Participatory cassava (*Manihot esculenta* Crantz) breeding for improved total carotenoids content and delayed postharvest physiological deterioration in Rwanda

By

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Abstract

Cassava (*Manihot esculenta* Crantz) is one of the important staple foods and plays a key role as a food security and income-generating crop for most smallholder farmers in sub-Saharan Africa. It is a multipurpose crop and could be a cheap source of starch in Rwanda. However, there are many factors that have an impact on its production, consumption and marketability. The main constraints in Rwandan cassava production are the lack of good high-yielding genotypes, with resistance to pests and diseases and reduced postharvest losses. The main goal of this study is to contribute to the increase of cassava productivity in Rwanda through participatory cassava breeding for high yielding cassava genotypes, with improved total carotene (TC) and delayed postharvest physiological deterioration (PPD). The review and feasibility study indicated that PPD is induced by wounds when storage roots are detached from the mother plant during harvesting, and it is accelerated by the reactive oxygen species (ROS), such as the oxygen ion (O_2) and peroxide $(O_2)_2$. The antioxidant properties of carotenoids help to extend the shelf-life of cassava storage roots. There are two types of phytoene synthase enzymes (PSY1 and PSY2) that regulate the accumulation of carotenoids in cassava, and recurrent selection can be used to improve cassava for increased TC and delayed PPD. A participatory appraisal identified the cassava production constraints as a lack of clean planting material, viral diseases, late bulking cultivars, drought, limited knowledge, weathered soils, insufficient fertilizers, land shortage, limited information, the lack of a market and effective storage techniques. PPD losses have been estimated at 11.9% of the total production per year. Piecemeal harvesting and the underground storage of roots were the main indigenous practices used to tackle the effects of PPD, while a change in colour and taste, rotting, difficulty in removing the skin and an increase of fibres in the flesh, were the methods used by farmers to assess PPD. Genetic variability for TC revealed that a high genetic variability (61.0%) and a variation of 98.2% were explained by genotypes, while 1.8% was due to an unknown origin. The TC had a very high heritability (H^2) of 99.2% and an expected genetic advance (GA%) of 159.6%, indicating the potential for improvement, using conventional breeding through simple recurrent selection. The PPD was negatively correlated with TC and dry matter content (DMC), indicating that the high TC and low DMC cultivars could have a delayed PPD. The genotype x environment (GxE) interaction analysis divulged that the % variation, due to the genotype for TC, was higher (96.0%) than the variation, due to the environment (1.7%) and the GxE interaction (2.4%), indicating less interaction effect of the environment on TC accumulation. An analysis of the genetic inheritance of TC and PPD indicated a significant variation between genotypes and families, which is essential for genetic diversity and for crop improvement through conventional breeding. The general combining ability (GCA) and

specific combining ability (SCA) were significant for most traits, indicating the possibility of improving cassava through recurrent selection, for most traits. The significant GCA and SCA for most traits indicated the role of additive and non-additive gene action. The high GCA/SCA ratio and % sum of square (SS) due to GCA, indicated that TC and PPD were more controlled by additive gene action. The F1 clones exhibited considerable phenotypic variability among families and progenies for the evaluated traits. Some progenies of F1 clones had a higher fresh root storage yield (FRSY), β-Carotene (β-C) and PPD tolerance than their parents, which was attributed to transgressive segregation and heterosis. The GxE for F1 clones revealed that the expression of β-C and PPD is genetic, with very few environmental effects. The GCA and SCA for F1 clones revealed that β-C and PPD were controlled by both additive and non-additive gene action. The GCA for parents indicated that the genotype Mavoka had a high positive GCA for β-C and FRSY and a high negative GCA for PPD and DMC, indicating that it is the best combiner in terms of FRSY, β-C and delayed PPD, and a bad combiner for DMC. This implies that improving β-C content in the cassava population, using Mavoka as a progenitor, could concurrently improve yield and delay PPD, but could reduce the dry matter content. The progenies from the family Mavoka x Garukunsubire expressed the highest positive heterosis for DMC and β-C. In terms of FRSY, the family Mavoka x Gahene had the highest positive mid-parent heterosis, while the family Garukunsubire x Gahene and Gahene x Gitamisi also expressed a positive heterosis, which was an indication that the Gahene genotype could be a good combiner for FRSY. The mid-parent heterosis for PPD was positive for the Garukunsubire x Gitamisi, Mavoka x Mushedile and Ndamiraba x Gitamisi families, while most families expressed negative heterosis. The feasibility study to introgress carotenoids into cassava indicated that it is possible to improve total carotene and dry matter concurrently, while the genetic studies revealed the concurrent improvement of yield, β-carotene and delayed PPD. This study gave an insight into the feasibility of improving the cassava population, using the farmers' preferred traits, and provided the basic foundation for a cassava breeding scheme in Rwanda. It generated improved total carotene clones, with delayed postharvest physiological deterioration (PPD) and high yield.

Declaration

I, **Athanase Nduwumuremyi**, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other person's data, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then
   a. their words have been rewritten, but the general information attributed to them has been referenced; and
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5. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in reference sections.

Signed…… ……Date……..……..

Athanase Nduwumuremyi
(Candidate)

As the candidate's supervisor, I agree to the submission of this thesis:

Signed:……Date……..

Prof. Rob Melis

(Principal supervisor)

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Dedication

I dedicate this thesis to my beloved wife Justine Mupenzi and our sons: Casey Ineza Rugero, Ervin Baruck Rugero and Sean Gadiel Rugero.

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General introduction

Cassava (*Manihot esculenta* Crantz) is a domesticated shrub from the Amazon Basin in South America (Leotard et al., 2009; Olsen and Schaal, 1999). It is widely cultivated in the tropical and sub-tropical lowland regions of the world, typically between 30°N and 30°S of the equator, and in areas where the annual mean temperature is greater than 18°C (Nassar and Ortiz, 2007). It was introduced in Africa by Portuguese sailors during the 16th century via the West African ports, from where it rapidly spread throughout the continent (Sayre et al., 2011). It was introduced into Rwanda around 1932 and an increase in production was observed after the 1994 genocide. However, since 2014, the yield has declined considerably, due to the pandemic of the cassava brown streak disease (CBSD) (Figure 1).

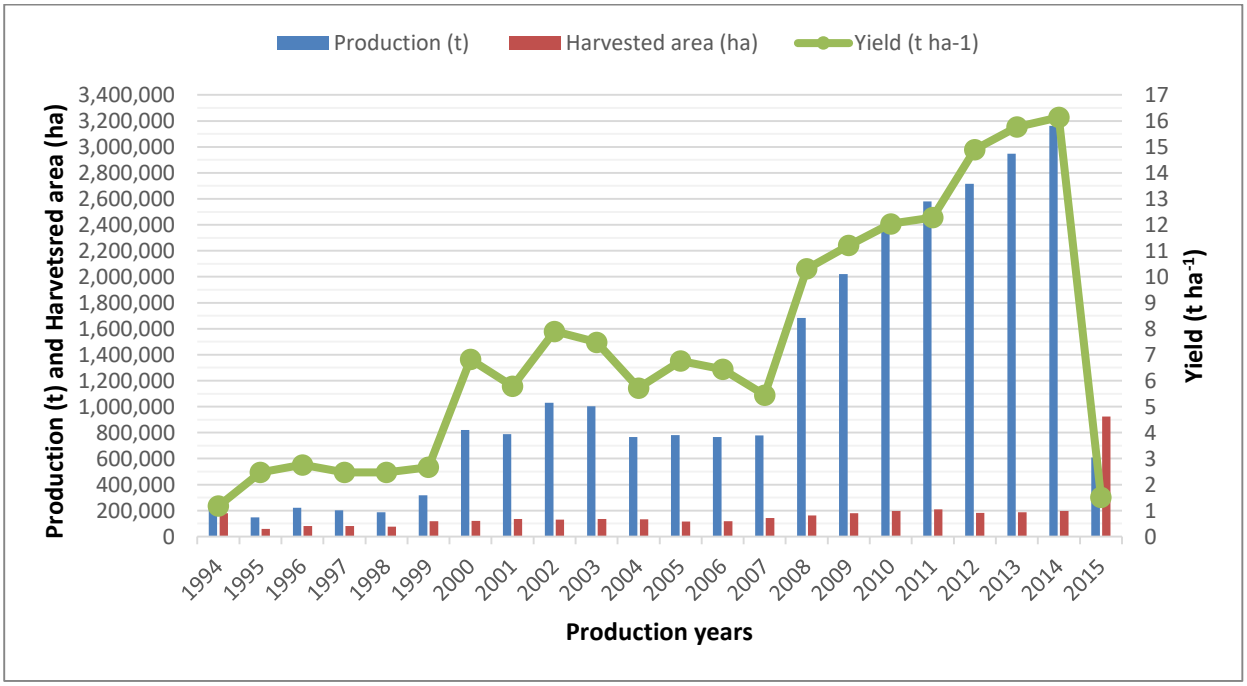

Figure 1: Cassava production trends in Rwanda, 1994-2015

Cassava is a staple food and an important source of calories for the approximately 500 million people living in developing countries (Bull et al., 2011). Among the crops providing calories, it is reported to occupy third place worldwide (FAO, 2008), after rice (*Oryza.* ssp. L) and wheat (*Triticum* spp. L). In 2014, the global production of cassava was 270 million tons, with Africa producing 54.3% and Rwanda producing 3.2 million tons (FAOSTAT, 2014). The potential yield of cassava is estimated at 90 t ha^{-1} of fresh mass under well-managed conditions (El-Sharkawy, 2004). However, the average yield of 10 to 15 t ha^{-1} achieved in sub-Saharan Africa

(SSA) is considerably lower (Sayre, 2011). The average yield varies from country to country, with a national average of 1.5 to 4 t ha^{-1} for Rwanda (NISR, 2016; Nahayo and Mutuyedata, 2012). This low yield is attributed to poor genotypes, biotic and abiotic factors (Night et al., 2011) and poor agronomic practices.

An estimated 250 million people in Africa are dependent on cassava as a primary food source (Howeler et al., 2013; Sayre et al., 2011) and it contributes over 500 kcal per day per person (FAO, 2010; Morante et al., 2010). Cassava is produced on marginal and sub-marginal lands in SSA by subsistence smallholder farmers (Howeler et al., 2013). It is an important staple food in Rwanda an excellent source of vegetable from its leaves, called “Isombe”, and it is currently being promoted as a cash crop since the establishment of cassava processing plants. Cassava is consumed in various forms (raw, paste or ugali, boiled for breakfast, mixed with beans, vegetables, etc) and its cooking and preparation methods vary from one individual to another (mixed with beans, boiled, paste or ugali, etc). In terms of production, it occupies the first place, followed by potatoes and sweet potatoes (FAOSTAT, 2014) and it reduces hunger and poverty in the country (Night et al., 2011).

Cassava is of high value in Rwanda when it comes to food security. It is efficient in carbohydrate production, is adapted to a wide range of environments and is tolerant to drought and acidic soils (FAO, 2010). It tolerates poor soils, requires less labour than other crops, and harvesting can be delayed by months, or even up to three years (Sayre, 2011). Being drought tolerant, cassava can be planted at any time of the year. However, its production is threatened by a lack of good varieties (high yielding, disease-free and resistant), its long growing cycle, low soil fertility, poor agronomic practices and postharvest losses. Moreover, the small land size, is an additional constraint for agricultural development, because it reduces the adoption of perennial crop as cassava, due to complications in the cropping system (rotation and intercropping practices).

As a staple food, cassava has numerous biotic and abiotic stresses that impact on its production, consumption and marketability (Bull et al., 2011). Furthermore, the poor infrastructure, combined with low storability, is the major obstacle in the value chain of cassava. Although initiatives by the IITA and CIAT, amongst others, have been successful in developing genotypes that are resistant, or tolerant, to various stress factors, the cassava brown streak disease (CBSD) remains a significant threat to its production in Rwanda. This disease occurs in isolation, or in combination with, the cassava mosaic disease (CMD), and cassava bacterial blight and pests (whiteflies: *Bemisia tabaci* and the cassava green mite: *Mononychellus tanajoa*) (Night et al., 2011), while other factors, such as poor agricultural

practices and post-harvest losses, present a considerable constraint to the attainment of a satisfactory yield by poorly-resourced farmers (Patil and Fauquet, 2009).

The short shelf-life of the cassava storage roots, which is caused by postharvest physiological deterioration (PPD), presents a major challenge for its increased production and utilization (Ceballos et al., 2004). PPD begins within 24 hours of the harvest, rapidly rendering the storage roots unpalatable, inedible and reducing their market value (Sánchez et al., 2006). With the poor road infrastructure and remote production sites, the short shelf-life severely limits marketing options and increases the likelihood of product losses and higher marketing costs. Conventional breeding and genetic engineering have been suggested as long-term strategies, to delay the onset of PPD (Salcedo and Siritunga, 2011). Other studies have reported the development of nutritious pro-Vitamin A carotenoids (pro-VAC) cassava genotypes, which may retard or inhibit the onset of PPP, due to the antioxidant properties of carotenoids (Morante et al., 2010; Sánchez et al., 2006; Zidenga et al., 2012). The aforementioned cassava production constraints, namely, the small land size, soil infertility, poor agricultural practices, the long growing cycle and the conservative attitude of farmers when it comes to adopting new genotypes, also hinder cassava production in Rwanda. Participatory breeding presents an alternative approach to changing the conservative behaviour of farmers, with regard to adopting new genotypes with farmer-desired traits. It is, therefore, the objective of this research to improve cassava production through participatory cassava breeding for high-yielding cultivars, with the purpose of improving the carotene content, delaying PPD, and incorporating the preferred traits of farmers.

Research goal and objectives

The main goal of this study is to contribute to an increase in cassava productivity through participatory cassava breeding for high yields in Rwanda and to improve cassava genotypes for carotenoids content and delayed postharvest physiological deterioration (PPD).

Specific objectives

The specific objectives of this study are:

1. To review the existing knowledge, principles and concepts for guiding in methodological development and feasibility of improving total carotenoids in cassava and delayed postharvest physiological deterioration,
2. To participatory assess cassava production constraints, farmers preferred traits and factors affecting the adoption of new genotypes in Rwanda,

3. To evaluate cassava genetic variability and the inter-relationship between yield and yield components and postharvest traits in Rwanda
4. To analysis the genotype x environment (GxE) interaction effects on cassava yield and postharvest traits in Rwanda,
5. To determine the genetic inheritance of cassava pulp colour and delayed postharvest physiological deterioration and undertake a diallel analysis of developed cassava genotypes with improved total carotenoids and delayed postharvest physiological deterioration at the early generation selection of F1 population
6. To determine the combining ability and heterosis for cassava β-carotene content and delayed postharvest physiological deterioration and farmers' preferred

The thesis is structured as follows:

Chapter I: Literature review.

Chapter II: Participatory appraisal of cassava production constraints, farmers preferred traits and factors affecting the adoption of new genotypes.

Chapter III: The genetic variability of cassava and the inter-relationship between yield and yield component and postharvest traits

Chapter IV: Genotype x environment interaction analysis of cassava yield and postharvest traits.

Chapter V: Genetic inheritance and diallel analysis of cassava pulp colour and delayed postharvest physiological deterioration at the early generation F1 seedling population.

Chapter VI: Combining ability and heterosis for cassava β-carotene and delayed postharvest physiological deterioration and farmers preferred traits at F1 clonal population.

Chapter VII: General overview of the research findings and implications for cassava breeding.

All the chapters, except for Chapters 1 and 7, follow the IMRAD format (Introduction, Materials and Methods, Results and Discussion)

Chapters 2 to 6 are written as scientific papers, in publishable format, and some of the text and references may therefore overlap.

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CHAPTER I

Literature review

Part of this chapter has been published by Athanase Nduwumuremyi[1,2,3], Rob Melis[1], Paul Shanahan[1] and Theodore Asiimwe[2] (2016). Introgression of antioxidant activity into cassava (Manihot esculenta C): an effective technique for extending fresh storage roots shelf-life. Plant Breeding 135: 1–8.

[1]African Centre for Crop Improvement (ACCI), University of KwaZulu-Natal, Private Bag X01, 3029, Scottsville, South Africa; [2]Rwanda Agriculture Board (RAB), PO Box 5016, Kigali, Rwanda; [3]Corresponding author, E-mail: nduwatha@gmail.com

Abstract

The postharvest physiological deterioration (PPD) of cassava is a main constraint that affects the crop's nutritional and economic value. PPD is induced by wounds that are created when detaching storage roots from the mother plant during harvesting. It is accelerated by the reactive oxygen species (ROS), such as the oxygen (O_2) and peroxide $(0_2)^{-2}$ ions. The carotenoids content and its antioxidant properties can help in extending the shelf-life of cassava storage roots. The primary mode of action of carotenoids as an antioxidant is to quench singlet oxygen. Cassava breeding was reported to successfully introgress carotenoids into cassava. The two types of the phytoene synthase (*PSY*) enzyme (*PSY1* and *PSY2*) are the key regulators of carotenoids accumulation in cassava. Carotenoids formation and accumulation in cassava storage roots are induced by a single nucleotide polymorphism in *PSY2*, which causes a non-conservative amino-acid exchange. This single nucleotide polymorphism in the *PSY* gene is co-segregated with β-carotene in cassava storage roots, a phenomenon that could help to unravel the mechanism of the introgression of carotenoids into cassava. This chapter investigates the feasibility of breeding for improving the quality of cassava landraces in developing countries.

Keywords: carotenoids; carotenoids quantification; carotenoids rapid screening method; conventional breeding; physiological postharvest deterioration; phytoene synthase; recurrent selection

1.1 Introduction

Cassava is a staple food and an important source of calories for the approximately 500-800 million people living in developing countries (Bull et al., 2011; Howeler et al., 2013). It is reported to occupy second place worldwide, for the production of starch, after maize (*Zea mays* L) (Howeler et al., 2013). However, post-harvest losses present a considerable limitation to its value chain development in developing countries. The short shelf-life of cassava storage roots, due to their primary and secondary deterioration, is a major challenge for increasing its production and utilization (Ceballos et al., 2004). The primary deterioration involves changes in the oxidative enzyme activities, and the generation of phenols (catechins and leucoanthocyanidins) which, at a later stage, polymerise to form condensed tannins (Uarrota et al., 2014).

The biochemical processes involved in the rapid deterioration of cassava are essentially wound-healing responses, which are well-known in many plant species (Cortés et al., 2002; Luna et al., 2011). This deterioration is commonly referred to as postharvest physiological deterioration (PPD). It is an abiotic response of a cassava storage root that is damaged during the harvesting process and is caused by the oxidation of phenolic compounds, in particular scopoletin (hydroxycoumarin, involved in plant defence), by a reactive oxygen species (chemical reactive molecules containing oxygen i.e. an oxygen ion and peroxide) which leads to oxidative stress (Buschmann et al., 2000; Reilly et al., 2007). The PPD begins within 24 hours after harvesting and rapidly renders the storage roots unpalatable, inedible and with a reduced market value (Sánchez et al., 2006). The PPD starts as a black-blue to black vascular discoloration (vascular streaking) and then spreads to the parenchyma (Figure 1.1)


a b c

Figure 1.1: PPD signs (a: no visible sign at harvest, b: 50% of PPD after three days, c: total PPD and bacterial rotting after one month)

Currently, PPD is scored visually, and due to different levels of injury caused on storage roots during the harvesting process, storage roots from one plant can score from 0 to 100% of the PPD scores. PPD scoring is therefore difficult and prone to experimental error (García et al., 2013). In addition, PPD scoring is a destructive procedure and requires that at least seven transversal slices are cut along the storage roots, which must be repeated on the storage roots

of the same cultivar, to minimize experimental errors. PPD experimental storage times do not always correspond to those found by farmers and consumers. Most of the available cassava cultivars in sub-Saharan Africa deteriorate after only three days. Weathley (1982), Sánchez et al. (2013) and Morante et al. (2010) evaluated PPD at different intervals after harvest (for example 3, 14 and 40 days after harvest). The time for deterioration beyond the edible state varies from one cultivar to another and depends on the storage conditions (room temperature, cool room and underground storage), consumer perceptions and the number of wounds incurred on the storage roots during the harvesting process.

Poor road infrastructure and remote production sites, as well as the short shelf-life, severely limit the marketing options and increase the likelihood of product losses and higher marketing costs. The rapid deterioration affects the economic value of the crop (Morante et al., 2010), with a recorded loss of 29%, 10% and 8%, respectively, in Africa, Latin America and Asia (Salcedo and Siritunga, 2011). In addition, due to the depreciation of deteriorated cassava, the economic losses can reach up to 90% (Westby, 2002).

PPD is mostly associated with other biochemical reactions. The storage root weight drops consistently after harvesting, due to respiration. Starch is gradually hydrolysed into sugars and hence starch is lost at a rate of 1% per day, which negatively affects starch properties, such as gel clarity, swelling power and gel viscosity (Sánchez et al., 2013). PPD is therefore a serious problem for the starch industry and affects the socio-economic status of farmers in cassava-growing areas.

Various approaches are being implemented to tackle PPD and to improve the shelf-life of cassava storage roots, including breeding (Morante et al., 2010) and biotechnology (Bull et al., 2011). Other approaches include physical techniques, such as underground storage, storage in boxes with moist sawdust and storage in bags, combined with the use of fungicides, as well as pruning plants before harvest, cold storage (2-4ºC), freezing or waxing the storage roots, to prevent access to oxygen and even chemical treatments (Ravi et al., 1996). However, the physical methods seem to generally be ineffective and impractical, because they are expensive and complicated, when handling large volumes of harvested roots (Sánchez et al., 2013).

Several studies have been conducted to deal with PPD in cassava. It has been discovered that reactive oxygen species (ROS) are involved in, and accelerate, the onset of PPD (Beeching et al., 2002; Reilly et al., 2004; Xu et al., 2013; Zidenga et al., 2012). The breeding of cassava that is enriched with carotenoids is not only interesting for developing nutritious cassava genotypes, but it also makes it more marketable, because of its reduced or delayed

PPD, as reported by Sánchez et al. (2006). The deterioration of cassava storage roots, due to PPD, involves oxidative stress (Xu et al., 2013; Zidenga et al., 2012). Thus, the antioxidant properties of the carotenoids may be the origin of delayed PPD in yellow cassava genotypes (Morante et al., 2010; Zidenga et al., 2012). The introgression of carotenoids into cassava through a breeding process could be regarded as a long-term strategy for increasing its shelf-life, as well as improving its other traits. This chapter therefore aims at reviewing the properties of carotenoids, the related genes and the feasibility of introgressing carotenoids in cassava, to extend the shelf-life of cassava.

1.2 Role of carotenoids in plant and human health

Carotenoids are natural tetraterpenoid pigments that have varied functions in plants and animals (Cazzonelli, 2011). They are synthesized by all photosynthetic organisms and some non-photosynthetic bacteria and fungi (Priya and Siva, 2014). Carotenoids are present in photosynthetic tissues of plants, where they play an essential role in photoreception and photoprotection and they also prevent photodamage in plants tissues. In non-photosynthetic tissues, they act as colorants, precursors for plant isoprenoid volatiles and signalling molecules (abscisic acid and strigolactones), nutritional antioxidants and Vitamin A precursors (Bouvier et al., 2005; Giuliano, 2014; Priya and Siva, 2014)

Carotenoids are among the best-known antioxidant phytochemicals, and are widely believed to contribute to the health-promoting properties of fruits and vegetables. As precursors of Vitamin A, and as antioxidants, carotenoids play a vital role in human nutrition (Priya and Siva, 2014). The nutritive importance of carotenoids is attributed to its conversion to Vitamin A. Carotenoids act as antioxidants that help prevent heart attacks and cancer, lower the risk of cataracts and muscular disorders, enhance the immune system and maintain skin health (Akinwale et al., 2010; Nassar et al., 2009; Priya and Siva, 2014). Carotenoids also have the vitaminic activity that is required for growth, reproduction, vision and the maintenance of the integrity of epithelial tissue (Akinwale et al., 2010).

The antioxidant properties of carotenoids may help to inhibit the onset of other diseases that are believed to be initiated by free radicals, such as atherosclerosis, age-related macular degeneration and multiple sclerosis (Edge et al., 1997). Furthermore, these properties are linked to the ability of carotenoids to quench singlet oxygen, to eliminate the deleterious effects of free radicals and to play a putative role in cancer prevention (Akinwale et al., 2010; Nassar et al., 2009; Priya and Siva, 2014; Uarrota et al., 2014).

The yellow colour derived from carotene in the preparation of cassava flour meal (commonly called ugali in East Africa), makes it more acceptable to consumers in African countries and it

is less expensive than adding palm oil to get the colour (Akinwale et al., 2010). In addition, they serve as the yellow, orange, and red pigments in many flowers and fruits, to attract insects for pollination and seed dispersal (Siva, 2007).

Though the β-carotene intake is different among men, women and children (WHO, 1998), the World Health Organisation (WHO) indicated that the average daily requirement of β-carotene recommended for an adult is 2.4 to 3.5 mg/day. Because of the nature of carotenoids, the mechanism with which the human body absorbs it and the different levels of β-carotene that are required for men, women and children, it is not easy to find the required quantity of β-carotene that is necessary for people living in rural areas in sub-Saharan Africa. Thus, breeding to improve carotenoids cassava can reverse the effects of a low intake of β-carotene in developing countries.

1.3 Carotenoids rapid screening methods

Present efforts to increase the nutritional value of cassava, including the pro-VAC content through conventional breeding, have generated thousands of new genotypes for evaluation in most developing countries. The quantification of carotenoids is complicated, due to its nature (Uarrota et al., 2014). In developing countries, simple screening, using colour scoring, is the common technique. The yellow and orange pigmentation in cassava storage roots indicate certain level of carotenoids content because the colour intensity is closely related to carotenoids (Sánchez et al., 2006). Therefore, the total carotenoids content and colour intensity are strongly and positively correlated. This suggests that simple screening, based on the visual scoring of colour, is adequate for the initial selection of the genotypes with a relatively high carotenoids content. However, this technique does not quantify the total carotenoids. It only separates the white and yellow or orange colours and is therefore not completely effective. To screen and quantify carotene content in cassava, spectrophotometric approaches, such as near infrared spectroscopy (NIRS), are used for semi-quantification or screening, while high performance liquid chromatography (HPLC) is used for the precise quantification of each individual carotenoid (α-carotene, β-carotene, lycopene, lutein, violaxanthin and zeaxanthin) (Esuma et al., 2012).

1.4 Carotenoids quantification methods

Carotenoids are highly variable molecules with a complex chemical structure and poor stability, which complicates their quantification (Uarrota et al., 2014). Carotenoids analysis is inherently difficult, because many carotenoids exist (Rodriguez-Amaya, 2010). The analytical method of carotenoids in sub-Saharan Africa is further constrained by the acquisition of standards and low concentrations of carotenoids in biological samples (tissue and serum).

Carotenoids quantification is usually based on a linear relationship between the weight of the standard injected and the resulting chromatogram peak area. However, there are no standards available for all carotenoids that are likely to be analysed or measured, and in many cases, only one isomeric form is commercially available (Rodriguez-Amaya, 2010; Uarrota et al., 2014).

Carotenoids extraction has been carried out with acetone, hexane, petroleum ether, methanol and ethanol. Among these solvents, only acetone can dissolve both carotenes and xanthophylls efficiently. Hexane and petroleum ether can dissolve carotenes, but not the xanthophylls, while methanol and ethanol can dissolve the xanthophylls efficiently, but not the carotenes (Rodriguez-Amaya, 2010). Tetrahydrofuran (THF) became a popular extracting solvent with the advent of high performance liquid chromatography (HPLC). It was shown to have excellent solubility for both β-carotene and lutein (Craft and Soares, 1992). For the traditional extraction of carotenoids, the use of a mixture of solvents, which is capable of dissolving both carotene and xanthophylls, could provide good results. However, this conventional method is destructive, produces a large amount of waste and is not environmentally friendly.

Near infrared spectroscopy (NIRS), resonance raman spectroscopy (RRS) and HPLC have the advantages of rapidity, simplicity, safety and low operational costs, while being non-destructive and environmentally friendly (Rodriguez-Amaya, 2010). For wet chemistry, in order to obtain good results, some precautionary measures have to be taken, both at the sample collection stage and in an analytical laboratory. Rodriguez-Amaya (1999; 2010) suggested that the analysis must be conducted within the shortest possible time, to prevent the isomerization and oxidation of carotenoids. In addition, oxygen must be excluded, there must be protection from light, high temperatures and contact with acid must be avoided, while high purity solvents must be used that are free from harmful impurities (e.g. peroxides). There must also be adequate storage conditions and the execution of the analysis must occur immediately after sample collection. The new approaches using NIRS (Davrieux et al., 2016 and Sánchez et al., 2014) provides accurate quantification of carotenoids.

1.5 The antioxidant properties of carotenoids

Carotenoids can be broadly split into two classes, namely, those that are pure hydrocarbons, containing no oxygen (α-carotene, β-carotene, lycopene), and xanthophylls that contain oxygen, such as lutein, violaxanthin, zeaxanthin (Priya and Siva, 2014). The antioxidant property of carotene is its major contribution to human foods. The antioxidant properties of carotenoids and other antioxidants, such as Vitamins E and C, may well depend on the oxygen concentrations present. β-carotene is an antioxidant at atmospheric oxygen concentrations

and it becomes a pro-oxidant in pure oxygen. Vitamin E plays a naturally-protective role against such pro-oxidant effects (Edge et al., 1997). The primary mode of action of carotenoids as antioxidants is to quench singlet oxygen (a reactive type of oxygen) (Rodriguez-Amaya, 2010).

Singlet oxygen can be generated by electronic energy transfer from the excited state (normally triplet state) of a sensitiser (SENS), to oxygen. In biological systems, sensitisers such as porphyrins, chlorophylls and riboflavin, can sensitise O_2 production and this can have deleterious effects, including DNA damage and lipid peroxidation (Azqueta and Collins, 2012; Edge et al., 1997; Palozza et al., 2003). These studies indicated that the β-carotene could inhibit photo-sensitised oxidation and it was therefore an efficient quencher of O_2. Electron exchange energy transfer quenching is the principal mechanism of carotenoids photoprotection against O_2 (Azqueta and Collins, 2012; Edge et al., 1997; Giuliano, 2014; Priya and Siva, 2014). Thus, the incorporation of the carotenoids into the liposomal membrane gives good protection against the effects of dye sensitisation, with β-carotene offering the best protection (Edge et al., 1997). The effect of dye sensitization increases sensitivity to excess sunlight for green and red plants. The excess sunlight can damage the plant cells responsible for photosynthesis, by triggering the release of unstable and highly reactive compounds, such as free radicals.

1.6 Carotenoids antioxidant property and postharvest physiological deterioration in cassava

Carotenoids can act as chain breaking antioxidants and thus protect cells and organisms against photoxidation (Azqueta and Collins, 2012; Edge et al., 1997; Palozza et al., 2003; Priya and Siva, 2014). The analysis of metabolites conducted five days after the cassava harvest, has recently revealed that PPD correlates negatively with phenolics and carotenoids, and positively with anthocyanins and flavonoids (Uarrota et al., 2014). The negative correlation between PPD, phenolics and carotenoids was possibly due to their antioxidant properties, while the positive correlation of PPD with anthocyanins and flavonoids, could be attributed to their pro-oxidant activities, which cause oxidative damage by reacting with various biomolecules, such as lipids, proteins and DNA (Procházková et al., 2011). According to Zidenga et al. (2012), the mechanical damage that occurs during harvesting operations initiates cyanogenesis, by bringing linamarin and linamarase into contact with each other. Cyanide (HCN) inhibits Complex IV in the mitochondrial electron transfer chain. The inhibition of Complex IV causes a burst of reactive oxygen species (ROS) production at Complexes I and III (Figure 1.2). This oxidative burst causes PPD. Overexpressing the mitochondrial alternative oxidase (*AOX*), which is insensitive to cyanide, prevents the overreduction of

Complexes I and III, thus lowering ROS production and delaying PPD. Alternative oxidase is a non-energy conserving terminal oxidase in the plant's mitochondrial electron transport chain (Vanlerberghe, 2013). A reduction of ROS to control PPD can also be achieved by the overexpression of ROS scavengers (Figure 1.2).

During the storage of cassava storage roots, the flavonoid, phenolic and carotenoids content changes. These changes are partly due to the de novo synthesis of those compounds and not to qualitative changes (Uarrota et al., 2014). The complexity of the changes occurring in cassava storage roots, in response to injury, commences as a non-specific response to wounding, during harvesting or root slicing, as a set of biochemical events take place to repair the damaged tissue. The increase in flavonoids may be related to the wound-healing responses (Uarrota et al., 2014). Reactive scavenging species and enzymes, such as superoxide dismutases (SOD), like MnSOD and Cu/ZnSOD, are activated as a protective form of the oxidative stress by cells over the PPD (Uarrota et al., 2014; Xu et al., 2013; Zidenga et al., 2012).

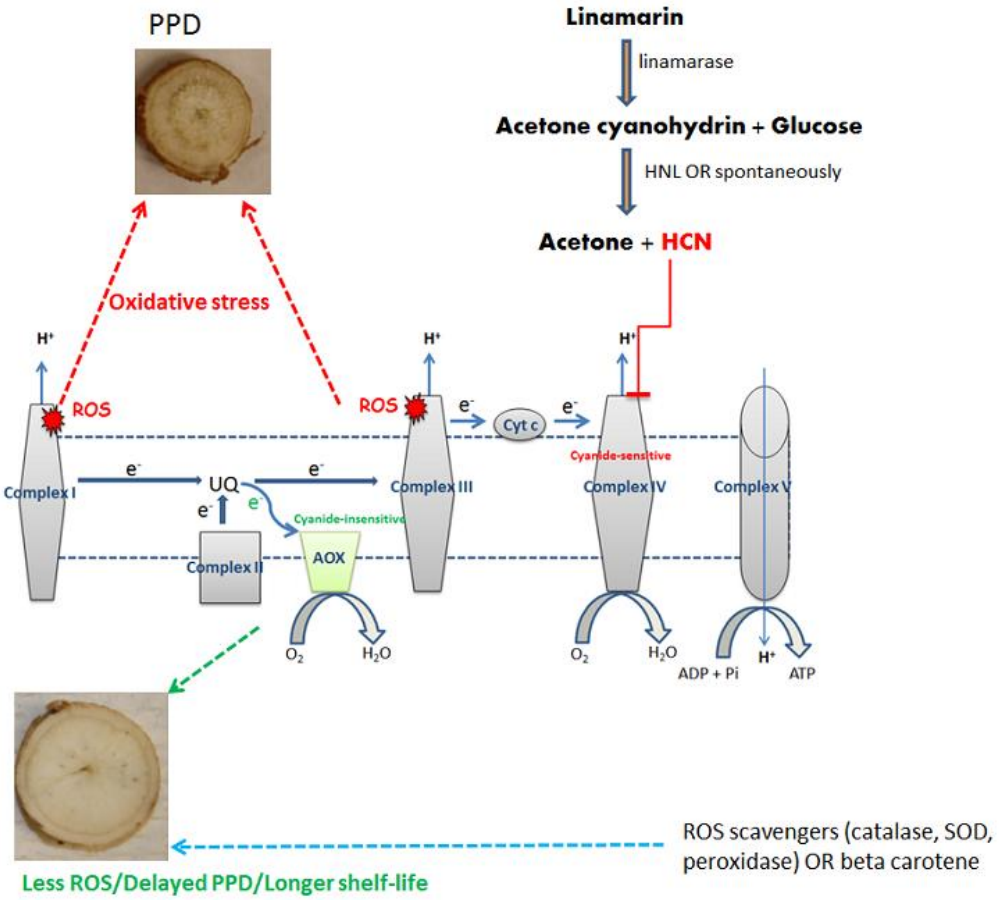

Figure 1.2: The mechanism and control of postharvest physiological deterioration in cassava storage roots (adapted from Zidenga et al., 2012)

Plants have nonenzymatic and enzymatic detoxification mechanisms to scavenge ROS. Nonenzymatic antioxidants include the major cellular redox buffers, ascorbate and glutathione, as well as tocopherol, flavonoids, alkaloids and carotenoids (Xu et al., 2013). Enzymatic ROS-scavenging mechanisms in plants include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione peroxidase. Several groups have addressed the overproduction of SOD in the chloroplasts as a means of enhancing tolerance to oxidative stress (Apel and Hirt, 2004; McKersie et al., 2000; Mittler et al., 2004; Xu et al., 2013)

Figure 1.3 illustrates the intrinsic relationship between reactive oxygen species (ROS) production, scavenging and homeostasis for regulating PPD in cassava storage roots.

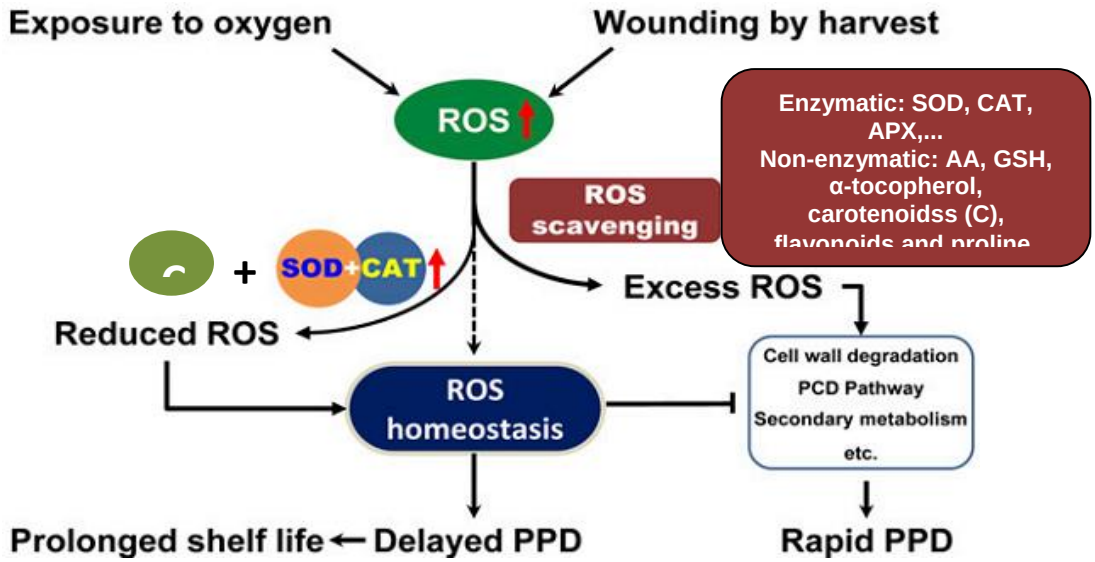

Figure 1.3: Illustration showing the intrinsic mechanism of ROS and PPD (adapted from Xu et al., 2013)

The exposure to oxygen or mechanical wounding during harvesting leads to increased ROS production in the storage root. The inefficient endogenous ROS scavenging of cassava results in excess ROS-inducing cell wall degradation, programmed cell death (PCD) and pathway and secondary metabolism, which trigger rapid PPD responses, and make it difficult to achieve stable ROS homeostasis in harvested cassava storage roots. The ectopic expression of SOD (superoxide dismutase) and CAT (catalase) associated with carotenoids (C) leads to the timely scavenging of excess ROS, thereby keeping the ROS homeostasis balanced and delaying the occurrence of PPD (Xu et al., 2013).

1.7 Carotenoids accumulation and responsible genes

Conventional breeding and genetic modification are both being applied to increase pro-VAC in food crops. It has been found that carotenoids accumulation is highly affected by genetic and environmental factors (Rodriguez-Amaya, 2010). A genetic study revealed that the flesh colour trait in cassava is controlled by two or more genes, which segregate together (Akinwale et al., 2010; Iglesias et al., 1997).

The yellow-fleshed cassava lines overexpress phytoene synthase (*PSY*), which is responsible for the yellow colour and the high-carotenoids content (Welsch et al., 2010). It has been discovered that only two *PSY* genes (*PSY1* and *PSY2*) are involved in carotenoids regulation in cassava (Arango et al., 2010). Both *PSY* genes present a similar contribution to carotenoids formation in cassava leaves. The *PSY2* predominantly regulates carotenoids content in the floral and root parts of cassava. Thus, *PSY2* plays a major role in carotenoids accumulation in most of the eaten parts of cassava. However, the carotenoids content in other plant species, such as maize, sorghum and rice, is regulated by three *PSY* genes (*PSY1*, *PSY2* and *PSY3*) (Li et al., 2009; Li et al., 2008). Their transcript levels vary, depending on the species, plant tissues and growth conditions/stress (drought and salinity, etc). For instance, in the leaves and endosperm of maize, the transcript level of *PSY1* is ten- to fifteen-fold over *PSY2,* while *PSY3* is four- to five-fold lower than in *PSY2* (Li et al., 2008), This is an inverse scenario for the root part of maize, where *PSY3* is four-fold higher than *PSY2* and ten-fold higher than *PSY1*. Therefore, the transcript levels of *PSY* genes, with their corresponding quantitative carotenoids accumulation in cassava storage roots, need further investigation.

The *PSY* is a key regulator of carotenoids biosynthesis and accumulation in many staple crops, including cassava (Giuliano, 2014; Palaisa et al., 2004; Pozniak et al., 2007). Recent findings reported that there is another protein in *Arabidopsis*, called ORANGE (OR) that controls carotenoids biosynthesis, by regulating PSY (Zhou et al., 2015). The genetic control of carotenogenesis must be investigated further, in order to guide breeders to improve the carotenoids content in staple crops.

The level of carotenoids accumulation in plant tissues is influenced by the expression level of *PSY*. It catalyses the specific reaction of prenyl lipid metabolism in the plastid, which is the first reaction in carotenogenesis. A single nucleotide polymorphism in PSY2, causing a non-conservative amino acid exchange, leads to the markedly increased carotenoids formation and accumulation in cassava storage roots (Welsch et al., 2010). This single nucleotide polymorphism in a PSY gene is co-segregated with high β-carotene levels in cassava storage roots, determining the colour of cassava flesh (white, yellow or orange). Polymorphism results

in a single amino acid change in a highly conserved region of the protein, which results in increased catalytic activity (Giuliano, 2014).

The allelic polymorphism is one of the two expressed phytoene synthase (PSY) genes that can enhance the flux of carbon through carotenogenesis, thus leading to the accumulation of coloured pro-VAC in storage roots (Welsch et al., 2010). However, the *PSY* genes from different plant sources differ greatly in their capacity to induce the accumulation of β-carotene in the endosperm of cereals (Giuliano, 2014). Thus, in some cases, the carotene desaturase *CrtI* had to be introduced, along with *PSY,* to increase the level of the carotenoids content in plant grains and roots (e.g. rice and potatoes) (Al-Babili et al., 2006; Diretto et al., 2007; Welsch et al., 2010). The discovery of the genes responsible for carotenoids synthesis and accumulation in cassava, provides an insight into a possible utilization of the gene expression and translation cascade in conventional and genetic engineering, to improve the nutritional value and storability of cassava.

1.8 Feasibility of introgressing carotenoids in cassava

Information coming from various studies (Morante et al., 2010; Reilly et al., 2007; Salcedo and Siritunga, 2011; Salcedo et al., 2010; Xu et al., 2013; Zidenga et al., 2012) indicates that cassava breeding, either through conventional means or genetic engineering, is an important tool that can be used to delay the onset of PPD. Recent studies have reported the development of nutritious pro-VAC cassava genotypes and that these genotypes, due to the antioxidant properties of carotenoids, may retard or inhibit the onset of PPD (Morante et al., 2010; Sánchez et al., 2006; Sánchez et al., 2013; Zidenga et al., 2012). It is known that cassava cultivars contain different concentrations of β-carotene, ranging from 0.1 to 3 mg kg^{1} fresh weight, the latter having bright yellow storage roots. This indicates that the genetic variability is important for breeding an improved level of β-carotene in cassava. Crop improvement through breeding depends on the availability of genetic variability and how easy this variability can be fixed in genotypes with good agronomic characteristics (Akinwale et al., 2010). Genetic mutation that breaks the sequence of β-carotene formation can be the cause of a high accumulation of carotenoids in cassava (Esuma et al., 2012).

To incorporate any traits into an existing variety, the mode of inheritance and the genes of the trait should be known, since this will determine the most appropriate breeding method to be used. It has been discovered that the inheritance of carotenoids in cassava storage roots is controlled by two genes (Chavez et al., 2000), the one controlling the transport of the precursor to the roots, and the other being responsible for the accumulation process. Akinwale et al. (2010) concluded that carotenoids synthesis and accumulation in cassava may be controlled by two or more genes. Chavez et al. (2000) reported further that epistasis affects carotenoids

synthesis and accumulation in cassava. Akinwale et al. (2010) also indicated that there are no maternal or cytoplasmic effects resulting from the inheritance of the carotenoids, hence any of the genotypes could be used as male or female parents in the crossing.

Provided that the total carotenoids content is a highly heritable trait (Ceballos et al., 2013), conventional breeding (Figure 1.4) can generate cassava cultivars with variable carotenoids derivative products, including β-carotene and other antioxidant products, such as lutein and zeaxanthin. In Brazil, through the domestication and selection of carotenoids-enriched cultivars, cassava landraces have acquired a large diversity in relation to many economic traits, such as a high content of carotenoids and excellent palatability, among other characters (Esuma et al., 2012). The level of β-carotene in cassava varieties can be improved through simple recurrent selection, which allows the increase of favourable alleles frequency, through the selection and recombination of breeding populations (Akinwale et al., 2010; Ceballos et al., 2013; Iglesias et al., 1997).

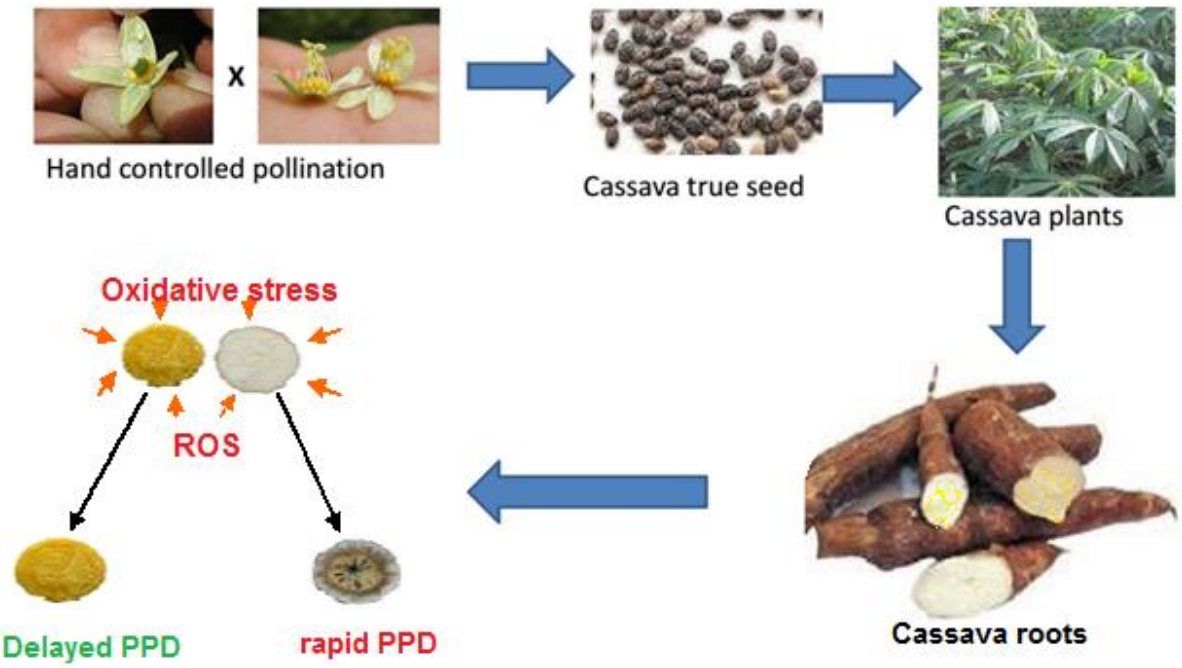

Figure 1.4: Conventional process for developing delayed PPD cassava cultivars

1.9 Current situation of carotenoids enriched-cassava cultivars

The International Centre for Tropical Agriculture (CIAT) has been trying to introgress the tolerance to PPD into cassava that is found in the wild relative *Manihot walkerae* Croizat (Bertram, 1993). As part of the HarvestPlus initiative to improve the nutritional quality of different crops, the cassava genotype GM905-66 was multiplied to provide storage roots for the improved nutritional value (bio-fortification) of cassava (Sayre, 2011). This genotype

showed no sign of PPD after being stored for two months at room temperature (Morante et al., 2010).

In recent years, the breeders at CIAT have produced highly-productive cassava varieties containing up to four-fold as much β-carotene as regular cassava. The International Institute of Tropical Agriculture *(*IITA) in Uganda evaluated 64 accessions and found that the improved accessions were much higher in total carotene (TC) than the landraces. As reported by Esuma et al. (2012), the IITA accessions had a higher mean total carotene (TC) (5.5 ± 2.01 µg/100g) and the landraces had the least mean TC (4.3 ± 1.32 µg/100g), with a skewness of 1.29 and -0.45, respectively. This breakthrough indicates that there is a possibility that the delayed PPD genotypes will become available to smallholder farmers in the near future. Siritunga and Salcedo (2011) recommended that further studies should focus on improving varieties, both through conventional breeding and genetic transformation. The hybridization between improved genotypes and landraces can drastically improve the shelf-life of cassava storage roots and it can consequently enhance the increased adoption of the crop in most cassava-growing countries.

1.10 Correlation between carotenoids and other important traits

The introduced carotenoids-enriched cassava varieties in East Africa, mostly in Rwanda, recorded a low adoption rate, due to low dry matter content and associated problems, such as drying difficulties, taste and aspects of cooking. The low dry matter content was also reported for the varieties tested in Uganda, where a study showed that carotenoids content correlated negatively (R^2=-0.46) with dry matter content (Esuma et al., 2012). This was also the case in Nigeria, where Akinwale et al. (2010) reported that the deeper the yellow colour of the cassava flesh, the lower the dry matter content. Genotypes with the highest carotene levels contain low dry matter, which affects the cooking quality (Akinwale et al., 2010; Ceballos et al., 2012; Esuma et al., 2012; Vimala et al., 2009). However, in a recent study, Ceballos et al. (2013), found a parallel gain of dry matter content (DMC) and carotenoids content in Latin American cassava, suggesting that simultaneously improvement of both traits is feasible if germplasm exchange happens. This finding will serve as an important input in possible future initiatives aimed at improving the carotenoids content of landraces, while preserving the dry matter content and cooking quality, which will increase the adoption rate of the developed varieties.

1.11 Conclusion

The reduced shelf-life of fresh cassava storage roots limits marketing options and increases the likelihood of product losses and higher marketing costs (Ceballos et al., 2004; Sánchez et

al., 2006; Zidenga et al., 2012). Recent studies have reported that PPD is accelerated by the reactive oxygen species (ROS), such as oxygen and peroxide ions (Sánchez et al., 2006; Sánchez et al., 2013; Xu et al., 2013; Zidenga et al., 2012). Thus, efforts in developing highly nutritious cassava could indirectly improve the shelf-life of fresh cassava storage roots by increasing the level of carotenoids, which acts as an antioxidant that is capable of quenching singlet oxygen (a reactive type of oxygen) (Rodriguez-Amaya, 2010).

Both conventional breeding and genetic engineering (genetic transformation) were reported to be effective in improving the level of the carotenoids content in cassava and other crops. However, the adoption of carotene-enriched yellow/ orange fleshed cassava is not feasible in most sub-Saharan African countries, due to the farmers' attitudes towards new technology, as well as the difficulties of integrating farmers into genetic engineering breeding or molecular breeding programs. Hence, participatory conventional breeding should be adopted as a cheap and effective approach for improving carotenoids in cassava and smoothing its adoption. To achieve this, an understanding of the attributes of carotenoids, the genes involved in its accumulation, as well as the effective screening and quantification methods, are of paramount importance. The finding that carotenoids is a highly heritable trait provides hope that conventional breeding, through recurrent selection, can be successful in developing cassava with a high carotenoids content, and ultimately, an effective way to extend the shelf-life of fresh cassava storage roots in developing countries.

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CHAPTER II

Participatory appraisal of cassava production constraints, farmers' preferred traits and factors affecting the adoption of new genotypes in Rwanda

This chapter was published by Athanase Nduwumuremyi[1,2], Rob Melis[1], Paul Shanahan[1] and Theodore Asiimwe[2], (2016). Participatory appraisal of preferred traits, production constraints and postharvest challenges for cassava farmers in Rwanda, Food Security (2016) 8:375–388.

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Abstract

Postharvest physiological deterioration (PPD) and late bulking are among the traits that make cassava an unattractive crop in many environments. This study aimed at assessing the main constraints of cassava production, the effects of late bulking, the losses due to PPD and the factors affecting the adoption of new cultivars in Rwanda. A participatory rural appraisal (PRA) and a baseline survey were conducted in March-May 2014 in three agro-ecological zones in the country, using a multistage sampling method. Cassava is grown on 0.29 ha, out of the total average land possession per household of 0.69 ha. The majority of cassava farmers (59.1%) practise intercropping, as their land holding is small. The average yield is 21.8 t ha^{-1}. Many constraints were identified, particularly the lack of clean cuttings, viral diseases, late bulking cultivars, drought, limited information and knowledge, weathered soils, insufficient fertilizers, land shortage, the lack of a market and effective storage techniques. Losses due to PPD have been estimated at 11.9% of the total production per year. Piecemeal harvesting and the underground storage of roots were the main practices used to delay PPD. A change in colour and taste, rotting, difficulty in removing the skin and an increase of fibres in the flesh, were the methods that farmers used for assessing PPD. The time for harvesting varied from district to district and was attributed to genetic x environment interactions. The use of late bulking varieties and the lack of other yielding crops resulted in reduced food availability and potential food crises. Farmers' preferences, information and extension services, performance, quality, market acceptability and cutting production, influenced the adoption of new cassava cultivars. Breeding objectives that target the end-user preferences, could therefore enhance the adoption of new cultivars.

Key words: carotenoids; cultivar adoption; end-user preferences; farming system; late bulking; physiological postharvest deterioration, storage techniques

2.1 Introduction

Cassava (*Manihot esculenta* Crantz) is the staple food for approximately 500-800 million people living in developing countries (Bull et al., 2011; Howeler et al., 2013), and worldwide, it is second only to maize (*Zea mays L*) for the production of starch (Howeler et al., 2013). In the developing world, cassava is amongst the top four most important crops, in terms of production, after rice (*Oryza sativa* L), maize and wheat (*Triticum spp.*). The potential yield of cassava is estimated at 90 t ha^{-1} of fresh storage roots, under well-managed conditions (El-Sharkawy, 2004). Cassava plays a key role as a food security and income-generating crop for many smallholder farmers in developing countries (Ceballos et al., 2004; El-Sharkawy, 2004; Tumuhimbise, 2013). In East Africa, cassava is eaten after boiling and processing to flour, to make porridge, local brew, ugali and bread, although sweet varieties lacking cyanogenic glycosides can be eaten raw (Kamau, 2006; Mkumbira et al., 2003; Were, 2011). Cassava is an important staple food in Rwanda and it is currently being promoted as a cash crop through the establishment of cassava processing plants. In addition to its storage root, its leaves are treated as a vegetable called “Isombe”. Cassava is consumed in various forms (raw, paste/bread or ugali, boiled for breakfast, mixed with beans, vegetables, etc) and its cooking and preparation methods vary from one individual to another (mixed with beans, boiled, as a paste or ugali, etc.). It occupies third place, after bananas and sweet potatoes, for reducing hunger and poverty in the country (FAOSTAT, 2011; Night et al., 2011). Cassava can be used as a cash crop in industries for the production of animal feed and the production of starch, as well as for use in the pharmaceutical and textile industries (Ceballos et al., 2004; El-Sharkawy, 2004).

Although cassava is a major food crop, its production is threatened by lack of good cultivars (early bulking, high yielding and disease resistant), low soil fertility, poor agronomic practices and postharvest losses. Postharvest losses are linked mainly to the short shelf-life of cassava storage roots, due to postharvest physiological deterioration (PPD), which presents a major challenge to its production and utilization (Ceballos et al., 2004). The PPD begins within 24 hours of harvesting and rapidly renders the storage roots unpalatable, inedible and it reduces their market value (Sánchez et al., 2006). With poor road infrastructure and remote production sites, the short shelf-life severely limits marketing options and increases the likelihood of product losses and higher marketing costs. Physical methods, such as underground storage, the use of fungicides, pruning plants before harvest and cold storage (2-4^{0}C) can be used to

limit PPD (Ravi et al., 1996). However, these techniques are ineffective and impractical, because they are too expensive and complicated, when handling large volumes of harvested storage roots (Sánchez et al., 2013). After harvesting, subsistence farmers need to store food for home consumption and, due to the limited land size, cassava cannot be stored underground (in the field) for long periods, thus there is a need for a technique that can extend its shelf-life for at least some weeks, and preferably several months. Conventional breeding and genetic engineering were suggested as long-term strategies to delay the onset of PPD (Salcedo and Siritunga, 2011). Recent studies elsewhere have reported the development of nutritious carotenoids (pro-Vitamin A with a yellow/orange flesh) cassava genotypes. Due to the antioxidant properties of carotenoids, these genotypes may retard or inhibit the onset of PPD (Morante et al., 2010; Sánchez et al., 2006; Zidenga et al., 2012). However, the hindrance factors for the adoption of yellow-fleshed cassava are still unclear in Rwanda.

Moreover, the high population pressure in Rwanda, resulting in the small land size of farms, can be a constraint in agricultural development, because it reduces the adoption of a long season or perennial crop, such as cassava, due to complications in the cropping system (rotation and intercropping practices) (Howeler et al., 2013). Farming systems and farmers' preferences vary from country to country and from one culture to another. In Rwanda, cassava is grown as a subsistence crop in ten out of twelve agro-ecological zones and the main farming system is intercropping. The type of farming system and the cassava variety preferences depend on the agro-socio environment, such as farm size, climate and crop utilization (Were, 2011). Cassava is grown as a monoculture on large commercial farms and in intercropping systems on small land holdings, for subsistence. Mbwika and Mayala (2001) reported that 46.9% and 15.0% of cassava is grown in intercropping and monocroping systems, respectively, while 38.1% is grown in mixed cropping systems in the country. Cassava is mainly intercropped with maize, beans, bananas, and occasionally with groundnuts or sweet potatoes (Mbwika and Mayala, 2001) and vegetable crops. Farmers preferred varieties based on traits, such as yield, dry matter content, taste, and early maturing or early bulking. The early bulking of cassava means a shortened growth period (Tumuhimbise et al., 2014) within which to accumulate starch and other yield components. Maximum cassava yields are obtained 12-15 months after planting (Hillocks and Jennings, 2003). However, the minimum growing cycle of cassava in Rwanda is around 10 months for the early bulking cultivars (personal observation). The organoleptic qualities (taste and texture) and the ability to cook quickly are important traits of cassava cultivars that are grown for food in most cassava growing countries. For instance, sweet cultivars are grown for raw consumption or for boiling, while bitter cultivars must undergo processing, to reduce the cyanide content.

Despite the popularity and importance of cassava, there is no operational breeding scheme in Rwanda. The cultivars that are grown are therefore mostly non-adapted exotics (introduced from other countries) and a few landraces that are susceptible to the most devastating viral diseases in the region, such as the cassava brown streak disease (CBSD) and the cassava mosaic disease (CMD). There is therefore a need to understand the hindrance factors of adoption and the appropriate varietal selection processes that could improve the cassava yield in the country. The national cassava program invests much effort in evaluating the adaptability of introduced germplasm and focuses on yield and disease resistance as the main traits, which do not necessarily match all critical preferences of farmers. Although there are some improved cultivars introduced from other countries, their adoption level is low and the factors affecting their adoption are still unclear. In addition, relying on varietal introduction, there is no room for participatory varietal selection in Rwanda. On the other hand, the current research conducted in East Africa shows that the limited involvement of end-users in the formal breeding process negatively affected the level of adoption of new cultivars (Kamau et al., 2011; Tumuhimbise, 2013; Were et al., 2014). Many breeding programs in developing countries fail, due to the lack of inclusion of participatory approaches, which negatively affects the level of adoption of newly-developed cultivars (Kamau et al., 2011; Were et al., 2012). Tumuhimbise (2013) reported that taste, cooking qualities and earliness are just a few of the dozens of crop traits of interest to smallholder farmers. Were et al. (2012) also reported that farmers have an indigenous knowledge that could be of value to cassava improvement processes and it could improve their adoption. In some countries, farmers are conservative, understanding the factors that affect the adoption of new genotypes could be important in enhancing adoption.

Participatory plant breeding (PPB) utilizes the farmers' skills in the identification and selection of their preferred traits, it breaks down the barrier between farmers and breeders, reduces the gap between variety development and adoption and improves the availability of planting materials to farmers (Kamau et al., 2011; Kanbar and Shashidhar, 2011; Smith et al., 2001). PPB is convenient for the adoption of new varieties, because farmers participate in the selection of parents and offspring. When farmers and breeders select the parents and new genotypes together, the breeding programs will be more efficient and effective (Ceccarelli, 2006). Participatory plant breeding utilizes many approaches, such as surveys and focus group discussions, in so-called "participatory rural appraisal" (PRA) approaches. The PRA relies heavily on the participation of the communities. This method is designed to enable local people to be involved, not only as sources of information, but as partners in gathering and analysing the information. These two approaches provide vital information on what is needed by farmers (Kamau et al., 2011; Were et al., 2012). The involvement of farmers at some breeding stages could change their conservative behaviour and promote the adoption of new genotypes that contain the preferred traits. Thus, the reorientation of cassava breeding and

the adoption of decentralised participatory breeding (Were et al., 2014) could enhance the adoption of new cultivars. Participatory rural appraisal (PRA), which is aimed at identifying gaps in cassava production, could help to build a strong foundation for a cassava breeding scheme in Rwanda.

2.1 Materials and methods

2.2.1 Study sites

This study was conducted in three major cassava-growing districts in Rwanda, namely, Bugesera, located in the Eastern Province, Kamonyi, located in Southern Province, and Gakenke, located in Northern Province (Figure 2.1). Geographically, Bugesera lies at 02°12′18″S 30°08′42″E, Kamonyi, at 2°0′0″S, 29°54′0″E and Gakenke at 1°42′0″S, 29°47′0″E (Figure 2.1).

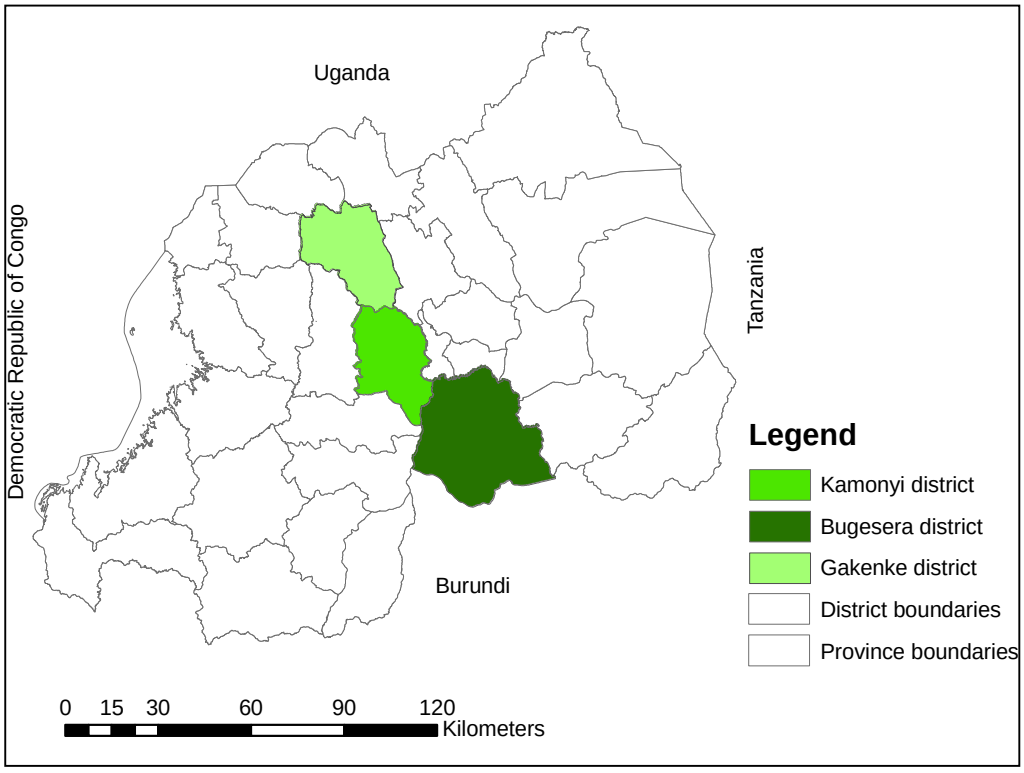

Figure 2.1: Map of Rwanda showing the study areas

The selection of these districts was based on cassava production levels, which could be influenced by different factors, including altitude, temperature, rainfall and type of soil (Table 2.1).

Table 2.1: Description of study area

Districts	Altitude (m)	Average precipitation	Temperature (ºC)	Soil type	Potential cassava production
Bugesera	1300-1500	900	18-30	Strongly altered clay	Very Good
Kamonyi	1400-1600	1050	16-30	Clay soils, schist	Good
Gakenke	1500-1900	1200	14-29	Diverse humic soils	Poor

2.2.2 Data collection

In order to assess cassava production constraints, preferred traits, factors affecting the adoption of new cassava cultivars, cassava market aspects, as well as losses due to postharvest physiological deterioration (PPD), data were collected through focus group discussions (FGD) and interviews with farmers. To facilitate data collection, FGD checklists and questionnaires were translated into the local language (Kinyarwanda). Three FGDs were conducted in each district, making it a total of nine for the whole study. Each FGD was composed of ten participants, namely, one district agronomist, one district extension officer, one cassava extension specialist from the Rwanda Agriculture Board (RAB), and seven farmer representatives from different groups of farmer field schools (FFSs). Some participants, mainly district and RAB staff, participated in all the FGDs within a district. Gender was balanced, with women taking up two-thirds of the group, because they are much more involved in agricultural activities than the men in the country.

Semi-structured questionnaires were developed that were to be administered to cassava farmers, cassava traders and processors. Sampling was done within FFSs at district level, where a total of 60 cassava farmers from each district were selected. Multistage sampling was performed, based on the cassava-growing areas, and three districts were chosen. Random sampling was done within the FFSs, where ten FFSs were selected from each district. Random sampling was also performed at household level, where six households were selected from each FFS, to participate in the interviews. This makes a total sampling size of 60 participants per district and 180 participants in total.

2.2.3 Data analysis

A pair-wise ranking matrix and scoring matrix (Andrew et al., 2007) were used to compute the data from PRA. Data for land size, land allocated to cassava, losses due to PPD, the time to harvest and cassava yield were analysed, using the post hoc test, ANOVA (Hilton and Armstrong, 2006). Other collected social data were screened and coded, to be analysed using

the Statistical Package for Social Sciences (SPSS), 16th Version. Percentages, means and cross tabulations are presented in the following Results section.

2.3 Results

2.3.1 Cassava farming system in Rwanda

2.3.1.1 Main food crops grown in Rwanda

Cassava, bean (*Phaseolus vulgaris* L), sweet potatoes (*Ipomoea batatas* L) and maize are the major crops grown in the study areas. In the Bugesera and Kamonyi Districts, cassava ranked first among 90.0% and 75.0% of respondents, respectively, while in the Gakenke District, it occupies fifth place after bananas (*Musa spp*. L), bean, maize and sweet potatoes. Maize occupies the second place in the Bugesera District, while bean occupies the same place in the Kamonyi and Gakenke Districts (Table 2.2). The places of other crops vary from district to district. Rice, coffee (*Coffea arabica* L), pineapple (*Ananas comosus* L) and sunflower (*Helianthus annuus* L) were ranked as minor crops in all districts.

Table 2.2: Ranking of cassava and other main crops in three districts of Rwanda (2014)

Ranking	Districts		
	Bugesera	**Kamonyi**	**Gakenke**
1st	Cassava (90.0%)	Cassava (75.0%)	Bananas (47.5%)
2nd	Maize (43.3%)	Bean (46.7%)	Bean (41.1%)
3rd	Sweet potatoes (40.0%)	Irish potato (38.1%)	Maize (33.3%)
4th	Bean (35.0%)	Soybean (33.3)	Sweet potatoes (28.8%)
5th	Groundnut (25.9)	Maize (30.0%)	Cassava (27.1%)
6th	Sorghum (23.5%)	Bananas (25.9%)	Vegetables (28.1%)
7th	Soybean (21.7%)	Taro (25.5%)	Taro (26.6%)

2.3.1.2 Land size and cassava yield

The land allocated to cassava differed significantly from district to district ($p < 0.001$) and, in general, it was greater than the land allocated to other crops. On average, the total land was 0.69 ha per household, while the average land allocated to cassava was 0.29 ha. The majority of farmers had a total land size of less than one ha and therefore practised intercropping systems (Table 2.3). Legumes (beans and soybeans) were the most common crops that were intercropped with cassava in the study area. However, some farmers mixed cassava with maize, pineapple, shrub crops and young trees, such as eucalyptus. The farmers with a land size larger than one ha, grew cassava as a monoculture (Table 2.3).

Table 2.3: Land size and cassava cropping system in Rwanda (2014)

Districts	Land size mean		Farming system		Estimated cassava Yield (t ha^{-1})
	Total Land size	Land allocated to cassava	Monoculture (%)	Intercropping (%)	
Bugesera	0.97	0.46	55.0	45.0	24.5
Kamonyi	0.52	0.23	43.3	56.6	24.2
Gakenke	0.56	0.20	33.3	66.7	16.7
Mean	**0.69**	**0.29**	**43.9**	**56.1**	**21.8**
LSD	**0.15**	**0.08**	**-**	**-**	**6.86**
P value	**<0.001**	**<0.001**	**-**	**-**	**0.045**

LSD= Least significant differences of means (5% level)

The yield of cassava varied significantly (p = 0.045) from district to district. Focus group discussions reported yields of 24.5, 24.2 and 16.7 t ha^{-1} in the Bugesera, Kamonyi and Gakenke Districts, respectively (Table 2.3).

2.3.1.3 Growing cycle, causes and effects of late bulking cultivars

Time to harvest varied significantly (p < 0.001) between districts and ranged from 6 to 24 months, but 16 months was the average bulking time for all districts. Early bulking cultivars (6-8 months) were reported in the Bugesera and Kamonyi Districts, while early bulking occurred at 12 months in the Gakenke District (Table 2.4).

Table 2.4: Time to harvest per district

Districts	Time to harvest (months)	Minimum time to harvest (months)	Maximum time to harvest (months)
Bugesera	13	6	24
Kamonyi	14	8	24
Gakenke	19	12	24
Mean	**16**	8.6	24
LSD	**1.444**	**-**	**-**
P value	**<0.001**	**-**	**-**

LSD= Least significant differences of means (5% level)

The first cause of the late harvest was the late bulking cultivars, which is inherent in cassava cultivars, as indicated by the majority of farmers in the study area. Agricultural practices and the cold environment were commonly reported to be the second cause of late bulking. Farmers from the Bugesera District suspected that planting cassava at the wrong time of the year (out of season) is the cause of late maturity (Table 2.5) and that this affected the yield. The best planting time in Rwanda is between September-October each year.

Table 2.5: Farmer perceptions on the causes and effects of late bulking cultivars

Perceptions	Districts			Mean (%)	Rank
	Bugesera (%)	Kamonyi (%)	Gakenke (%)		
Causes of late bulking					
Agricultural practices/ farming system	75.7	100.0	25.1	66.9	1
Cold climate and storms	10.5	100	73.1	61.2	2
Drought	97.3	-	-	32.4	3
Planting at wrong time	10.5	-	-	3.5	4
Effects of late bulking					
Losses of other crops' yields	75.4	87.5	99.4	87.4	1
Food crisis (lack of food, malnutrition and prolonged hunger)	73.9	59.2	16.7	49.9	2
Poverty	38.9	33.5	-	24.1	3
Crop rotation impediment	-	-	62.8	20.9	4
Delayed return of investment	6.9	15.4	14.9	12.4	5
Lack of cuttings	5.0	4.3	6.4	5.2	6

Food crises (the shortage of food, malnutrition and prolonged hunger), lack of cuttings, the loss of other crop yields and the delayed return on investments, were commonly reported as the main effects of late bulking cassava cultivars. Farmers in the Bugesera and Kamonyi Districts also highlighted poverty and crop rotation as being impediments (Table 2.5).

2.3.1.4 Availability of clean cuttings

The majority of farmers (an average of 66.6% in all districts) confirmed that the availability of clean cuttings was a problem. The sources of clean cuttings were research institutes, farmers' groups, cooperatives and NGOs (Figure 2.2). Farmers lacked a source of clean cuttings from neighbouring farmers and their own previous cassava crops (Figure 2.2). However, farmers were unsure of the health status of the available cuttings.

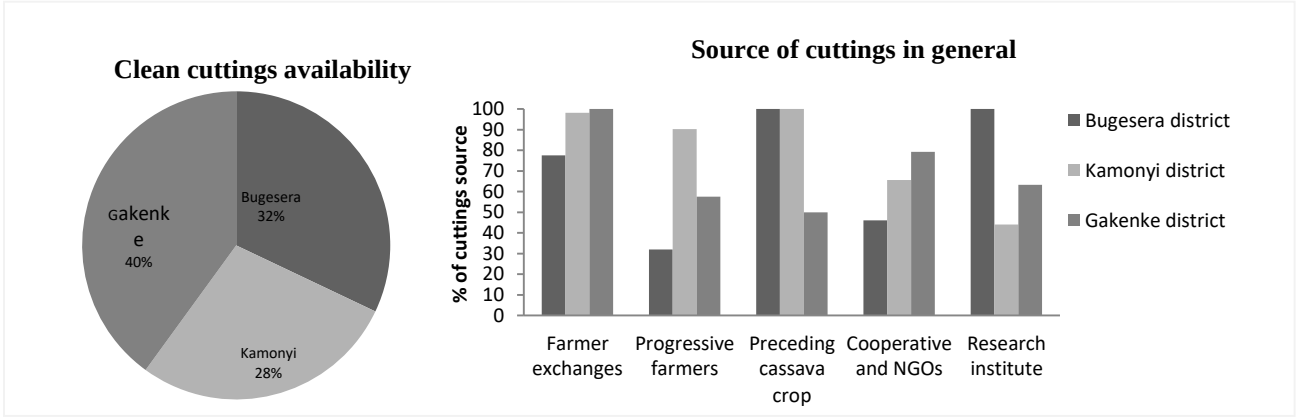

Figure 2.2: Availability of clean cuttings

2.3.2 Cassava production constraints in Rwanda

The majority of farmers (99.4%) confirmed the presence of cassava production constraints. The lack of clean cuttings, late bulking cassava cultivars and diseases, especially CBSD and CMD, were the main constraints of cassava production. Drought was the second most challenging constraint in the Bugesera District, while the lack of clean cuttings was ranked the same in the Kamonyi and Gakenke Districts (Table 2.6).

Table 2.6: Constraints to cassava production per district

Rank of constraints	Constraints per districts		
	Bugesera (98.3%)	**Kamonyi (100%)**	**Gakenke (100%)**
1st	Lack of clean cuttings	Late bulking cassava cultivars	Diseases (CBSD and CMD)
2nd	Drought	Lack of clean cuttings	Lack of clean cuttings
3rd	Weathered soils	Diseases (CBSD and CMD)	Insufficient fertilizers
4th	Insufficient fertilizers	Small land size	Late bulking cassava cultivars
5th	Limited access to information	Insufficient fertilizers	Limited access to information
6th	Lack of market	Weathered soils	Storage of fresh and dried cassava
7th	Theft and animal damage	Limited access to information	Agriculture policy of crop regionalization
8th	Small land size	Storage of fresh and dried cassava	Cold climate
Other minor constraints	Limited knowledge on cassava cropping systems	Limited knowledge on cassava cropping system	Heavy rainfall and storms
	Agricultural policy of crop regionalization	-	-

CBSD: cassava brown streak disease; CMD: cassava mosaic disease

The other major challenging constraints, per ranking order, were weathered soils, insufficient fertilizers, small land size, limited information and access to information, and lack of a market, storage of fresh and dried cassava, theft and animal damage and agricultural policy of crop regionalization. Several minor cassava production constraints, such as cold environment, heavy rainfall and storms, as well as limited knowledge on cassava cropping, were identified by farmers and varied from district to district (Table 2.6).

2.3.3 Postharvest physiological deterioration of cassava

2.3.3.1 Fresh root cassava storage constraints and storage techniques

A total of 96.7%, 98.3% and 70.7% of farmers in the Bugesera, Kamonyi and Gakenke Districts, respectively, confirmed the lack of effective storage techniques (Figure 2.3).

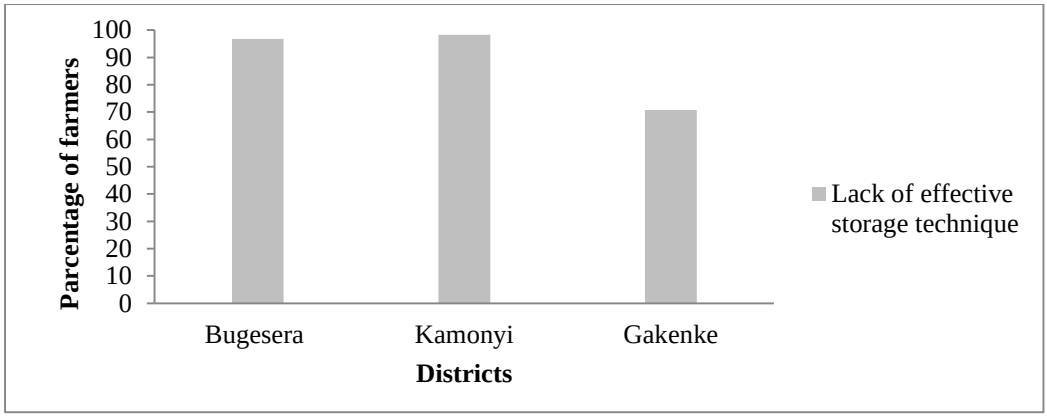

Figure 2.3: Effective cassava storage constraints

The traditional technique of storing cassava as flour i.e. drying and processing cassava storage roots to flour, was the most widely used technique to deal with PPD, and this was confirmed by the majority of farmers (89.2%) across the districts. Piecemeal harvesting (gradual harvesting) can conserve cassava storage roots for more than one year, as indicated by 71.9% of farmers interviewed in the study area. However, once harvested, the underground storage of cassava roots in the soil (interment) conserves the storage roots for only four days, on average, which was confirmed by 53.8% of the farmers (Table 2.7). Less common techniques highlighted by farmers in the Kamonyi District were the storage of peeled cassava in water and precooking it. However, the latter cannot conserve cassava storage roots for more than three days.

Table 2.7: Traditional storage techniques of cassava fresh storage roots in Rwanda

Storage techniques	Districts			Means (%)	Rank
	Bugesera (%)	Kamonyi (%)	Gakenke (%)		
Storage of flour (drying and processing in flour)	87.5	80.0	100	89.2	1
In the field (piecemeal harvesting)	72.7	55.9	87.0	71.9	2
Interment in the soil of harvested roots	60.0	56.9	44.4	53.8	3
Precooking	-	25.9	-	25.9	4
Dumping in water	-	17.6	-	17.6	5

2.3.3.2 Local methods for detecting PPD in Rwanda

The colour change of the cassava flesh was the most common method for measuring PPD, as reported by 89.8%, 80.7% and 86.4% of the farmers interviewed in the Bugesera, Kamonyi and Gakenke Districts, respectively. Rotting of cassava flesh and changing taste were

classified as second and third most common methods used to assess PPD damage (Table 2.8). However, FGDs indicated that local methods used to detect PPD included the difficulty experienced when removing the skin (peeling) and an increase in fibres in the cassava flesh.

Table 2.8: Local methods to detect PPD damage

PPD measuring methods	Districts			Mean (%)
	Bugesera	Kamonyi	Gakenke	
Colour change of cassava flesh	89.8%	80.7%	86.4%	85.6%
Rotting of cassava flesh	55.6%	28.3%	43.8%	42.6%
Changing taste of cassava flesh	11.1%	79.2%	18.8%	36.4%
Unclassified methods				
Difficult to remove cassava skin (peeling)	Yes	Yes	No	-
Increase of fibers in cassava flesh	No	No	Yes	-

2.3.3.3 Losses due to PPD and value given to damaged cassava storage roots

There was no significant difference (p = 0.259) in cassava production losses due to PPD in the study area. Losses of 12.6%, 10.3% and 13.3%, respectively, were reported in the Bugesera, Kamonyi and Gakenke Districts. The PPD appeared approximately three days after harvest (Table 2.9).

Table 2.9: PPD appearance after harvest and PPD losses in Rwanda 2014

Districts	PPD appearance after harvest (days)	PPD losses %
Bugesera	3.0	12.6
Kamonyi	2.9	10.3
Gakenke	2.7	13.8
Mean	**2.9**	**11.9**
P value	**-**	**0.26**

In case of total deterioration due to PPD, the deteriorated roots processed into flour and given to poor families of a lower social class, or used to feed animals, especially pigs. Some farmers in the Kamonyi and Gakenke Districts indicated that deteriorated cassava was processed into flour for constructing their houses (i.e. for painting and mixing with cements). However, 44.1%, 41.0% and 14.5%, respectively, of farmers in the Bugesera, Gakenke and Kamonyi Districts, reported that damaged cassava was considered to be garbage (Table 2.10).

Table 2.10: Place and value given to deteriorated cassava due to PPD

Value given to damaged storage roots	Districts			Mean (%)	Rank
	Bugesera (%)	Kamonyi (%)	Gakenke (%)		
Drying and processing in flour	26.7	87.5	40.5	51.6	1
Garbage	44.1	14.5	41.0	33.2	2
Food for the poor	14.3	41.8	23.1	26.4	3
Flour processing for painting houses	-	50.0	23.1	24.4	4
Animal feed	45.8	-	23.1	23.0	5

2.3.3.4 Availability of PPD tolerant cassava cultivars in Rwanda

Although PPD normally begins to appear after 24 hours, the farmers indicated that some cultivars showed evidence of PPD only after three days, which they saw as an indication of PPD tolerance. The common popular cultivars with delayed PPD were Rwizihiza, Mavoka, Cyizere, Seruruseke and Mbakungahaze (Table 2.11). Most of these cassava cultivars are assumed to have high yield, be disease resistant and possibly improved dry matter, carotenoids content and other valuable traits. Some landraces (Gahene, Nyiramabuye, Rutanihisha, Yangwe, Rwicabana and Gapfutsi) were also reported to tolerate PPD. These landraces are bitter, with a high cyanide content, which could delay microbial attacks. The only sweet landraces tolerant to PPD were Gacyalicyali and Mushedile. However, farmers indicated that none of the cultivars tolerate PPD beyond three days, under normal conditions of storage i.e. at room temperature. This shows that there is no cultivar in Rwanda that is tolerant to PPD, compared to cultivars in other countries, which can withstand PPD from one week, to several weeks.

Table 2.11: Some cassava cultivars in which symptoms of PPD were delayed

Delayed PPD cultivars	Districts			PPD delayed time to symptom expression (days)[a]
	Bugesera (%)	Kamonyi (%)	Gakenke (%)	
Gehene	46.5	-	-	3
Nyiramabuye	3.4			1
Rwizihiza	33.7	56.2	6.7	3
Mavoka	26.0	10.7	50.7	3
Gitamisi	21.0	-	36.0	2
Cyizere	48.4	100.0	8.0	2
Improved cultivars	13.1	-	-	3
Rutanihisha	9.6	40.6	-	2
Seruruseke	42.8	33.3	75.0	3
Yangwe	7.2	-	13.3	3
Gacyalicyali	-	20.8	28.0	2
Rwicabana	-		12.0	3
Gapfutsi	21.6	-	-	3
Mushedile	3.8	-	-	1
Mbakungahaze	18.8	34.7	45.0	2

[a] *measured from 24 hours after harvesting*

2.3.4 Farmer preferred cassava traits in Rwanda

The adoption of newly-introduced cassava cultivars must correspond to the preferences of farmers and consumers. In order of importance, the preferred cassava traits were sweet taste, high yield, good quality ugali (viscosity and colour), resistance to pest and diseases, early bulking, being multipurpose, good colour of the flesh and flour, many clients, delayed PPD, dry matter content, good odour/ smell, long storage in the field, many cuttings produced and good cookability (Table 2.12).

Table 2.12: Consumers and farmers' traits preference

Preferred cassava traits	Districts			Mean (%)	Rank
	Bugesera (%)	Kamonyi (%)	Gakenke (%)		
Sweet taste	95.3	60.7	100.0	85.3	1
High yield	80.0	72.8	64.5	72.4	2
Good ugali (good quality: taste, colour and viscosity)	91.2	38.3	74.8	68.1	3
Resistance to diseases	50.8	40.5	80.3	57.2	4
Early bulking	47.0	53.8	53.4	51.4	5
Multipurpose	32.6	100.0	17.8	50.1	6
Good colour of flesh (preferably white colour)	30.7	94.4	5.3	43.5	7
Many clients (level of acceptability at market)	69.6	21.2	8.3	33.0	8
Delayed to PPD	-	65.0	6.2	23.7	9
Dry matter content	16.5	21.5	30.5	22.8	10
Good odour	42.9	18.6	-	20.5	11
Long storage in field	26.5	3.3	6.2	12.0	12
Many cuttings produced	13.7	-	3.5	5.7	13
Cooked well (cookability)	3.4	-	4.2	2.5	14

2.3.5 Farmer perceptions on yellow-fleshed cassava

All farmers confirmed the availability of two yellow cassava cultivars. However, the majority of them (95%, 55% and 52.3% in the Bugesera, Kamonyi and Gakenke Districts, respectively) disliked yellow-fleshed cassava. They highlighted some reasons for its unpopularity, namely, drying problem (low dry matter), bad colour of the flour, lack of taste, rapid rotting, the carotene odour from volatile carotenoids compounds, low demand (few clients), poor storage in the field (spoiled when kept in the field) and the fact that it did not cook well. However, 33% of the respondents like yellow-fleshed cassava cultivars for its early bulking, its resistance to CMD, its high yield, good ugali, its multipurpose use (eaten as raw or processed into flour for ugali), its Vitamin A content and its cookability (Table 2.13).

Table 2.13: Farmers' perceptions on yellow-fleshed cassava

Preferences of yellow cassava cultivar	Districts Bugesera (%)	Kamonyi (%)	Gakenke (%)	Mean (%)	Rank
High preference	5.0	45.0	47.7	32.6	-
Less preference	95.0	55.0	52.3	67.4	-
Reasons for high preference					
Early bulking	100.0	100.0	62.7	87.6	1
Resistant to pest and diseases	66.7	51.9	28.0	48.9	2
High yield	33.3	22.2	52.9	36.1	3
Good ugali	7.7	76.8	13.6	32.7	4
Multipurpose	-	40.7	-	13.7	5
Sweetness	-	-	21.6	7.2	6
Vitamin A	-	8.3	12.0	6.8	7
Cooked well		-	9.1	3.0	8
Reasons for less preference					
Drying problem	16.7	100	16.0	44.2	1
Bad colour	10.9	75.8	44.6	43.8	2
Without taste	77.0	10.0	42.9	43.3	3
Rapid rotting in the field	38.8	6.1	60.0	35.0	4
Carotene odour	26.0	40.0	28.6	31.5	5
Fewer clients	54.2	30.0	8.0	30.7	6
Low dry matter	38.9	-	-	13.0	8
Poor storage in the field	14.5	25.0	-	13.2	7
Not cooking well	15.2		2.9	6.0	9

2.3.6 Factors influencing adoption of new cassava genotypes

Focus group discussions revealed some factors that affect the adoption of new cassava cultivars. Pair-wise ranking listed the factors in descending order, namely: farmer preferences, information and extension services, performance (yield, early bulking, disease resistant), quality (cooking aspect, taste, dry matter, viscosity of ugali, colour of ugali), market acceptability and stake production. These proved to be the main factors that influence whether farmers adopt or reject the newly-introduced cassava genotypes in Rwanda (Table 2.14).

Table 2.14: Pair wise ranking of factors affecting adoption of new cassava cultivar in three districts of Rwanda

Factors	Ranking per district			
	Bugesera	Kamonyi	Gakenke	Overall rank
Farmer consultation before development of a new cultivar	1	2	1	1
Performance (yield, early bulking, diseases resistant)	3	1	2	2
Quality (cooking aspect, taste, dry matter, viscosity of Ugali)	4	3	3	3
Market acceptability	2	4	6	4
Information and extension services	6	5	4	5
Stake production	5	6	5	6

2.4 Discussion and conclusion

This study aimed at identifying the main constraints of cassava production, the preferred traits of farmers, the effects of late bulking cultivars, losses due to PPD, and factors affecting the adoption of new genotypes. An understanding of these aspects in the cassava farming system provides guidelines and objectives for the cassava breeding program in Rwanda.

The study found that cassava is one of the main food crops in the country, but its place varies from district to district. This agrees with Stephen and Lecumberri (2011), who reported that cassava, beans, maize, bananas and sweet potatoes are the main food crops grown in Rwanda. The findings showed that the average farm size was 0.69 ha, with an average of 0.29 ha being allocated to cassava per household. However, many farmers possess a farm size of less than the average (0.6 ha). This is corroborated by Rurangwa (2013), who reported that the majority of Rwandan households have less than 0.2 ha. The land allocated to cassava was greater than for other crops and the predominance of intercropping was attributed to the small size of land available. This is a result of the dense population of the country (407 people/km^2, according to Rwanda's National Institute of Statistics in 2012), which is the greatest in the SSA region (Rurangwa, 2013). Land in the study area was fragmented, necessitating subsistence farming. Subsistence farming was associated with the large spacing (1 x 1 m/10000 plants per ha) required for cassava and the need for food diversification. Households with small plots tended to practise intercropping, in contrast to those with larger plots, which mostly practised monoculture. This is corroborated by Mbwika and Mayala (2001) who found that the majority of cassava farmers in Rwanda practised mixed cropping. Many scientists (El-Sharkawy, 2004; Munga, 2008; Were et al., 2012) report that cassava is grown by small-scale farmers in intercropping and mixed cropping systems in developing countries.

The results revealed that the lack of clean cuttings, the occurrence of pests and diseases (especially CBSD and CMD) and late bulking cultivars, were the main constraints of cassava

production in the study area. These findings agree with Tumuhimbise (2013), who showed that virus diseases, such as CBSD and CMD, are the most challenging constraints in Uganda, followed by the lack of early bulking cultivars, which was reported by Kamau (2006) to be a challenging constraint of cassava production in the East African region.

The majority of farmers confirmed the lack of effective storage techniques. This was linked to postharvest physiological deterioration (PPD), which starts a few hours after harvest. Most cassava production is marketed as fresh roots for consumption, freshly-boiled cassava, or for processing, which means that they need to be free from any deterioration (PPD and bacterial rots). PPD differs from bacterial rot in that there is a change in colour of the flesh, due to physiological activity. This begins within 24 hours after harvest and starts as blue-black to black vascular discoloration (vascular streaking). It then spreads to the parenchyma, thereby rendering the storage root unpalatable, due to its flavour, odour and colour, and therefore unmarketable (Morante et al., 2010; Reilly et al., 2007; Sánchez et al., 2006). Ceballos et al. (2004) also reported that this results in a short shelf-life and presents major challenges in developing countries, when it comes to increasing production and utilization. Tackling the effects of PPD needs much effort; therefore, the private sector and government must invest in infrastructures and cassava processing plants, in order to reduce the time between harvest, marketing and the initiation of processing activities.

The traditional measures used in the study area to tackle losses caused by PPD were to keep cassava storage roots in the field, using gradual (piecemeal) harvesting and the underground storage, which conserves the roots for at least four days. The former can conserve cassava storage roots for approximately one year, but it can also compromise agriculture practices (rotation), because it occupies land for longer periods. Sayre (2011) reported that harvesting cassava can be delayed by months, or even up to three years. On the other hand, cassava storage roots that are kept in the field for long periods can become woody and their quality and flavour may be affected.

The traditional ways in which farmers assess PPD include noting a change in colour and taste, rotting, difficulty in removing skin (peeling) and increased fibre. The difficulty in peeling could be attributed to an increase in fibres, which may be a defence mechanism of cassava against bacterial attack, after wounding (Kpémoua et al., 1996; Luna et al., 2011; Uarrota et al., 2014).

The study found that, on average, 11.9% of cassava production losses occur due to PPD. FAO (2000) reported total postharvest losses of 29%, 10% and 8% in Africa, Latin America and Asia, respectively. Rwandan farmers consider cassava to be totally deteriorated approximately three days after harvest. This may be the result of a bacterial attack, which can cause total rotting of cassava flesh, leading to a total financial loss. For non-bacterial PPD,

farmers minimize the financial loss by feeding the affected cassava to animals (especially pigs) (Ubalua, 2007), by selling it at a low price as food to poor families of a lower social class, and by processing the flour, for use in cement and house paints. Okafor (2008) reported that cassava flour performs satisfactorily as a water reducing admixture in concrete. Cassava starch prevents floor cracks, by stopping the formation of calcium silicate hydrates (CSH) in concrete mixtures, and is mainly responsible for adding quick strength to the quality of standard cement (Abalaka, 2012).

The significant differences in harvesting time observed in the districts, was attributed to the genotype x environment interactions and ecological differences among districts. The majority of farmers reported that bulking time is an inherent characteristic of the cultivar and the growing environment. The food crisis (lack of some important foods at specific times and locations, which leads to malnutrition) and the lack of other crop yields, were linked to the late bulking cultivars occupying land for long periods of time. These findings agree with those of Tumuhimbise (2013) and Kamau (2011), who reported that the late bulking cultivars occupy land for extended periods and that the land can consequently not be effectively utilised for the sequential cultivation of other crops. Zidenga et al. (2012) reported that the land may need to be released for other uses, in a semi-commercial setting. The lack of cuttings was also perceived as a result of late bulking cultivars.

A sweet taste, high yield, good ugali (good quality taste, colour and ugali viscosity), resistance to diseases, early bulking and multipurpose uses, were the most preferred traits, according to the farmers. These traits, except the multipurpose trait, were reported by Tumuhimbise (2013) in Uganda, where farmers selected cultivars that focused largely on high fresh root yield, early bulking, resistance to pests and diseases, and sweetness. The needs of farmers, the behaviour of consumers, the priorities of farmers, the production environment, the available transformation technologies and the farming systems should be some of the factors that dictate cassava selection.

The introduction and breeding of a yellow-fleshed cassava cultivar could be an effective way of reducing postharvest losses caused by PPD. Many scientists (Bayoumi et al., 2010; Sánchez et al., 2006; Sánchez et al., 2013; Xu et al., 2013; Zidenga et al., 2012) reported that carotenoids could delay the onset of PPD, owing to their antioxidant properties (Morante et al., 2010; Zidenga et al., 2012). Despite the availability of some yellow-fleshed cassava in the country, their adoption is hampered by their yellow/orange colour being linked to traits, such as low dry matter content and carotene odour, when consumed fresh or when boiled. However, the carotene odour disappeared when improved carotenoids cultivars were processed into flour, in order to produce cassava paste or ugali. This odour could originate

from volatile compounds that are derived from boiling (heating) cassava storage roots with a high carotenoids content. Rios et al. (2008) and Zepka et al. (2014) reported that oxidation and thermal treatment cause the degradation of carotenoids, which influence the aroma and flavour of the products that contain them. This explains the non-adoption of yellow cultivars with high β-carotene content. Salcedo and Siritunga (2011) reported that several agronomic traits, such as starch and dry matter, are negatively correlated with PPD and Wenham (1995) confirmed that delayed PPD was associated with reduced dry matter content.

In conclusion, this study revealed the main constraints of cassava production and the losses caused by PPD, the farmers' preferred traits, as well as the factors affecting the adoption of new cultivars in Rwanda. The lack of clean cuttings, the occurrence of pests and diseases (CBSD and CMD) and late bulking cultivars, are associated with an 11.9% loss, due to PPD, and this handicaps the cassava sector in the country. The newly-introduced yellow cultivars, which are resistant to CMD, have early bulking (eight months) and a high yield could be an option, to reverse the main constraints reported by farmers. However, the lack of a local participatory breeding program has affected the adoption of introduced cultivars. Participatory plant breeding and involving farmers at some stages of the breeding process, could promote the ownership of the developed cultivars and could consequently enhance their adoption. The development and adoption of the improved carotene cultivars for paste (ugali), early bulking, disease resistance and delayed PPD, are expected to promote the cassava sector and improve the livelihood of cassava growers in Rwanda.

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CHAPTER III

Cassava genetic variability and inter-relationship between yield and yield component and postharvest traits in Rwanda

Abstract

Genetic variability is the backbone of crop improvement. However, breeding for some traits progresses slowly, due to insufficient information on genetic variability among the populations. This study aimed at examining the extent of genetic variability in cassava for yield, yield components and postharvest physiological deterioration (PPD). During 2014 and 2015, experiments were conducted in five contrasting environments in Rwanda. The data collected were based on the cassava farmers' preferred traits and they were subjected to the variance analysis, using GenStat 17th Edition. The results showed a high genetic variability (61.0%) among 30 genotypes that were collected across the country. The lowest determinant coefficient (R^2) was 0.734 for the dry storage root yield (DSRY) and the highest (0.982) was recorded for total carotenoids (TC), indicating that 73.4% of the DSRY variation was due to genotype, 26.6% of the variation was from an unknown origin, while 98.2% of the TC variation was explained by genotypes and only 1.8% was due to an unknown origin. Similarly, TC had a very high heritability (H^2) of 99.2% and an expected genetic advance (GA %) of 159.65%. The phenotypic variance coefficient (PCV %) for all traits was higher than the genotypic variance coefficient (GCV %), except for the viral diseases traits (CMD and CBSD). The high H^2 (%) and GA (%) for carotenoids content was an indication that conventional breeding could improve the carotenoids content in cassava, using simple recurrent selection. The PPD, evaluated at four different stages (1, 3, 7 and 30 days after harvest), showed a significant (p<0.05) negative correlation with TC and dry matter content (DMC), indicating that the high TC and low DMC cultivars could have a delayed PPD. Two out of the 30 genotypes were yellow-fleshed cultivars (Garukunsubire and Mavoka, with 1.84 to 2.32 µg/g TC, respectively), could form the basis for improved carotenoids content for cassava and could contribute to the development of delayed PPD cultivars in Rwanda. The information generated by this study will guide cassava breeders in improving the landrace population, based on the genetic variability observed in the country.

Key words: Broad sense heritability, Carotenoids content, Genetic advance, Genotypic variance coefficient, Phenotypic variance coefficient, Physiological postharvest deterioration, Viral diseases

3.1 Introduction

Cassava plays a key role as a food security and income-generating crop for many smallholder farmers in developing countries (Ceballos et al., 2004; El-Sharkawy, 2004; Sewando, 2014; Tumuhimbise, 2013). An estimated 250 million people are dependent on cassava as a primary source of food in Africa (Sayre et al., 2011), and it contributes over 500 kcal per day per person (FAO, 2010; Morante et al., 2010). Cassava is produced mostly by smallholder farmers on marginal and sub-marginal lands in Africa. Cassava tolerates poor soils, requires less labour than other crops, and harvesting can be delayed by months, or even up to three years (Sayre, 2011). However, cassava as a staple food has numerous biotic, abiotic and physiological stresses that impact on its production, consumption and marketability (Bull et al., 2011). Viral diseases, such as the cassava mosaic disease (CMD) and the cassava brown streak disease (CBSD), cassava bacterial blight, pests (whiteflies: *Bemisia tabaci*; and cassava green mite: *Mononychellus tanajoa*) (Night et al., 2011), and other factors, such as poor agricultural practices and post-harvest losses, present considerable constraints to the attainment of a satisfactory yield by resource-poor farmers (Patil and Fauquet, 2009). Crop improvement could play a vital role in overcoming all the stress factors in cassava production.

Genetic variability is the backbone of crop improvement and it is very important when selecting suitable genotypes for crop improvement (Hakeem et al., 2013; Sinha and Mishra, 2015; Tumuhimbise et al., 2015). Cassava has considerable genetic variability for different agronomic traits (Kundy et al., 2015), though the potential of this variability has not yet been fully explored (Ntawuruhunga and Dixon, 2010). Crop improvement through breeding depends on the availability of genetic variability and how these desired traits can be fixed in genotypes with good agronomic characteristics (Akinwale et al., 2010). According to Kundy et al. (2015), the improvement of many traits can significantly be achieved through selection that is based on several components, rather than one component.

In order to develop a successful breeding program, cassava breeders need a good knowledge of the genetic variability of various traits (Tumuhimbise et al., 2015). This knowledge is essential in assisting breeders to estimate genetic parameters for quantitative traits and their correlation, which permits smooth parental selection, based on genetic variability (Bello et al., 2012; McAdam et al., 2014). Understanding the information on the genetic variability within a population is an important requirement for crop improvement, because it predicts the possibility of phenotypic selection for significant genetic gain (Bello et al., 2012; Tumuhimbise et al., 2015). In addition, it can allow the selection of parental combinations and the formation of heterotic groups for good genetic gain (Turyagyenda et al., 2012), based on adequate genetic variability.

Farmers prefer cultivars with a high storage root yield, which is one of the main goals in cassava improvement (Ntawuruhunga and Dixon, 2010). However, cassava yield is a complex trait that is controlled by many quantitative genes and its expression is highly variable (Kundy et al., 2015; Shi et al., 2009) and difficult to assess in large populations, compared to other phenotypically observable traits (Ntawuruhunga and Dixon, 2010). Thus, the correlation between various components that contribute to the yield increase, must be analysed. Various studies (Kundy et al., 2015; McAdam et al., 2014; Ntawuruhunga and Dixon, 2010) have indicated that storage root yield is genetically influenced by the storage root number per plant, the storage root size, the harvest index, the stem girth, and the canopy width. The success of selection for high yield depends largely on the nature and the extent of available heritable yield components in populations (Tumuhimbise, 2013).

Though high cassava storage root yield is the farmer's number one preference, in most cassava production areas, postharvest losses are still high and affect cassava production. This is due to the physiological nature of the cassava storage root, which starts to deteriorate immediately after harvesting (Beeching et al., 2002; Sánchez et al., 2006). The rapid deterioration affects the economic value of cassava (Morante et al., 2010) with a recorded loss of 29%, 10% and 8%, respectively, in Africa, Latin America and Asia (Salcedo and Siritunga, 2011). Postharvest losses affect the nutritional and economical value of cassava, and the economic losses, due to the depreciation of deteriorated cassava, which can reach up to 90% (Westby, 2002). Therefore, postharvest physiological deterioration (PPD) could hinder the adoption of cassava in remote areas that have a poor infrastructure for cassava processing.

Like other quantitatively inherited traits, PPD has relationships with other traits, such as dry matter and carotenoids content (Beeching et al., 2002; Sánchez et al., 2006). Morante et al. (2010) indicated that there is limited progress in improving the tolerance to PPD, through genetic enhancement. However, exploring the large genetic variability of cassava carotenoids and dry matter content, as reported by Esuma et al. (2012) and Ceballos et al. (2013), could help in the breeding of delayed PPD cultivars. Morante et al. (2010) reported that most of the germplasm is generally discarded in the early stages of selection in a breeding program, which could reduce the genetic variability for PPD. Tumuhimbise et al. (2015) reported that a large portion of phenotypic variance in PPD, which is accounted for by the genotypic component, indicates large genetic variability for PPD in Ugandan germplasm. The genetic variability for PPD in cassava, as well as the inter-relationship between PPD and other important traits, needs further study. There is a need to estimate genetic variability, heritability, expected genetic advance for PPD resistance, and the correlation between the yield and postharvest traits of cassava genotypes. This study therefore aimed at examining the extent of genetic variability in cassava for yield and yield components, and PPD and its related traits.

3.2 Material and methods

3.2.1 Experimental site

The experiments were conducted at five locations, namely, at the KaramaI, Muhanga and Karama II research stations, and on the farmers' cooperative farms, at Kamonyi and Gakenke Districts. The experimental fields were selected, based on altitude, and were established at altitudes ranging from 1338 to 1875 m above sea level (asl) (Table 3.1). The geographic coordinates were recorded from the centre-point of the experiment field.

Table 3.1: Geographical coordinates of experiment locations

Location	Longitude	Latitude	Altitude	Province
Karamal	2º15'54.126''S	30º15'22.46''E	1338	South-Eastern
Karama II	2º 15'59.2308''S	30º15'21.32''E	1336	South-Eastern
Muhanga	2º04'12.274''S	29º43'24.69''E	1875	West-Southern
Kamonyi	2º02'41.049''S	29º54'34.25''E	1637	East-Southern
Gakenke	1º40'57.084''S	29º47'39.29''E	1614	South-Northern

Soil and climatic factors indicated that the experiment fields were diverse (Table 3.2).

Table 3.2: Soil and climatic parameters of experimental locations

Parameters	Locations				
	Karamal	KaramaII	Kamonyi	Muhanga	Gakenke
Soil parameters					
pH	5.8	5.6	5.7	5.7	5.4
Available P (mg kg^{-1})	3.2	3.4	3.2	4.8	3.9
Exch K (cmol kg^{-1})	0.75	0.79	0.56	0.58	0.48
Total N (%)	0.28	0.24	0.12	0.38	0.21
Organic C (%)	1.71	1.69	1.18	3.06	2.8
Exch Ca(cmol kg^{-1})	2.33	2.28	2.05	3.06	2.65
Exch Mg(cmol kg^{-1})	0.32	0.31	0.64	0.14	0.28
Exch Na(cmol kg^{-1})	0.02	0.03	0.08	0.04	0.01
CEC (cmol kg^{-1})	10.82	10.6	8.74	16.3	14.2
Clay (%)	71.2	67.1	28.3	67.5	63.1
Sand (%)	26.1	28.3	61.1	28.3	23.4
Silt (%)	2.7	4.6	10.6	4.2	13.5
Climatic parameter*					
Rainfal (mm)	889	914	1134	1222	1298
Av min temperature (Cº)	15.2	15.1	14.4	13.2	13.1
Av max temperature (Cº)	30.2	29.8	29.8	28.7	26.8

**the data sourced from nearby weather station,*

3.2.2 Experimental germplasm

Thirty cassava genotypes (Table 3.3) were selected from research institutes, farmers' cooperatives and private farms. The selection of genotypes was purposefully conducted through consultative discussions between local scientists, researchers and farmers. The main

traits for selection were high yield, CMD and CBSD resistance and the yellow-flesh colour (only two yellow-fleshed genotypes were available and collected).

Table 3.3: Cassava genotypes evaluated at five locations in Rwanda (2014/2015)

Nº of genotypes	Code of genotypes	Name of genotypes	Colour of flesh
1	G1	Mavoka	Yellow
2	G2	Garukansubire	Yellow
3	G3	Gahene	White
4	G4	Mushedile	White
5	G5	Kibombwe	White
6	G6	Ndamirabana	White
7	G7	Gitamisi	White
8	G8	Rwizihiza	White
9	G9	Cyizere	White
10	G10	Kwatamumpare	White
11	G11	Creolina	White
12	G12	Gacyacyali	White
13	G13	Serukuseke	Cream
14	G14	PDB/10	White
15	G15	Kavumu	White
16	G16	PDB/11	White
17	G17	NAS3OP/4	White
18	G18	MH98/0105	White
19	G19	Bukarasa	White
20	G20	Gikorumunyu	White
21	G21	Bereryinkumi	White
22	G22	Mbakungahaze	White
23	G23	Nyirakarasi	White
24	G24	MM96/2536	White
25	G25	MM96/0669	White
26	G26	MM96/0316OP/21	White
27	G27	Mbagarumbuse	White
28	G28	Gapfutsi	White
29	G29	Rwicabana	White
30	G30	Wadada	White

3.2.3 Experimental design and management

The experiments were laid in 5 x 6 alpha design, with two replicates. Cuttings of 25 cm lengths, with at least four nodes, were taken from mature cassava and planted horizontally in a flat seedbed at a spacing of 1 x 1 m, giving a population density of 10 000 plants ha^{-1}. Each plot comprised three rows with eight plants each, which made a total of 24 plants per plot. The data were collected from the inner rows, while the outer rows served as border rows, to minimize the competitive genetic effects. The plots and blocks were separated by 1.5 m and 2 m alleys, respectively, to reduce inter-plot and inter-block plant competition. The trials were weeded manually and no fertilizers and irrigation were applied.

3.2.4 Data collection

The data were collected from four randomly-selected plants from each plot. The data collected included the following: storage root number (SRN), storage root size (SRS), storage root mass (SRM), shoot mass (STM), total biomass mass (TBM), harvest index (HI), dry matter content (DMC), fresh storage root yield (FSRY), dry storage root yield (DSRY), cassava mosaic and cassava brown streak disease severity (CMD-S and CBSD-S), cassava brown streak disease root necrosis (CBSD-RN), total carotenoids (TC) and postharvest physiological deterioration (PPD).

Data on CMD and CBSD severity (CMD-S and CBSD-S) were collected from the leaves and stems, six months after planting, while CBSD root necrosis (CBSD-RN) was collected at harvest, using the 1-5 scale, namely: 1 = no symptoms on leaves, stems and roots; 2 = slight chlorotic spots on leaves and stems, necrosis in roots; 3 = moderate chlorotic spots on leaves and/or stems, necrosis in roots; 4 = severe chlorotic spots on leaves and/or stems/necrosis in roots; 5 = very severe chlorotic spots on leaves and/or stems/necrosis in roots (Hahn et al., 1980; Hillocks et al., 1996; Rwegasira and Rey, 2012). Total carotenoids was analysed, using a spectrophotometric procedure proposed by Rodriguez-Amaya and Kimura (2004), where total carotenoids content (µg g^{-1}) was calculated, using the following formula:

$$\mathbf{TC}(\boldsymbol{\mu g\ g^{-1}}) = \frac{\mathbf{A\ x\ volume\ (mL) x\ 10^4}}{\mathbf{A_{1cm}^{1\%}\ x\ sample\ weight\ (g)}}$$

Where A is the absorbance; volume is the total volume of extract (25 mL); and $A_{1cm}^{1\%}$ is the absorption coefficient of β- carotene in petroleum ether (PE).

The method developed by CIAT (Morante et al., 2010; Zidenga et al., 2012) was used to evaluate PPD, where the proximal and distal ends of the cassava storage roots were removed immediately after harvest. The proximal ends were exposed to the air and the distal ends were covered by using food plastic wrappers. The room temperature ranged from 21-28ºC and the relative humidity was 70-80%. The assessment was conducted at 1, 3, 7 and 30 days after harvest (PPD-1, PPD-3, PPD-7 and PPD-30, respectively), using the score 1-10 to represent the discoloration, where score 1 = 10%, 2 = 20%,….., 10 = 100% (Chávez et al., 2005; Wheatley et al., 1985) on the transversal 10 slices that were 2 cm thick. They were cut along each storage root and, at each data collection, two storage roots were cut to score the slices, and the mean score from 20 slices per genotypes was calculated.

FSRY expressed in t ha^{-1} was estimated using the formula:

$$\mathbf{FSRY}\left(\mathbf{t\ ha^{-1}}\right) = \frac{\mathbf{SRMx10\ 000}}{\mathbf{4}}\mathbf{x\ 1000}$$

HI was obtained using the formula:

$\mathbf{HI} = \frac{\mathbf{SRM}}{\mathbf{TBM}}$, where SRM is the storage root mass, TBM represents the total biomass mass.

DMC (%) was determined using the oven-drying method. Cassava storage roots from the individual four plants were washed, sliced into small pieces that had been picked randomly from apical, distal and middle sections of the storage root, and 100 g were dried in an oven for 48 hours at 80^{o}C to reach constant weight. The samples were reweighed to obtain the dry mass and the DMC (%) was then calculated, using the formula: $\mathrm{DMC}\ (\%) = \frac{\mathrm{DM}}{\mathrm{FM}} \mathrm{x} 100$, where DM is the dry mass of the sample, and FM is the fresh mass of the sample.

DSRY (t ha^{-1}) was obtained by using the formula:

$$\mathbf{DSRY\ t\ ha^{-1}} = \frac{\mathbf{DMC}\ (\%)\mathbf{x}\ \mathbf{FSRY}\left(\mathbf{t\ ha^{-1}}\right)}{\mathbf{100}}$$

3.2.5 Data analysis

The analysis of variance (ANOVA) to determine the differences between genotypes was performed, using the REML analysis in GenStat 17th Edition. Using Hartley's F_{max}, test (Ott and Longnecker, 2008), the locations variance was not significant (p >0.05), thus a combined ANOVA across the locations was performed. During statistical analysis, the genotype was considered as a fixed effect, while locations and replicates were random effects, using the following model:

$$\mathbf{Pijk} = \mu + \mathbf{gi} + \mathbf{lj} + \mathbf{glij} + \mathbf{eijk,}$$

Where Pijk is phenotypic value due to genotype i in j replicates and k location; μ is population mean; gi is genotype effects; lj is locations effects (environments); glij is genotype x environment interaction effects; eijk is environment error associated to genotype i, environment j and replications k.

The genotype and environment effects were considered random in the statistical model (Payne et al., 2011), in order to estimate the genotype, environment and their interaction variance components for each trait. The phenotypic variance component for each trait was partitioned into observational components of variance, as reported by Hallauer et al. (2010).

$\delta^2_p = \delta^2_G + \delta^2_E + \delta^2_{GE}$, where σ^2p is phenotypic variance; δ^2_G variance due to genotype; δ^2_E is variance due to environment; δ^2_{GE} is variance due to genotype x environment interaction.

The broad sense heritability (H^2) was calculated as the % ratio of genotypic and phenotypic variances as follows; $H^2 = \frac{\delta^2_G}{\delta^2_p} \text{ x } 100$

To determine the response of traits to selection and the magnitude of variation responsive to selection, the phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were calculated, using the method of Burton and de Vane (1953) and Shabanimofrad et al. (2013): $\text{PCV } (\%) = \frac{\sqrt{\delta^2_p}}{\overline{X}} \text{ x } 100$, $\text{GCV } (\%) = \frac{\sqrt{\delta^2_G}}{\overline{X}} \text{ x } 100$, where $\overline{X}$ represents the mean of each trait.

The expected genetic advance (GA) under selection for each trait was calculated, according to Singh and Chaudhary (1985), as follows: $\text{GA} = i \text{ x } \delta_p \text{x } H^2$, where *i* is the selection differential, which varied with selection intensity (5% intensity was used at which I = 2.06). The δ_p is the phenotypic standard deviation and H^2 is the heritability in a broad sense.

The expected GA (%) of the mean was calculated according to the Shukla et al. (2006): $\mathbf{GA}\ (\%) = \frac{\mathbf{GA}}{\overline{\mathbf{X}}}\mathbf{x100}$

The phenotypic correlation using simple correlations and a principal component (PC) biplot were used to compare the traits. In the PC biplot, the angles between the biplot axes represent the correlations between the variables, and lines in opposite directions indicate negative correlation (Simon and Payne, 2104). The closer the angle is to 90, or 270 degrees, the smaller the correlation. An angle of 0 or 180 degrees reflects a correlation of 1 or -1, respectively (Kohler and Luniak, 2005).

3.3 Results

3.3.1 Combined analysis of variance for yield and yield components, viral diseases and postharvest traits at five locations

Genotypes mean squares (MS) were significantly different (*P*<0.001) for all traits. Locations MS also were significantly different (*P*<0.001) for all traits (Table 3.4). Genotype x locations interactions were significant (*P*<0.05) only for some traits, namely, SRN, DMC, CMD-S, CBSD-S, PPD-3 and PPD-7. Replicates MS also were significant (*P*<0.05) for some traits, such as SRN, FSRY, DSRY, HI, PPD-7 and PPD-3. The determinant coefficient (R^2) ranged from 0.734 for DSRY to 0.982 for total carotenoids (Table 3.4).

Table 3.4: Combined analysis variances of cassava yield and yield components, viral diseases and postharvest traits at five locations in Rwanda

Source of variation	DF	Mean squares					
		SRN	FSRY	DMC	DSRY	HI	CMD-S
Rep	1	30.56**	569.50***	13.52	53.32***	0.080**	0.48
Loc	4	33.42***	514.03***	41.71***	43.45***	0.18***	6.14***
Gen	29	28.73***	171.04***	74.39***	14.81***	0.06***	13.38***
Rep.Loc	4	0.70	303.31***	3.28	24.17***	0.002	1.37
Rep.Gen	29	4.91	54.51	4.38	4.94	0.01	1.38*
Loc.Gen	116	6.07*	47.94	6.51**	4.13	0.01	2.23***
Rep.Loc.Gen	116	3.94	48.13	3.77	4.30	0.01	0.77
R^2		0.80	0.74	0.88	0.73	0.78	0.89
CV (%)		43.92	70.59	6.41	70.70	32.38	29.88

Table 3.4: Continued

Source of variation	DF	Mean squares					
		CBSD-S	CBSD-RN	TC	PPD-3	PPD-7	PPD-30
Rep	1	1.61	0.30	0.001	2499.9***	2945.3***	34.7
Loc	4	107.11***	72.32***	0.248***	5567.5***	3200.1***	859.0***
Gen	29	1.90***	3.11***	1.952***	1158.6***	1886.9***	1028.4***
Rep.Loc	4	6.25***	0.95	0.094***	356.6*	1250.5***	120.7
Rep.Gen	29	0.96*	0.82	0.037***	198.4*	424.6***	348.6**
Loc.Gen	116	0.86*	0.97	0.012	355*	441.4***	175.9
Rep.Loc.Gen	116	0.57	0.72	0.009	111.4	160.7	165.3
R^2		0.90	0.86	0.982	0.892	0.882	0.770
CV (%)		23.49	34.30	17.07	43.03	30.05	15.11

*R^2=$(1-\frac{\text{SS err}}{\text{SS tot}})$: coefficient of determination, DF = degrees of freedom; FSRY = fresh storage root yield (t ha^{-1}); HI = harvest index; DMC = dry mass content (%); DSRY = dry storage root yield (t ha^{-1}); SRN = storage root number plant^{-1}; PPD-3, -7, -30 = postharvest physiological deterioration (%) after 3, 7 and 30 days respectively, CMD-S= cassava mosaic disease severity, CBSD-RN = cassava brown streak disease root necrosis scored on a scale of 1 -5; TC=Total carotenoids, CV = coefficient of variation (%); * = P<0.05; ** = P<0.01; *** = P<0.001*

3.3.2 Genetic variability and inter-relations for dry matter, total carotenoids and PPD

The genotypic variance component at five environments was higher for all traits, except PPD-3, compared to the variance for environment and genotype x environment interactions. The broad sense heritability (H^2) for those traits ranged from moderate to very high, with PPD-30 and TC leading the group with 100 and 99.2%, respectively (Table 3.5). The PCV for DMC and TC were higher than the GCV for both traits, while GCV was higher than PCV for PPD evaluated at three different periods after harvest (1, 3, and 7). A very high expected GA (% of mean) was recorded for TC (Table 3.5).

Table 3.5: Variance components for three postharvest traits scored over five environments

Traits	δ^2_g	δ^2_p	δ^2_{GE}	δ^2_E	$\overline{X}$	GCV(%)	PCV(%)	$H^2_{(\%)}$	GA(%)
DMC	6.79	8.66	1.28	0.59	30.30	8.6	9.7	78.4	15.7
TC	0.19	0.20	0.00	0.00	*0.57*	77.9	78.2	99.2	159.6
PPD-1	21.53	41.90	9.95	10.42	11.40	56.8	40.7	51.4	60.1
PPD-3	80.40	269.50	102.2	86.90	35.19	46.6	25.5	29.8	28.7
PPD-7	144.6	281.60	91.00	46.00	59.03	28.4	20.4	51.3	30.1
PPD30	85.2	85.20	-11.40	11.40	91.29	10.1	10.1	100.0	20.8

DMC: dry matter content, TC: total carotene, PPD-1: postharvest physiological deterioration after one day, PPD-3: postharvest physiological deterioration after three days, PPD-7: postharvest physiological deterioration after seven days, PPD-30: postharvest physiological deterioration after thirty days, δ^2_g: genotypic variance, δ^2_p: phenotypic variance, δ^2_E: environment variance, δ^2_{GE}: GxE interaction variance, GCV: genotypic coefficients of variation; PCV: phenotypic coefficients of variation; H^2: broad sense heritability; GA (%): genetic advance % of the mean

Correlations between DMC, TC and PPD were highly significant (p<0.001). The correlation between TC and DMC and PPD was negative and significant, while the correlation for DMC and PPD scored at 1, 3, 7 and 30 days after harvest was positive and significant (p<0.001) (Table 3.6). The Pearson's correlation indicates a strong negative relationship between TC and DMC, while the correlation between DMC and PPD was positively weak to moderate (r= 0.2 - 0.4).

Table 3.6: Correlation matrix of three postharvest traits of cassava

Traits	DMC	TC	PPD-1	PPD-3	PPD-7	PPD-30
DMC	-					
TC	-0.4158***	-				
PPD-1	0.4842***	-0.1748***	-			
PPD-3	0.3496***	-0.1974***	0.4863***	-		
PPD-7	0.3368***	-0.2312***	0.417***	0.7961***	-	
PPD-30	0.2885***	-0.293***	0.2536***	0.4064***	0.5228***	-

*DMC: dry matter content, TC: total carotene, PPD-1: postharvest physiological deterioration after one day, PPD-3: postharvest physiological deterioration after three days, PPD-7: postharvest physiological deterioration after seven days, PPD-30: postharvest physiological deterioration after thirty days, *: significance level at 5% where * = p<0.05; ** = p<0.01; *** = p<0.001*

The PC biplot explained 94.7% of the inter-relationship between postharvest traits. The TC line had a negative direction on PC-2, compared to the PPD-1, PPD-3, PPD-7 and DMC lines, which indicated a negative inter-relationship between TC and PPD (Figure 3.1). The inter-relationship between TC and PPD-30 also was explained by the opposite direction of PPD-30 line vs TC line.

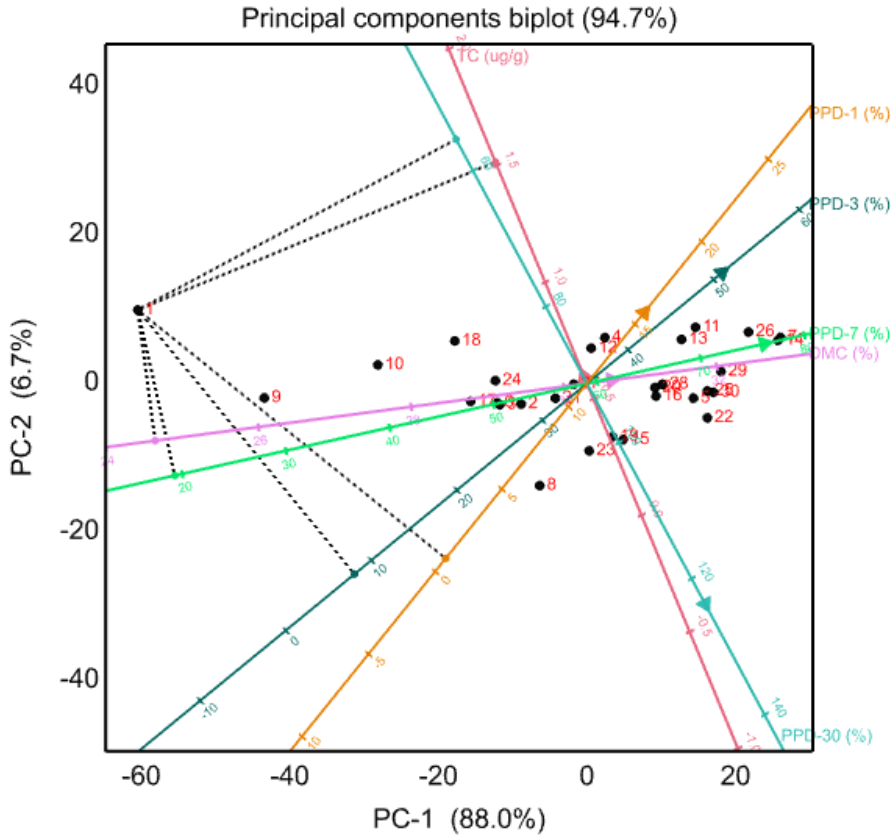

Figure 3.1: PC biplot explaining interrelation between postharvest traits

The 30 genotypes evaluated at five environments had significant (p<0.001) variability on selected postharvest traits. The TC varied from 0.3-2.32 µg g-1 among genotypes, where G1, G2 and G13 recorded a high TC of 2.32, 1.84 and 0.94 µg g-1, respectively (Table 3.7). The colours of flesh of those three genotypes were yellow for G1 and G2, and cream for G13 (Table 3.3). The genotype with the highest TC had the lowest DMC of 25.2%. The genotypes with high DMC (%) were G19, G7, G8, G27 and G3 with 35.2%, 33.8%, 33.8%, 33.5% and 33.2%, respectively. The PPD evaluated at different periods showed that G1, followed by G17 and G18, were the best genotypes with the lowest PPD rate, compared to the other genotypes (Table 3.7). The rank, based on the average rank across five environments, indicated that G1 (Mavoka, a yellow-fleshed root), G18, G14, G16 and G25 were the best genotypes in terms of storability of cassava storage root at ambient room conditions.

Table 3.7: Performance and ranking of genotypes for TC, DMC and PPD across five environments

Genotypes	Cassava genotypes performance										Over all rank
	TC (µg g^{-1})	Rank	DMC (%)	Rank	PPD-3 (%)	Rank	PPD-7 (%)	Rank	PPD-30 (%)	Rank	
G1	2.32	1	25.2	30	6.6	1	20.5	1	55.0	1	1
G10	0.37	22	31.0	14	31.0	8	49.0	5	91.0	12	8
G11	0.38	19	28.8	22	26.0	6	52.0	10	88.0	7	12
G12	0.38	20	30.2	18	39.0	18	63.0	18	86.5	8	22
G13	0.94	3	30.5	17	44.0	26	67.0	22	100.0	23	20
G14	0.67	5	29.0	21	26.0	4	51.0	6	88.0	11	3
G15	0.44	15	33.0	6	53.0	30	77.0	27	98.0	18	25
G16	0.47	12	31.4	12	27.5	9	52.0	8	100.0	23	4
G17	0.38	20	26.7	26	11.5	2	29.5	1	73.0	3	7
G18	0.84	4	26.5	27	22.0	3	39.0	3	77.0	2	2
G19	0.35	23	35.2	1	45.0	25	71.0	24	91.0	12	21
G2	1.84	2	27.5	25	36.5	15	60.0	19	88.0	9	15
G20	0.44	16	30.8	15	47.0	26	67.0	21	92.5	14	24
G21	0.33	27	32.4	8	50.0	29	80.0	30	97.0	15	30
G22	0.48	11	28.2	23	36.0	16	61.0	15	100.0	23	19
G23	0.33	26	31.1	13	42.5	24	62.0	20	98.0	18	26
G24	0.53	8	26.0	28	24.0	4	50.5	7	85.5	10	6
G25	0.52	9	28.0	24	31.7	10	47.5	4	78.0	5	5
G26	0.55	6	30.6	16	31.5	11	63.0	22	98.0	18	13
G27	0.45	13	33.5	4	40.0	19	64.0	14	96.3	22	11
G28	0.54	7	29.1	20	35.5	14	55.0	9	91.0	15	9
G29	0.39	17	25.7	29	34.0	12	78.0	27	100.0	23	28
G3	0.30	30	33.2	5	33.5	13	55.0	13	100.0	23	16
G30	0.32	29	29.2	19	27.0	7	52.0	10	85.0	4	18
G4	0.35	24	32.0	11	43.0	20	69.0	17	100.0	23	23
G5	0.35	24	32.2	10	48.0	28	74.0	27	96.0	17	29
G6	0.45	14	32.4	9	36.0	17	56.0	12	91.0	5	9
G7	0.39	18	33.8	2	42.0	21	61.0	16	98.0	18	14
G8	0.52	10	33.8	3	44.0	22	72.0	26	98.0	23	16
G9	0.32	28	32.4	7	42.0	23	73.0	24	99.0	23	26
Mean	***0.57***	***-***	***30.30***	***-***	***35.19***	***-***	***59.03***	***-***	***91.29***	***-***	***-***
LSD	***0.10***	***-***	***1.98***	***-***	***13.34***	***-***	***15.62***	***-***	***12.15***	***-***	***-***
P value	***<0.001***	***-***	***<0.001***	***-***	***<0.001***	***-***	***<0.001***	***-***	***<0.001***	***-***	***-***
CV (%)	***20.31***	***-***	***7.42***	***-***	***43.03***	***-***	***30.05***	***-***	***15.11***	***-***	***-***

G: Genotype, LSD: least significant difference, CV: coefficient of variation, DMC: dry matter content, TC: total carotenoids, PPD-1: postharvest physiological deterioration after one day, PPD-3: postharvest physiological deterioration after three days, PPD-7: postharvest physiological deterioration after seven days, PPD-30: postharvest physiological deterioration after thirty days

3.3.3 Genetic variability and interrelationship of cassava yield and yield components

Genotypic variance components were higher than environment and GxE interaction variance components for RN, FSRY, DMC, DSRY and HI. Inversely, the environment variance component was higher than the genotypic variance for TB (Table 3.8). The PCV (%) was higher than GCV (%) for all traits. The H^2 (%) ranged from 20.5% for TB, to 93.1% for DSRY. As expected for TB, all traits recorded moderate to high H^2 (%). The expected GA (% of mean) was high for DSRY, FSRY and RN, with 70.0%, 68.4% and 54.0%, respectively.

Table 3.8: Variance components for yield and yield component traits scored over five environments

Traits	δ^2_g	δ^2_p	δ^2_{GE}	δ^2_E	$\overline{X}$	GCV(%)	PCV(%)	H^2(%)	GA(%)
RN	2.265	3.649	0.928	0.456	4.520	33.3	42.3	62.1	54.1
TB	35.100	170.400	6.400	128.900	26.690	22.2	48.9	20.6	20.8
FSRY	12.310	14.230	-5.850	7.770	9.828	35.7	38.4	86.5	68.4
DMC	6.788	8.658	1.283	0.587	30.300	8.6	9.7	78.4	15.7
DSRY	1.068	1.147	-0.576	0.655	2.933	35.2	36.5	93.1	70.0
HI	0.004	0.009	0.001	0.003	0.316	21.2	29.4	52.1	31.7

RN: root number, TB: Total biomass, FSRY: fresh storage root yield (t ha^{-1}), DMC: dry matter content, DSRY: dry storage root yield (t ha^{-1}); HI: harvest index, δ^2_g: Genotypic variance, δ^2_p: phenotypic variance, δ^2_E: environment variance, δ^2_{GE}: GxE interaction variance, GCV: genotypic coefficients of variation; PCV: phenotypic coefficients of variation; H^2: broad sense heritability; GA (%): genetic advance % of the mean

Correlations matrix of yield and yield components revealed a significance (p<0.001) correlation between yield and yield component traits. The Pearson's correlation indicated that RN correlated moderately and positively with TB, FRSY, HI and DRSY (Table 3.9), while the DMC correlated negatively with all yield and yield components traits, which indicated that dry matter could affect the final yield.

Table 3.9: Correlation matrix of yield and yield components traits of cassava

Traits	RN	TB	FRSY	DMC	DRSY	HI
RN	-					
TB	0.6647***	-				
FSRY	0.669***	0.7716***	-			
DMC	-0.0331ns	-0.0616ns	-0.1529**	-		
DSRY	0.5169***	0.209***	0.5502***	-0.1049ns	-	
HI	0.669***	0.7716***	1.000***	-0.1529***	0.5502***	-

*RN: root number, TB: Total biomass, FSRY: fresh storage root yield (t ha^{-1}), DMC: dry matter content, DSRY: dry storage root yield (t ha^{-1}); HI: harvest index, *: significance level at 5% where * = p<0.05; ** = p<0.01; *** = p<0.001*

The principal component (PC) biplot explained 95.9% (PC1 and PC2 represent 87.9% and 8.0%, respectively) of the inter-relationships and revealed the inter-relationship between yield and yield components traits (Figure 3.2). The DMC (%) had a negative direction on PC1, while HI, RN, FSRY and DSRY had a positive direction on the same PC, which indicates the negative coefficients expressing the negative inter-relations between DMC (%) with the other traits. All traits have a positive direction on PC2, expect TB, which is on flat direction vs PC2, indicating the neutral direction and negligible interrelation coefficients with DMC (%).

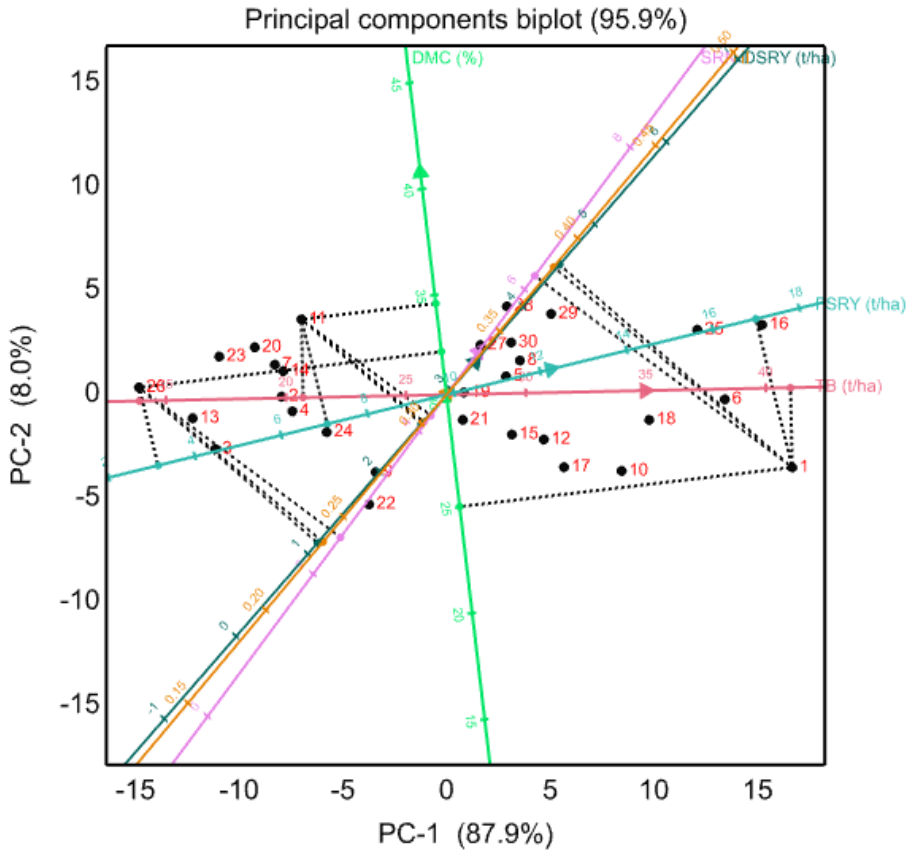

Figure 3.2: PC biplot explaining correlation between yield and yield components traits

There were significant (p<0.001) differences for all yield and yield component traits. The average mean across five environments indicated that G23, G13, G7, G25 and G16 were the top five genotypes, while G20, G11, G5, G29 and G15 were the poorest performing genotypes in terms of RN (Table 3.10). The G1, G14, G23, G4, G25 had a high TB. In terms of FRSY, the yield ranged from 3.5 - 19.4 t ha^{-1}; G23 had the highest yield of 19.4 t ha^{-1}, followed by G1 and G4, with yields of 18.1 and 16.7 t ha^{-1}, respectively. The lowest yielding genotype was G5, with 3.5 t ha^{-1}. Similarly, for the DSRY, the genotype G23 recorded the highest yield of 6.0 t ha^{-1}, while G5 had the lowest yield of 1.1 t ha^{-1}. The HI varied from 0.4 to 0.1. Thirteen genotypes had a similar HI of 0.4, while the genotype with lowest HI was G20, with 0.1 (Table 3.10). The averaged rank of 30 genotypes tested at five environments revealed that G23, G4, G7, G14 and G8 were the best-performing genotypes in terms of yield and yield components.

Table 3.10: Performance and ranking of genotypes for yield and yield components across five environments

Genotypes	Cassava genotypes performance												Overall rank
	RN	Rank	TB (t ha^{-1})	Rank	FSRY (t ha^{-1})	Rank	DMC (%)	Rank	DSRY (t ha^{-1})	Rank	HI	Rank	
G1	4.8	11	40.7	1	18.1	2	25.17	30	4.6	3	0.4	5	7
G10	3.8	21	19.3	25	6.9	19	30.95	14	2.1	20	0.3	19	21
G11	2.6	28	16.1	29	5.9	25	28.79	22	1.7	27	0.3	22	29
G12	4.7	12	20.2	22	6.0	24	30.16	18	1.8	24	0.2	23	23
G13	7.1	2	28.9	13	11.1	11	30.47	17	3.4	11	0.4	3	10
G14	5.4	8	39.3	2	14.5	4	28.95	21	4.2	4	0.3	16	4
G15	1.9	30	20.1	23	5.8	26	33.04	6	2.0	22	0.2	24	26
G16	6.4	5	30.1	9	10.8	13	31.40	12	3.4	12	0.3	15	9
G17	4.2	18	22.5	19	9.6	17	26.66	26	2.6	18	0.4	8	19
G18	3.5	22	35.6	6	11.0	12	26.45	27	2.6	17	0.2	29	17
G19	2.9	25	22.2	20	4.9	28	35.20	1	1.8	26	0.2	26	24
G2	5.2	10	30.1	10	12.7	7	27.46	25	3.5	10	0.4	6	13
G20	1.9	29	16.3	28	4.0	29	30.75	15	1.2	29	0.1	30	30
G21	3.0	24	20.0	24	6.4	22	32.41	8	2.1	21	0.2	27	22
G22	4.7	13	29.7	11	10.6	15	28.16	23	2.9	15	0.3	20	14
G23	9.4	1	38.2	3	19.4	1	31.06	13	6.0	1	0.4	1	1
G24	5.5	7	30.9	8	12.9	5	26.00	28	3.4	13	0.4	9	12
G25	6.7	4	35.7	5	12.7	6	28.03	24	3.6	8	0.4	4	6
G26	4.0	19	28.3	15	8.8	18	30.64	16	2.6	16	0.3	17	15
G27	4.4	14	18.9	26	5.5	27	33.50	4	1.9	23	0.3	14	18
G28	3.9	20	27.1	17	10.6	14	29.05	20	3.1	14	0.4	12	16
G29	3.1	23	23.9	18	6.6	20	25.71	29	1.7	28	0.2	28	27
G3	2.8	27	16.5	27	6.6	21	33.21	5	2.2	19	0.4	10	20
G30	4.2	17	22.0	21	6.2	23	29.18	19	1.8	25	0.3	21	25
G4	5.3	9	36.8	4	16.7	3	32.04	11	5.3	2	0.4	2	2
G5	2.8	26	13.7	30	3.5	30	32.23	10	1.1	30	0.2	25	28
G6	4.3	15	27.5	16	12.1	9	32.40	9	3.9	7	0.4	7	11
G7	6.9	3	28.8	14	12.0	10	33.79	2	4.1	6	0.4	13	3
G8	6.2	6	32.3	7	10.4	16	33.76	3	3.5	9	0.4	11	5
G9	4.2	16	29.1	12	12.4	8	32.44	7	4.1	5	0.3	18	8
Mean	***4.5***	***-***	***26.7***	***-***	***9.8***	***-***	***30.30***	***-***	***2.9***	***-***	***0.3***	***-***	***-***
LSD	***1.97***	***-***	***12.75***	***-***	***6.15***	***-***	***1.98***	***-***	***1.83***	***-***	***0.10***	***-***	***-***
P value	***<0.001***	***-***	***<0.001***	***-***	***<0.001***	***-***	***<0.001***	***-***	***<0.001***	***-***	***<0.001***	***-***	***-***
CV (%)	***49.45***	***-***	***54.27***	***-***	***71.05***	***-***	***7.42***	***-***	***70.66***	***-***	***34.27***	***-***	***-***

G: Genotype, LSD: least significant difference, CV: coefficient of variation, RN: root number, TB: Total biomass, FSRY: fresh storage root yield (t ha^{-1}), DMC: dry matter content, DSRY: dry storage root yield (t ha^{-1}); HI: harvest index,

3.3.4 Genetic variability and inter-relationships for severity of viral diseases

The variance components analysis for CMD and CBSD revealed that the genotypic variance component was higher, compared to the environment and GxE interaction variance components for CMD-S traits. On the contrary, the environment variance component was higher than the genotypic variance components for both evaluated traits of CBSD (CBSD-S and CBSD-RN) (Table 3.11). The PCV (%) was higher than GCV (%) for both diseases. The H^2 was 60.4% for CMD, 14.1% for CBSD-RN and 5.5% for CBSD-S. A similar observation was noted for GA (%), where CMD had a higher GA (%), compared to the two severities of CBSD. This implies that breeding for CBSD could progress slowly.

Table 3.11: Variance components for viral diseases scored over five environments

Traits	δ^2_g	δ^2_p	δ^2_{GE}	δ^2_E	Mean	GCV(%)	PCV(%)	$H^2_{(\%)}$	GA(%)
CMD-S	1.12	1.85	0.67	0.07	2.93	36.0	46.3	60.4	57.7
CBSD-S	0.10	1.90	0.03	1.77	3.23	10.0	42.7	5.5	5.0
CBSD-RN	0.21	1.52	0.12	1.19	2.47	18.7	49.9	14.1	14.6

CMD-S= cassava mosaic disease severity, CBSD-RN = cassava brown streak disease root necrosis scored on a scale of 1 -5, δ^2_g: Genotypic variance, δ^2_p: phenotypic variance, δ^2_E: environment variance, δ^2_{GE}: GxE interaction variance, GCV: genotypic coefficients of variation; PCV: phenotypic coefficients of variation; H^2: broad sense heritability; GA (%): genetic advance % of the mean

The Pearson's correlation showed a significant (p<0.001) negative correlation between CMS-S and FSRY. Similarly, both traits of CBSD (CBSD-S and CBSD-RN) indicated a significant (p<0.05) negative correlation with the FSRY (Table 3.12). CMS-S does not present a significant correlation with the two traits of CBSD, while the two CBSD traits were strongly and positively correlated (p<0.001).

Table 3.12: Correlation matrix for viral diseases and FRSY of cassava

Traits	CMD-S	CBSD-S	CBSD-RN	FRSY
CMD-S	-			
CBSD-S	0.0558ns	-		
CBSD-RN	0.0731ns	0.7177***	-	
FSRY	-0.2573***	-0.189***	-0.2048***	-

*CMD-S= cassava mosaic disease severity, CBSD-RN = cassava brown streak disease root necrosis scored on a scale of 1 -5, *: significance level at 5% where * = p<0.05; ** = p<0.01; *** = p<0.001*

The PC biplot for multivariate analysis revealed the inter-relationship between CMD and CBSD traits. The PC 1 accounted for 92.8% of the inter-relationship between viral disease and FSRY (the FSRY served as an indication of how viral disease affects the cassava yield in general). The PC2 accounted for only 4.7 % of the inter-relationships. The FSRY presented a negative direction (negative coefficients) with both PCs (Figure 3.3), which indicated that all viral diseases negatively affected the cassava yield.

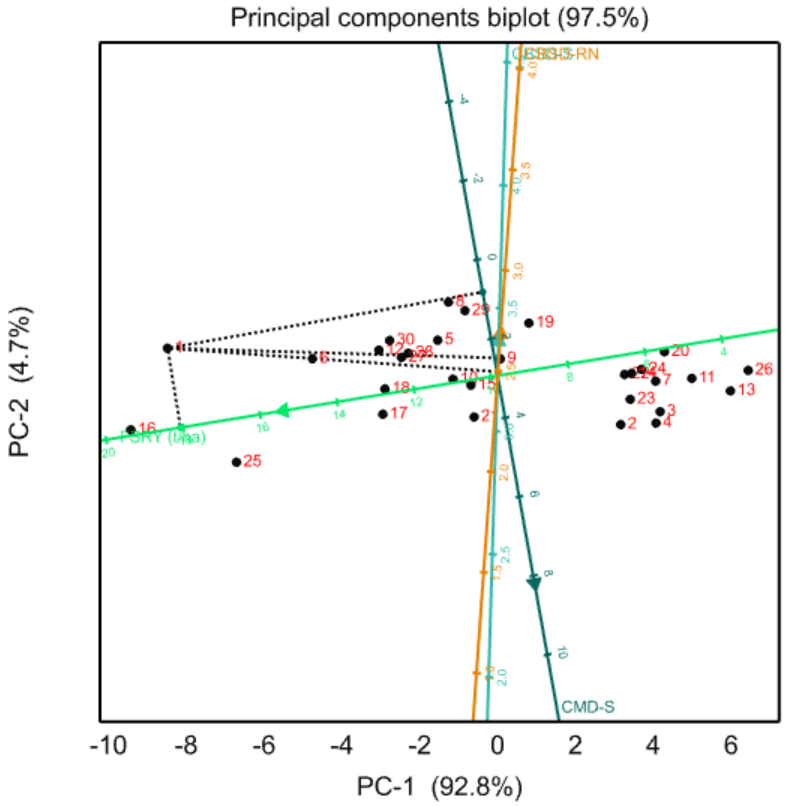

Figure 3.3: PC biplot explaining correlation between viral diseases and FRSY of cassava

The severity of CMD and CBSD differed significantly between the genotypes. Only two genotypes (G1 and G16) did not show CMD symptoms, six months after cassava planting (Table 3.13). The symptoms of both CBSD traits were present in all genotypes, with significantly (p<0.001) different severities. The viral diseases ranking across five environments indicated that G1, G16, G8, G13 and G2 were more resistant to CMD compared to other genotypes. The genotype G4 (Mushedile, a landrace grown mainly in the western province of Rwanda) showed the lowest CBSD severity in the storage roots.

Table 3.13: Performance and ranking of genotypes for CMD-S and CBSD-S and CBSD-RN across five environments

Genotypes	Cassava genotypes performance						Overall
	CMD-S	Rank	CBSD-S	Rank	CBSD-RN	Rank	rank
G1	1.0	1	3.7	26	3.1	25	16
G10	4.7	29	3.3	16	2.2	10	27
G11	4.6	28	3.0	6	2.8	20	20
G12	4.9	30	3.0	6	2.9	21	24
G13	1.5	4	2.6	3	1.7	3	1
G14	1.8	7	3.7	26	2.6	17	18
G15	3.7	21	3.1	12	2.3	11	17
G16	1.0	1	3.6	24	3.2	28	14
G17	2.1	11	2.1	1	1.3	2	2
G18	2.9	14	3.0	6	3.0	24	8
G19	3.9	23	3.7	26	2.4	12	29
G2	1.7	5	3.3	16	2.1	8	6
G20	4.3	27	3.6	24	2.0	6	29
G21	3.5	19	4.1	30	2.0	6	28
G22	3.1	16	3.5	22	2.4	12	23
G23	2.8	13	3.2	14	2.6	17	12
G24	3.4	17	3.0	6	2.4	12	7
G25	2.7	12	3.3	16	1.9	4	9
G26	1.9	8	4.0	29	2.9	21	25
G27	2.9	14	2.9	5	2.1	8	4
G28	3.9	23	3.1	12	2.5	16	19
G29	3.5	19	2.8	4	3.1	25	10
G3	4.2	26	3.2	14	2.9	21	26
G30	3.4	17	3.4	20	2.4	12	22
G4	3.7	21	2.4	2	1.2	1	5
G5	4.0	25	3.0	6	3.3	29	20
G6	1.9	8	3.0	6	1.9	4	3
G7	2.0	10	3.3	16	2.6	17	11
G8	1.3	3	3.5	22	3.3	29	15
G9	1.7	5	3.4	20	3.1	25	13
Mean	***2.9***	***-***	***3.2***	***-***	***2.5***	***-***	***-***
LSD	***1.074***	***-***	***0.7599***	***-***	***0.8097***	***-***	***-***
P value	***<0.001***	***-***	***<0.001***	***-***	***<0.001***	***-***	***-***
CV (%)	***41.56***	***-***	***26.74***	***-***	***37.14***	***-***	***-***

G: Genotype, LSD: least significant difference, CV: coefficient of variation, CMD-S= cassava mosaic disease severity, CBSD-RN = cassava brown streak disease root necrosis scored on a scale of 1 -5.

3.4 Principal component analysis of cassava traits' contribution to genotype variations

The principal components analysis on selected postharvest, viral diseases and yield and yield components traits indicated that the first three principal components explained 60.9% of the total variation. Only four components were presented, because their eigenvalues were <1. The PC1 (29.24%) explained more variation than PC2, PC3 and PC4, which accounted for a variation of 20.09%, 11.65% and 8.45%, respectively. For PC1, the FSRY, DSRY, TB, SRN, and HI contributed positively to the variation, while for the PPD-3, PPD-7, CMD-S, DMC and PPD-1, much of variation was accounted for by PC2 (Table 3.14). The variation due to PC3,

positively contributed to only two traits, namely CBSD-S and PPD-30, while the TC and CMD-S contributed negatively to the PC4 variation. In contrast, the HI and CBSD-RN contributed positively to the variation due to PC4.

Table 3.14: Principal component analysis of cassava trait contribution to genotype variations

Traits	Principal Component (PC)			
	PC1	PC2	PC3	PC4
FSRY	0.942	0.047	0.007	0.047
DSRY	0.905	-0.028	-0.010	0.130
TB	0.869	-0.176	-0.072	-0.135
SRN	0.830	-0.046	0.002	0.017
HI	0.436	0.161	-0.158	0.427
PPD-3	-0.131	0.903	-0.059	0.097
PPD-7	-0.060	0.894	-0.270	0.087
PPD-1	-0.061	0.662	0.115	-0.029
DMC	0.090	0.579	0.169	-0.239
PPD-30	-0.034	0.015	0.924	0.087
CBSD-S	-0.049	-0.042	0.901	0.056
CBSD-RN	-0.037	-0.152	0.111	0.785
TC	-0.193	-0.119	-0.061	-0.606
CMD-S	0.172	0.452	0.138	-0.497
Eigen value	4.093	2.812	1.631	1.183
Percentage variation	29.2	20.1	11.6	8.5
Cumulative percentage variation	29.2	49.3	61.0	69.4

RN: root number, TB: Total biomass, FSRY: fresh storage root yield (t ha^{-1}), DMC: dry matter content, DSRY: dry storage root yield (t ha^{-1}); HI: harvest index, TC: total carotenoids, PPD-1: postharvest physiological deterioration after one day, PPD-3: postharvest physiological deterioration after three days, PPD-7: postharvest physiological deterioration after seven days, PPD-30: postharvest physiological deterioration after thirty days, CMD-S= cassava mosaic disease severity, CBSD-RN = cassava brown streak disease root necrosis scored on a scale of 1 -5.

3.5 Discussion and conclusions

This study aimed at examining the extent of genetic variability in cassava for yield and yield components, viral disease severity and physiological postharvest deterioration. To achieve this objective, many traits, such as SRN, TB, HI, DMC, FSRY, DSRY, CMD-S, CBSD-S, CBSD-RN, TC, and PPD (PPD-1, PPD-3, PPD-7 and PPD-30), were recorded. It is expected that the information generated by this study will guide future cassava breeders to improve landrace populations, based on the genetic variability for farmers' preferred traits in Rwanda, such as high yield, resistance to viral diseases and delayed PPD cultivars, in addition to other important traits.

The significant variation of the mean squares of genotypes observed for all traits indicated that genotypes were significantly different; thus a genetic advance could be achieved by the hybridization of the evaluated genotypes. Locations were also significantly different, which could be attributed to the environmental effects on the genotype performance of different traits. The significant difference observed between replicates could be attributed to the soil variation. This is in agreement with Tumuhimbise (2015), who reported a variation among genotypes,

which will result in genetic advance through crossing contrasting genotypes. The significant variation among environments can affect the performance of various cassava traits (Ntawuruhunga and Dixon, 2010; Ssemakula and Dixon, 2007), due to the unpredictable features of the environment.

The determinant coefficient (R^2) ranged from 0.734 for DSRY, to 0.982 for total carotenoids, indicating that 73% of the variation of DSRY was due to genotypes, while 27% variation was of unknown origin. Total carotenoids variation was explained as 98% by the genotype variation and only 2% was due to an unknown origin. This implies that carotenoids could be selected phenotypically, based on storage root flesh colour. Similarly, Ceballos et al. (2013) reported the possibility of improving carotenoids in cassava conventionally in Africa, in parallel with DMC. Njenga et al. (2010) reported that 98% of the variability in carotene content can be explained phenotypically by the variability in colour of the cassava storage root.

The higher genotypic variance components observed for yield, CMD and CBSD severity and postharvest traits (RN, FSRY, DMC, DSRY, HI, TC, CMD, PPD-1, PPD-7 and PPD-30), compared to the variances for environment and GxE interactions components, indicated the great variability of genotypes. The H^2(%) for those traits ranged from moderate to high, the high H^2(%) indicated a considerable genetic variation among the 30 cassava genotypes that were unaffected by the environment, which implies that a substantial genetic advance could be achieved by the hybridization of the genotypes. The highest heritability (>50%) was observed on RN, FSRY, DMC, DSRY, HI, TC, CMD, PPD-1, PPD-7 and PPD-30, and could be attributed to the high genetic variability of genotypes evaluated in this study. The high heritability of carotenoids content that was found in cassava storage roots in this study, agrees with the findings of Morillo-C et al. (2012) and Ceballos et al. (2013), who reported a high narrow sense heritability of carotenoids in cassava storage roots.

The high genetic advance (%) for the traits recorded >50% of H^2, indicated that good progress could be made in improving the traits. The substantial genetic advance through conventional breeding for most important cassava traits was reported by Ceballos et al. (2013), Boakye et al. (2013) and Tumuhimbise et al. (2015). These findings agree with Pradeepkumar et al. (2001), Kalia and Sood (2005) and Okwuagwu et al. (2008), who reported that the high heritability estimates, along with high genetic advance for most of the cassava traits. The heritability alone could not be used for selection, due to the occurrence of non-additive variance, which implies that genetic advance, as a percentage of the mean, becomes a useful indicator of the progress that can be expected as a result of the selection of a population for specific traits.

The PCV was higher than its corresponding GCV for DMC and TC, which indicated the significant role of the environment in the expression of these traits. These findings agree with those of Ntawuruhunga and Dixon (2010), Manu-Aduening et al. (2013) and Tumuhimbise et al. (2015). In contrast, for the PPD, the GCV was higher than PCV, which indicated little effect of the environment on PPD expression. The latter finding agrees with that of Tumuhimbise et al. (2015), who reported a low environment effect on PPD expression. The high GCV suggests a higher selection progress in this population, while low GCV values indicate reduced genetic variability (Okwuagwu et al., 2008).

The Pearson's correlation and PC biplot analysis, as a procedure used on multivariate analysis for genetic variability studies, indicated that TC correlated negatively with PPD and DMC. The negative relationship between TC and PPD could be attributed to the antioxidant properties present in carotenoids. Several authors have reported that carotenoids have antioxidant properties (Azqueta and Collins, 2012; Edge et al., 1997; Priya and Siva, 2014; Rodriguez-Amaya, 2010; Uarrota et al., 2014), which could delay the onset of PPD. The negative relationship between TC and PPD agreed with the reports, indicating that carotenoids is negatively correlated with PPD (Sánchez et al., 2006; Sánchez et al., 2013; Uarrota et al., 2014; Xu et al., 2013; Zidenga et al., 2012). The findings on the correlation between DMC and PPD corroborate those of Chávez et al. (2005), Sánchez et al. (2006) and Morante et al. (2010), who reported that DMC correlates positively weak with PPD. The DMC correlated negatively with TC, which indicated challenges for the improvement of carotenoids-enriched cassava, because the negatively-correlated traits are not easily improved in parallel. All enriched carotenoids cultivars were recently introduced into the country from IITA and could face adaptation problems to the local conditions, which could consequently impair the simultaneous improvement of TC and DMC. However, Ceballos et al. (2013) reported simultaneous gains for TC and DMC through rapid recurrent selection.

The main principal component (PC1) and second principal component (PC2) contributed much value towards the total variation of the traits, and were as high as 29.2% and 20.1%, respectively, for the genotypes evaluated in this study. TC negatively contributed to the total genetic variation, as indicated by PCA, which could be attributed to the low number of locally-available carotenoids-enriched cultivars. In view of the role of carotenoids in human nutrition and their ability to delay PPD, there is a need to conventionally improve the local cassava population, in order to increase its genetic variability towards carotenoids content.

In conclusion, this study revealed a high genetic variability (61.0%), with high broad sense heritability and GA (% of mean) for the important cassava traits evaluated in Rwanda. This is an opportunity for the breeders to improve landraces, based on phenotypic selection. Though

some traits showed negative inter-relations among them, such as TC and DMC, the high heritability of TC indicated that selecting genotypes enriched in carotenoids can improve the cassava population in Rwanda and, consequently, the storability of cassava storage root will be achieved. The low genetic variability of TC (only G1 and G2 are yellow-fleshed cassava) in the local cassava population can be improved by inter-mating the two genotypes with available landraces in diallel and other factorial mating designs. This study suggests further exploration on the extent of combining ability of locally-available enriched carotenoids genotypes and landraces, in order to improve the carotenoids content and delay the PPD of cassava in Rwanda.

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CHAPTER IV

Genotype x environment interaction effects analysis of cassava yield and postharvest traits in Rwanda

Abstract

The genotype x environment interaction (GEI) effects complicate selection of, and recommendations for high performance genotypes. The general or specific adaptation of cultivar traits is the main goal of breeders. This study analysed the GEI effects of postharvest (total carotene – TC, postharvest physiological deterioration - PPD), viral disease severity and yield traits of 30 cassava genotypes. The experiments were conducted in the 2014/15 season at five different locations in Rwanda. The collected data were analysed using the additive main effects and multiplicative interaction (AMMI) model, AMMI stability value (ASV) and genotype stability index (GSI) analysis. The results indicated that all traits were significantly affected by genotypes. The TC, PPD and viral disease traits (cassava mosaic severity - CMD-S, cassava brown streak disease on leaves stem CBSD-LS and root necrosis CBSD-RN) were significantly affected by the environment. The GEI was insignificant for TC, but significant for viral disease traits, and PPD evaluated three days after harvesting. The interactive principal component axis (IPCA1) was significantly different for all traits, while IPCA2 was significantly different for dry matter content (DMC), CMD-S and PPD. The % sum of square (SS) of variation due to genotypes was higher than % SS variation due to the environment for all traits, except CBSD-RN and CBSD-S, indicating the influence of the environment on the severity of the viral diseases. The % variation due to the genotype for TC was higher (96%) than the variation due to the environment (1.7%) and GxE interaction (2.4%), indicating less interaction effect of environments on TC accumulation. The ASV indicated that G1 (a higher TC genotype) was an unstable genotype for TC, while the GSI ranked the same genotype as the most stable for PPD. The AMMI biplot indicated that G1 had a general adaptation to all locations, and delayed the onset of PPD more than other genotypes. In terms of fresh storage root yield (FSRY) and CMD-S, G1 was the ideal genotype for all environments. The correlation between TC and PPD was significantly negative, indicating the possible effect of carotenoids in delaying the onset of PPD.

Keywords: additive main effects and multiplicative interaction; genotype adaptation; genotype stability index; physiological postharvest deterioration; total carotenoids content

4.1 Introduction

Cassava is among the food security crops in developing countries that are located in the tropical and sub-tropical lowland regions of the world. It is efficient in carbohydrate production, adapted to a wide range of environments and tolerant to drought and acidic soils (FAO, 2010). It tolerates poor soils, requires less labour than other crops, and harvesting can be delayed by months, or even up to three years (Sayre, 2011). It is a food security crop that is generally grown for subsistence by smallholder farmers on marginal and sub-marginal lands in Africa (Sayre, 2011). Although cassava grows well in different environments, its production varies from genotype to genotype, and from one environment to another. This variability is attributed to the inherent genotype properties, environmental conditions and GxE interactions (Falconer and Mackay, 1996). During varietal selection, breeders aim to select high yielding genotypes, which are stable across all environments (Akinwale et al., 2011). The GxE interactions could complicate the selection process (Bondari, 2003; Ding et al., 2007; Kvitschal et al., 2009; Tumuhimbise, 2013; Tumuhimbise et al., 2014), therefore, the breeders must conduct multi-environment tests to study the effects of GxE interactions. It is important for breeders to understand the effects of GxE interactions for the varietal recommendation of a specific genotype for a specific environment.

The GxE interaction is a result of the differential response of genotypes across environments (Malosetti et al., 2013). The phenotypic characteristics of an individual are determined by the effects of genotype and the environment, which are not always additive, because of GxE interactions (Akinwale et al., 2011; Falconer and Mackay, 1996). Good progress of a breeding program depends on the degree and nature of genotypic and non-genotypic variation for various characteristics (Safavi et al., 2015). Complex traits, such as yield, carotenoids content and PPD, could be greatly influenced by various environmental conditions (Cummings, 2015; Wu et al., 2012). The yield performance of cassava depends on genetic and environmental factors, thus understanding these factors helps breeders to select stable performing genotypes, which requires specific statistical methods and tools.

The GxE interactions in multi-environment trials complicates the analysis and interpretation of the generated results, and can consequently lead to low efficiency in the selection of the best stable genotypes (Agyeman et al., 2015). To determine the significance and magnitude of GxE interaction, various methods and techniques have been suggested (Agyeman et al., 2015; Booyse, 2014; Gauch et al., 2008; Kvitschal et al., 2009; Yan et al., 2007). According to Booyse (2014), the additive main effects and multiplicative interaction (AMMI), the genotype main effects and genotype x environment interaction biplot (GGE), cluster analysis, principal component analysis and linear discriminant (canonical variate) analysis, are the most common multivariate statistical methods used to investigate GxE interactions. According to Agyeman

et al. (2015), the AMMI and GGE biplot analyses are two methods that are widely used to overcome the difficulties in the data analysis of multi-environment trials. The AMMI analysis method is the most accurate in detailing the specific adaptations of cassava genotypes to favourable and unfavourable environments (Kvitschal et al., 2009). The AMMI model estimates the magnitude and significance of the GxE interaction effects of each genotypes' response, by using a single model, combining the analysis of variance for the main effects of genotypes and environments, as well as the principal component analysis (PCA) for the GxE interaction (Kang and Gauch, 1996). The GGE biplot provides more information with regards to environments and genotype performance than the AMMI biplot analysis (Agyeman et al., 2015). However, the GGE biplot method is unable to separate the genotype effects from the GEI effects, which is not the case in the AMMI (Gauch et al., 2008).

Most local cassava breeding programs select the genotypes based on yield and yield component traits, which are of importance to farmers. However, the adoption of cultivars depends on various factors, including social-economic and environmental conditions (Hahn et al., 1992). The postharvest physiological deterioration and total carotenoids content are generally ignored by cassava breeders in sub-Saharan Africa (SSA), which could explain the considerable postharvest losses and low β-carotene content of available cassava cultivars in the region. Therefore, the analysis of GxE interaction involving postharvest traits, yield and yield components, and viral diseases traits were conducted in this study, using the AMMI biplot analysis: i) to investigate the significance and magnitude of GxE interactions of 30 cassava genotypes for yield and postharvest traits, and ii) to identify the most stable and high yielding genotypes at five contrasting environments in Rwanda.

4.2 Material and methods

4.2.1 Experimental site

The description of experimental sites, their geographic coordinates and soil and climatic parameters were described previously in Chapter III (Tables 3.1 and 3.2).

4.2.2 Experimental germplasm

The germplasm used in this experiment is described in Table 3.3 of Chapter III.

4.2.3 Experimental design and management

The experiments were laid in 5 x 6 alpha designs, with two replicates. Cuttings of 25 cm lengths, with at least four nodes, were taken from mature cassava, and planted horizontally in a flat seedbed at a spacing of 1 x 1 m, giving a population density of 10 000 plants ha^{-1}. Each

plot was comprised of three rows with eight plants each, which made a total of 24 plants per plot. The data were collected from the inner rows, while the outer rows served as border rows, to minimize the competitive genetic effects. The plots and blocks were separated by 1.5 m and 2 m alleys, respectively, to reduce inter-plot and inter-block plant competition. The trials were weeded manually and no fertilizers and irrigation water were applied.

4.2.4 Data collection

The data were collected from four randomly-selected and hand-uprooted plants from each plot. The data collected included the following: storage root number (SRN), storage root size (SRS), storage root mass (SRM), shoot mass (STM), total biomass mass (TBM), harvest index (HI), dry mass content (DMC), fresh storage root yield (FSRY), dry storage root yield (DSRY), cassava mosaic and cassava brown streak disease severity on leaves and stem (CMD-S and CBSD-LS), cassava brown streak disease root necrosis (CBSD-RN), total carotenoids (TC) and postharvest physiological deterioration (PPD).

Data on CMD and CBSD severity (CMD-S and CBSD-LN) were collected from the leaves and stems six months after planting, while CBSD storage root necrosis (CBSD-RN) was collected at harvest, using the 1-5, scale: 1 = no symptoms on leaves, stems and storage roots, 2 = slight chlorotic spots on leaves and stems/necrosis in storage roots, 3 = moderate chlorotic spots on leaves and or stems/necrosis in storage roots, 4 = severe chlorotic spots on leaves and or stems/necrosis in storage roots, and 5 = very severe chlorotic spots on leaves and or stems/necrosis in storage roots (Hahn et al., 1980; Hillocks et al., 1996; Rwegasira and Rey, 2012). Total carotenoids was analysed from homogeneous representative sample of 15 g per genotype, using the spectrophotometric procedure proposed by Rodriguez-Amaya and Kimura (2004), where total carotenoids content (µg g^{-1}) was calculated, using the following formula:

$$\mathbf{TC}(\boldsymbol{\mu g\ g^{-1}}) = \frac{\mathbf{A\ x\ volume\ (mL) x\ 10^4}}{\mathbf{A_{1cm}^{1\%}\ x\ sample\ weight\ (g)}}$$

Where A is the absorbance; volume is the total volume of extract (25 mL); and $A_{1cm}^{1\%}$ is the absorption coefficient of β- carotene in petroleum ether (PE).

To evaluate PPD, the method developed by CIAT (Morante et al., 2010; Zidenga et al., 2012) was used with modification, where the proximal and distal ends of cassava storage roots were removed immediately after harvest. The proximal ends were exposed to the air and the distal ends of the storage root were covered, using food plastic wrappers. The room temperature ranged from 21-28^{o}C, and the relative humidity was 70-80%. The assessment was conducted

at 3, 7 and 30 days after harvest (PPD-3, PPD-7 and PPD-30, respectively) on ten transversal slices of 2 cm thick cut along each storage root, using the score of 1-10 to represent the discoloration, where score 1 = 10%, 2 = 20%,….., 10 = 100% (Chávez et al., 2005; Wheatley et al., 1985). At each data collection, two storage roots were cut to score the slices, and the mean score from 20 slices per genotype, was calculated.

FSRY expressed in t ha^{-1} was estimated using the formula: $\mathrm{FSRY}\ (\mathrm{t\ ha}^{-1}) = \frac{\frac{\mathrm{SRM\ x\ 10\ 000}}{4}}{1000}$, where SRM is the storage root mass, 10000 represents the total number of plants per ha, while 4 is the plants sampled.

HI was obtained using the formula: $\mathrm{HI} = \frac{\mathrm{SRM}}{\mathrm{TBM}}$, where SRM is the storage root mass, and TBM represent total biomass mass.

DMC (%) was determined using the oven drying method. Cassava storage roots from the four plants were washed, sliced into small pieces picked randomly from apical, distal and middle sections of the storage root, and 100 g were dried in an oven for 48 hours at 80^{o}C. The samples were reweighed to obtain the dry mass, and DMC (%) was then calculated, using the formula:

$\mathrm{DMC}\ (\%) = \frac{\mathrm{DM}}{\mathrm{FM}}\mathrm{x}100$, where DM is the dry mass of the sample, FM is the fresh mass of the sample.

DSRY (t ha^{-1}) was obtained by using the formula: $\mathrm{DSRY}\ (\mathrm{t\ ha}^{-1}) = \frac{\mathrm{DMC}\ (\%)\ \mathrm{x\ FSRY}\ (\mathrm{t\ ha}^{-1})}{100}$

4.2.5 Data analysis

The analysis of variance (ANOVA) was performed for each location, using GenStat 17th Edition. Then the Hartley's F_{max} test for variance homogeneity (Ott and Longnecker, 2008) was conducted to reveal the homogeneity of variance across locations. The combined AMMI analysis was performed across locations, using the model suggested by Gauch and Zobel (1996) below:

$$\mathbf{Y}ij = \boldsymbol{\mu} + \mathbf{g}i + \mathbf{e}j + \sum_{n=1}^{N} \boldsymbol{\lambda_n \alpha_{in} \gamma_{jn}} + \boldsymbol{\rho_{ge}} + \boldsymbol{\varepsilon_{ij}}$$

Where: $\mathrm{Y}ij$: yield of genotypes; μ: grand mean; gi: genotypic main effect; ej: environmental main effect; N: number of PCA axes considered; λ_n: singular value of the nth PCA axis; α_{in}: scores for the ith genotype on the nth axis; and γ_{jn}: scores for the jth; ρ_{ge}: residual for IPCAs not fitted; ε_{ij}: error term.

The AMMI stability value (ASV) proposed by Purchase et al. (2000) was used to quantify and rank genotypes according to their yield stability. Although there are other statistical methods that are widely used to measure stability, the ASV statistic is the most suitable for the AMMI analysis (Farshadfar, 2008). The ASV has been defined as the distance from the coordinate point to the origin in a two-dimensional scatterplot of the first interaction principal component axis (IPCA1) scores, against the second interaction principal component axis (IPCA2) (Farshadfar et al., 2012; Purchase et al., 2000). The IPCA1 accounts for most of the GE variation, and the IPCA1 scores are weighted by the ratio of IPCA1 SS (from AMMI ANOVA) to IPCA2 SS in the ASV formula below:

$$\mathbf{ASV} = \sqrt{\left[\frac{\mathbf{SSIPCA1}}{\mathbf{SSIPCA2}}(\mathbf{IPCA1\ score})\right]^{2} + (\mathbf{IPCA2\ score})^{2}}$$

The lower the ASV, the more stable a genotype is.

Genotype selection across environments entails various complementary techniques. In this study, ranking based on genotypes performance, AMMI stability value (ASV) and genotype stability index (GSI) were used to determine the best performing stable genotypes across five environments. The GSI simultaneously selects genotypes for performance and stability (Farshadfar, 2008). The GSI is calculated, based on the ASV and yield performance rank of genotypes, as per the following equation:

$$\mathrm{GSI}i = \mathrm{RASV}i + \mathrm{RY}i$$

Where: GSI*i* is the genotype stability index for the ith genotype across environments for each trait; RASV*i* represents the rank of the ith genotype across environments, based on ASV; and RY*i* is rank of the ith genotype, based on mean performance across environments. A genotype with the lowest GSI for a specific trait is considered to be the best for combined performance and stability across environments (Farshadfar, 2008; Farshadfar et al., 2012). The sum of GSI rank for all traits was calculated, to identify the most stable genotypes for all traits and environments, and the genotype with the smallest rank sum was considered to be the best across traits.

4.3 Results

4.3.1 Effects of environment and genotype interactions on trait variations

The results of the combined analysis of variance for genotypes showed significant (p<0.001) differences for all traits. The DMC, HI, CBSD-S, CBSD-RN, PPD-3 and PPD-30 were significantly (p<0.001) influenced by environments. The TC and CMD-S were significantly

(p<0.05) affected by environments. Blocks showed significant differences for some traits, such as FSRY, DSRY, CMD-S, CBSD-LS, TC, PPD-3 and PPD-7, which indicates the possibility of a soil characteristic variation between blocks, which affects the expression of these traits (Table 4.1). The GxE interaction also indicated significant differences (p<0.05) for some traits, namely, DMC, CMD-S, CBSD-LS, cassava brown streak disease root necrosis (CBSD-RN) and PPD-3, which demonstrates the combined effects of genotypes and environments on the expression of those traits.

The interaction principal component analysis (IPCA1) was significantly different (p<0.05) for all traits, while IPCA2 was significantly different for DMC, CMD-S, PPD-3 and PPD-7 (Table 4.1). The significant IPCA2 justified the use of the AMMI2 model for those traits, but the AMMI1 model was also applied to the significant traits with IPCA1 only. The % SS variation due to genotype, was higher than the % SS variation due to environment for all traits, except CBSD-RN and CBSD-S, which explains the effect of the environment on the expression of CBSD. The % SS variation for FSRY and DSRY showed that the GxE interaction had a higher % SS variation, compared to the % SS for genotypes and environments separately, which indicates the influence of GxE interaction on the expression of such traits.

The GxE interaction variation, partitioned to IPCA1 and IPCA2, showed that IPCA1 accounted for a much higher % SS variation than IPCA2 and the residual. The IPCA1 captured almost double the % SS variation, compared to IPCA2 for all traits, except the postharvest physiological deterioration evaluated after 30 days (PPD-30), which was explained 100% by IPCA1 (Table 4.1). The residual % SS variation for FRSY was higher, compared to that of the other traits, which indicated that FRSY is influenced by many factors, the 22.5% variation of which was due to unknown factors in the GxE interactions. The % variation due to genotype for TC was higher (96%) than the variation due to environment (1.7%) and GxE interaction (2.4%). The GxE interaction variation, partitioned into principal components, indicated that IPCA1 counted 80.8%, IPCA2 counted 18.4%, while the residual was 0.8% of all variations (Table 4.1).

Table 4.1: Combined AMMI analysis for nine traits of 30 cassava genotypes evaluated at five locations in Rwanda in 2014-2015

Source of variation	Mean squares											
	DF	FSRY	DMC	DSRY	HI	CMD-S	CBSD-S	CBSD-RN	TC	PPD-3	PPD-7	PPD-30
Treatments	149	84.4***	20.67***	7.26**	0.02653***	4.321***	3.92***	3.313***	0.3959***	651***	797***	360.1***
Genotypes (G)	29	171***	74.39***	14.81***	0.05827***	14.196***	1.9***	3.057***	1.9516***	1159***	1887***	1028.4***
Locations (E)	4	514	41.71***	43.45	0.17766***	4.547*	107.11***	72.775***	0.2479*	5568***	3200	859***
Block	5	356.5***	5.33	30***	0.0174	1.617	5.32***	0.913	0.0754***	785***	1589***	103.5
Interactions (GEI)	116	47.9	6.51**	4.13	0.0134	1.845***	0.86*	0.982*	0.012	355***	441***	175.9
IPCA 1	32	93.4**	15.72***	8.4**	0.02786***	4.018***	1.74***	1.91***	0.0315**	774***	906***	438.7***
IPCA 2	30	43.9	7.09*	3.45	0.0128	1.831**	0.97	1.285	0.0077	441***	546***	143.1
Residuals	26	23.2	0.03	1.51	0.0006	0.565	0	0	0.0005	59	108	38.3
Error	145	49.4	3.9	4.43	0.0104	0.837	0.65	0.734	0.0148	129	213	202
Source of variation	**Sum of squares**											
	DF	FSRY	DMC	DSRY	HI	CMD-S	CBSD-S	CBSD-RN	TC	PPD-3	PPD-7	PPD-30
Treatments	149	12577	3079	1082.1	3.953	643.9	583.6	493.7	58.98	97054	118720	53660
Genotypes (G)	29	4960	2157	429.5	1.69	411.7	55.2	88.7	56.6	33601	54720	29823
Locations (E)	4	2056	167	173.8	0.711	18.2	428.5	291.1	0.99	22270	12801	3436
Block	5	1783	27	150	0.087	8.1	26.6	4.6	0.38	3926	7947	518
Interactions (GEI)	116	5561	755	478.8	1.553	214	99.9	113.9	1.39	41183	51200	20402
IPCA 1	32	2989	503	268.8	0.892	128.6	55.7	61.1	1.01	24755	28996	14038
IPCA 2	30	1317	213	103.4	0.383	54.9	29.2	38.6	0.23	13221	16376	4294
Residuals	26	1254	1	39.2	0.017	30.5	0	0	0.01	3207	5828	2069
Error	145	7164	565	641.9	1.505	121.4	94.4	106.4	2.15	18677	30952	29284
% variation due to G		39.4	70.1	39.7	42.7	50.7	9.5	18	96	34.6	46.1	55.6
% variation due to E		16.3	5.4	16.1	18	32.4	73.4	59	1.7	22.9	10.8	6.4
% Variation due to GE		44.2	24.5	44.2	39.3	16.9	17.1	23.1	2.4	42.4	43.1	38
% GEI due to IPCA1		53.76	70.15	65.34	69.04	60.1	65.61	61.28	80.8	60.1	56.6	68.8
% GEI due to IPCA2		23.69	29.71	25.13	29.64	25.7	34.39	38.72	18.4	32.1	32	21
% residual		22.55	0.14	9.53	1.32	14.3	0	0	0.8	7.8	11.4	10.1

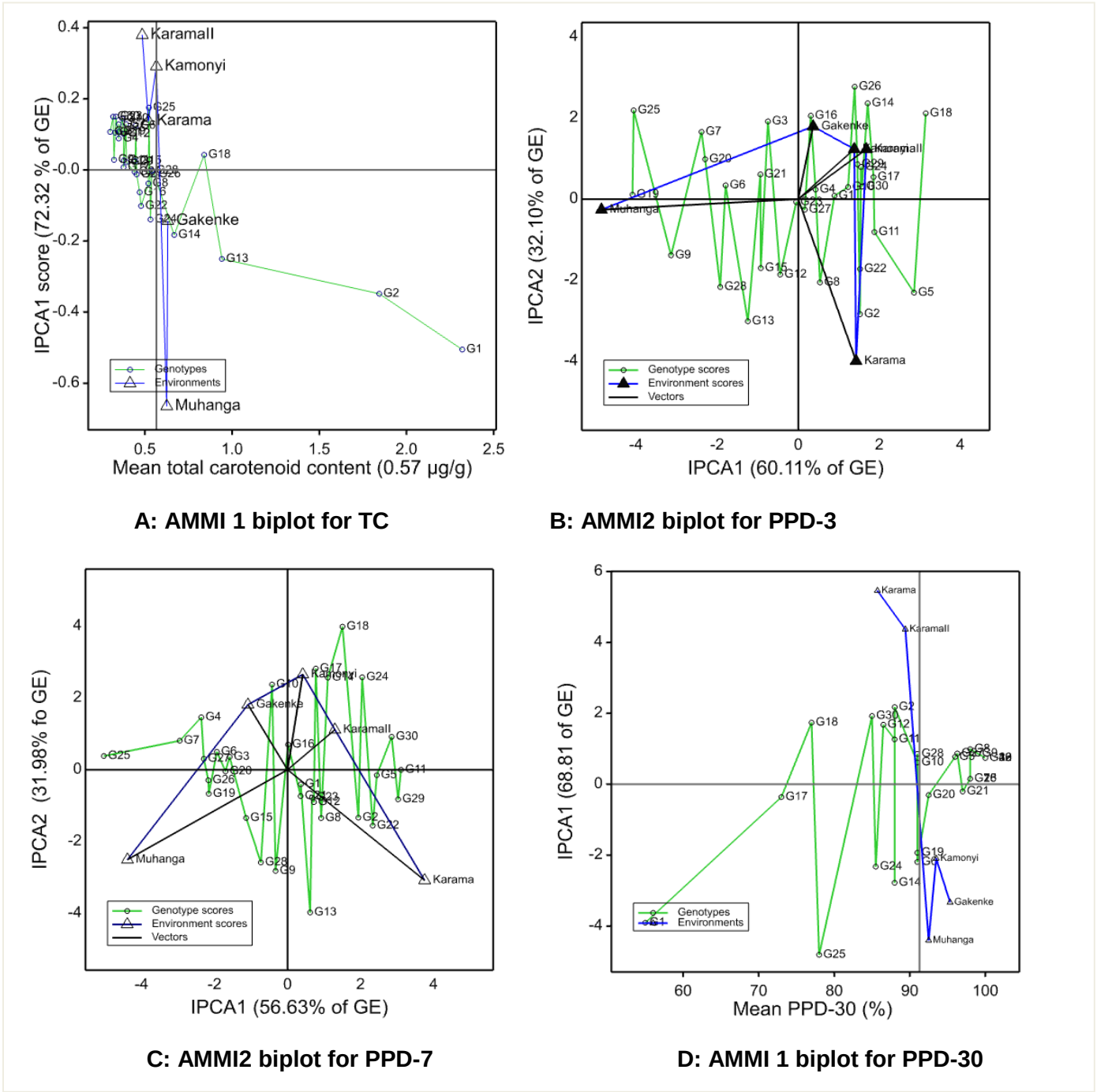
*IPCA1= interaction principal component axes one, IPCA2= interaction principal component axes two, FSRY = fresh storage root yield (t ha^{-1}); HI = harvest index; DMC = dry mass content (%); DSRY = dry storage root yield (t ha^{-1}); PPD-3, -7, -30 = postharvest physiological deterioration (%) after 3, 7 and 30 days respectively, CMD-S= cassava mosaic disease severity scored on a scale of 1 -5, CBSD-RN = cassava brown streak disease root necrosis scored on a scale of 1 -5, CBSD-LS=cassava brown streak disease on leaves and stem scored on a scale of 1 -5, TC=total carotene (µg 100g^{-1}); significance level * = P<0.05; ** = P<0.01; *** = P<0.001*

4.3.2 Effects of genotypes and environment interaction on postharvest traits across environments

The IPCA2 was significant for PPD-3 and PPD-7, and not significant for TC and PPD-30, hence the AMMI1 model was used for TC and PPD-30, while the AMMI2 model was used for PPD-3 and PPD-7. The AMMI1 indicated that most variations for TC and PPD-30 were accounted for by PC1 (72.3% and 68.8%, respectively, of the GxE interaction) (Figure 4.1 A and D). The distribution of genotypes in AMMI1 biplot 4.1 indicated that most of genotypes are scattered closer to the origin (the centre of the biplot), indicating less interaction with the environment for TC. The genotype G18 exhibited good general adaptation, with a mean greater than the general mean, and its IPCA score is close to zero for TC for all environments, while G1, G2, G13 exhibited specific adaptation for the Muhanga location, with a higher value than general mean and a large value of IPCA. (Figure 4.1 A). The genotype selection index (GSI) revealed that the carotenoids-enriched genotypes were not stable, where G1, G2 and G13 had higher carotenoids content, while G28 was the most stable, followed by G26, G18, G27 and G29 (Table 4.2). The most unstable genotypes were G30, G23, G3, G10 and G5 and these genotypes also had a low carotenoids content (Table 4.2). Figure 4.1 D indicated that G17 had good general adaptation for PPD-30, but it had less PPD damage than the general mean and an IPCA score of close to zero. The genotype points were more scattered than the location points, indicating that the variability due to genotypes is higher than the location variability. Though G1 was unstable across locations, it showed a delayed PPD-30, compared to other genotypes.

The AMMI2 biplots for PPD-3 and PPD-7 indicated that most of the genotypes were scattered far from the biplot centre, showing that most genotypes were unstable (Figure.4.1 B and C). In Figure 4.1 B for PPD-3, genotypes G1, G4, G23 and G27 were scattered close to the origin of the biplot (0, 0), indicating less interaction with the locations scattered away from the biplot centre and exhibiting maximum interaction with locations. The mean rank and GSI rank showed that G1 is the best genotype to withstand PPD-3 (Table 4.2). Based on the projection judgement of genotype points on the environment for PPD-3, the genotypes G18, G14, G26 and G16 had a positive interaction with the Gakenke, Kamonyi and KaramaII locations (Figure 4.1), hence indicating a specific adaptability to these locations. Genotypes G2, G5, G22, G8 and G11 showed a specific adaptability to the Karama location, while G9, 23 and G28 were specifically adapted to the Muhanga location (Figure 4.1 B). For the PPD-7, genotypes G1 and G16 were closer to the biplot origin (0,0) indicating a general adaptation to locations, and these genotypes were ranked first and second, respectively, by GSI (Table 4.2). The projection of genotype points on environments for PPD-7 indicated that genotypes G18, G24 and G30

had a positive interaction with the KaramaII and Kamonyi locations (Figure.4.1 C), revealing their specific adaptability to both locations. Based on the length of the vectors from the origin, the Muhanga and Karama locations exhibited a high interaction with genotypes for PPD-3 and PPD-7 (Figure.4.1 B and C).



A: AMMI 1 biplot for TC

B: AMMI2 biplot for PPD-3

C: AMMI2 biplot for PPD-7

D: AMMI 1 biplot for PPD-30

Figure 4.1: AMMI 1 biplot A for TCC and D for PPD-30 and AMMI2 biplot B for PPD-3 and C for PPD-7

Table 4.2: Ranking of 30 genotypes over five environments for postharvest traits

Genotypes	TC						PPD-3					
	Means	Rank	ASV	AVS Rank	GSI	GSI Rank	Means	Rank	ASV	AVS Rank	GSI	GSI Rank
G1	2.32	1	2.22	30	31	14	6.6	1	1.69	4	5	1
G2	1.84	2	1.54	29	31	14	36.5	17	4.06	22	39	22
G3	0.3	30	0.47	17	47	28	33.5	12	2.39	10	22	8
G4	0.35	24	0.39	12	36	21	43	23	0.84	3	26	13
G5	0.35	24	0.56	20	44	26	48	28	5.87	26	54	29
G6	0.45	14	0.55	19	33	18	36	15	3.4	16	31	17
G7	0.39	18	0.56	21	39	23	42	20	4.81	25	45	26
G8	0.52	10	0.17	10	20	5	44	24	2.31	8	32	19
G9	0.32	28	0.13	9	37	22	42	20	6.09	27	47	27
G10	0.37	22	0.63	23	45	27	31	9	2.34	9	18	3
G11	0.38	19	0.12	6	25	11	26	5	3.64	18	23	9
G12	0.38	20	0.45	14	34	19	39	18	2.06	6	24	11
G13	0.94	3	1.1	28	31	14	44	24	3.84	20	44	25
G14	0.67	5	0.82	27	32	17	26	5	4.02	21	26	13
G15	0.44	15	0.12	7	22	8	53	30	2.45	11	41	23
G16	0.47	12	0.36	11	23	9	27.5	8	2.15	7	15	2
G17	0.38	20	0.04	1	21	7	11.5	2	3.55	17	19	5
G18	0.84	4	0.42	13	17	3	22	3	6.31	28	31	17
G19	0.35	23	0.48	18	41	24	45	26	7.72	29	55	30
G20	0.44	16	0.12	8	24	10	47	27	4.45	24	51	28
G21	0.33	27	0.46	16	43	25	50	29	1.87	5	34	20
G22	0.48	11	0.45	15	26	12	36	15	3.37	15	30	16
G23	0.33	26	0.66	24	50	29	42.5	22	0.12	1	23	9
G24	0.53	8	0.62	22	30	13	24	4	3.03	14	18	3
G25	0.52	9	0.78	26	35	20	31.7	11	7.98	30	41	23
G26	0.55	6	0.11	4	10	2	31.5	10	3.84	19	29	15
G27	0.45	13	0.11	5	18	4	40	19	0.4	2	21	7
G28	0.54	7	0.08	2	9	1	35.5	14	4.24	23	37	21
G29	0.39	17	0.11	3	20	5	34	13	2.89	12	25	12
G30	0.32	29	0.66	25	54	30	27	7	2.98	13	20	6

Table 4.2: Continued

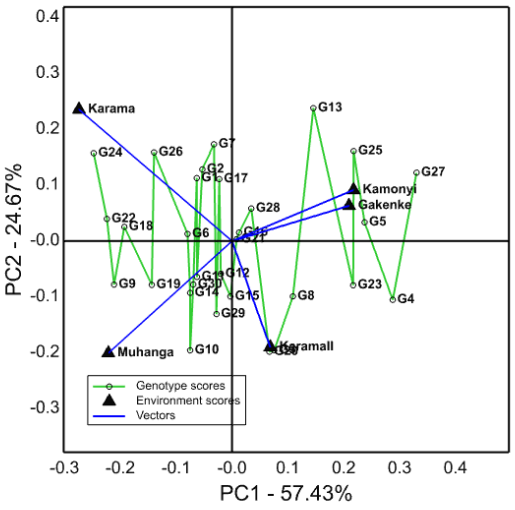
Genotypes	PPD-7						PPD-30					
	Means	Rank	AVS	AVS Rank	GSI	GSI Rank	Means	Rank	AVS	AVS Rank	GSI	GSI Rank
G1	20.5	1	0.77	2	3	1	55	1	12.78	29	30	14
G2	60	14	3.72	16	30	12	88	8	7.12	25	33	17
G3	55	11	2.86	9	20	5	100	25	2.46	8	33	17
G4	69	23	4.47	23	46	28	100	25	2.46	8	33	17
G5	74	27	4.37	21	48	29	96	16	2.66	15	31	15
G6	56	13	3.49	15	28	10	91	11	7.92	27	38	28
G7	61	15	5.34	27	42	26	98	19	0.65	1	20	3
G8	72	25	2.13	6	31	14	98	19	3.39	19	38	28
G9	73	26	2.9	10	36	21	99	24	2.92	16	40	30
G10	49	5	2.51	8	13	3	91	11	2.02	7	18	2
G11	52	8	5.55	29	37	22	88	8	4.15	20	28	10
G12	63	18	1.58	5	23	9	87	7	5.51	21	28	10
G13	67	21	4.16	20	41	25	100	25	2.46	8	33	17
G14	51	7	3.25	14	21	6	88	8	9.09	28	36	27
G15	77	28	2.44	7	35	19	98	19	0.65	1	20	3
G16	52	8	0.71	1	9	2	100	25	2.46	8	33	17
G17	29.5	2	3.16	13	15	4	73	2	2.65	14	16	1
G18	39	3	4.83	25	28	10	77	3	6.91	24	27	9
G19	71	24	3.91	18	42	26	91	11	6.32	22	33	17
G20	67	21	3.04	12	33	15	93	15	1.01	6	21	7
G21	80	30	0.98	3	33	15	97	18	0.79	5	23	8
G22	61	15	4.46	22	37	22	100	25	2.46	8	33	17
G23	62	17	1.43	4	21	6	98	19	0.65	1	20	3
G24	50.5	6	4.48	24	30	12	86	6	7.61	26	32	16
G25	47.5	4	8.99	30	34	17	78	4	15.8	30	34	25
G26	63	18	3.88	17	35	19	98	19	0.65	1	20	3
G27	64	20	4.11	19	39	24	96	17	3	17	34	25
G28	55	11	2.91	11	22	8	91	11	3.25	18	29	13
G29	78	29	5.47	28	57	30	100	25	2.46	8	33	17
G30	52	8	5.18	26	34	17	85	5	6.51	23	28	10

ASV= AMMI stability value, G= genotype; GSI = genotype selection index, PPD-3=postharvest physiological deterioration after three days, PPD-7= postharvest physiological deterioration after seven days, PPD-30= postharvest physiological deterioration after thirty days, TC= total carotenoid (µg g^{-1})

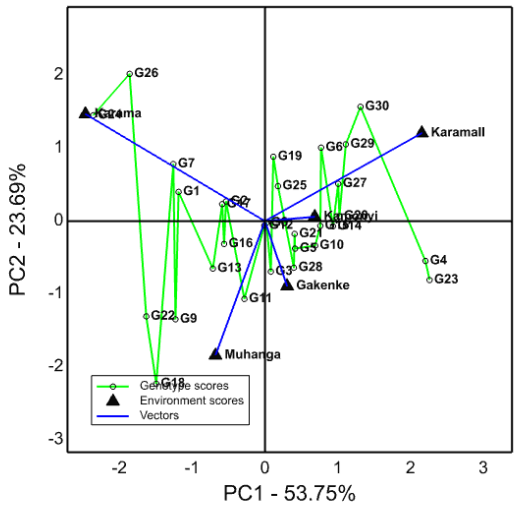
4.3.3 Effects of genotypes and environment interaction on yield traits across environments

The IPCA2 was significant for all yield traits (HI, FRSY, DMC and DRSY), thus the AMMI2 model was used to analyse the GxE interaction on yield and yield components across five environments. The AMMI2 for all yield traits showed that most of the genotypes were scattered far from the biplot centre (0,0), indicating that most genotypes were unstable (Figure 4.2). The AMMI stability value (ASV) is the distance in two dimensional scatterplots of IPCA1 and IPCA2 scores, which are measured by using the theorem of Pythagoras (Purchase et al. 2000); the genotypes with the lowest ASV are the most stable genotypes. In terms of FRSY, the AMMI biplot and ASV indicated that G8 and G12 were the most stable genotypes, while the most unstable genotypes were G23 and G24 (Figure 4.2 B and Table 4.3). ASV quantifies GxE interaction variation, but does not indicate the best genotype (high yield and stable). The genotype selection index (GSI) combines both genotype stability and high yield, giving a useful method to determine the ideal genotypes. Based on GSI, genotypes G25, G2, G8, G14 and G1 were ideal for all environments for FRSY (Table 4.3). The distance from biplot origin (0, 0) indicated that the Gankeke and Kamonyi locations had the lowest interaction with genotypes for FRSY (Figure 4.2 B).

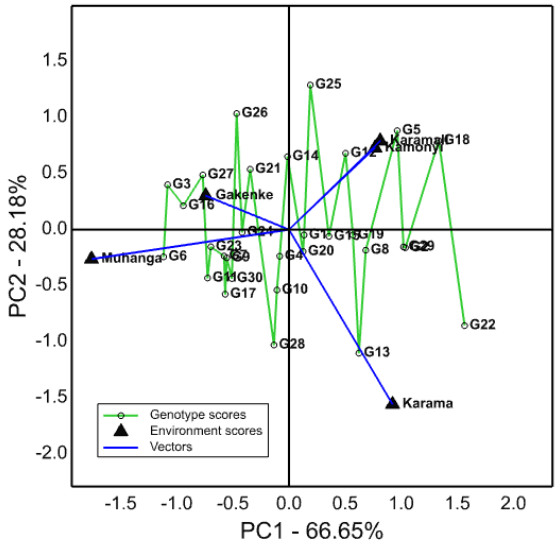
The AMMI biplot and ASV indicated that G21 and G16 were the most stable genotypes (Figure.4.2 A and Table.4.3), while the GSI showed that G17, G2, G1, G28 and G16 were the most ideal genotypes for HI. The high dry matter content (DMC) is among the consumers' preferred cassava traits, and genotypes G4 and G1 were most stable for DMC, as shown by ASV and the AMMI biplot, while the GSI indicated that G15, G4, G19, G21 and G7 were the ideal genotypes (Figure 4.2 C and Table.4.3). In terms of dry storage root yield (DSRY), the AMMI biplot and ASV indicated that genotypes G12 and G8 were most stable, while the GSI revealed G8, G25, G28, G16 and G4 as ideal genotypes (Figure 4.2 D and Table 4.3). Based on the distance from the biplot origin (0, 0), the highest GxE interaction for DRSY was found at the Karama and KaramaII locations (Figure 4.2.D).



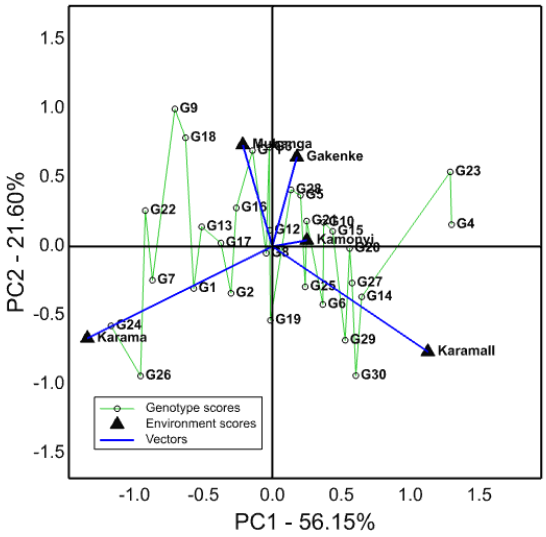
A: AMMI 1 biplot for HI



B: AMMI 1 biplot for FSRY



C: AMMI1 biplot for DM



D: AMMI1 biplot for DSRY

Figure 4.2: AMMI 1 biplot A for HI, B for FSRY, C for DM and D for DSRY

Table 4.3: Overall mean and ranking of 30 genotypes over five environments for yield and yield components

Genotypes	Harvest Index						Fresh Storage Root Yield (t ha^{-1})					
	Means	Rank	ASV	AVS Rank	GSI	GSI Rank	Means	Rank	ASV	AVS Rank	GSI	GSI Rank
G1	0.393	5	0.187	11	16	3	18.112	2	2.744	20	22	5
G2	0.391	6	0.18	9	15	2	12.708	7	1.252	9	16	2
G3	0.363	10	0.265	17	27	11	6.593	21	0.723	4	25	8
G4	0.432	2	0.684	29	31	18	16.746	3	5.078	28	31	15
G5	0.238	25	0.558	27	52	30	3.488	30	1.021	7	37	22
G6	0.388	7	0.187	12	19	6	12.148	9	2.037	16	25	8
G7	0.355	13	0.191	13	26	9	11.996	10	2.985	22	32	17
G8	0.356	11	0.275	18	29	13	10.41	16	0.055	1	17	3
G9	0.283	18	0.502	23	41	24	12.439	8	3.123	23	31	15
G10	0.283	19	0.264	16	35	20	6.916	19	1.615	13	32	17
G11	0.259	22	0.161	8	30	15	5.879	25	1.255	10	35	20
G12	0.241	23	0.076	3	26	9	6.038	24	0.064	2	26	10
G13	0.411	3	0.418	21	24	7	11.128	11	1.762	15	26	10
G14	0.334	16	0.199	14	30	15	14.465	4	2.141	17	21	4
G15	0.241	24	0.1	5	29	13	5.835	26	1.744	14	40	24
G16	0.336	15	0.033	2	17	5	10.786	13	1.321	11	24	7
G17	0.387	8	0.124	6	14	1	9.593	17	1.363	12	29	13
G18	0.198	29	0.454	22	51	29	10.991	12	4.091	26	38	23
G19	0.237	26	0.346	19	45	26	4.931	28	0.929	5	33	19
G20	0.142	30	0.254	15	45	26	3.988	29	2.344	18	47	29
G21	0.234	27	0.02	1	28	12	6.428	22	0.951	6	28	12
G22	0.279	20	0.527	25	45	26	10.552	15	3.958	25	40	24
G23	0.439	1	0.516	24	25	8	19.371	1	5.241	29	30	14
G24	0.378	9	0.603	28	37	22	12.9	5	5.593	30	35	20
G25	0.396	4	0.536	26	30	15	12.719	6	0.635	3	9	1
G26	0.286	17	0.365	20	37	22	8.843	18	4.716	27	45	27
G27	0.338	14	0.786	30	44	25	5.463	27	2.362	19	46	28
G28	0.356	12	0.1	4	16	3	10.607	14	1.115	8	22	5
G29	0.233	28	0.147	7	35	20	6.622	20	2.756	21	41	26
G30	0.271	21	0.181	10	31	18	6.152	23	3.395	24	47	29

Table 4.3: Continued

Genotypes	Dry matter content (%)						Dry Storage Root Yield (t ha^{-1})					
	Means	Rank	ASV	AVS Rank	GSI	GSI Rank	Means	Rank	ASV	AVS Rank	GSI	GSI Rank
G1	25.17	30	0.324	2	32	14	4.565	3	1.526	18	21	4
G2	27.46	25	2.477	26	51	27	3.455	10	0.857	11	21	4
G3	33.21	5	2.609	27	32	14	2.215	19	0.728	8	27	10
G4	32.04	11	0.31	1	12	1	5.347	2	3.411	29	31	16
G5	32.23	10	2.463	25	35	19	1.107	30	0.654	5	35	20
G6	32.4	9	2.674	28	37	22	3.941	7	1.05	14	21	4
G7	33.78	2	1.398	15	17	5	4.098	6	2.292	25	31	16
G8	33.76	3	1.64	18	21	9	3.496	9	0.128	1	10	1
G9	32.44	7	1.346	11	18	6	4.106	5	2.108	24	29	13
G10	30.95	14	0.601	4	18	6	2.092	20	0.992	13	33	19
G11	28.79	22	1.782	20	42	25	1.739	27	0.795	10	37	21
G12	30.16	18	1.383	14	32	14	1.78	24	0.129	2	26	9
G13	30.46	17	1.853	21	38	24	3.408	11	1.351	16	27	10
G14	28.95	21	0.656	5	26	10	4.187	4	1.737	21	25	8
G15	33.04	6	0.85	6	12	1	1.96	22	1.153	15	37	21
G16	31.4	12	2.252	23	35	19	3.393	12	0.741	9	21	4
G17	26.66	26	1.473	16	42	25	2.57	18	0.979	12	30	15
G18	26.45	27	3.278	29	56	30	2.585	17	1.831	22	39	23
G19	35.2	1	1.357	12	13	3	1.75	26	0.541	3	29	13
G20	30.75	15	0.357	3	18	6	1.209	29	1.47	17	46	28
G21	32.41	8	0.986	7	15	4	2.085	21	0.678	6	27	10
G22	28.16	23	3.826	30	53	28	2.878	15	2.429	26	41	24
G23	31.06	13	1.666	19	32	14	6.048	1	3.426	30	31	16
G24	26	28	0.997	8	36	21	3.388	13	3.121	28	41	24
G25	28.03	24	1.377	13	37	22	3.565	8	0.691	7	15	2
G26	30.64	16	1.524	17	33	18	2.622	16	2.678	27	43	27
G27	33.5	4	1.895	22	26	10	1.857	23	1.539	19	42	26
G28	29.05	20	1.085	9	29	12	3.084	14	0.545	4	18	3
G29	25.71	29	2.436	24	53	28	1.7	28	1.545	20	48	29
G30	29.18	19	1.29	10	29	12	1.753	25	1.847	23	48	29

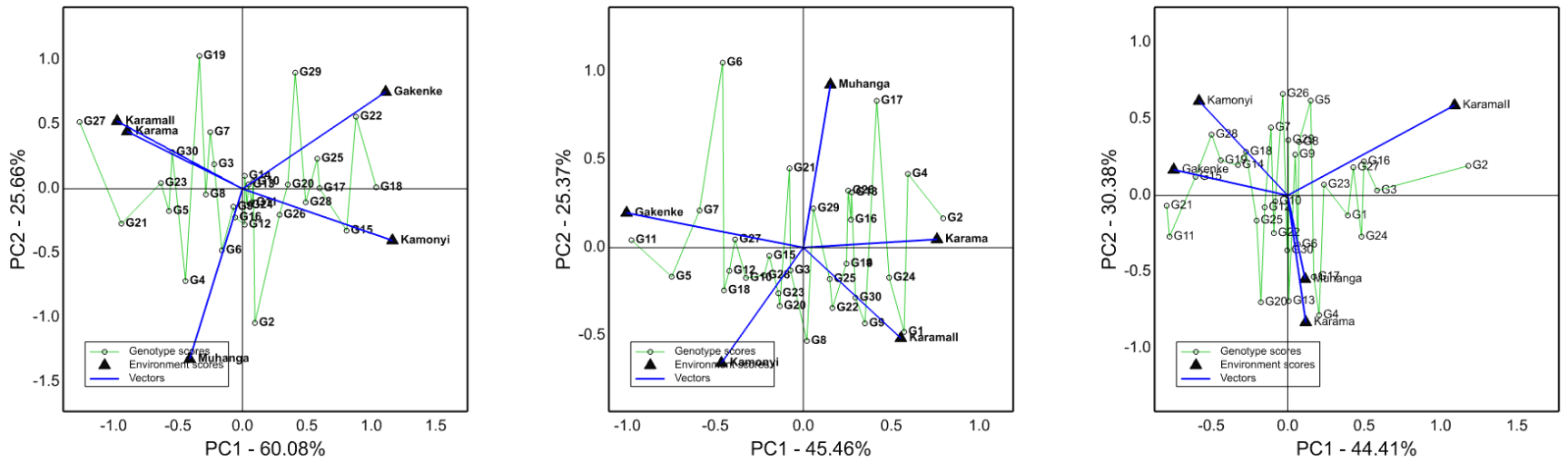
ASV= AMMI stability value, DMC: dry matter content, DSRY: dry storage root yield (t ha^{-1}), G= genotype, GSI = genotype selection index, FSRY: fresh storage root yield (t ha^{-1}), HI: harvest index

4.3.4 Effects of genotypes and environment interaction on CMD and CBSD severity across environments

The IPCA 2 was significant for CMD and CBSD severity; hence, the AMMI2 model was adopted to analysis the GxE interaction. Most genotypes were scattered far away from the AMMI biplot centre (0, 0) for the cassava mosaic disease (CMD) and the cassava brown streak disease (CBSD) severity, indicating that most of genotypes were unstable across five environments (Figure.4.3). For CMD-S, the ASV and AMMI biplot indicated that G14, G13, G24, G11 and G10 were the most stable genotypes, but not with the lowest severity, while the GSI revealed that G1, G13, G16 had the lowest severity and were the most stable genotypes (Table 4.4). The distance from biplot origin (0,0) indicated that G27 and G19 were positively interacting with Karama and Karama II, indicating their specific adaptation to such locations. Genotypes G29 and G22 had a specific adaptation to Gakenke location, G2 and G15 were specifically adapted to the Kamonyi location, while G4 and G21 presented a positive interaction with Muhanga location, showing specific adaptation (Figure.4.3 A).

The CBSD severity on leaves and stems (CBSD-LS), analysed using ASV and the AMMI biplot, indicated that G3, G29, G23, G15 and G25 were the most stable genotypes (Figure 4.3 B), while GSI revealed that G29, G3, G15, G13 and G28 were among the genotypes that had low severity and stability (Table .4.4). The genotypes and environments falling in the same biplot sector interact positively; a genotype with a high positive interaction in a specific environment indicates a specific adaptation to such an environment. Thus, G6 had a specific adaptation to the Gakenke location, G17 showed a specific adaptation to Muhanga, G1 had a specific adaptation to KaramaII, while G5 presented specific adaptability to the Karama location (Figure.4.3 B).

The CBSD necrosis on storage root (CBSD-RN) causes a considerable loss of products and affects food security for cassava farmers in East African countries. The ASV and AMMI biplot determined the stability and adaptability of genotypes for CBSD-RN, and showed that G10, G12, G9, G22 and G2 were the most stable genotypes across five environments (Figure.4.3 C). The GSI analysis divulged that G25, G6, G22, G30 and G10 were among the genotypes with the lowest severity and good stability. However, G2 was specifically adapted to the Karama location, G28 adapted to the Kamonyi and Gakenke locations, while G4 was specifically adapted to the Karama and KaramaII locations (Figure.4.3 C).



A: AMMI 1 biplot for CMD-S **B: AMMI 1 biplot for CBSD-LS** **C: AMMI 1 biplot for CBSD-RN**

Figure 4.3: AMMI 1 biplot A for CMD-S, B for CBSD-LS, C for CBSD-RN

Table 4.4: Ranking of 30 genotypes over five environments for viral diseases in 2014-2015

Genotypes	CMD-S						CBSD-LS						CBSD-RN					
	Means	Rank	ASV	ASV Rank	GSI	GSI Rank	Means	Rank	ASV	ASV Rank	GSI	GSI Rank	Means	Rank	ASV	ASV Rank	GSI	GSI Rank
G1	1	1	0.261	7	8	2	3.1	21	1.142	25	46	30	2.3	16	0.595	15	31	15
G2	1.7	5	1.074	16	21	9	3	16	1.445	29	45	29	1.9	6	1.755	30	36	20
G3	4.2	26	0.552	10	36	16	2.9	11	0.185	1	12	2	2.6	22	0.864	26	48	28
G4	3.5	18	1.268	18	36	16	2	1	1.153	26	27	14	1	1	0.846	25	26	11
G5	4	25	1.358	22	47	28	2.8	7	1.36	28	35	20	2.8	25	0.664	17	42	26
G6	1.7	5	0.612	11	16	7	3	16	1.344	27	43	25	1.8	5	0.337	5	10	2
G7	1.9	10	0.738	14	24	11	3.1	21	1.086	23	44	28	2.4	18	0.476	11	29	14
G8	1.3	3	0.673	12	15	6	3.1	21	0.537	13	34	18	3.1	29	0.369	10	39	23
G9	1.7	5	0.216	6	11	5	3	16	0.767	19	35	20	3.1	29	0.28	3	32	16
G10	4.7	29	0.208	5	34	14	3.3	26	0.614	17	43	25	2.2	14	0.125	1	15	4
G11	4.5	28	0.204	4	32	13	2.9	11	1.763	30	41	23	2.6	22	1.172	29	51	29
G12	4.9	30	0.284	9	39	19	2.6	3	0.77	20	23	8	2.4	18	0.235	2	20	7
G13	1.3	3	0.116	2	5	1	2.6	3	0.588	15	18	4	1.7	3	0.697	20	23	8
G14	1.8	9	0.11	1	10	4	3.2	24	0.452	8	32	16	2.1	10	0.52	13	23	8
G15	3.7	21	1.938	26	47	28	2.9	11	0.352	4	15	3	2.1	10	0.896	27	37	21
G16	1	1	0.261	7	8	2	2.9	11	0.514	12	23	8	2.9	28	0.77	23	51	29
G17	1.7	5	1.414	24	29	12	2.1	2	1.13	24	26	13	1.3	2	0.593	14	16	6
G18	2.8	13	2.453	29	42	23	2.6	3	0.85	21	24	11	2.8	25	0.494	12	37	21
G19	3.9	22	1.306	19	41	22	3.2	24	0.452	8	32	16	2.2	14	0.686	19	33	17
G20	4.3	27	0.834	15	42	23	3.4	28	0.413	6	34	18	2	7	0.75	21	28	13
G21	3.6	20	2.235	28	48	30	4	30	0.476	11	41	23	2	7	1.169	28	35	19
G22	3.1	16	2.157	27	43	26	3.3	26	0.457	10	36	22	2.1	10	0.282	4	14	3
G23	2.8	13	1.495	25	38	18	3	16	0.366	5	21	7	2.3	16	0.357	7	23	8
G24	3.1	16	0.153	3	19	8	2.8	7	0.898	22	29	15	2.4	18	0.763	22	40	24
G25	2.4	12	1.392	23	35	15	3	16	0.325	3	19	6	1.7	3	0.342	6	9	1
G26	1.9	10	0.71	13	23	10	3.6	29	0.564	14	43	25	2.6	22	0.672	18	40	24
G27	2.9	15	3.032	30	45	27	2.7	6	0.701	18	24	11	2.1	10	0.66	16	26	11
G28	3.9	22	1.165	17	39	19	2.9	11	0.434	7	18	4	2.5	21	0.836	24	45	27
G29	3.5	18	1.328	21	39	19	2.8	7	0.247	2	9	1	2.8	25	0.365	9	34	18
G30	3.9	22	1.319	20	42	23	2.8	7	0.607	16	23	8	2	7	0.362	8	15	4

ASV= AMMI stability value, CMD-S= cassava mosaic disease severity scored on a scale of 1 -5, CBSD-RN = cassava brown streak disease root necrosis scored on a scale of 1 -5, CBSD-LS=cassava brown streak disease on leaves and stem scored on a scale of 1 -5, G= genotype; GSI = genotype selection index

4.4 Phenotypic correlation of important cassava traits

The Pearson's correlation showed significant correlation between FSRY and other traits, except PPD-3 (Table.4.5). A negative correlation was observed between FSRY and DMC, CMD-S, CBSD-RN and CBSD-LS, indicating the influence of these traits on the overall yield of cassava. The correlation matrix revealed a significant negative correlation between harvest index with viral diseases (CMD-S, CBSD-RN and CBSD-LS), indicating the effects of viral diseases on HI. Dry matter content had a significant negative correlation with DRSY and TC, hence these traits could be influenced by DMC. There was a significant positive correlation between of CBSD-LS and CBSD-RN, indicating the influence of CBSD-LS on the presence of CBSD-RN. CBSD-RN and PPD cause cassava postharvest loss, due to storage root necrosis and physiological deterioration, and the correlation analysis revealed a significant negative correlation between both traits, suggesting that CBSD-RN symptoms could mask the PPD signs. The correlation between TC and PPD-3 was significantly negative, indicating the possible effects of carotenoids in delaying the onset of PPD.

Table 4.5: Phenotypic correlation between yield, postharvest and viral disease traits

Traits	FSRY	HI	DMC	DSRY	CMD-S	CBSD-LS	CBSD-RN	TC	PPD-3
FSRY	1								
HI	0.550***	1							
DMC	-0.153**	-0.105	1						
DSRY	0.982***	0.546***	-0.005***	1					
CMS-S	-0.276***	-0.309***	0.070	-0.267***	1				
CBSD-LS	-0.192***	-0.207***	0.131	-0.170**	0.070	1			
CBSD-RN	-0.202***	-0.274***	0.092	-0.184	0.089	0.640***	1		
TC	0.227***	0.195***	-0.416***	0.143	-0.328***	-0.013	-0.058	1	
PPD-3	-0.008	0.052	0.350***	0.049	0.021	-0.193***	-0.164***	-0.197***	1

*FSRY = fresh storage root yield (t ha^{-1}); HI = harvest index; DMC = dry mass content (%);DSRY: dry storage root yield (t ha^{-1}); CMD-S= cassava mosaic disease severity scored on a scale of 1 -5, CBSD-RN = cassava brown streak disease root necrosis scored on a scale of 1 -5, CBSD-LS=cassava brown streak disease on leaves and stem scored on a scale of 1 -5, TC: total carotenoids, PPD-3: postharvest physiological deterioration after three days, PPD-7: postharvest physiological deterioration after seven days, significance level; * = P<0.05; ** = P<0.01; *** = P<0.001.*

4.5 Discussion and conclusions

The AMMI analysis of 30 cassava genotypes revealed significant genotype effects for all evaluated traits, indicating the presence of genetic variation in Rwandan germplasm. The observed genetic variation implies that good progress for improved cassava for various traits could be achieved by selection and hybridization. The environments significantly affected most traits evaluated in this study, indicating the importance of conducting multi-location trials to identify the generally stable genotypes and genotypes specifically adapted to certain environments. To select and recommend high yielding and stable genotypes for various environments, the analysis of multi-location trials often identify GxE interaction, which causes

difficulties in interpretation (Agyeman et al., 2015). This is overcome by using the AMMI biplot analysis, a method that is widely used for multi-location trial data analysis (Agyeman et al., 2015; Noerwijati et al., 2014; Sholihin, 2015) to determine the stability of genotypes across environments.

The GxE interaction significantly affected traits such as DMC, CMD-S, CBSD-S and CBSD-RN, demonstrating the combined effects of environment and genotype for the expression of such traits. The effect of GxE interaction on DMC corroborates with studies by Ssemakula and Dixon (2007), who reported on the influence of environment on cassava dry matter content. On the contrary, Benesi et al. (2005) reported that DMC is not highly controlled by environments and they suggested it was controlled by one, or a few, major genes. The GxE interaction for CMD-S indicated that genotypes respond differently to CMD in various environments, explaining the need for a specific adaptation analysis for the trait, as reported by Ssemakula and Dixon (2007). The significant GxE interaction for CBSD expression suggests a quantitative nature of a multi-gene trait, as reported by Pariyo et al. (2015). The findings on the GxE interaction for yield and viral disease severity traits agreed with several studies (Baiyeri et al., 2008; Njoku et al., 2015; Ntawuruhunga and Dixon, 2010; Tumuhimbise et al., 2015), which reported that GxE interaction analysis is important to recommend genotypes with adequate adaptation to target environments. The insignificant GxE interaction for PPD-30, FSRY, DSRY, HI and TC highlighted the stable performance of genotypes for these traits across various locations. The stability performance of genotypes for PPD at various environments was reported by Tumuhimbise et al. (2015) in Uganda.

The TC had 96%, 2.4% and 1.7% variation, respectively, due to genotype, GxE interaction and environment. The AMMI1 biplot indicated a low interaction of environment with TC. The low interaction between environment and TC expression can be explained by the qualitative nature of this trait, as most qualitative traits are generally controlled by a few genes and are less prone to environmental effects (Ssemakula et al., 2007). The observed high variation due to genotypes and low environmental effects, and the relatively low GxE interaction for TC, indicated that it would require evaluation over only a few environments, to determine and recommend stable and high performing genotypes for TC. These findings agree with those of Rodriguez-Amaya (2010) and Ssemakula and Dixon (2007), who reported high genetic effects for carotenoids accumulation. Iglesius et al. (1997), Ssemakula et al. (2007) and Akinwale et al. (2010) reported similar findings, speculating that carotenoids accumulation in cassava is controlled by a few genes, which implies that a few environments suffice for the evaluation and selection of carotenoids-enriched cassava clones.

ASV and GSI determine the stability and performance of genotypes evaluated at various environments (multi-locations). The genotypes with the highest carotenoids content were specifically adapted to some environments. For instance, G1, a genotype with high carotenoids, was specifically adapted to Muhanga. Though unstable across environments, this genotype delayed PPD-30 more than other genotypes across locations. Thus, the hybridization of G1 with other cassava genotypes could improve the level of carotenoids content and the postharvest quality of cassava, including the delayed onset of physiological postharvest deterioration. The significant correlation between CBSD-LS and CBSD-RN was an indication of the effects of CBSD-LS on storage root quality and yield. The correlation analysis confirmed that there is a negative correlation between PPD and total carotenoids content. This corroborates the finding of Sánchez et al. (2006), Morante et al. (2010) and Uarrota et al. (2014), who reported that PPD correlates negatively with carotenoids, indicating that carotenoids could delay the onset of PPD. The mechanism by which carotenoids delays the onset of PPD was reported by several scientists (Azqueta and Collins, 2012; Edge et al., 1997; Palozza et al., 2003; Priya and Siva, 2014; Rodriguez-Amaya, 2010; Xu et al., 2013), who indicated that as non-enzymatic antioxidants, carotenoidss can act as chain-breaking antioxidants and thus protect cells and organisms against photoxidation, by quenching singlet oxygen (a reactive type of oxygen).

In conclusion, this study indicated that the genotype effects observed for all traits explained a wide genetic variation among the cassava genotypes in Rwanda, hence selection and hybridization can result in good progress in the development of improved cassava for postharvest quality. The GxE interaction for TC and FSRY was not significant, implying that few complications in selection for TC and FSRY over different locations can be expected. The PPD scored three and seven days after harvest, were affected by GxE interaction, indicating that selection for delayed PPD could be complicated by the GxE interaction, and hence the selection and recommendation for delayed PPD genotypes must be done with caution.

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CHAPTER V

Genetic inheritance and diallel analysis of cassava pulp colour and delayed postharvest physiological deterioration at early generation selection of F1 seedling population

Abstract

Early selection at seedling stage for quantitative and qualitative traits of cassava could shorten varietal release time and could help to reduce viral disease build-up in successive generations. Genetic inheritance analysis provides the essential breeding information for the development of improved cultivars. This study aimed to develop F1 segregating cassava populations and to determine the inheritance mode of pulp colour and other important traits at the F1 seedlings stage. Fifteen families were generated from a 6x6 half diallel established and evaluated at the Karama research station in Rwanda. The 15 F1 families exhibited significant variation between genotypes and families, indicating genetic diversity that is essential for crop improvement through conventional breeding. General combining ability (GCA) was significant ($p<0.01$) for all traits, except for the cassava brown streak disease on leaves (CBSD-L), while the specific combining ability (SCA) was significant ($p<0.01$) for all evaluated traits (height, CBSD-L: cassava brown streak disease on leaves, CBSD-S: cassava brown streak disease on stem, CBSD-RN: cassava brown streak disease root necrosis, SRN: storage root number, SRL: storage root length, SRG: storage root girth, FSRY: fresh storage root yield, HI: harvest index, DMC: dry matter content, pulp colour, and PPD: physiological postharvest deterioration). The significant GCA indicated the possibility of improving cassava through recurrent selection for most of the evaluated traits. Based on the significance and direction of GCA, genotypes G2 and G7 were good general combiners for improving fresh storage root yield, while G1 and G2 were good general combiners for improving carotenoids (yellow/orange pulp colour) and delayed physiological postharvest deterioration. The significance of GCA and SCA effects for most traits indicated the role of both additive and non-additive gene action in controlling most of the cassava traits. The highest GCA/SCA ratio and % sum of square (SS) due to GCA were recorded for CBSD-RN, SRN, FSRY, HI, pulp colour and PPD, indicating that these traits were mostly controlled by additive gene action. The first three principal components (PCs) were most important and explained 71.2% of total variation among families for all traits, which indicated the potential for success of early selection for all traits. This information is very important and can contribute to shortening the breeding cycle for pulp colour (as carotenoids indicator), PPD and other important traits.

Key words: additive and non-additive gene action; combining ability; physiological postharvest deterioration

5.1 Introduction

Cassava is a multipurpose crop in developing countries and a cheap source of starch across sub-Saharan Africa (SSA). It is an amphidiploid allopolyploid (2n = 36 chromosomes), has a regular bivalent pairing and behaves as a diploid (El-Sharkawy, 2004). Recently the diploid nature of cassava was confirmed by Aiemnaka et al. (2012). The cassava plant is monoecious, bearing separate male and female flowers on the same plant (Chavarriaga-Aguirre and Halsey, 2005). It is classified as heterozygous, because it is a cross-pollinated plant (Jennings and Iglesias, 2002). However, the duration of flowering on the same plant is variable and can last up to two months, which can cause self- and sib-fertilization, hence there is a need for controlled pollination.

Cassava is the cheapest source of calories in many countries of SSA. Its storage roots contain significant amounts of various vitamins and proteins. In addition, its starch can be used in industries, such as food manufacturing, pharmaceuticals, textiles, plywood, paper and adhesives, and as feedstock to produce ethanol biofuel (FAO, 2013). In Rwanda, cassava is among the important staple foods, and it has a double role in nutrition, by providing starch from its roots and protein from green leaves. Furthermore, it has the ability to provide a piecemeal harvest which can be extended from eight months to two years. Thus, it is one of the most reliable food security crops in the country.

Despite being a food security crop and cheap source of calories, it is exposed to production losses, due to postharvest physiological deterioration (PPD) and cassava brown streak disease root necrosis (CBSD-RN) in the country. The PPD is an abiotic response of cassava storage roots damaged during harvesting process, which induces a progressive loss of production, while its secondary stage results in bacterial attack, causing a total loss of production. It has been reported that introgressing carotenoids content, with its antioxidant properties, in cassava, can help in extending the shelf-life of storage roots for some days (Sánchez et al., 2006; Nduwumuremyi et al., 2016a). The primary mode of action of carotenoids as antioxidants is to quench singlet oxygen (Rodriguez-Amaya, 2010). Though conventional breeding (recurrent selection) is feasible to improve the carotenoids content (Ceballos et al, 2013) in most developing countries, there is the challenge of quantifying carotenoids in the thousands of new genotypes that are generated. The yellow colour intensity of the pulp is highly correlated to the carotenoids content in cassava storage roots (Chávez et al., 2005). A simple screening method, using colour scoring, can be used for the initial

selection (seedlings F1) of the storage roots of cassava genotypes with a relatively high total carotenoids content.

The CBSD-RN is caused by the attack of a virus affecting the above-ground parts and extending to the storage roots. Depending on the level of infestation, CBSD-RN can cause up to a 100% loss of total production. The speed of build-up of the virus differs from one genotype to another, which can explain the resistance and/or tolerance levels. The heterogeneous nature of cassava results in the wide and unpredictable diversity of F1 seedlings, which is interesting for breeders, but presents difficulties in propagation (Ceballos et al., 2004). The genetic diversity of F1 seedlings can produce hybrids possessing all the important characteristics, including high yield, disease resistance, high level of carotenoids, and delayed physiological postharvest deterioration. The vegetative propagation of cassava through stem cuttings easily transmits viral diseases (Sastry, 2013). The early selection in a segregating F1 population can help to shorten the breeding scheme. The early selection in F1 seedlings is generally based on high heritability traits, such as plant height, branching habits, flowering, and certain diseases (Ceballos et al., 2004). However, in some areas seedlings produce many storage roots; hence, the fresh storage root yield, harvest index, dry mass content, carotenoids content and PPD can be selected at the F1 seedling stage. The high-heritability traits can be selected for in early stage of cassava evaluation. Njenga (2014) suggested that carotene content can be selected in the early stages of the breeding cycle.

To develop F1 hybrids, the half-diallel mating design is one of the most used designs in cassava breeding for generating half-sib offspring in genetic studies, as it helps to identify the good general (parents) and specific combiners (Ram, 2014). According to Griffing (1956b), the analysis of diallels uses the random or fixed model and one of four methods (1 = parents, one set of F1s and reciprocals; 2 = parents and one set of F1s without reciprocals; 3 = one set of F1s and the reciprocals are included; and 4 = only one set of F1s). Method 4 is the most common in the diallel crossing systems. A random model is useful for estimating GCA and SCA variances. In contrast, when parents are considered fixed effects, the aim is to measure the GCA effect for each parent and the SCA effect for each pair of parents. The diallel with selfs and reciprocals is neither practical nor useful, as cassava does not possess maternal effects, selfing fixed genes in homozygous state, but it does not contribute to the recombination of genes between different parents, and recombination is achieved by crossing in one direction making reciprocals unnecessary (Acquaah, 2012). The fixed model of Methods 3 or 4 is the most appropriate for obtaining unbiased estimates of combining abilities and gene action (Shattuck et al., 1993). This method is most suitable when there are no genotypic reciprocal effects (Griffing, 1956a).

This study was conducted: (1) to develop an F1 cassava population segregating for improved carotenoids content, delayed postharvest physiological deterioration and other important traits; (2) to analyze a half-diallel crossing population for the general combining ability (GCA) and specific combining ability (SCA) effects for cassava pulp colour, delayed postharvest physiological deterioration and other important traits; and (3) to determine the genetic inheritance of cassava pulp colour, delayed postharvest physiological deterioration and other important traits.

5.2 Material and methods

5.2.1 Germplasm selection and hybridization

Twelve genotypes (Table 5.1) were selected from research institutes, farmer's cooperatives and private farms. The selection of genotypes was done in a participatory manner through consultative discussion between local scientists and farmers. The main traits for selection were high yield, cassava brown streak disease (CBSD) resistance and pulp colour. The parents were planted in crossing block paired rows at the Karama research station, located at 2°15'54.126''S, 30°15'22.4619''E, with an altitude of 1338 m asl. Among the twelve parents, only six parents produced flowers and showed genetic compatibility. Due to the high pressure of viral diseases, coupled with the lack of irrigation facilities, four parents did not produce flowers and for another two parents the abortion rate was high, an indication of sterility and genetic incompatibility. Hence a 6 x 6 half- diallel was produced to generate fifteen families. Hand pollination was performed following the procedure proposed by Kawano (1980). Approximately three months after hand pollination, the botanical seeds were harvested and stored for about three months, to break the dormancy. Seeds were soaked overnight in gibberellic acid to enhance germination. The soil was vapour sterilized. Seeds were planted in a small screenhouse made from transparent plastic, to create favourable conditions for germination. The temperature inside the screenhouse ranged from 28 to 38°C.

Table 5.1: List of parental germplasm

Nº	Code of genotypes	Name of genotypes	Type	Yield	CMD	CBSD	Pulp colour
1	G1	Mavoka	Improved	High	Resistant	Susceptible	Yellow
2	G2	Garukansubire	Improved	High	Resistant	Susceptible	Yellow
3	G3	Gahene	Landrace	High	Susceptible	Susceptible	White
4	G4	Mushedile	Landrace	High	Susceptible	Tolerant	White
5	G5	Kibombwe	Landrace	Medium	Susceptible	Susceptible	White
6	G6	Ndamirabana	Improved	High	Resistant	Susceptible	White
7	G7	Gitamisi	Landrace	High	Susceptible	Tolerant	White
8	G8	Rwizihiza	Improved	High	Resistant	Susceptible	White
9	G9	Cyizere	Improved	High	Resistant	Susceptible	White
10	G10	Kwatamumpare	Landrace	Medium	Susceptible	Susceptible	White
11	G11	Creolina	Landrace	Medium	Susceptible	Susceptible	White
12	G12	Gacyacyali	Landrace	Low	Susceptible	Susceptible	White

5.2.2 Experimental design and management

The fifteen F1 seedling families were planted in November 2014 in a randomised complete block design (RCBD). Each family comprised of 33 selected seedlings that were divided into three groups of 11 seedlings each. Each group was planted on one line, representing one replicate. Planting spacing was 1 x 1 m, which gives a population density of 10,000 plants ha^{-1}. Regular weeding was performed and no fertilizers or pesticides were applied.

5.3.4 Data collection

The selected twelve cassava traits recorded were as follows: height, CBSD-L: cassava brown streak disease on leaves; CBSD-S: cassava brown streak disease on stem; CBSD-RN: cassava brown streak disease root necrosis; SRN: storage root number; SRL: storage root length; SRG: storage root girth; FSRY: fresh storage root yield; HI: harvest index; DMC: dry matter content, pulp colour; and PPD: physiological postharvest deterioration. These were recorded for the F1 seedlings to analyse the phenotypic differences among generated families. The height (cm) was measured as the distance from the ground to the shoot tip. The CBSD-L, CBSD-S and CBSD-RN were scored on a scale of 1 to 5 where: 1 = no visible necrosis, and 5 = severe necrosis (Hillocks et al., 1996). The storage roots per plant were counted and weighed to obtain the storage root number (SRN). The SRL (cm) was measured as the length between the ends of a storage root, while SRG (cm) was measured as the circumference at the widest point of the mid-section of the storage root. The FSRY (t ha^{-1}) was estimated from the storage root mass of each plant. To estimate FRSY (kg ha^{-1}), the following formula was used:

$$\mathbf{FRSY}\left(\mathbf{kg\ ha^{-1}}\right) = \mathbf{SRM}\left(\mathbf{kg\ plant^{-1}}\right)\mathbf{X}\ \frac{\mathbf{10000}}{\mathbf{1000}}$$

Harvest index (HI) was determined by expressing the fresh storage root mass (kg plant $^{-1}$) as a proportion of total biomass (kg plant $^{-1}$) (Fukuda et al. 2010). The DMC was determined using the specific gravity method (Chávez et al., 2005; Kawano et al., 1987) together with the following formula:

$$\textbf{DMC } (\%) = \left(\frac{WA}{WA-WW} X158.3\right) - 142$$

Where WA and WW are weight measured in the air and water, respectively.

The pulp colour, to estimate the total carotenoids content level, was determined by using a 1-4 scale (1: white, 2: cream, 3: yellow, 4: orange). The PPD was determined by using the method developed by CIAT (Morante et al., 2010; Zidenga et al., 2012) with some moditifcation, whereby the proximal and distal ends of cassava storage roots were removed immediately after harvest. The proximal ends were exposed to the air and the distal ends were covered by food plastic wrappers. The storage room temperature ranged from 21 to 28^{o} C, and the relative humidity ranged from 70 to 80%. The assessment was conducted at seven days after harvest, using a score of 1-10 to represent the discoloration, where score 1 = 10%, 2 = 20%,….., 10 = 100% (Chávez et al., 2005; Wheatley et al., 1985) on ten transversal slices of 2 cm thick, cut along each storage root. Two storage roots were cut to score the slices and a mean score obtained from 20 slices per individual plant.

5.2.5 Data analysis

Data collected from the individual plants of a family were averaged for statistical analyses. The analysis of data was done using SAS Version 9.3 (SAS Institute Inc, 2011). The Griffing's (1956b) diallel Method 4 (crosses only), Model 1 (fixed effects) was used to estimate the GCA and SCA effects:

$$\boldsymbol{Yij = \mu + vij + bk + eijkl,}$$

where, *Yij* = observed value of the cross between parent i and j; μ = overall mean; vij = F1 hybrid effect; $vij = gi + gj + Sij$ where, *gi* = GCA of the parent i; *gj* = GCA of the parent j; *sij* = SCA of the cross between parents i and j;
bk = effect of the kth block; and *eijkl* = experimental error.

The GCA and SCA effects were estimated according to Griffing's (1956b) Method 4, Model 1 using the DIALLEL-SAS05 program developed by Zhang et al. (2005). The significance of combining ability effects was determined by using the t-test at 0.05, 0.01 and 0.001 levels of probability, adapted from the combining ability analysis of variance output. The GCA and SCA

effects for each trait were determined from the percentage of a families' sum of squares (SS) due to GCA and SCA (Tumuhimbise et al., 2014). The importance of GCA and SCA effects was calculated using GCA/SCA ratio. A ratio greater than one (unity) indicates that additive effects are more important than non-additive effects in the inheritance of a selected trait, while a ratio smaller than one indicates that dominance effects are more important in the inheritance of a selected trait. The principal component analysis using GenStat software (Payne et al., 2011) determined components loadings to explain variation among traits.

5.3 Results

The seedlings derived from F1 botanical seeds were evaluated for the selected traits. The traits evaluated were height, cassava brown streak disease on leaves (CBSD-L), cassava brown streak disease on stem (CBSD-S), cassava brown streak disease root necrosis (CBSD-RN), storage root number (SRN), storage root length (SRL), storage root girth (SRG), fresh storage root yield (FSRY), harvest index (HI), dry mass content (DMC), pulp colour and postharvest physiological deterioration (PPD) one week after harvest.

5.3.1 Performance of fifteen families for selected important traits

The fifteen families were highly significant (p<0.001) in terms of performance, except for the CBSD-L, CBSD-S and HI traits, which were significant at a statistical level of p<0.05. The mean performance of the fifteen families is presented in Table 5.2. The families G1xG2, G1xG3, G1xG6, G1xG7, G2xG6, G2xG7 and G4xG7 were the tallest, compared to the families' average. The families G1xG2, G1xG4, G2xG6, G3xG4, G3xG6, G4xG7, and G6xG7 had the lowest CBSD-L. The families G1xG2, G1xG3, G2xG4, G2xG6, G2xG7, G3xG4, G4xG6, G4xG7 and G6xG7 showed the lowest CBSD-S, while the families G1xG2, G1xG3, G1xG6 and G2xG7 had the highest CBSD-RN. The families G1xG2 and G6xG7 did not show CBSD symptoms on leaves and stems. The family average for FSRY was 25.3 t ha^{-1}. The yield of seven families, G1xG2, G1xG3, G2xG3, G2xG4, G2xG7, G4xG7 and G6xG7, was above the average. In terms of pulp colour, the families G1xG2, G1xG4, G1xG6, G1xG7, G2xG4, G2xG6 and G2xG7 showed pulp colour ranging from bright yellow to deep yellow, and these families had a low PPD, compared to the families' average (Table 5.2).

Table 5.2: Mean performance of F1 seedlings in 15 families

Family	Height (cm)	CBSD-L	CBSD-S	CBSD-RN	SRN	SRL (cm)	SRG (cm)	FSRY (t ha^{-1})	HI	DMC (%)	Pulp colour	PPD - 7 (%)
G1 x G2	198.61	1.00	1.09	1.76	10.09	31.36	18.63	26.10	0.40	31.32	2.85	25.03
G1 x G3	219.85	1.12	1.25	1.57	10.09	32.54	17.45	27.30	0.37	35.70	1.82	28.79
G1 x G4	189.21	1.05	1.35	1.39	8.15	19.73	14.32	16.73	0.51	34.62	2.46	25.12
G1 x G6	213.27	1.11	1.52	1.97	9.79	34.97	16.73	22.00	0.45	35.81	2.70	19.54
G1 x G7	198.85	1.17	1.91	1.33	9.88	32.97	17.18	21.05	0.47	37.81	3.82	12.27
G2x G3	169.76	1.25	1.46	1.15	9.76	25.85	14.46	26.57	0.38	37.55	1.97	21.21
G2 x G4	186.42	1.22	1.16	1.36	9.30	25.30	13.49	31.86	0.31	32.00	2.12	17.12
G2 x G6	211.06	1.08	1.22	1.36	10.30	33.67	15.91	20.48	0.51	34.99	2.76	21.36
G2 x G7	216.09	1.20	1.10	1.55	12.79	37.97	16.91	43.85	0.30	35.26	2.52	20.00
G3 x G4	196.63	1.09	1.23	1.24	9.30	26.21	12.79	16.96	0.55	35.62	1.24	39.70
G3 x G6	196.19	1.01	1.32	1.39	8.27	29.39	15.58	13.85	0.62	36.34	1.12	43.64
G3 x G7	188.30	1.13	1.28	1.27	9.37	32.21	14.60	20.68	0.45	34.43	1.00	34.09
G4 x G6	176.75	1.12	1.16	1.30	8.76	25.82	12.82	20.77	0.47	36.62	1.09	34.85
G4 x G7	200.88	1.03	1.09	1.27	10.70	33.33	15.42	37.67	0.30	34.76	1.06	35.30
G6 x G7	194.46	1.00	1.09	1.09	10.06	27.36	15.24	33.81	0.30	36.41	1.06	35.61
Mean	***197.09***	***1.11***	***1.28***	***1.40***	***9.77***	***29.91***	***15.44***	***25.31***	***0.43***	***36.62***	***1.97***	***27.58***
LSD(5%)	***16.46***	***0.15***	***0.35***	***0.21***	***1.32***	***4.36***	***0.96***	***11.35***	***0.15***	***3.5***	***0.3***	***4.4***
P value	***<0.001***	***0.0306***	***0.006***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***0.002***	***<0.001***	***<0.001***	***<0.001***

Cassava brown streak disease on leaves=CBSD-L), cassava brown streak disease on stem=CBSD-S), cassava brown streak disease root necrosis=CBSD-RN), storage root number=SRN, storage root length=SRL, storage root girth=SRG, fresh storage root yield=FSRY (t ha^{-1}), harvest index=HI, dry mass content=DMC (%) and postharvest physiological deterioration after one week of harvest=PPD-7(%)

5.3.2 Combined analysis of variance of diallel crosses and gene action for selected traits

The analysis of variance indicated that variation between families was significantly for all evaluated traits at various significance levels (0.05, 0.01 and 0.001). A high significant difference (p<0.001) was observed for all traits, except CBSD-L, CBSD-S and HI. The ANOVA of diallel revealed a significant difference for the general and specific combining ability (GCA and SCA) effects for most evaluated traits. The GCA effect was highly significant (p<0.001) for height, CBSD-RN, SRN, SRL, SRG, FSRY, DMC, pulp colour and PPD; the GCA for HI and CBSD-S were significant at p<0.01 and p <0.05, while it was insignificant for CBSD-L (Table 5.3). The SCA effect was significantly different for all evaluated traits. Most of the traits had significant GCA and SCA effects indicating the effects of both additive and non-additive gene action for their expression.

The ratio GCA/ SCA for CBSD-RN, SRN, FSRY, HI, pulp colour and PPD was more than one (one unity). In addition, these traits indicated >40% of SS due to GCA. The highest % of SS due to GCA was recorded for FSRY (75.5%), PPD (74.2%), CBSD-RN (46.6%), SRN (45.6%), pulp colour (41.6%), HI (40.7%), respectively. In contrast, the highest % of SS due to SCA was recorded for height **(**55.8%), SRG (55.8%), SRL (43.4%) and CBSD-S (42.2%), respectively (Table 5.3).

Table 5.3: Combined analysis of variance of 6x6 half-diallel for selected traits in F1 cassava seedlings

Source of variation	DF	Height (cm)	CBSD-L	CBSD-S	CBSD-RN	SRN	SRL (cm)
Family	14	595.25***	0.02*	0.14**	0.16***	3.65***	68.29***
GCA	5	432.63**	0.02	0.13*	0.25***	6.27***	91.63***
SCA	9	685.59***	0.02*	0.15**	0.11***	2.20**	55.33***
Error	28	98.60	0.01	0.04	0.02	0.63	6.79
R-Square		0.75	0.56	0.62	0.83	0.75	0.83
CV (%)		5.04	7.68	15.99	9.00	8.01	8.74
GCA/SCA (SS ratio)		0.35	0.40	0.48	1.28	1.58	0.92
% SS due to GCA		19.58	16.02	20.24	46.69	45.66	39.97
% SS due to SCA		55.86	39.99	42.21	36.36	28.82	43.44
% SS due to error		24.55	43.99	37.55	16.94	25.52	16.59

Table 5.3: Continued

Source of variation	DF	SRG (cm)	FSRY (t ha^{-1})	HI	DMC (%)	Pulp colour	PPD - 7
Family	14	9.01***	211.57***	0.03**	31.44***	2.26***	253.49***
GCA	5	20.46***	346.29***	0.04**	46.90***	4.83***	480.48***
SCA	9	2.65***	136.73*	0.03**	22.85***	0.83***	127.38***
Error	28	0.33	46.06	0.01	4.39	0.03	6.93
R-Square		0.93	0.70	0.66	0.78	0.97	0.95
CV (%)		3.74	26.35	20.54	5.65	9.10	9.38
GCA/SCA (SS ratio)		0.35	4.29	1.41	0.90	1.14	3.23
% SS due to GCA		19.58	75.56	40.72	31.25	41.65	74.24
% SS due to SCA		55.86	17.61	28.94	34.76	36.53	22.98
% SS due to error		24.55	6.83	30.33	33.99	21.82	2.78

GCA=General combining ability, SCA=specific combining ability, SS=sum of squares, CV=coefficient of variation, Cassava brown streak disease on leaves=CBSD-L), cassava brown streak disease on stem=CBSD-S), cassava brown streak disease root necrosis=CBSD-RN), storage root number=SRN, storage root length=SRL, storage root girth=SRG, fresh storage root yield=FSRY (t ha^{-1}), harvest index=HI, dry mass content=DMC (%) and postharvest physiological deterioration after one week of harvest=PPD-7(%)

5.3.3 General combining ability effects

The general combining ability effects indicated direction and compared the performance of parents in generating progenies with good characteristic (good combiners). The GCA effects for G1 (Mavoka) was positive and highly significant (p<0.001) for CBSD-S, CBSD-RN, SRG, DMC, pulp colour, and negatively and highly significant for PPD-7. The parent G2 was highly significant and positive for SRN, SRG, pulp colour and highly significant and negative for PPD-7 (Table 5.4). In contrast to the first two parents, the G3 was highly significant and negative for SRG and pulp colour, while it was highly significant and positive for PPD-7. The G4 had a highly significant negative GCA effects for SRL, SRG, DMC and pulp colour, and a highly significant positive GCA for PPD-7. The G6 had a highly significant negative and positive GCA effects for pulp colour and PPD-7, respectively. The G7 had a highly significant positive GCA for SRN, SRL, SRG, FSRY, and a highly significant negative GCA effects for CBSD-S (Table 5.4).

Table 5.4: General combining ability effects for cassava selected traits

Parents	Height (cm)	CBSD-L	CBSD-S	CBSD-RN	SRN	SRL (cm)
G1	8.59**	-0.02	0.26***	0.26***	-0.22	0.5
G2	-0.88	0.06*	0.04	0.04	0.84***	1.15
G3	-3.68	0.02	-0.09**	-0.09**	-0.52*	-0.84
G4	-8.89**	0	-0.11**	-0.11**	-0.67**	-4.79***
G6	1.57	-0.05*	0.03	0.03	-0.42*	0.41
G7	3.28	0	-0.12***	-0.12	0.98***	3.57***
SE	***2.62***	***0.02***	***0.03***	***0.03***	***0.21***	***0.69***

Table 5.4: Continued

Parents	SRG (cm)	FSRY (t ha^{-1})	HI	DMC (%)	Pulp colour	PPD-7 (%)
G1	1.78***	-3.35	0.02	3.05***	0.95***	-6.78***
G2	0.55***	5.58**	-0.06*	-0.49	0.59***	-8.29***
G3	-0.57***	-5.30**	0.06*	1.64**	-0.68***	7.39***
G4	-2.09***	-0.64	0	-2.37***	-0.47***	3.55***
G6	-0.23	-3.91*	0.06*	-0.73	-0.28***	4.28***
G7	0.55***	7.63***	-0.08**	-1.1	-0.10*	-0.15
SE	*0.15*	*1.76*	*0.02*	*0.55*	*0.05*	*0.68*

Standard error=SE, cassava brown streak disease on leaves=CBSD-L), cassava brown streak disease on stem=CBSD-S), cassava brown streak disease root necrosis=CBSD-RN), storage root number=SRN, storage root length=SRL, storage root girth=SRG, fresh storage root yield=FSRY (t ha^{-1}), harvest index=HI, dry mass content=DMC (%) and postharvest physiological deterioration after one week of harvest=PPD-7(%)

5.3.4 Specific combining ability effects

Two families (1x3 and 2x3) had a significant (p<0.001) positive SCA effects for height, while one family (1x2) had a highly significant (p<0.001) negative SCA effects for CBSD-L. The CBSD-S and CBSD-RN had the same magnitude of SCA effects, whereby five families (1x6, 2x3, 1x7, 2x7 and 6x7) showed a highly significant SCA effects. The families 1x3 and 1x7, and 2x6, and 3x7 had a significant SCA effects at p<0.001, and p<0.01, and p<0.05, respectively, for the FRSY. The families 1x3, 1x4, 1x6, 3x7, 4x6 and 1x2 had a significant SCA effects at a different significance level (Table 5.5) for DMC. The families 1x2, 1x3, 2x6, 3x4, 1x7, 4x7 and 5x7 exhibited a high significant (p<0.001) SCA effects for pulp colour, while the seven families (1x2, 1x6, 2x3, 2x4, 3x6, 1x7 and 4x7) revealed a high significant (p<0.001) SCA effects for PPD-7 (Table 5.5).

Table 5.5: Specific combining ability effects for important cassava traits

Families	Height (cm)	CBSD-L	CBSD-S	CBSD-RN	SRN	SRL (cm)
1x2	-6.19	-0.14***	0.06	0.06	-0.31	-0.2
1x3	17.86***	0.01	0.01	0.01	1.05**	2.97*
1x4	-7.58	-0.03	-0.16**	-0.16**	-0.74*	-5.89***
1x6	6.03	0.08	0.29***	0.29***	0.65	4.14***
2x3	-22.78***	0.07	-0.20***	-0.20***	-0.34	-4.37***
2x4	-0.9	0.07	0.03	0.03	-0.65	-0.96
2x6	13.28**	-0.03	-0.11	-0.11	0.11	2.2
3x4	12.11**	-0.03	0.04	0.04	0.71*	1.93
3x6	1.21	-0.06	0.06	0.06	-0.56	-0.09
1x7	-10.11	0.09*	-0.20***	-0.2***	-0.66	-1.01
2x7	16.6	0.04	0.23***	0.23***	1.19**	3.34**
3x7	-8.39	0.01	0.09	0.09	-0.87*	-0.43
4x6	13.02	-0.07	0.02	0.02	-0.07	-0.29
4x7	9.39	-0.08	0.1	0.1	0.61	4.64***
5x7	-7.49	-0.05	-0.21***	-0.21***	-0.27	-6.53***
SE	***4.44***	***0.04***	***0.06***	***0.06***	***0.35***	***1.17***

Table 5.5: Continued

Family	SRG (cm)	FSRY (t ha^{-1})	HI	DMC (%)	Pulp colour	PPD-7(%)
1x2	0.86**	-1.44	0.02	2.15*	-0.66***	12.52***
1x3	0.81**	10.63***	-0.14**	4.40***	-0.42***	0.61
1x4	-0.82**	-4.59	0.06	-2.68**	0.01	0.77
1x6	-0.27	3.95	-0.05	-3.12**	0.07	-5.53***
2x3	-0.96***	0.99	-0.05	-0.21	0.09	-5.46***
2x4	-0.42	1.61	-0.06	-1.76	0.03	-5.72***
2x6	0.14	-6.50*	0.09*	-0.41	0.48***	-2.21
3x4	0.01	-2.41	0.06	-0.27	0.42***	1.18
3x6	0.94***	-2.25	0.08	-1.19	0.11	4.40***
1x7	-0.58*	-8.54**	0.10*	-0.75	1.00***	-8.37***
2x7	0.37	5.34	0.01	0.23	0.06	0.86
3x7	-0.80**	-6.96*	0.04	-2.72**	-0.19*	-0.72
4x6	0.31	-0.01	0.02	-3.10**	0.13	0.56
4x7	1.53***	5.38	-0.05	1.62	-0.34***	4.33***
5x7	-0.51	4.79	-0.10*	1.62	-0.53***	3.90**
SE	***0.26***	***2.98***	***0.04***	***0.93***	***0.08***	***1.16***

Standard error=SE, cassava brown streak disease on leaves=CBSD-L), cassava brown streak disease on stem=CBSD-S), cassava brown streak disease root necrosis=CBSD-RN), storage root number=SRN, storage root length=SRL, storage root girth=SRG, fresh storage root yield=FSRY (t ha^{-1}), harvest index=HI, dry mass content=DMC (%) and postharvest physiological deterioration after one week of harvest=PPD-7(%)

5.3.5 Contribution of traits to variability of fifteen families

The principal components (PC) analysis revealed that the first three principal components accounted for 71.2% of the total variation of the fifteen families. The PC1 explained 34.9% of the total variation, whereby SRN, SRL, SRG, height, FSRY, CBSD-RN, pulp colour had a considerable positive contribution, while HI and PPD showed a negative contribution. The PC2 showed 20.4% of the total variation, in which pulp colour, CBSD-S and HI contributed positively much of the variation (Table 5.6). The PPD and DMC expressed a considerable positive variation to the PC3 (15.9%), while the CBSD-L contributed negatively. The fourth PC

indicated 7% of the total variation, in which CBSD-L and DMC were the most contributing factors (Table 5.6).

Table 5.6: Principal components analysis of 12 selected traits in F1 seedlings of 15 families

Traits	Principal Component (PC)			
	PC1	**PC2**	**PC3**	**PC4**
SRN	0.810523	-0.39457	-0.02922	0.177492
SRL	0.807107	-0.09515	0.224897	0.062593
SRG	0.784913	0.321997	0.360499	-0.02214
Height	0.701026	-0.0019	0.374577	-0.03153
FSRY	0.678931	-0.64836	-0.20782	-0.01957
HI	-0.5921	0.563496	0.297321	0.105727
CBSD-RN	0.509106	0.423682	0.363	-0.28471
Pulp colour	0.552686	0.700638	-0.29786	-0.1403
CBSD-S	0.004925	0.679898	-0.34837	0.03644
CBSD-L	0.188924	0.039291	-0.68549	0.502723
PPD	-0.58441	-0.44672	0.594831	0.06883
DMC	0.134719	0.328554	0.537597	0.657233
Eigen value	4.18748	2.448028	1.907872	0.839962
Percentage variation	34.89566	20.40023	15.89893	6.99968
Cumulative percentage variation	34.89566	55.29589	71.19483	78.19451

Cassava brown streak disease on leaves=CBSD-L), cassava brown streak disease on stem=CBSD-S), cassava brown streak disease root necrosis=CBSD-RN), storage root number=SRN, storage root length=SRL, storage root girth=SRG, fresh storage root yield=FSRY (t ha^{-1}), harvest index=HI, dry mass content=DMC (%) and postharvest physiological deterioration =PPD, Principal component =PC

5.4 Discussion and conclusion

The genetic variability and inheritance mode of targeted traits in a cassava breeding program are the keys for achieving good progress. An understanding of the genetic inheritance of such traits is very important in order to develop a cassava breeding strategy. Firstly, this study aimed to develop F1 cassava populations segregating for pulp colour, delayed postharvest physiological deterioration and other important traits. Secondly, it aimed to analyse the diallel data for general combining ability (GCA) and specific combining ability (SCA) effects, and finally, to determine genetic inheritance of the above-mentioned traits.

The F1 segregating populations, comprising of fifteen families, exhibited significant differences for all evaluated traits. This indicates considerable genetic variation among the generated progenies, as well as their parents. The significant variability observed between families was evidence of successful diallel mating. A wide genetic variability in cassava was reported by many scientists (Kundy et al., 2015; Laila et al., 2015; Njenga et al., 2010; Ntawuruhunga and Dixon, 2010; Tumuhimbise et al., 2015). This implies that hybridization and selection for important traits in cassava can result in good progress in generating improved cultivars.

The diallel analysis for combining ability revealed significant general and specific combining ability effects of most evaluated traits. The general combining ability (GCA) effects was significant for all traits, except CBSD-L, while the SCA effects was significant for all evaluated traits. The significant GCA effects indicated the possibility of improving cassava through recurrent selection. Griffing (1956b) indicated that the GCA variance indicates additive effects, while SCA variance contains non-additive effects. Sharma et al. (2013) reported that GCA is mainly the result of additive gene effects and additive × additive interactions, while SCA is the consequence of dominance, epistatic deviation and genotype × environmental interactions. The significant GCA and SCA effects found in this study corroborate the finding of Chipeta et al. (2015), who reported the significance of GCA and SCA effects for most cassava traits. The parents with considerable significant GCA effects for the desirable traits, either positive or negative, could be considered as good combiners, depending on the nature of the targeted traits. The parents G2 and G7 had a significant positive GCA for FSRY, indicating that they are good general combiners for FSRY. The G1 and G2 had a significant positive GCA effects for improved carotenoids (yellow/ orange pulp colour), and a significant negative GCA effects for PPD, indicating that they are good general combiners for both traits. The last two parents, having deep yellow colour, could generate offspring with a high carotenoids content and a delayed PPD, because of the antioxidant properties reported in high carotenoids genotypes. Sánchez et al. (2006), Morante et al. (2010) and Zidenga et al. (2012) reported that carotenoids delays the onset of PPD due to its antioxidant properties.

The CBSD-RN, SRN, FSRY (t ha^{-1}), HI, pulp colour and PPD recorded the highest GCA/ SCA ratio and % SS due to GCA, indicating a considerable contribution of additive gene action in controlling these traits. This agrees with the findings of Tumuhimbise (2013), who reported the predominance of additive gene action in expression of FSRY, HI and CBSD-RN. Njenga et al. (2014) reported that additive gene action plays an important role in the inheritance of pulp colour of the cassava storage root, while the findings on PPD corroborate those of Thompson (2013), who reported that PPD is controlled by multiple genes acting additively.

The contribution of traits to the variability of the fifteen families using PC analysis (PCA), indicated that the first three PCs were most important and explained 71.2% of the total variation among families. The evaluated twelve traits (SRN, SRL, SRG, height, FSRY, CBSD-RN, pulp colour, HI, PPD, CBSD-S, DMC and CBSD-L) indicated the possibility of selection for these traits at an early stage of the cassava breeding process. This can contribute to shortening of varietal release and saving resources. Tumuhimbise (2013) reported that the early selection of cassava (at the seedling stage) could save time and resources in breeding programs. In addition, it can reduce degeneration, due to the accumulation of viruses and yield

losses reported in vegetatively-propagated crops in successive cycles of propagation (Torrance, 2015; Sastry, 2013).

In conclusion, the fifteen F1 families exhibited significant variation between parents and families, which indicated the significant genetic diversity that is essential for crop improvement through conventional breeding. The significant GCA effects indicated the possibility of improving cassava through recurrent selection for most traits. Based on the significance and direction of GCA effects, genotypes G2 and G7 could be used as good general combiners for improving fresh storage root yield, while G1 and G2 could be good combiners for improved carotenoids (yellow/orange pulp colour) and delayed physiological postharvest deterioration. The significant GCA and SCA effects for most of evaluated characteristics in this study confirmed the role of additive and non-additive gene action in cassava traits expression. The high significance of SCA effects for traits, explained the greater importance of dominance effects than the additive gene effects for most cassava traits. However, the high GCA/ SCA ratio and % SS, due to GCA for CBSD-RN, SRN, FSRY, HI, pulp colour and PPD, indicated that these traits are controlled by the additive gene action. The PCA indicated that early selection is possible and that it could shorten the varietal release period and reduce the degeneration and yield losses due to the build-up of viruses in successive cycles of cassava propagation.

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Chapter VI

Combining ability and heterosis for cassava β-carotene, delayed postharvest physiological deterioration and farmers' preferred traits

Abstract

Combining ability and heterosis generate important information to assist in generating improved cassava recombinants. This study aimed at determining the combining ability and heterosis for cassava traits evaluated in F1 clones, generated from a half-diallel (6 x 6) mating design. The F1 clones exhibited considerable agronomic and morphological variability between families and offsprings. The best F1 clones produced a higher fresh root storage yield (FRSY) (44.2 t ha^{-1}) than the best parent (26.3 t ha^{-1}). Similarly, the best F1 progenies had a higher amount of β-carotene (β-C) of 6.12 mg 100 g^{-1} against 1.32 mg 100 g^{-1} of the best parent. This could be attributed to the recombination of additive alleles and epistasis. The environments did not exhibit a significant influence on the expression of β-C and postharvest physiological deterioration (PPD), indicating that the expression of such traits is mostly genetically determined. The general combining ability (GCA) and specific combining ability (SCA) effects for both β-C and PPD were highly significant, indicating the role of both additive and non-additive gene action in controlling such traits. The storage root traits (cassava brown streak disease root necrosis, CBSD-RN, β-C and PPD) were highly influenced (over 50% of variability) by GCA effects, indicating that such traits are predominantly controlled by additive gene action. The disease and yield traits (cassava mosaic -CMD, cassava brown streak disease on leave and stem -CBSD-L, -CBSD-S, total biomass -TB, FSRY, harvest index -HI and dry matter content -DMC) were considerably influenced (over 50% of variability) by SCA effects, indicating a predominance of non-additive gene action in controlling these traits. The GCA indicated that genotypes Mavoka and Garukunsubire had a significant desirable and positive GCA for β-C, an undesirable significant and negative GCA for DMC, and desirable significant negative GCA for PPD. This implies that improving β-C in cassava population. using Mavoka and Garukunsubire as progenitors, could concurrently improve yield and delay PPD. However, this should be done carefully, so as not to reduce the DMC. The F1 progenies from the family Mavoka x Garukunsubire expressed the highest positive heterosis for CMD, DMC and β-C. The high positive heterosis for DMC in this family could be linked to transgressive segregation, because one of the parents was a poor combiner for DMC. This study generated new clones with an improved β-C, FRSY, delayed PPD and other farmers' preferred traits, and it provided the foundation for a cassava breeding program in Rwanda.

Key words: transgressive segregation, additive gene action, non-additive gene action, general combining ability, specific combining ability, cassava brown streak disease, dry matter content

6.1 Introduction

Cassava is a food security crop and generates cash income for smallholder farmers in many countries of tropical and subtropical Africa, Asia, and Latin America. In Africa, its annual per capita consumption is around 80 kg per person (IITA, 2016). In sub-Saharan Africa (SSA), cassava is mainly a subsistence crop for small-scale farmers. In Rwanda, the preferred cassava traits are a sweet taste, a high yield, good quality ugali (viscosity and colour), resistance to pest and diseases, early bulking, multipurpose, good colour of flesh and flour, delayed post-harvest physiological deterioration (PPD), high dry matter content, good odour/ smell, long storage ability in the field, many cuttings produced and good cookability (Nduwumuremyi et al., 2016b).

The viral diseases and postharvest losses are the most serious challenges for cassava production in developing countries. Cassava brown streak disease (CBSD) and cassava mosaic disease (CMD) affect the cassava yield and storage root quality in most parts of East Africa (Ephraim et al., 2015; Rwegasira and Rey, 2012). In addition, PPD causes significant postharvest losses, as the storage root perish rapidly (Kiaya, 2014). Cassava breeding is the most sustainable strategy to generate new high yielding recombinants that are resistant to diseases, with delayed physiological deterioration. During the breeding process, information generated on the combining ability and heterosis assists in the development of new improved recombinants (Mendes et al., 2015; Zhao et al., 2016). At present, there is limited genetic information on the combining ability and heterosis for yield, postharvest and disease traits, and other important cassava traits in Rwanda.

To generate new recombinants, the half-diallel mating design is most commonly used by cassava breeders (Nduwumuremyi et al., 2013; Tumuhimbise, 2013). The diallel analysis provides information on the general combining ability (GCA), the specific combining ability (SCA) and heterosis (Glover et al., 2005). The combining ability indicates the capacity to transmit characteristic from parents to offspring (Upadhyay and Jaiswal, 2015). A knowledge of the combining ability helps to determine gene action and the identification/selection of the

best genotypes (parents) for hybridization, as well as the identification/selection of the best combinations (crosses) for population improvement. This information is very important for designing suitable breeding strategies for cassava improvement. Therefore, this study aimed at:

(i) determining the combining ability of six cassava genotypes and the heterosis of fifteen cassava families for cassava pulp colour and delayed physiological postharvest deterioration, and other farmers preferred traits; and

(ii) selecting promising high yielding cassava clones with improved carotenoids and delayed physiological postharvest deterioration, and other farmer preferred traits.

6.2 Materials and methods

6.2.1 Experiment location

The experiment was conducted at two locations, namely, the Karama and Muhanga research centres. Karama is located at 2°16' 0.927''S, 30°15' 20.693''E, with an altitude of 1332 m above sea level m (asl), while Muhanga is located at 2°04'9.842''S, 29°43' 9.842''E, with an altitude of 1879 masl. The two locations experience bimodal rainfall with different amounts of rain and temperatures. The Muhanga location is cooler and receives a higher amount of rain than the Karama location (Table 6.1).

Table 6.1: Soil and climatic parameters of experimental locations

Parameters	Locations	
	Karama	Muhanga
Soil parameters		
pH	5.4	5.9
Available P (mg kg^{-1})	3.1	4.3
Exch K (cmol kg^{-1})	0.78	0.58
Total N (%)	0.23	0.38
Organic C (%)	1.69	3.06
Exch Ca (cmol kg^{-1})	2.36	3.06
Exch Mg (cmol kg^{-1})	0.38	0.14
Exch Na (cmol kg^{-1})	0.04	0.03
CEC (cmol kg^{-1})	10.82	16.3
Clay (%)	71.2	67.5
Sand (%)	26.1	28.3
Silt (%)	2.7	4.2
Climatic parameter*		
Rainfal (mm)	895	1320
Av min temperature (C°)	14.6	12.0
Av max temperature (C°)	30.8	28.4

**the data sourced from nearby weather station,*

6.2.2 Germplasm

The six parents described in the Chapter V (see Table 5.1) constituted the crossing block of 6 x 6 half-diallel mating design, and generated 15 families of 33 full-sib plants each. In this clonal evaluation trial, the total numbers of genotypes were 450, derived from 15 families. Within each family, 30 full-sib plants, producing enough cuttings (at least 12), were selected from the 33 full-sib plants. The 12 cuttings were subdivided into two groups of six cuttings each, to undergo trials at two locations (environments).

6.2.3 Experimental design and management

The 450 genotypes (clones) selected from the seedling trial were planted in October 2015 at two locations, in randomised complete block design (RCBD) with three replications. One plot represented one family and had 60 plants (two plants per clone and 30 clones per family) whereas one block had 900 plants (60 plants per plot and 15 families/ or 15 plots per block). The plant spacing between and within the rows was 1 x 1 m, indicating a population density of 10000 plants ha^{-1}. The distance between blocks was 2 m, to minimize competition. In addition to the clonal trial, the six parents were evaluated along their offspring in another RCBD. The weeding was conducted regularly and ridging was performed once, three months after planting, while no fertilizers, pesticides and water irrigation were applied.

6.3.4 Data collection

The trials were harvested at nine months after planting as described by Tumuhimbise (2013), who reported that most cassava genotypes attain a relatively high early fresh storage root yield at nine months after planting in Uganda. The data were collected on each individual plant for cassava mosaic disease (CMD), cassava brown streak disease (CBSD) severity, and cassava brown streak disease root necrosis (CBSD-RN), scored on a scale of 1 -5, where: 1 = no symptoms; and 5 = very severe symptoms (Hillocks et al. 1996). The fresh root storage yield (FSRY) (t ha^{-1}) was estimated from storage root mass per plant. To estimate FRSY, the storage root mass (SRM) was used following the formula:

$$\mathbf{FRSY}\left(\mathbf{kg\ ha^{-1}}\right) = \mathbf{SRM}\left(\mathbf{kg\ plant^{-1}}\right)\mathbf{X}\ \frac{\mathbf{10000}}{\mathbf{1000}}.$$

The harvest index (HI) was determined by expressing fresh storage root mass (kg $plant^{-1}$) as proportion of total biomass (kg $plant^{-1}$) (Fukuda et al., 2010). The DMC was determined by using the specific gravity method (Chávez et al., 2005; Fukuda et al., 2010; Kawano et al., 1987), as per the following formula:

$$\mathbf{DMC}\ (\%) = \left(\frac{\mathbf{WA}}{\mathbf{WA\text{-}WW}}\mathbf{X158.3}\right)\mathbf{-142}$$

Where WA and WW are weight measured in air and water, respectively.

The β-carotene (β-C) was estimated using the colour chart that was used in estimating β-C in sweet potatoes, as described by Burgos et al. (2009). The PPD was determined using the method developed by Wheatley et al. (1985) with some modification. The proximal and distal ends of cassava storage roots were removed immediately after harvesting. Proximal ends were exposed to the air and distal ends of the storage root were covered using food plastic wrappers. The room temperature ranged from 22-28^{o} C and the relative humidity was between 70-80%. The assessment was conducted seven days after harvest, using the score 1-10 to represent the discoloration, where 1 = 10%, 2 = 20%, 10 = 100% (Chávez et al., 2005; Wheatley et al., 1985). The two storage roots per genotype were cut into ten transversal slices, each 2 cm thick, and the mean score obtained from the 20 slices from the two selected storage roots.

6.2.5 Data analysis

Data collected from individual plant, which constitute a family, were averaged for statistical analysis. The analysis of data was done using SAS studio (University edition). The Griffing's (1956b) diallel Method 4, Model 1 for fixed effects was fitted, to estimate the GCA and SCA effects:

$$\boldsymbol{Yij = \mu + vij + bk + eijkl}$$

Where, *Yij* = observed value of the cross between parent i and j;
μ = overall mean; $vij =$ F1 hybrid effect, $vij = gi + gj + Sij$ where, *gi* = GCA of the parent i; *gj* = GCA of the parent j; *sij* = SCA of the cross between parents i and j; *bk* = effect of the kth block; and *eijkl* = experimental error.

The GCA and SCA effects were estimated according to Griffing's (1956b) Method 4, Model 1 using the DIALLEL-SAS05 program developed by Zhang et al. (2005). The significance of combining ability effects was determined using t-test at 0.05, 0.01 and 0.001 levels of probability. The GCA and SCA effects for each trait were determined from the percentage of families' sum of squares (SS) due to GCA and SCA (Tumuhimbise et al., 2014). The relative importance of the GCA and SCA effects for each trait was determined from the percentage of the families' sum of squares (SS) (Tumuhimbise, 2013; Were et al., 2012). The mid-parent (MP) and best parent (BP) heterosis was analysed, using the formula MPH $(\%) = \frac{(\text{F1-MP})}{\text{MP}}$ X 100, and BPH $(\%) = \frac{(\text{F1-BP})}{\text{BP}}$ X 100. The selection of the best clones for advancement was done by using the selection index (SI) proposed by Ceballos et al. (2004), with some

modifications. $\mathrm{SI} = (\mathrm{FRSY} * 5) + (\beta\mathrm{-Carotene} * 4) - (\mathrm{PPD} * 3) - (\mathrm{CBSD} - \mathrm{RN} * 2)$, and the variables were standardized, using the following formula: $\mathrm{Xi} = (\mathrm{Xi} - \mu)/\ \mathrm{St.\,Dev}$.

6.3 Results

6.3.1 Descriptive statistics of ten important selected traits of F1 cassava clones

The ten important cassava traits evaluated in this study, showed a considerable variation among the F1 clones of fifteen families generated through 6 x 6 half diallel mating design. The FSRY, β-C and TBM were highly skewed, while the CMD-S and HI were moderately skewed, explaining the genetic diversity among the generated F1 clones. The FSRY ranged from 2.0 to 44.2 t ha^{-1} with an average of 8.7 t ha^{-1}, while DMC ranged from 26.1 to 42.1%, with an average of 34.0%. The β-C ranged from 0.001 to 1.88 mg 100 g^{-1} with an average of 0.34 mg 100 g^{-1}, while the PPD evaluated showed a deterioration of 10 to 60.5% after one week (Table 6.2).

Figure 6.1: **New developed clones with high carotenoids and delayed PPD. A**: Flowers covered to avoid free cross pollination, **B**: new clone with high yield, **C**: Deep yellow of cassava pulp, **D and E**: Orange and or pink of cassava pulp, **F**: Orange fleshed cooked cassava

Table 6.2: Summary statistics of 10 traits measured in clonal F1 of 15 cassava families evaluated at two sites

Traits	Mean	SE	SD	Kurtosis	Skewness	Minimum	Maximum
CMD-S	1.49	0.05	0.44	1.21	0.98	1.00	3.10
CBSD-L	2.01	0.03	0.31	-0.30	-0.27	1.20	2.67
CBSD-S	2.18	0.05	0.46	0.04	-0.25	1.00	3.29
CBSD-RN	2.14	0.09	0.87	-1.51	0.14	1.00	3.50
FSRY	8.70	0.67	6.32	10.17	2.47	1.98	44.20
TB	2.66	0.11	1.02	1.15	1.09	1.07	6.45
HI	0.30	0.01	0.10	1.99	0.78	0.10	0.69
DMC	33.97	0.35	3.36	-0.09	-0.49	26.10	42.10
β-C	0.32	0.05	0.45	2.83	1.79	0.001	1.88
PPD	33.85	1.51	14.37	-0.91	0.26	10.00	60.55

SE: standard Error, SD: standard deviation, CMD-S: cassava mosaic disease severity, CBSD-L: cassava brown streak disease on leaves, CBSD-S: cassava brown streak disease on stem, CBSD-RN: cassava brown streak disease root necrosis, FSRY: fresh storage root yield, TB: total biomass, HI: harvest index, DMC: dry matter content, β-C: β-Carotene, PPD: physiological postharvest deterioration

6.3.2 Diallel analysis for cassava β-carotene, delayed postharvest physiological deterioration and farmers' preferred traits

The environment (E) significantly (p<0.001) influenced the expression of all traits, except CMD, DMC and PPD. The families exhibited significant differences for CBSD-S, FSRY and HI at p<0.05 and at p<0.001 for the remaining traits. The effects due to families were further partitioned into two components, namely, the effects due to GCA and SCA. The GCA was significant (p<0.05) for CMD and DMC, and significant (p<0.001) for β-C and PPD. The SCA was significant for β-C and PPD at p <0.001, CMD and DMC at p <0.01, and TB and CBSD-L at p <0.05.

The relative importance of additive and non-additive gene effects for the expression of the studied traits was partitioned into GCA and SCA effects, expressed as a percentage of the sum of squares. The variability of the trait expression among families indicated that the pulp traits (CBSD-RN, β-C and PPD), were highly influenced (over 50% of variability) by the GCA effects, while CMD, CBSD-L, CBSD-S, TB, FSRY, HI and DMC, leaves and yield traits were considerably influenced (over 50% of variability) by the SCA effects (Table 6.3).

Table 6.3: Combined analysis of variance for important traits of 15 families of cassava clones generated from 6 x 6 half-diallel

Source of variation	Mean squares					
	DF	CMD	CBSD-L	CBSD-S	CBSDN	TB
Environments (E)	1	0.06	1.20***	5.34***	56.64***	25.26***
Families	14	0.42***	0.16***	0.19*	0.21***	1.73***
E x Families	14	0.36***	0.18***	0.34***	0.36***	1.77***
GCA	5	0.37*	0.09	0.24	0.32	0.68
SCA	9	0.44**	0.18*	0.16	0.14	2.31*
Error	74	0.10	0.04	0.09	0.05	0.32
CV (%)		22.03	10.01	13.89	10.06	21.3
% SS due to GCA		31.6	22.6	46.3	55.8	14.1
% SS due to SCA		68.4	77.4	53.7	44.1	85.9

Table 6.3: Continued

Source of variation	Mean squares					
	DF	FSRY	HI	DMC	β-C	PPD
Environments (E)	1	705.31***	0.21***	0.64	0.35***	57.36
Families	14	51.27*	0.01*	26.08***	1.00***	912.69***
E. Families	14	58.28**	0.00	15.45*	0.13***	35.50
GCA	5	34.16	0.01	23.13*	2.31***	1564.96***
SCA	9	60.78	0.00	27.71**	0.27***	550.32***
Error	74	21.24	0.00	7.38	0.02	84.46
CV (%)		52.9	24.12	7.99	46.72	27.15
% SS due to GCA		23.8	42.1	31.7	89.5	61.2
% SS due to SCA		76.2	57.9	68.3	10.5	38.5

GCA: general combining ability, SCA: specific combining ability, CV: coefficient of variation, %SS: percentage sum of squares, CMD-S: cassava mosaic disease severity, CBSD-L: cassava brown streak disease on leaves, CBSD-S: cassava brown streak disease on stem, CBSD-RN: cassava brown streak disease root necrosis, FSRY: fresh storage root yield, TB: total biomass, HI: harvest index, DMC: dry matter content, β-C: β-Carotene, PPD: physiological postharvest deterioration

6.3.3 General combining ability effects for cassava β-carotene, delayed postharvest physiological deterioration and other farmers' preferred traits

The GCA effects of ten important selected traits of six cassava parents were analysed across two locations. Mavoka had a significant ($p<0.001$) positive GCA effects for β-C, an undesirable significant ($p<0.01$) negative GCA effects for DMC, and a desirable significant ($p<0.001$) negative GCA effects for PPD. Garukunsubire also presented a significant ($p<0.001$) positive GCA effects for β-C and a significant ($p<0.001$) negative GCA effects for PPD. The GCA effects for Mavoka and Garukunsubire indicates the ability of both parents to improve the level of β-C and delayed PPD. Gahene, Ndamirabana and Gitamisi showed a significant ($p<0.001$) negative GCA effects for β-C and a positive GCA effects for PPD, while Mushedile also had significant negative GCA effects for β-C (Table 6.4). In addition, Mavoka had the highest mean of β-C, followed by Garukunsubire.

Table 6.4: Means and general combining ability effects for important traits of 15 families of cassava clones generated from 6 x 6 half-diallel

Parents	CMD		CBSD-L		CBSD-S		CBSD-RN		FSRY(t ha^{-1})	
	$\overline{X}$	GCA	$\overline{X}$	GCA	$\overline{X}$	GCA	$\overline{X}$	GCA	$\overline{X}$	GCA
Mavoka	1	-0.10	4	0.05	3	0.06	3	0.16	17.3	1.41
Garukunsubire	1	0.02	4.5	0.00	4	-0.07	4.5	-0.16	12.6	0.72
Gahene	4.5	0.02	4	-0.02	4	0.00	4	-0.08	2.4	-0.85
Mushedile	3.5	0.22**	2.5	-0.10*	1	-0.14	1	-0.00	26.3	-1.81
Ndamirabana	1.5	-0.05	2	0.01	3	0.00	2	0.08	19.3	-0.20
Gitamisi	3	-0.11	3	0.07	2	0.14	3	0.00	9.13	0.73
Means	2.4		3.3		2.8		2.9		14.5	
SE	0.3		0.2		0.2		0.3		1.9	

Table 6.4: Continued

Parents	TB		HI		DMC (%)		βC (mg 100g^{-1})		PPD (%)	
	$\overline{X}$	GCA	$\overline{X}$	GCA	$\overline{X}$	GCA	$\overline{X}$	GCA	$\overline{X}$	GCA
Mavoka	5.5	0.15	0.31	0.00	26.0	-1.72**	1.32	12.27***	10.	-11.72***
Garukunsubire	5.5	0.15	0.23	0.01	29.4	0.23	0.03	6.59***	55.	-7.30***
Gahene	0.9	-0.24	0.27	0.00	33.1	0.68	0	-7.01***	50.	5.24**
Mushedile	5.6	-0.15	0.47	-0.04*	32.5	1.12*	0	-4.01***	55.	9.98***
Ndamirabana	5.2	-0.00	0.37	-0.00	31.3	-0.25	0	-4.01***	40.	1.27
Gitamisi	4.5	0.09	0.2	0.01	33.5	-0.06	0	-3.82***	50.	2.52
Means	***4.5***		***0.31***		***30.9***		***0.25***		***43.3***	
SE	***0.4***		***0.02***		***0.6***		***0.12***		***3.8***	

SE: standard error, $\overline{X}$;means, GCA: general combining ability, CMD-S: cassava mosaic disease severity, CBSD-L: cassava brown streak disease on leaves, CBSD-S: cassava brown streak disease on stem, CBSD-RN: cassava brown streak disease root necrosis, FSRY: fresh storage root yield, TB: total biomass, HI: harvest index, DMC: dry matter content, β-C: β-Carotene, PPD: physiological postharvest deterioration

6.3.4 Specific combining ability effects for cassava β-Carotene, delayed postharvest physiological deterioration and farmers' preferred traits

The mean performance and SCA effects were analysed for fifteen families grown over two locations. The family Garukunsubire x Gahene had a desirable significant ($p<0.05$) negative SCA effects for CMD, and no CMD symptoms. The families Mavoka x Mushedile and Gahene x Ndamirabana recorded the least CBSD-L (1.7) with a significant ($p<0.05$) negative SCA effects. The family Garukunsubire x Gahene had a positive SCA effects for FSRY, and the highest average FSRY (13.7 t ha^{-1}), while the family Mavoka x Mushedile had the lowest average FSRY (3.9 t ha^{-1}), with a significant ($p<0.05$) negative SCA effects (-4.40). The family Garukunsubire x Gahene had the highest average of TB per plant (3.63 kg) and a significant ($p<0.05$) positive SCA effects, while the family Garukunsubire x Ndamirabana showed the highest HI (0.34), with a significant positive SCA effects. The family Mavoka x Garukunsubire had the highest average of DMC (35.9%), with a high significant ($p<0.001$) positive SCA effects, while the family Mavoka x Ndamirabana recorded the lowest average of DMC (28.4%), with a significant ($p<0.001$) negative SCA effects (-3.62). The family of Mavoka x Garukunsubire had a significant ($p<0.001$) positive SCA effects for β-Carotene, and the highest average β-C. The family Mavoka x Ndamirabana had the least PPD (13.3%) recorded after one week of storage (Table 6.5).

Table 6.5: Means and specific combining ability effects for important traits of 15 families of cassava clones generated from 6x6 half-diallel

Families	CMD-S		CBSD-L		CBSD-S		CBSD-RN		FSRY (t ha^{-1})	
	$\overline{X}$	SCA	$\overline{X}$	SCA	$\overline{X}$	SCA	$\overline{X}$	SCA	$\overline{X}$	SCA
G1xG2	1.39	-0.01	1.98	-0.07	2.16	0	2.18	0.05	7.28	-3.55
G1xG3	1.46	0.05	2.18	0.14	2.14	-0.1	2.3	0.08	13.13	3.87
G1xG4	1.84	0.24*	1.73	-0.22*	2.12	0.02	2.13	-0.16	3.9	-4.40*
G1xG6	1.31	-0.02	2.08	0.01	2.28	0.02	2.4	0.02	11.19	1.27
G1xG7	1	-0.26*	2.27	0.13	2.44	0.05	2.3	0	13.66	2.8
G2xG3	1.26	-0.27*	2.12	0.13	2.27	0.16	2.01	0.12	6.36	-2.2
G2xG4	1.94	0.21	1.79	-0.1	1.86	-0.09	1.98	0.02	11.7	4.08*
G2xG6	1.44	-0.02	2	-0.01	2.13	0.01	1.82	-0.23	10.36	1.14
G2xG7	1.5	0.1	2.15	0.06	2.16	-0.08	2	0.03	10.68	0.52
G3xG4	1.56	-0.16	1.96	0.08	2.18	0.14	2.02	-0.02	5.94	-0.09
G3xG6	1.87	0.41**	1.77	-0.22*	1.9	-0.29*	1.99	-0.14	8.16	0.52
G3xG7	1.36	-0.02	1.9	-0.15	2.41	0.08	2.02	-0.03	6.48	-2.09
G4xG6	1.22	0.42***	2.17	-0.26**	2.16	-0.11	2.47	-0.25	6.03	0.65
G4xG7	1.72	0.13	1.94	-0.02	1.98	-0.19	2.05	-0.08	8.67	1.05
G6xG7	1.36	0.05	2.06	-0.03	2.46	0.13	2.31	0.08	6.93	-2.29
Means	***1.48***		***2.01***		***2.18***		***2.13***		***8.7***	***0.09***
SE	***0.046***		***0.032***		***0.048***		***0.092***		***0.666***	
P Value	***<.001***		***<.001***		***<.001***		***<.001***		***<.001***	

Table 6.5: Continued

Families	TB (kg plant^{-1})		HI		DMC (%)		β-C (mg 100g^{-1})		PPD (%)	
	$\overline{X}$	SCA	$\overline{X}$	SCA	$\overline{X}$	SCA	$\overline{X}$	SCA	$\overline{X}$	SCA
G1xG2	2.39	-0.57	0.29	-0.03	35.9	3.42***	1.47	5.49***	32.5	17.68***
G1xG3	3.2	0.63*	0.34	0.02	33.28	0.35	0.26	-3.97***	24	-3.36
G1xG4	1.62	-1.02*	0.23	-0.02	34.65	1.28	0.63	-0.36	35.83	3.72
G1xG6	3.04	0.23	0.33	0.02	28.36	-3.62***	0.46	-2.94**	13.33	-10.06***
G1xG7	3.63	0.72*	0.33	0	30.75	-1.42	0.79	1.78	16.66	-7.98**
G2xG3	2.25	-0.31	0.27	-0.04	34.04	-0.84	0.20	-1.48	24.16	-7.61**
G2xG4	3.29	0.63*	0.34	0.06*	33.26	-2.06*	0.32	-1.54	34.16	-2.35
G2xG6	2.91	0.1	0.33	0.01	33.36	-0.58	0.50	1.02	25	-2.81
G2xG7	3.05	0.14	0.32	-0.01	34.21	0.07	0.19	-3.49***	24.16	-4.9
G3xG4	2.41	0.16	0.23	-0.01	35.87	0.09	0.00	1.85	50	0.92
G3xG6	2.45	0.04	0.32	0.02	35.5	1.1	0.00	1.85	50.17	9.81***
G3xG7	1.98	-0.52	0.32	0	33.86	-0.71	0.00	1.74	41.85	0.23
G4xG6	2.57	-0.08	0.21	0.04	35.7	-0.86	0.00	-0.08	39.16	5.94*
G4xG7	2.73	0.14	0.29	0.02	34.85	-0.17	0.00	-0.03	50	3.64
G6xG7	2.26	-0.47	0.29	-0.02	35.88	2.24*	0.00	-0.01	46.66	9.01**
Means	***2.65***		***0.29***		***33.96***		***0.32***		***33.84***	***0.79***
SE	***0.108***		***0.01***		***0.35***		***0.049***		***1.514***	
P Value	***<.001***		***<.001***		***<.001***		***<.001***		***<.001***	

SE: standard error, $\overline{X}$:means, G1: Mavoka, G2: Garukunsubire, G3: Gahene, G4: Mushedile, G6: Ndamirabana, G7: Gitamisi, SCA: specific combining ability, CMD-S: cassava mosaic disease severity, CBSD-L: cassava brown streak disease on leaves, CBSD-S: cassava brown streak disease on stem, CBSD-RN: cassava brown streak disease root necrosis, FSRY: fresh storage root yield, TB: total biomass, HI: harvest index, DMC: dry matter content, β-C: β-Carotene, PPD: physiological postharvest deterioration

6.3.5 Estimates of mid-parent heterosis of selected traits of cassava clones across two locations

The family Mavoka x Garukunsubire expressed the highest positive heterosis for CMD, DMC and β-C. Out of fifteen families, only three families (Movaka x Mushedile, Mushedile x Ndamirabana and Mushedile x Gitamisi) had a desirable positive mid-parent heterosis for CBSD-S and CBSD-RN resistance. This indicates that Mushedile could be the better parent

for improving cassava resistance to CBSD. In terms of FRSY, the families Mavoka x Gahene, Garukunsubire x Gahene and Gahene x Gitamisi had the highest positive mid-parent heterosis, while the remaining families expressed a negative heterosis for FRSY (Table 6.6). The heterosis for β-C was positively higher for all families with parent Garukunsubire, indicating that it could be a good combiner to improve β-C. The mid-parent heterosis for PPD was positive for the families Garukunsubire x Gitamisi, Mavoka x Mushedile and Ndamiraba x Gitamisi, while most of families expressed the desirable negative heterosis (Table 6.6).

Table 6.6: Mid-parents heterosis for important cassava traits evaluated at clonal stage across two sites

Families	CMD		CBSD-S		CBSD-RN		FSRY (t ha^{-1})	
	Mean	MPH	Mean	MPH	Mean	MPH	Mean	MPH
G1xG2	1.39	39.33	2.16	-38	2.18	-41.65	7.28	-51.12
G1xG3	1.46	-46.89	2.14	-38.62	2.30	-34.22	13.13	33.71
G1xG4	1.84	-17.83	2.12	6.29	2.13	6.62	3.90	-82.05
G1xG6	1.31	4.89	2.28	-23.92	2.40	-3.61	11.19	-38.73
G1xG7	1.00	-50	2.44	-2.05	2.30	-23.27	13.66	3.56
G2xG3	1.26	-54.06	2.27	-43.05	2.01	-52.63	6.36	-14.89
G2xG4	1.94	-13.36	1.86	-25.36	1.98	-27.68	11.70	-39.75
G2xG6	1.44	15.56	2.13	-39.12	1.82	-43.88	10.36	-34.9
G2xG7	1.50	-25	2.16	-27.86	2.00	-33.02	10.68	-1.54
G3xG4	1.56	-60.89	2.18	-12.68	2.02	-19.01	5.94	-58.53
G3xG6	1.87	-37.39	1.90	-45.67	1.99	-33.45	8.16	-24.67
G3xG7	1.36	-63.56	2.41	-19.58	2.02	-42.03	6.48	12.43
G4xG6	1.22	-45.37	2.16	8.02	2.47	65.17	6.03	-73.52
G4xG7	1.72	-47.06	1.98	32.49	2.05	2.68	8.67	-50.99
G6xG7	1.36	-39.27	2.46	-1.43	2.31	-7.51	6.93	-51.18
G1	1		3		3		17.3	
G2	1		4		4.5		12.6	
G3	4.5		4		4		2.4	
G4	3.5		1		1		26.3	
G5	1.5		3		2		19.3	
G6	3		2		3		9.13	

MPH: mid-parent heterosis, G1: Mavoka, G2: Garukunsubire, G3: Gahene, G4: Mushedile, G6: Ndamirabana, G7: Gitamisi, SCA: specific combining ability, CMD-S: cassava mosaic disease severity, CBSD-S: cassava brown streak disease on stem, CBSD-RN: cassava brown streak disease root necrosis, FSRY: fresh storage root yield,

Table 6.6: Continued

Families	HI		DMC (%)		β-C (mg 100 g^{-1})		PPD (%)	
	Mean	MPH	Mean	MPH	Mean	MPH	Mean	MPH
G1xG2	0.29	8.14	35.90	29.64	1.47	117.8	32.50	0
G1xG3	0.34	18.22	33.28	12.72	0.26	-60.6	24.00	-20
G1xG4	0.23	-38.96	34.65	18.54	0.63	-4.6	35.83	10.26
G1xG6	0.33	-1.79	28.36	-0.86	0.46	-30.4	13.33	-46.67
G1xG7	0.33	31.53	30.75	3.46	0.79	19.6	16.66	-44.44
G2xG3	0.27	11.73	34.04	8.94	0.20	1190.3	24.16	-53.97
G2xG4	0.34	-1.97	33.26	7.44	0.32	1964.5	34.16	-37.88
G2xG6	0.33	11.52	33.36	10	0.50	3125.8	25.00	-47.37
G2xG7	0.32	49.76	34.21	8.79	0.19	1125.8	24.16	-53.97
G3xG4	0.23	-34.86	35.87	9.4	0.00	0.0	50.00	-4.76
G3xG6	0.32	3.36	35.50	10.38	0.00	0.0	50.17	11.5
G3xG7	0.32	38.85	33.86	1.78	0.00	0.0	41.85	-16.3
G4xG6	0.21	-48.53	35.70	12.02	0.00	0.0	39.16	-17.54
G4xG7	0.29	-11.91	34.85	5.67	0.00	0.0	50.00	-4.76
G6xG7	0.29	3.49	35.88	10.91	0.00	0.0	46.66	3.7
G1	0.31		26		1.32		10	
G2	0.23		29.4		0.03		55	
G3	0.27		33.1		0		50	
G4	0.47		32.5		0		55	
G6	0.37		31.3		0		40	
G7	0.2		33.5		0		50	

MPH: mid-parent heterosis, G1: Mavoka, G2: Garukunsubire, G3: Gahene, G4: Mushedile, G6: Ndamirabana, G7: Gitamisi, SCA: specific combining ability, HI: harvest index, DMC: dry matter content, β-C: β-Carotene, PPD: physiological postharvest deterioration

6.3.6 Selection of F1 clones based on farmer preferred traits

The selection of genotypes was performed using a selection index that was based on the key four traits (FRSY, CBSD-RN, β-carotene and PPD) across two locations. The clone 183 from the family Garukunsubire x Gitamisi had the highest FRSY, followed by clone 115 generated from the family Mavoka x Gahene with a yield of 45.6 t ha^{-1} and 44.0 t ha^{-1}, respectively. In terms of FSRY, fourteen clones out of the top twenty selected clones performed beyond the better parent (Table 6.7). The highest β-carotene (6.12 mg 100 g^{-1}) was observed from two clones, 670 and 93, generated from families Mavoka x Garukunsubire and Mavoka x Gitamisi, respectively. The same clones expressed the lowest postharvest physiological deterioration (5%) after one week of storage at room temperature. Unfortunately, several of the top twenty clones had storage root necrosis due to cassava brown streak disease.

Table 6.7: Mean performance, best parent heterosis and ranking based on fresh storage root yield, cassava brown streak disease, β-carotene and delayed postharvest physiological deterioration of top best 20 clones

Clones	Pedigree	FSRY		CBSD-RN		β-Carotene		PPD		SI
		$\bar{X}$	BPH	$\bar{X}$	BPH	$\bar{X}$	BPH	$\bar{X}$	BPH	
426	G1xG7	41.7	141.0	4.0	33.3	1.65	25.0	8	-73.3	185.6
96	G1xG7	40.5	134.1	3.0	0.0	1.65	25.0	8	-73.3	183.6
670	G1xG2	31.4	81.5	2.0	-33.3	6.12	363.6	5	-83.3	161.5
115	G1xG3	44.0	154.3	1.0	-66.7	0.03	-97.7	30	0.0	160.3
401	G1xG6	30.3	75.1	2.0	-33.3	1.32	0.0	10	-66.7	131.6
183	G2xG7	45.6	136.3	3.0	0.0	0.00	-99.3	60	9.1	100.2
216	G1xG7	23.6	36.4	2.0	-33.3	1.32	0.0	10	-66.7	98.1
93	G1xG7	23.8	37.6	1.0	-66.7	0.15	-88.6	15	-50.0	89.6
272	G1xG7	25.5	47.4	1.0	-66.7	0.15	-88.6	20	-33.3	88.1
78	G1xG3	19.0	9.8	1.0	-66.7	1.32	0.0	10	-66.7	79.1
52	G1xG3	17.9	3.5	1.0	-66.7	1.65	25.0	8	-73.3	78.6
52	G1xG3	19.5	12.7	2.0	-33.3	1.32	0.0	10	-66.7	77.6
79	G1xG6	26.8	54.9	4.0	33.3	0.03	-97.7	30	0.0	62.3
79	G1xG6	26.8	54.9	4.0	33.3	0.03	-97.7	30	0.0	62.3
50	G1xG3	15.5	-10.4	2.0	-33.3	1.32	0.0	10	-66.7	57.6
423	G1xG3	15.5	-10.4	1.0	-66.7	0.15	-88.6	15	-50.0	48.1
93	G1xG7	9.5	-45.1	4.0	33.3	6.12	363.6	5	-83.3	44.0
25	G1xG2	10.3	-40.5	1.0	-66.7	1.65	25.0	8	-73.3	40.6
50	G1xG3	13.9	-19.7	1.0	-66.7	0.15	-88.6	15	-50.0	40.1
288	G1xG2	13.0	-24.9	1.0	-66.7	0.15	-88.6	15	-50.0	35.6

BPH: best parent heterosis, SI: selection index, G1: Mavoka, G2: Garukunsubire, G3: Gahene, G4: Mushedile, G6: Ndamirabana, G7: Gitamisi, CBSD-RN: cassava brown streak, FSRY: fresh storage root yield, PPD: postharvest physiological deterioration

6.4 Discussion and conclusions

This study was conducted on 450 clones of fifteen families generated from 6x6 half-diallel mating design. The F1 clones exhibited considerable phenotypic variability among families and progenies for the evaluated traits, such as FSRY, β-C, DMC, TBM, CMD-S, HI, CBSD-S, CBSD-RN and PPD. Some F1 clones produced higher FRSY than the best parents; the lowest FRSY was 1.98 t ha^{-1}, while the highest was 44.20 t ha^{-1}. Similarly, some F1 progenies had higher amounts of β-C and higher PPD tolerance than their parents, which could be attributed to the transgressive segregation and heterosis, which are desirable for the improvement of most cassava traits. Similar findings reported by Tumuhimbise (2013) and Njenga et al. (2014) indicated that some cassava progenies outperformed their parents in terms of various traits, including FRSY and pulp/flesh colour (an indication of β-C content).

The environments did not exhibit a significant influence on the expression of β-C and PPD, indicating that the expression of such traits is mostly controlled by the plant genes. Tumuhimbise et al. (2015) reported a low environmental effect on PPD expression, while the low environmental effects on β-C agrees with the findings of many authors (Akinwale et al., 2011; Rodriguez-Amaya, 2010; Ssemakula and Dixon, 2007), who indicated that the accumulation of β-C is predominately governed by genetic effect, with a low GxE interaction.

The families' mean squares exhibited a significant difference for all traits, indicating significant variation among families. The ExFamilies interaction effects were significant for most traits, except HI and PPD, indicating that they are unstable, and that selection for these two traits cannot be performed solely at one location. The remaining traits were stable and could be selected at each location. The GxE interaction effects indicated that most traits had a stable performance across two locations. These findings agree with many authors (Baiyeri et al., 2008; Njoku et al., 2015; Ntawuruhunga and Dixon, 2010; Tumuhimbise et al., 2015; Were et al., 2012), who reported significant GxE interaction effects for most agronomic and morphological cassava traits. The number of sites was low because of the small number of stakes available and further studies on GxE interaction are needed

The GCA and SCA effects for both β-C and PPD were highly significant, indicating the role of additive and non-additive gene action in controlling such traits. The relative importance of additive and non-additive gene effects revealed that the pulp traits (CBSD-RN, β-C and PPD) were highly influenced (over 50% of variability) by GCA effects, indicating that such traits are predominantly controlled by additive gene action. A similar finding was reported by Tumuhimbise (2013) and Kulembeka et al. (2012), who indicated that CBSD-RN severity and PPD are predominantly controlled by additive gene action. The β-C was controlled by additive gene action, which is desirable as the trait that can be improved through recurrent selection. This is supported by Njenga et al. (2014) who reported that the pulp colour of the cassava storage root is positively controlled by additive gene action. Ceballos et al. (2013) and Nduwumuremyi et al. (2016a) reported that carotene can be selected through recurrent selection in the cassava breeding scheme. The GCA results indicated that the pulp traits are highly heritable and should react positively to selection. This agrees with Perkes et al. (2013), who reported that the traits with a predominance of additive gene action are highly heritable and react positively to selection.

The viral diseases and yield traits (CMD, CBSD-L, CBSD-S, TB, FSRY, HI and DMC) were considerably influenced (over 50% of variability) by SCA effects, which showed a predominance of non-additive gene action in controlling these traits. Several authors (Chipeta et al., 2015; Kulembeka et al., 2012; Tumuhimbise, 2013; Were et al., 2012) reported on the non-additive gene action for FRSY and most of the cassava traits. The non-additive gene action found for CMD disagreed with Tumuhimbise (2013) and Parkes et al. (2013), who reported that CMD resistance is predominantly controlled by additive gene effects.

The GCA effects for parents indicated that genotype Mavoka had a significant desirable positive GCA for β-C and FRSY, an undesirable significant negative GCA for DMC, and a desirable significant negative GCA for PPD. The genotype Garukunsubire presented similar

attributes, indicating the ability of both parents to improve the level of β-Carotene and delayed PPD, when combined in a hybridization scheme. The improvement of β-C content in the cassava population using Mavoka as progenitor, could be used to concurrently improve yield and delayed PPD, but could lead to a reduction in the dry matter content. The findings on the negative correlation between carotenoids and dry matter was reported by many authors (Akinwale et al., 2010; Ceballos et al., 2012; Esuma et al., 2012; Nduwumuremyi et al., 2016a; Vimala et al., 2009), which could negatively affect the farmers' adoption. The antioxidant properties of carotenoids could delay the PPD by protecting the wounded part of the storage root against reactive oxygen, as reported by many authors (Azqueta and Collins, 2012; Edge et al., 1997; Giuliano, 2014; Nduwumuremyi et al., 2016a; Priya and Siva, 2014; Xu et al., 2013; Zidenga et al., 2012), and could promote the adoption of improved carotenoids cassava clones.

In terms of CMD, the clones from the families Garukunsubire x Gahene and Garukunsubire x Mushedile had the desirable high negative SCA, indicating that Garukunsubire could be a good combiner for CMD resistance. The individuals from the family Mushedile x Ndamirabaha, followed by Mavoka x Mushedile and Garukunsubire x Gitamisi, had a desirable high negative SCA (-0.26, -0.22, respectively) for CBSD-L. The family Mavoka x Garukunsubire had the highest average DMC (35.9%) and a desirable significant positive SCA effects while the family Mavoka x Ndamirabana recorded the lowest average of DMC (28.3%), and an undesirable significant negative SCA effects (-3.62). The family Mavoka x Garukunsubire had the highest average β-Carotene, with a desirable positive SCA effects, while the family Mavoka x Ndamirabana had the lowest PPD (13.3%) after one week of storage and a desirable negative SCA effect (-10.06).

The progenies from family Mavoka x Garukunsubire expressed the highest positive heterosis for CMD, DMC and β-C. The high positive heterosis for DMC in this family is an interesting scenario, which could be linked to transgressive segregation, because one of the parents was a bad combiner for DMC. The progenies from three families (Movaka x Mushedile, Mushedile x Ndamirabana and Mushedile x Gitamisi) had a desirable positive mid-parent heterosis for CBSD-S and CBSD-RN resistance, indicating that Mushedile could be used for improving cassava resistance to CBSD. In terms of FRSY, the families Mavoka x Gahene, Garukunsubire x Gahene and Gahene x Gitamisi had the highest positive mid-parent heterosis, indicating that Gahene could be a good combiner for FRSY. The mid-parent heterosis for PPD was positive for the families Garukunsubire x Gitamisi, Mavoka x Mushedile and Ndamiraba x Gitamisi, while most of the families expressed negative heterosis. The heterosis for FRSY, DMC, CMD, CBSD, β-C and PPD indicates the genetic diversity of the parents used.

In conclusion, good progress was made to improve β-C, FRSY, delayed PPD and other important cassava traits using the available wide genetic diversity of the cassava. This study gave an insight into the feasibility of improvement of the above traits, and provides the foundation for a cassava breeding program for Rwanda. The selection of the genotypes for advancement was performed using a selection index. Unfortunately, most of the selected top twenty clones had storage root necrosis, due to cassava brown streak disease. Therefore, more investigation is needed to identify new sources of resistance to CBSD and the development of a protocol for the rapid multiplication of cuttings to facilitate the dissemination of newly- developed cassava hybrids.

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CHAPTER VII

General overview of the research findings and implications for cassava breeding

7.1 Introduction

Cassava is among the important staple foods and plays a key role as a food security and an income-generating crop for most smallholder farmers in tropical and subtropical developing countries (Ceballos et al., 2004; El-Sharkawy, 2004; Tumuhimbise, 2013). It is a multipurpose crop and a cheap source of starch in Rwanda. However, there are many factors that impact on its production, consumption and marketability. The main constraint in cassava production in the country is the lack of good genotypes with high yield, that are resistant to pests and diseases and that have minimum postharvest losses. The improvement of cassava through breeding approaches is the key for addressing the most challenging constraint factors in the development of cassava in the country.

The main goal of this study was to contribute to the increase of cassava productivity through participatory cassava breeding for high-yielding cassava genotypes, with improved carotenoids content and delayed postharvest physiological deterioration (PPD) for Rwanda. This was achieved through the following activities:

1. A review of the existing knowledge, principles and concepts for guiding the methodological development of improved carotenoids cassava with a delayed physiological postharvest deterioration;
2. A participatory appraisal of the preferred traits, production constraints and postharvest challenges of cassava farmers;
3. An evaluation of cassava genetic variability for total carotenoids and the farmers' preferred traits;
4. An analysis of genotype x environment (GxE) effects on total carotenoids content and farmers' preferred traits; and
5. The development of F1 clones and the determination of the genetic inheritance of cassava families for high yield, improved carotenoids and delayed PPD.

7.2 Summary of the findings

Review of the existing knowledge and feasibility of improving carotenoids content and delaying physiological postharvest deterioration

- PPD is induced by wounds, when detaching storage roots from the mother plant during harvesting;
- It is accelerated by the reactive oxygen species (ROS), such as oxygen ion (O^{2-}) and peroxide (O_2^{2-});
- Carotenoids content and its antioxidant properties help to extend the shelf-life of cassava storage roots;
- The two types of phytoene synthase (PSY) enzymes (PSY1 and PSY2) are key regulators of carotenoids accumulation in cassava;
- Carotenoids is a highly heritable trait, which provides hope that conventional breeding through recurrent selection can be successful in improving the carotenoids content of cassava; and
- Consequently, it can effectively extend the shelf-life of fresh cassava storage roots in developing countries.

Participatory appraisal of farmer preferred traits, production constraints and postharvest challenges for cassava farmers

- Cassava is grown on 0.29 ha, out the total average land possession per household of 0.69 ha in the study area;
- The majority of cassava farmers (59.1%) practice intercropping;
- The average yield was 21.8 t ha^{-1};
- The constraints, per order of importance are: lack of clean cuttings, viral diseases, late bulking cultivars, drought, limited knowledge, weathered soils, insufficient fertilizers, land shortage, limited information, lack of market and effective storage techniques;
- The losses due to PPD were estimated at 11.9% of the total production per year;
- A piecemeal harvesting and the underground storage of roots were the main practices used to tackle the effects of PPD;

- A change in colour and taste, rotting, difficulty to remove skin and an increase in fibres in the flesh were the methods used by farmers to assess PPD; and
- Farmers' preferences influenced the adoption of new cassava cultivars.

Cassava genetic variability for total carotenoids and farmers' preferred traits

- A high genetic variability (61.0%) exists among the 30 genotypes collected across the country;
- The 98.2% of total carotene (TC) variation was explained by genotypes and only 1.8% were due to an unknown origin;
- Total carotene had a high heritability (H^2) estimates of 99.2% and an expected genetic advance (GA %) of 159.6 %;
- The high H^2 estimate (%) and GA (%) for TC indicated that conventional breeding could improve carotenoids in cassava, using simple recurrent selection; and
- The PPD was negatively correlated with TC and dry matter content (DMC), indicating that the high TC and low DMC cultivars could have a delayed PPD.

GxE effects on total carotenoids content and farmers' preferred traits

- The TC, PPD and viral diseases traits were significantly affected by the environment;
- The % variation due to genotype for TC was higher (96%) than the variation due to environment (1.7 %) and GxE interaction (2.4 %), indicating less interaction effect of environment on TC accumulation; and
- The Mavoka cultivar was generally adapted to all locations, and had a higher carotenoids content with delayed onset of PPD than other genotypes. Thus, it could be a good genetic source for improving the carotenoids content and extending the shelf-life in cassava.

Diallel analysis and genetic inheritance of total carotenoids and delayed postharvest physiological deterioration

- The fifteen F1 families exhibited a significant variation between the genotypes and families, indicating the significant genetic diversity essential for crop improvement through conventional breeding;

- The general combining ability (GCA) effects was significant ($p<0.01$) for all traits, except for cassava brown streak disease on leaves (CBSD-L), while specific combining ability (SCA) effects were significantly different ($p<0.01$) for all evaluated traits (height, CBSD-L: cassava brown streak disease on leaves, CBSD-S: cassava brown streak disease on stem, CBSD-RN: cassava brown streak disease root necrosis, SRN: storage root number, SRL: storage root length, SRG: storage root girth, FSRY: fresh storage root yield, HI: harvest index, DMC: dry matter content, pulp colour, and PPD: physiological postharvest deterioration);
- The significant GCA effects indicated the possibility of improving cassava through recurrent selection for most of evaluated traits. Based on the significance and direction of GCA effects, the parents G2 and G7 were the best general combiners for improved fresh storage root yield, while the parents G1 and G2 were the best general combiners for improved carotenoids (yellow/orange pulp colour) and delayed physiological postharvest deterioration;
- The significant GCA and SCA effects for most traits indicated the role of both additive and non-additive gene action in expressing most of the cassava traits;
- The highest GCA/ SCA ratio and % sum of square (SS) due to GCA were recorded for CBSD-RN, SRN, FSRY, HI, pulp colour and PPD, indicating that these traits were primarily controlled by additive gene action; and
- The first three principal components (PCs) were most important and explained 71.2% of total variation among families for all traits, which indicated the possible success of early selection for all traits.

Combining ability effects and heterosis for cassava β-Carotene and delayed postharvest physiological deterioration and farmers' preferred traits at F1 clonal evaluation

- The F1 clones exhibited considerable phenotypic variability among families and offspring for the evaluated traits, such as FSRY, β-C, DMC, TBM, CMD-S, HI, CBSD-S, CBSD-RN and PPD. Some F1 clones produced higher FRSY, β-C and PPD tolerance than their parents, and this could be attributed to the transgressive segregation and heterosis, which are desirable for the improvement of most cassava traits;
- The environments did not exhibit a significant influence on the expression of β-C and PPD, indicating that the expression of such traits is mostly genetically controlled;
- The environments x families' interaction effects were significant for most traits, except HI and PPD, indicating that they are unstable, and the selection for these two traits

cannot be performed solely at each location. The remaining traits were stable and could be selected at each location;

- The GCA and SCA effects for both β-C and PPD were highly significant, indicating the role of additive and non-additive gene action in controlling such traits;
- The GCA effects for parents showed that the genotype Mavoka had a high positive GCA effects for β-C and FRSY, and a high negative GCA effects for PPD and DMC, indicating that it is the best combiner in terms of FRSY, β-C and delayed PPD and a poor combiner for DMC, as it can reduce the dry matter content. This implies that improving β-C content in the cassava population, using Mavoka as a progenitor, could concurrently improve the yield and delay PPD, but it would reduce the dry matter content;
- The individuals from the family Mavoka x Garukunsubire expressed the highest positive heterosis for CMD, DMC and β-C. In terms of FRSY, the families Mavoka x Gahene, Garukunsubire x Gahene and Gahene x Gitamisi had the highest positive mid-parent heterosis, indicating that Gahene could be a good combiner for improving FRSY. The mid-parent heterosis for a delayed PPD was positive for the families Garukunsubire x Gitamisi, Mavoka x Mushedile and Ndamiraba x Gitamisi; and
- This study gave a detailed insight into the opportunities for developing improved cassava cultivars for Rwanda. A selection index was used to identify the most promising new clones (Ceballos et al., 2013). Unfortunately, several of the selected top twenty clones had storage root necrosis, due to cassava brown streak disease.

7.3 Implication of the findings and further research

The present study gave insight into the feasibility improvement of the cassava population, and provided the foundation for a cassava breeding scheme in Rwanda. It generated improved carotenoids clones with a delayed postharvest physiological deterioration (PPD) and high yield. The study to introgress high carotenoids content into cassava indicated that it will be possible to concurrently improve carotenoids and dry matter, while the genetic studies revealed the concurrent improvement of yield, β-carotene and delayed PPD. As several of the selected top twenty clones had storage root necrosis, due to cassava brown streak disease, there is a need to screen for CBSD and CMD resistance in subsequent selection stages (multi-location trials, on-farm and demonstration trials of selected promising clones), and to initiate breeding for CBSD and CMD resistance in the country. In addition, there is a need to develop a rapid multiplication protocol for disease-free cassava stakes. The involvement of cassava stakeholders (extension service, farmers, cooperatives, processors, traders, etc) will ensure the quick and sustainable adoption of improved cassava varieties.

7.4 References

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