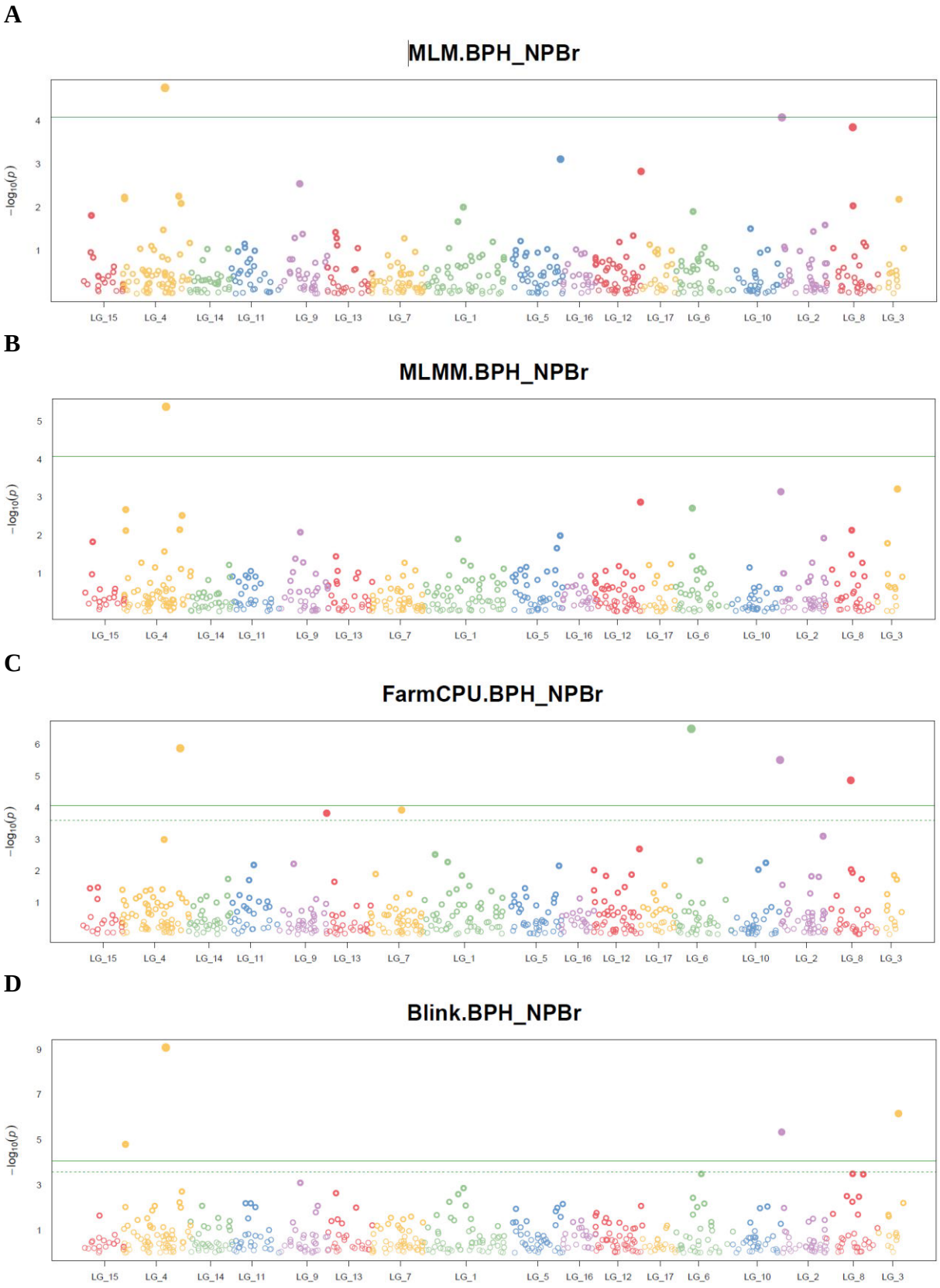

Figure 7.13. Genome association analysis of best-parent heterosis for the number of primary branches in 266 hybrids of *Gynandropsis gynandra* using (A) MLN, (B) MMLM, (C) FarmCPU and (D) BLINK algorithm of GAPIT.

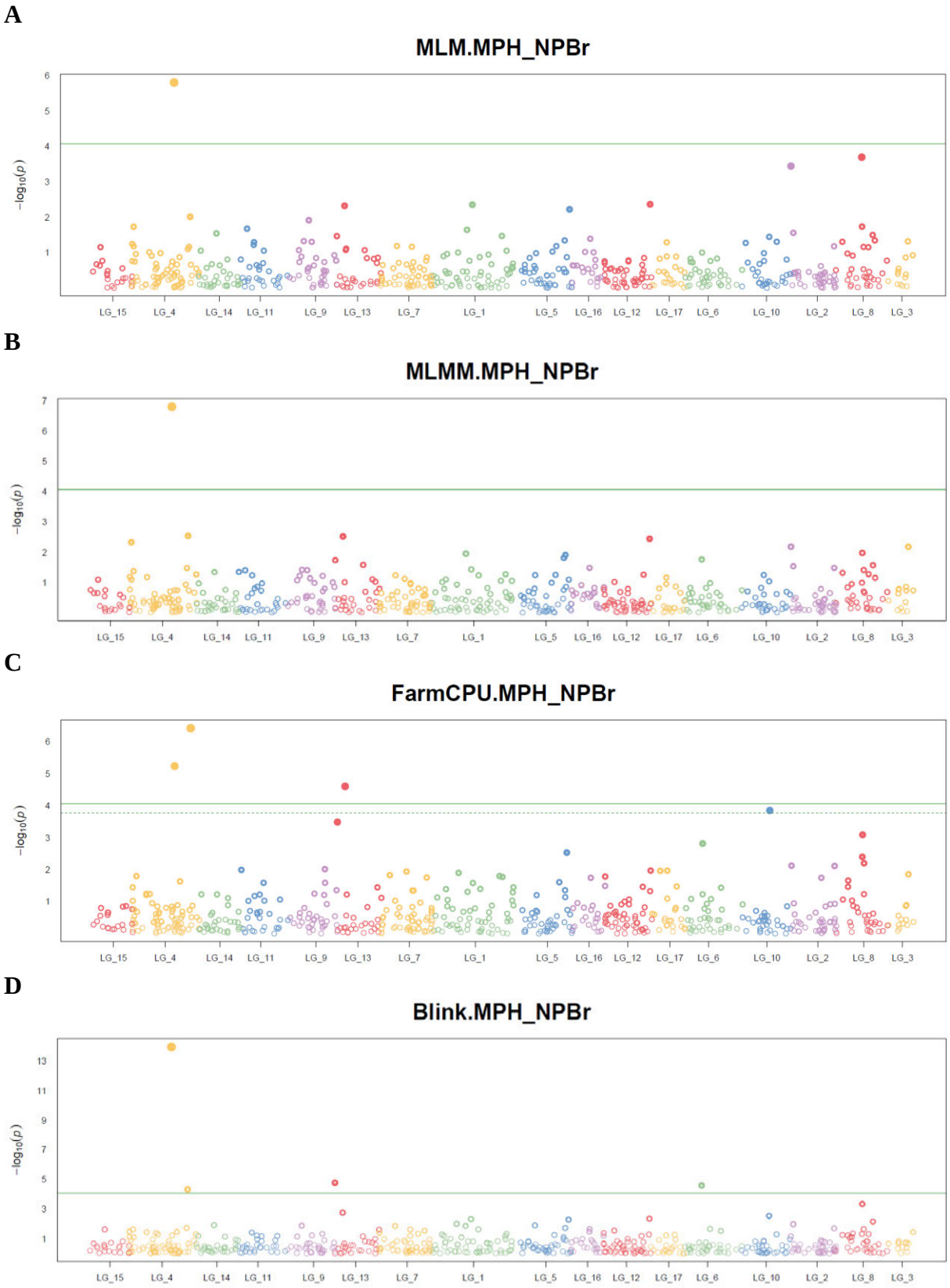

Figure 7.14. Genome association analysis of mid-parent heterosis for the number of primary branches in 266 hybrids of *Gynandropsis gynandra* using (A) MLN, (B) MMLM, (C) FarmCPU and (D) BLINK algorithm of GAPIT.

7.4 Discussion

7.4.1 Genetic diversity among advanced lines of *Gynandropsis gynandra*

The 38 parental lines were clustered into three groups associated with geographical origin. The three groups were the Asian, East/Southern, and West African groups. This aligned with previous reports on genome differentiation among accessions of *G. gynandra* (Sogbohossou 2019). Sogbohossou (2019) used a set of 752 305 SNPs to depict genome-wide differentiation among the accessions. The subset of 594 DArT-SNP markers used in the present study represents a key step towards the development of the core set of markers that can be used in the rapid assessment of genetic diversity among accessions of *G. gynandra*. These markers can be used in establishing KASP markers for diversity analysis. On the other hand, local adaptation could have shaped the observed genetic structure in the species and therefore determined the performance of the genotypes. The relatedness observed between Asian and West African genotypes may explain their grouping in the same group for mineral content, showing that genes controlling these traits were shared between the population. Additionally, low genetic flow among these populations might also be attributable to limited exchange among local communities and the existence of physical barriers for natural seed dissemination. The 594 markers represent a good set of markers to understand genes controlling various traits in the species.

7.4.2 Genome-wide association with heterosis in *Gynandropsis gynandra*

We identified eight markers associated with heterosis levels in *G. gynandra*. Similarly, markers associated with the level of heterosis were also reported in several plant species, such as *Arabidopsis* (Yang et al. 2017), maize (Liu et al. 2020c), rice (Huang et al. 2015; Lin et al. 2020; Yang et al. 2021), and cotton (Sarfraz et al. 2021). Some of these markers had positive effects, while others had negative effects. More importantly, MABiomLa1 in linkage group 13 was associated with mid-parent heterosis for edible fresh biomass and best-parent heterosis in spider plant, showing its pleiotropic nature. The effect of this marker was negative for both traits, supporting the positive association observed between leaf area and biomass productivity in the species and the fact that biomass and leaf area were the only traits displaying positive heterosis in the species. We also observed that several markers were associated with both mid- and best heterosis. This was the case for MANBrch1 and MAFlow1 for the number of primary branches and time to 50% flowering, respectively. In contrast, we did not identify markers

associated with combining ability, which might be due to the low number of parental lines used in the present study. Therefore, developing and "immortalized F2", recombinant inbred lines (RILs) and backcross populations will be useful.

Overall, the genes behind the identified markers were not identified as the reference genome was still under development. In addition, we did not identify the genes and their functions associated with these markers, which is required to fully understand their roles. This could be done through integrated approach combining phenomics, genomics and transcriptomics as used by Li et al. (2016) in rice. Linkage disequilibrium of the markers was not assessed and is ongoing. Also, multi-traits GWAS on the six traits could be investigated and are being assessed. The current analysis was performed across environments, and future analysis will be then performed per environment to decipher potential marker × environment interaction effects. The identified markers need to be validated using different populations. To this end, multiple populations including F_2 populations, "immortalized F_2" by paired-cross recombinant inbred lines (RILs), cross between RIL populations and the backcross population (RILBC1), chromosome segment substitution lines (CSSLs), residual heterozygosity offspring (RH-F_2), new diverse hybrid crosses or F1 lines and multiple-hybrid populations (MHPs) (Liu et al. 2020b) could be generated. Specifically, efforts are ongoing to develop MAGIC populations, to assemble natural populations and to develop new diverse hybrid crosses or F1 lines and multiple-hybrid populations using new lines and diallel mating design. Additionally, assessing the potential influence of the observed clustering on heterosis expression in the species could play a key role in deciphering genes associated with heterosis and contribute to uncovering the genetic mechanism controlling heterosis in plant species.

7.5 Conclusion

The present study confirmed at the molecular level the existence of three groups among the advanced lines based on biomass and related traits. Additionally, the subset of 594 markers used is powerful in revealing the genetic diversity among genotypes of spide plants and will further narrow to establish fast and cost-effective markers for genetic diversity studies. We also identified eight markers associated with the amount of heterosis in the species for flowering time, total and edible biomass, leaf area and number of primary branches. A pleiotropic marker MABiomLa1 was identified and affected both edible biomass and leaf area with negative effects. This study paves way for further studies on heterosis in the species as well as the identification of the extent of environmental influence on heterosis expression in the species.

References

Abukutsa-Onyango MO (2007) The diversity of cultivated African leafy vegetables in three communities in western Kenya. Afr J Food Agric Nutr Dev 7 (3):1-15. 10.18697/ajfand.14.IPGRI1-3

Bernal-Vasquez A-M, Utz H-F, Piepho H-P (2016) Outlier detection methods for generalized lattices: a case study on the transition from ANOVA to REML. Theor Appl Genet 129 (4):787-804. 10.1007/s00122-016-2666-6

Blum A (2013) Heterosis, stress, and the environment: a possible road map towards the general improvement of crop yield. Journal of experimental botany 64 (16):4829-4837

Boyles RE, Cooper EA, Myers MT, Brenton Z, Rauh BL, Morris GP, Kresovich S (2016) Genome-wide association studies of grain yield components in diverse sorghum germplasm. The plant genome 9 (2)

Burghardt LT, Young ND, Tiffin P (2017) A Guide to Genome-Wide Association Mapping in Plants. Current Protocols in Plant Biology:22-38

Butler D, Cullis B, Gilmour A, Gogel B, Thompson R (2017) ASReml-R Reference Manual Version 4. . VSN International Ltd Hemel Hempstead, HP1 1ES. UK

Damesa TM, Möhring J, Worku M, Piepho HP (2017) One step at a time: Stage-wise analysis of a series of experiments. Agronomy Journal 109 (3):845-857

Darwin C (1877) The effects of cross and self fertilisation in the vegetable kingdom. D. Appleton,

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular ecology 14 (8):2611-2620

Francis RM (2017) pophelper: an R package and web app to analyse and visualize population structure. Molecular Ecology Resources 17 (1):27-32. https://doi.org/10.1111/1755-0998.12509

Fritsche-Neto R, Galli G, Borges KLR, Costa-Neto G, Alves FC, Sabadin F, Lyra DH, Morais PPP, Braatz de Andrade LR, Granato I, Crossa J (2021) Optimizing Genomic-Enabled Prediction in Small-Scale Maize Hybrid Breeding Programs: A Roadmap Review. Frontiers in Plant Science 12. 10.3389/fpls.2021.658267

Fujimoto R, Uezono K, Ishikura S, Osabe K, Peacock WJ, Dennis ES (2018) Recent research on the mechanism of heterosis is important for crop and vegetable breeding systems. Breeding science 68:145-158

Gruber B, Unmack PJ, Berry OF, Georges A (2018) dartr: An r package to facilitate analysis of SNP data generated from reduced representation genome sequencing. Molecular Ecology Resources 18 (3):691-699. https://doi.org/10.1111/1755-0998.12745

Grzebelus D, Iorizzo M, Senalik D, Ellison S, Cavagnaro P, Macko-Podgorni A, Heller-Uszynska K, Kilian A, Nothnagel T, Allender C, Simon PW, Baranski R (2014) Diversity, genetic mapping, and signatures of domestication in the carrot (Daucus carota L.) genome, as revealed by Diversity Arrays Technology (DArT) markers. Molecular Breeding 33 (3):625-637. 10.1007/s11032-013-9979-9

Hochholdinger F, Baldauf JA (2018) Heterosis in plants. Curr Biol 28 (18):R1089-R1092. https://doi.org/10.1016/j.cub.2018.06.041

Holland JB, Nyquist WE, Cervantes-Martínez CT (2003) Estimating and interpreting heritability for plant breeding: an update. Plant Breed Rev 22:9-112. 10.1002/9780470650202.ch2

Huang X, Yang S, Gong J, Zhao Y, Feng Q, Gong H, Li W, Zhan Q, Cheng B, Xia J, Chen N, Hao Z, Liu K, Zhu C, Huang T, Zhao Q, Zhang L, Fan D, Zhou C, Lu Y, Weng Q, Wang Z-X, Li J, Han B (2015) Genomic analysis of hybrid rice varieties reveals numerous superior alleles that contribute to heterosis. Nat Commun 6:6258. 10.1038/ncomms7258. https://www.nature.com/articles/ncomms7258#supplementary-information

Isik F, Holland J, Maltecca C (2017) Genetic data analysis for plant and animal breeding, vol 400. Springer. https://doi.org/10.1007/978-3-319-55177-7

Kamvar ZN, Tabima JF, Grünwald NJ (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281. 10.7717/peerj.281

Katabuchi M (2015) LeafArea: an R package for rapid digital image analysis of leaf area. Ecol Res 30 (6):1073-1077. 10.1007/s11284-015-1307-x

Kilian A, Wenzl P, Huttner E, Carling J, Xia L, Blois H, Caig V, Heller-Uszynska K, Jaccoud D, Hopper C, Aschenbrenner-Kilian M, Evers M, Peng K, Cayla C, Hok P, Uszynski

G (2012) Diversity Arrays Technology: A Generic Genome Profiling Technology on Open Platforms. In: Pompanon F, Bonin A (eds) Data Production and Analysis in Population Genomics: Methods and Protocols. Humana Press, Totowa, NJ, pp 67-89. doi:10.1007/978-1-61779-870-2_5

Kwarteng AO, Abogoom J, Adu Amoah R, Nyadanu D, Ghunney T, Nyam KC, Ziyaaba JZ, Danso EO, Asiedu DD, Whyte T (2018) Current knowledge and breeding perspectives for the spider plant (*Cleome gynandra* L.): a potential for enhanced breeding of the plant in Africa. Genet Resour Crop Evol 65 (5):1529-1550. 10.1007/s10722-018-0626-5

Labroo MR, Studer AJ, Rutkoski JE (2021) Heterosis and Hybrid Crop Breeding: A Multidisciplinary Review. Frontiers in Genetics 12. 10.3389/fgene.2021.643761

Li D, Huang Z, Song S, Xin Y, Mao D, Lv Q, Zhou M, Tian D, Tang M, Wu Q, Liu X, Chen T, Song X, Fu X, Zhao B, Liang C, Li A, Liu G, Li S, Hu S, Cao X, Yu J, Yuan L, Chen C, Zhu L (2016) Integrated analysis of phenome, genome, and transcriptome of hybrid rice uncovered multiple heterosis-related loci for yield increase. Proceedings of the National Academy of Sciences 113 (41):E6026. 10.1073/pnas.1610115113

Lin T, Zhou C, Chen G, Yu J, Wu W, Ge Y, Liu X, Li J, Jiang X, Tang W, Tian Y, Zhao Z, Zhu C, Wang C, Wan J (2020) Heterosis-associated genes confer high yield in super hybrid rice. Theoretical and Applied Genetics 133 (12):3287-3297. 10.1007/s00122-020-03669-y

Liu H, Wang Q, Chen M, Ding Y, Yang X, Liu J, Li X, Zhou C, Tian Q, Lu Y, Fan D, Shi J, Zhang L, Kang C, Sun M, Li F, Wu Y, Zhang Y, Liu B, Zhao XY, Feng Q, Yang J, Han B, Lai J, Zhang XS, Huang X (2020a) Genome-wide identification and analysis of heterotic loci in three maize hybrids. Plant Biotechnol J 18 (1):185-194. 10.1111/pbi.13186

Liu J, Li M, Zhang Q, Wei X, Huang X (2020b) Exploring the molecular basis of heterosis for plant breeding. J Integr Plant Biol 62:287-298. https://doi.org/10.1111/jipb.12804

Liu Y, Lin Y, Gao S, Li Z, Ma J, Deng M, Chen G, Wei Y, Zheng Y (2017) A genome-wide association study of 23 agronomic traits in Chinese wheat landraces. The Plant Journal 91 (5):861-873. doi:10.1111/tpj.13614

Liu Y, Yi Q, Hou X, Hu Y, Li Y, Yu G, Liu H, Zhang J, Huang Y (2020c) Identification of quantitative trait loci for kernel-related traits and the heterosis for these traits in maize (*Zea mays* L.). Mol Genet Genom 295 (1):121-133. 10.1007/s00438-019-01608-1

Longin CFH, Gowda M, Mühleisen J, Ebmeyer E, Kazman E, Schachschneider R, Schacht J, Kirchhoff M, Zhao Y, Reif JC (2013) Hybrid wheat: quantitative genetic parameters and consequences for the design of breeding programs. Theor Appl Genet 126 (11):2791-2801. 10.1007/s00122-013-2172-z

Mogga M, Sibiya J, Shimelis H, Lamo J, Yao N (2018) Diversity analysis and genome-wide association studies of grain shape and eating quality traits in rice (Oryza sativa L.) using DArT markers. PLOS ONE 13 (6):e0198012. 10.1371/journal.pone.0198012

Onyango CM, Kunyanga CN, Ontita EG, Narla RD, Kimenju JW (2013) Current status on production and utilization of spider plant (*Cleome gynandra* L.) an underutilized leafy vegetable in Kenya. Genet Resour Crop Evol 60:2183-2189. 10.1007/s10722-013-0036-7

Paradis E (2010) pegas: an R package for population genetics with an integrated–modular approach. Bioinformatics 26 (3):419-420. 10.1093/bioinformatics/btp696

Pearson O (1983) Heterosis in vegetable crops. In: Heterosis. Springer, pp 138-188

Piepho HP, Möhring J, Melchinger AE, Büchse A (2008) BLUP for phenotypic selection in plant breeding and variety testing. Euphytica 161 (1):209-228. 10.1007/s10681-007-9449-8

Pritchard JK, Stephens M, Rosenberg NA, Donnelly P (2000) Association Mapping in Structured Populations. The American Journal of Human Genetics 67 (1):170-181. https://doi.org/10.1086/302959

R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Rasheed A, Hao Y, Xia X, Khan A, Xu Y, Varshney RK, He Z (2017) Crop Breeding Chips and Genotyping Platforms: Progress, Challenges, and Perspectives. Molecular Plant 10 (8):1047-1064. https://doi.org/10.1016/j.molp.2017.06.008

Sarfraz Z, Iqbal MS, Geng X, Iqbal MS, Nazir MF, Ahmed H, He S, Jia Y, Pan Z, Sun G, Ahmad S, Wang Q, Qin H, Liu J, Liu H, Yang J, Ma Z, Xu D, Yang J, Zhang J, Li Z, Cai Z, Zhang X, Zhang X, Huang A, Yi X, Zhou G, Li L, Zhu H, Pang B, Wang L, Sun

J, Du X (2021) GWAS Mediated Elucidation of Heterosis for Metric Traits in Cotton (Gossypium hirsutum L.) Across Multiple Environments. Front Plant Sci 12:565552. 10.3389/fpls.2021.565552

Schnable PS, Springer NM (2013) Progress Toward Understanding Heterosis in Crop Plants. 64 (1):71-88. 10.1146/annurev-arplant-042110-103827

Schouten HJ, van de Weg WE, Carling J, Khan SA, McKay SJ, van Kaauwen MPW, Wittenberg AHJ, Koehorst-van Putten HJJ, Noordijk Y, Gao Z, Rees DJG, Van Dyk MM, Jaccoud D, Considine MJ, Kilian A (2012) Diversity arrays technology (DArT) markers in apple for genetic linkage maps. Molecular Breeding 29 (3):645-660. 10.1007/s11032-011-9579-5

Schwarzwälder L, Thorwarth P, Zhao Y, Reif JC, Longin CFH (2022) Hybrid wheat: quantitative genetic parameters and heterosis for quality and rheological traits as well as baking volume. Theoretical and Applied Genetics. 10.1007/s00122-022-04039-6

Self SG, Liang K-Y (1987) Asymptotic Properties of Maximum Likelihood Estimators and Likelihood Ratio Tests under Nonstandard Conditions. J Am Stat Assoc 82 (398):605-610. 10.1080/01621459.1987.10478472

Shull GH (1908) The Composition of a Field of Maize. Journal of Heredity os-4 (1):296-301. 10.1093/jhered/os-4.1.296 %J Journal of Heredity

So Y-S, Edwards J (2011) Predictive Ability Assessment of Linear Mixed Models in Multienvironment Trials in Corn. Crop Science 51 (2):542-552. https://doi.org/10.2135/cropsci2010.06.0338

Sogbohossou DEO (2019) Orphan no more : Ethnobotany and genetic analysis of leaf yield and secondary metabolites content in *Gynandropsis gynandra* (Cleomaceae). PhD Thesis, Wageningen University, Wageningen

Sogbohossou EOD, Achigan-Dako EG, Maundu P, Solberg S, Deguenon EMS, Mumm RH, Hale I, Van Deynze A, Schranz ME (2018) A roadmap for breeding orphan leafy vegetable species: a case study of *Gynandropsis gynandra* (Cleomaceae). Hortic Res 5 (1):2. 10.1038/s41438-017-0001-2

Sprague GF, Tatum LA (1942) General vs. Specific Combining Ability in Single Crosses of Corn. Agronomy Journal 34 (10):923-932. https://doi.org/10.2134/agronj1942.00021962003400100008x

VanRaden, P.M. (2008). Efficient methods to compute genomic predictions. *J Dairy Sci* 91(11)**,** 4414-4423. doi: 10.3168/jds.2007-0980.

Varshney RK, Terauchi R, McCouch SR (2014) Harvesting the Promising Fruits of Genomics: Applying Genome Sequencing Technologies to Crop Breeding. PLOS Biology 12 (6):e1001883. 10.1371/journal.pbio.1001883

Wang H, Xu C, Liu X, Guo Z, Xu X, Wang S, Xie C, Li W-X, Zou C, Xu YJSr (2017) Development of a multiple-hybrid population for genome-wide association studies: theoretical consideration and genetic mapping of flowering traits in maize. 7:40239

Wang J, Zhang Z (2021) GAPIT Version 3: Boosting Power and Accuracy for Genomic Association and Prediction. Genomics, Proteomics & Bioinformatics. https://doi.org/10.1016/j.gpb.2021.08.005

Wehner TJTg, heterosis eo (1999) Heterosis in vegetable crops.387-397

Wenzl P, Carling J, Kudrna D, Jaccoud D, Huttner E, Kleinhofs A, Kilian A (2004) Diversity Arrays Technology (DArT) for whole-genome profiling of barley. Proceedings of the National Academy of Sciences of the United States of America 101 (26):9915-9920. 10.1073/pnas.0401076101

Wittenberg AHJ, Lee Tvd, Cayla C, Kilian A, Visser RGF, Schouten HJ (2005) Validation of the high-throughput marker technology DArT using the model plant Arabidopsis thaliana. Molecular Genetics and Genomics 274 (1):30-39. 10.1007/s00438-005-1145-6

Xiao Y, Liu H, Wu L, Warburton M, Yan J (2017) Genome-wide Association Studies in Maize: Praise and Stargaze. Molecular Plant 10 (3):359-374. 10.1016/j.molp.2016.12.008

Xu Y, Li P, Zou C, Lu Y, Xie C, Zhang X, Prasanna BM, Olsen MS (2017) Enhancing genetic gain in the era of molecular breeding. Journal of Experimental Botany 68 (11):2641-2666. 10.1093/jxb/erx135

Yang M, Wang X, Ren D, Huang H, Xu M, He G, Deng XW (2017) Genomic architecture of biomass heterosis in *Arabidopsis*. Proc Natl Acad Sci USA 114 (30):8101-8106

Yang N, Lu Y, Yang X, Huang J, Zhou Y, Ali F, Wen W, Liu J, Li J, Yan J (2014) Genome Wide Association Studies Using a New Nonparametric Model Reveal the Genetic Architecture of 17 Agronomic Traits in an Enlarged Maize Association Panel. PLOS Genetics 10 (9):e1004573. 10.1371/journal.pgen.1004573

Yang W, Zhang F, Zafar S, Wang J, Lu H, Naveed S, Lou J, Xu J (2021) Genetic dissection of heterosis of indica–japonica by introgression line, recombinant inbred line and their testcross populations. Scientific Reports 11 (1):10265. 10.1038/s41598-021-89691-6

Zohoungbogbo HPF, Blalogoe JS, Sogbohossou EDO, Houdegbe CA, Achigan-Dako EG (2018) Tips for successful crosses in Cleome. Laboratory of Genetics Horticulture and Seed Science, Cotonou, Benin. doi:10.13140/RG.2.2.11977.90723

CHAPTER 8
General discussion and implications of the study

8.1 Context and farmers' preferred traits in *Gynandropsis gynandra*

Gynandropsis gynandra is an important nutritious leafy vegetable and medicinal plant for local communities and pharmaceutical industries. Farmers are searching for improved varieties characterised by high leaf yield and related traits (plant height and the number of leaves), broad leaves, late flowering, good germination and resistance to pests and diseases (Sogbohossou et al. 2018b; Sogbohossou et al. 2018a; Cleome Consortium 2017; Mutoro 2019; Ndinya et al. 2020; Kiebre et al. 2015). Tremendous achievements have been made in recent decades in understanding phenotypic variability, seed germination, agronomic practices, molecular genetic diversity, selection of improved cultivars, reproductive biology, gene discovery, genomics resources, pharmaceutical properties and participatory varietal selection (Chapter 2) (Achigan-Dako et al. 2021). Despite significant genetic diversity found in the species, genetic gain in the species is still low due to limited knowledge on the biomass productivity of large collections and improved lines, genetics of inheritance of key functional traits (nutrients, biomass and related traits, etc.) and genes controlling the functional traits, thus hindering the implementation of sustainable and successful breeding programs. The present study generated information that contributes to filling this gap by understanding the genetic diversity, the genetic mechanism controlling inheritance, combining ability, level of heterosis of mineral contents, biomass and related traits in *G. gynandra*. However, some farmers preferred traits (resistance to pests and diseases, drought) and retailers and consumers desired traits (good appearance, colour, superior taste and aroma, high nutritional value, long shelf life, affordability and medicinal attributes) were not investigated in this study and future studies should address them.

8.2 Genetic pools in *Gynandropsis gynandra* for use in breeding program

Understanding the genetic variability within a plant species is paramount in implementing a sustainable breeding program. Studies on phenotypic variability in biomass and related traits (Chapter 3) and minerals content (Chapter 4) among four selfing generations of advanced lines of Cleome (*Gynandropsis gynandra* L. (Briq.) confirmed the existence of significant genetic

variability in the species (Sogbohossou et al. 2019; Chataika et al. 2021; Sogbohossou et al. 2020; Blalogoe et al. 2020; Omondi et al. 2017a; Omondi et al. 2017b; Reeves et al. 2018). This variability was structured into three groups based on biomass productivity (Chapter 3) and included Asian, West African, and East/Southern African groups. More importantly, the molecular data identified the same three distinct genetic groups using a subset of 38 lines (Chapter 7). Though three nutritional groups were identified regarding the mineral content (Houdegbe et al. 2022), two major groups were perceptible and included East/Southern African and Asian/West African. All together showed the existence of three gene pools in the species, including Asian, West African, and East/Southern African groups. These groups were linked to their region of origin, strengthening the hypothesis of geographical signature in cleome genetic diversity (Sogbohossou et al. 2019; Blalogoe et al. 2020). The characteristics of these three gene pools were presented in Table 8.1. The identified gene pools are key for developing high-yielding and nutrient-dense improved populations and cultivars and implementing successful breeding programs. Elite genotypes from these groups could be involved in the variety testing procedure (tests for distinctiveness, uniformity and stability (DUS) and value for cultivation and use (VCU)) for cultivar registration and release.

Table 8.1. Biomass and nutritional characteristics of the three genetic groups of *G. gynandra*

Groups	**Biomass potential**	**Nutritional potential**
Asian	▪ low biomass productivity ▪ high harvest index ▪ less vigorous plants ▪ moderate number of short primary branches ▪ low dry matter content ▪ relative late flowering time ▪ small leaves	▪ high calcium ▪ high magnesium ▪ high manganese ▪ low phosphorus ▪ low copper ▪ low zinc
West African	▪ high biomass productivity ▪ high harvest index ▪ moderate vigorous plants ▪ low number of long primary branches ▪ high dry matter content ▪ early flowering ▪ medium leaf size	▪ high calcium ▪ high magnesium ▪ high manganese ▪ low phosphorus ▪ low copper ▪ low zinc
East/Southern African	▪ high biomass productivity ▪ low harvest index ▪ more vigorous plants ▪ high number of short primary branches ▪ moderate dry matter content. ▪ late flowering, ▪ broad leaves	▪ low calcium ▪ low magnesium ▪ low manganese ▪ high phosphorus ▪ high copper ▪ high zinc content

However, these gene pools observed might be improved with increased germplasm collection for other species' distribution areas (including America, Oceania, Asia, Africa) since only genotypes from Asia and Africa were investigated in the present study. Such a large collection could be used in gene discovery studies in the species and the identification of genes associated with local adaptation in the species because of the geographical signature in the species. To this end, methods such as genome-wide association studies (GWAS) and QTL mapping can be employed, particularly with ongoing efforts to release the genome of *G. gynandra* (Hoang et al. 2022) as well as a subset of 594 markers used in the present study. These markers are well distributed across the genome of the species and could be used in developing a low cost-effective platform for diversity analysis, such as KASP markers.

8.3 Breeding for high yield and leaf mineral-dense content in *G. gynandra*

Knowledge on the inheritance of target traits is crucial in designing the breeding program and selecting the type of cultivars to be developed. In our search for understanding the genetics of inheritance of minerals content (Chapter 5) and biomass and related traits (Chapter 6), we observed that both additive and non-additive gene action controlled minerals content and biomass and related traits in the species. Specifically, minerals content was predominantly controlled by non-additive gene action, while biomass and related traits (except dry matter content) were mainly governed by additive gene action. This revealed that different gene action controls agronomic and mineral traits in the species. More importantly, the average hybrid performance was higher than that of the parents for all biomass and related traits (Chapter 6) and several minerals content (zinc, potassium, phosphorus and manganese), indicating hybrid vigour, which is the first report in the species. Consequently, breeding for higher biomass and nutritious cultivars in the species should consider both gene action and recurrent reciprocal selection will be a sound breeding method with a focus on hybrid cultivars to better exploit the hybrid vigour. Multiple crossing programs could be implemented, and synthetics, composites and population improvements could be developed to ensure access to improved cultivars by smallholder farmers. Therefore, breeding strategies should focus on (i) selecting parents with good general combing ability, followed by (ii) selection based on specific combining ability. In that vein, good general combiners for multiple traits for mineral contents (P14, P24, P04, P18, P31, P29, P21, P22, and P27) and for biomass and related traits (M22, M1, M16, M10, M2, M18, M20, F15, F11, F16, F1, F2 and F3) were identified. Crosses with promising hybrid

vigour were P04xP26, P18xP16, P20xP19, P18xP01, and P25xP28 (mineral contents), F10xM8, F9xM3, F10xM22, F14xM10, F3xM21 and F3xM22, (agronomic traits). These identified parents and crosses are excellent and valuable candidates and resources for developing improved populations for research and breeding purposes. The identified combiners could be used for developing improved populations such as recombinant inbred lines (RILs), multiparent advanced generation intercross (MAGIC) and nested association mapping (NAM), F2 populations, and backcrossing populations for genetic and breeding purposes. These populations will be used to decipher genes associated with farmers' preferred traits and nutritional and phytochemical contents.

Genotype × year or genotype × environment interaction variance was significant for all biomass and related traits (Chapter 3 and Chapter 6), and minerals content (Chapter 3), which might translate to phenotypic plasticity in the species. This is showing that these traits were influenced not only by the genotype but also the interaction between genotype and year or environment. Potential environmental factors that might influence these traits could include the temperature, the relative humidity and light intensity (photoperiods). The significance of year or environment implies that investigated traits might vary with year or environment. In addition, the significant genotype × year interaction indicated that the genotypes' performance was not consistent across environments, and selection should consider the interaction effect when selecting genotypes. However, we did not investigate the genotype by environment interaction and the level of phenotypic plasticity in the present study and are already included in the ongoing activities of our breeding team.

High broad-sense heritability and expected genetic gain (> 20%) at a selection intensity of 5% for all most traits showed that selection will have a positive effect on species performance. Also, significant association between biomass and related traits, offering the possibility for simultaneous multiple trait selection, especially farmers' preferred traits such as biomass yield, leaf size, flowering time and the number of branches. Breeders can therefore use specific plant material from specific geographical origins to enhance their breeding programs' genetic gain.

8.4 Marker assisted selection for biomass and related traits heterosis in *Gynandropsis gynandra*

Our study is the first to reveal markers associated with heterosis in *G. gynandra*. This study contributes to the ongoing efforts to understand molecular mechanisms controlling heterosis in

plant, which is still unclear. Using GWAS, six markers were identified with two markers for best parent heterosis (BPH) and one for mid parent heterosis (MPH) for flowering, a single marker for MPH for edible biomass, one marker for total fresh biomass and one marker for both BPH and MPH for the number of primary branches. No consistent markers associated with combining ability were observed for general combining ability and might be due to the low number of parents and the density of markers used. Furthermore, the identified markers represent an important resource for marker-assisted selection in the species for better exploitation of heterosis but need to be validated although QTL mapping and the use of a larger natural populations. The use of transcriptomic approaches will be insightful, particularly in the validation of these marker effects as well as depicting additional genes controlling heterosis in the species.

8.5 Implications and perspectives of the study

In summary, future studies should focus on (i) assessing the extent of genotype-by-environment interaction and phenotypic plasticity in the species; (ii) evaluating the association between mineral content and biomass and related traits in *G. gynandra*; (iii) determining the physiological basis of heterosis and combining ability in *G. gynandra*; (iv) assessing the level of maternal effect and epistasis on the inheritance of mineral content and biomass and related traits in the species; (v) identifying the genetic mechanism controlling other important traits such as vitamins, secondary metabolites, fatty acids and proteins; and (vi) assessing the diversity associated with proteins and fatty acid content in the species. In the era of genomics, the potential of genomic selection in predicting hybrid performance should be assessed together with machine learning techniques. For genomic selection, the multi-traits random forest (RF), the multi-traits genomic best linear unbiased predictor (GBLUP), and the multi-traits partial least squares (PLS) methods could be investigated. The potential of the machine and deep learning methods, including Multilayer Perceptron (MLP) and Convolutional Neural Network (CNN) (deep learning methods), Support Vector Machine (SVM) and Random Forests (RF) (machine learning methods) could be explored. Speed breeding will enhance the species' genetic gain by increasing the number of generations per year and reducing the breeding cycle. The usefulness of high-throughput phenotyping should also be assessed. Furthermore, gene editing is a promising tool that can be implemented in the species using the best identified lines. On the other hand, the performance of best lines and crosses should be assessed under different agronomic practices given the variability in agricultural practices among farmers from different

regions. Specifically, genotype × environment × management (G × E × M) should be investigated. Here, management could refer agricultural practices, biotic and abiotic stress. The pressing biotic stress includes insect pests such aphids and diseases. Regarding abiotic stress, heat and drought are the most pressing. Studies on abiotic stress were initiated, and the results are promising.

References

Achigan-Dako EG, Sogbohossou DEO, Houdegbe CA, Salaou MA, Sohindji FS, Blalogoe J, Chataika BY, Zohoungbogbo HF, Adje CAO, Fassinou Hotegni NV, Francisco R, Abukutsa-Onyango MO, Schranz ME (2021) Ten years of *Gynandropsis gynandra* research for improvement of nutrient-rich leaf consumption: lessons learnt and way forwards. Annu Rev Plant Biol 4 (3):767-812. doi:10.1002/9781119312994.apr0774

Blalogoe JS, Odindo AO, Sogbohossou EOD, Sibiya J, Achigan-Dako EG (2020) Origin-dependence of variation in seed morphology, mineral composition and germination percentage in *Gynandropsis gynandra* (L.) Briq. accessions from Africa and Asia. BMC Plant Biol 20 (1):168. doi:10.1186/s12870-020-02364-w

Chataika BY, Akundabweni LS-M, Houdegbe AC, Achigan-Dako EG, Sibiya J, Masamba K (2021) Dietary phytochemical screening of spider plant (*Gynandropsis gynandra* (L.) Briq.) accessions from Africa and Asia to identify genotypes for use in nutraceutical breeding. Front Sustain Food Syst 5 (344). doi:10.3389/fsufs.2021.733909

Cleome Consortium (2017) Utilizing the genome of *Cleome gynandra* for the development of improved cultivars for West and East African markets - Annual progress report 2016. Cleome Consortium, Cotonou

Hoang NV, Sogbohossou EOD, Xiong W, Simpson CJC, Singh P, van den Bergh E, Zhu X-G, Brautigam A, Weber APM, van Haarst JC, Schijlen EGWM, Hendre PS, Deynze AV, Achigan-Dako EG, Hibberd JM, Schranz ME (2022) The genome of *Gynandropsis gynandra* provides insights into whole-genome duplications and the evolution of C_4 photosynthesis in Cleomaceae. bioRxiv:2022.2007.2009.499295. doi:10.1101/2022.07.09.499295

Houdegbe AC, Achigan-Dako EG, Sogbohossou E, Schranz ME, Odindo AO, Sibiya J (2022) Leaf elemental composition analysis in spider plant [*Gynandropsis gynandra* L.(Briq.)]

differentiates three nutritional groups. Front Plant Sci 13:841226. doi:10.3389/fpls.2022.841226

Kiebre Z, Bationo Kando P, Sawadogo N, Sawadogo M, Zongo J-D (2015) Selection phenotypic interests for the cultivation of the plant *Cleome gynandra* L. in the vegetable garderns in Burkina-Faso. J Exp Biol Agric Sci 3 (3):288-297. doi:10.18006/2015.3(3).288.297

Mutoro K (2019) Analysis of preference attributes for spider plant genotypes in Kenya: Implications for breeders and farmers. Int J Hortic Floric 7 (8):001-004. doi:10.46882/IJHF/1092

Ndinya C, Onyango E, Dinssa F, Odendo M, Simon JE, Weller S, Thuranira E, Nyabinda N, Maiyo N (2020) Participatory variety selection of three african leafy vegetables in Western Kenya. J Med Active Plants 9 (3):145-156. doi:10.7275/mkq0-3p85

Omondi EO, Debener T, Linde M, Abukutsa-Onyango M, Dinssa FF, Winkelmann T (2017a) Mating biology, nuclear DNA content and genetic diversity in spider plant (Cleome gynandra) germplasm from various African countries. Plant Breed 136 (4):578-589. doi:10.1111/pbr.12485

Omondi EO, Engels C, Nambafu G, Schreiner M, Neugart S, Abukutsa-Onyango M, Winkelmann T (2017b) Nutritional compound analysis and morphological characterization of spider plant (*Cleome gynandra*)-an African indigenous leafy vegetable. Food Res Int 100:284-295. doi:10.1016/j.foodres.2017.06.050

Reeves G, Singh P, Rossberg TA, Sogbohossou EOD, Schranz ME, Hibberd JM (2018) Natural variation within a species for traits underpinning C4 photosynthesis. Plant Physiol 177 (2):504. doi:10.1104/pp.18.00168

Sogbohossou EOD, Achigan-Dako EG, Maundu P, Solberg S, Deguenon EMS, Mumm RH, Hale I, Van Deynze A, Schranz ME (2018a) A roadmap for breeding orphan leafy vegetable species: a case study of *Gynandropsis gynandra* (Cleomaceae). Hortic Res 5 (1):2. doi:10.1038/s41438-017-0001-2

Sogbohossou EOD, Achigan-Dako EG, Mumm R, de Vos RCH, Schranz ME (2020) Natural variation in specialised metabolites production in the leafy vegetable spider plant (*Gynandropsis gynandra* L. (Briq.)) in Africa and Asia. Phytochemistry 178:112468. doi:10.1016/j.phytochem.2020.112468

Sogbohossou EOD, Achigan-Dako EG, van Andel T, Schranz ME (2018b) Drivers of management of spider plant (*Gynandropsis gynandra*) across different socio-linguistic groups in Benin and Togo. Econ Bot:1-25. doi:10.1007/s12231-018-9423-5

Sogbohossou EOD, Kortekaas D, Achigan-Dako EG, Maundu P, Stoilova T, Van Deynze A, de Vos RCH, Schranz ME (2019) Association between vitamin content, plant morphology and geographical origin in a worldwide collection of the orphan crop *Gynandropsis gynandra* (Cleomaceae). Planta 250:933–947. doi:10.1007/s00425-019-03142-1