EVALUATING SEED QUALITY AND PERFORMANCE OF LOW AND HIGH PHYTIC ACID MAIZE (*Zea mays* L.) UNDER VARYING PHOSPHORUS RATES AND WATER REGIMES IN DRYLAND CONDITIONS

by

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DECLARATION

- **Mohammed Abdalla Elgorashi Bakhite,** declares that;
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GENERAL ABSTRACT

Maize (*Zea mays*) being the staple crop for many communities in Sub-Saharan Africa and also used for animal feeding, a considerable effort has been made to improve quality and yield. In recent times plant breeders have focused on reducing phytic acid (PA) on maize seeds to improve grain nutritional quality. Although studies of low phytic acid genes have been reported in temperate maize, the current research was based on the tropical genetic background which was screened for low phytic acid. This is the first report for applied breeding of the trait in the tropical maize. Little is known on the agronomy and responses of tropical maize specifically to water stress and its response to phosphorus application. No studies provided on the seed quality performance and response of low phytic acid maize to water stress and phosphorus application. Reducing Phytic acid of tropical maize could have negative effects on seed quality and yield. The primary objective of this study was to compare the performance of low phytic acid (LPA) maize seeds of tropical origin with three other varieties i.e. high phytic acid (HPA) of tropical origin, SC701 and LS8520 based on seed quality and water stress.

The study consisted of two maize synthetic populations differing in phytic acid (PA) content (from the African Center of Crop Improvement (ACCI)); namely, LPA and HPA synthetic populations. Both the LPA and HPA synthetic populations were derived from a tropical second generation (F2) population and were selected based on their phytic acid (PA) content. They were produced at the Ukulinga Research Farm, University of KwaZulu-Natal (29°40'05.7"S 30°24'20.9"E), in Pietermaritzburg, South Africa. These two maize synthetic populations were compared with two

commercial maize varieties (from McDonald Seeds), white maize (SC701) and yellow maize LS 8520 R (484) which in this study was coded LS8520. All seeds used in this study were produced under identical production conditions and in the same growing season, thereby ensuring that the seeds were of the same physiological age.

The first experiment investigated the characterisation of LPA maize varieties for seed germination and vigour. Seed quality was evaluated using the standard germination test and accelerate aging test together with electric conductivity test (EC). The second experiment was conducted as a pot trial to investigate the effect of exogenous phosphorus application on seed quality and yield of low phytic acid maize varieties. In third experiment, a field study over two seasons (2015/2016 and 2016/2017) was conducted at Ukulinga Research Farm in Pietermaritzburg, under dryland conditions. The objective of the experiment was to evaluate the newly produced ACCI's two genetically synthetic maize populations of LPA and HPA under dryland field condition and compare them with the commercial white and yellow tropical maize varieties their germination, growth, yield and yield components. Lastly, an experiment under controlled conditions on photosynthetic efficiency and yield responses of LPA and HPA maize tropical lines to deficit irrigation. The study was carried out under controlled environment conditions. The objective was to evaluate the photosynthetic efficiency of low phytic acid (LPA) and high phytic acid (HPA) tropical maize varieties grown under water-stressed conditions.

The results of the first experiment indicated that the performance of LPA varieties was comparable to those of commercially produced varieties. This study suggests that the combination of LPA lines

of tropical origin used in this study was satisfactory to meet the minimum seed quality parameters particularly seed germination and vigour. The results for phosphorus application showed that the application of phosphorus improved the growth, flowering and yield of LPA and other varieties as well. The mean germination time (MGT), germination vigour index (GVI), electrical conductivity (EC) ($\mu S\ g^{-1}$), root length, shoot length and the root-shoot ratio of the harvested seeds after phosphorus application were also improved. When the LPA and HPA tropical maize exposed to dryland environments the results revealed that the SC701 variety outperformed the other three varietiess in growth and yield. It was concluded that the LPA maize performed lower under field conditions. In the water stress trial, results showed that LPA maize varieties recorded higher values of stomatal conductance (*gs*) and transpiration rate (*T*) compared to HPA and SC701 for both normal and water stress conditions. HPA showed a significantly ($P < 0.001$) higher value of photosynthetic rate (*A*) than LPA and SC701 for all the water stress treatments. HPA and SC701 were comparable in most of chlorophyll fluorescence parameters. With regard to yield, HPA showed increased performance in terms of overall yield and seed weight, and this suggests that HPA varieties are less sensitive to water stress than LPA maize varieties. The results from this study have proven that LPA maize varieties are sensitive to limited conditions and further research under a wide range of environmental conditions is required.

Overall, the results indicated that the seed germination of LPA was comparable to other varieties but the yield remains low. There is a need to conduct more experiments to demonstrate the performance of LPA under field conditions in response to phosphorus application and water stress. These differences between the traits could help breed programs. The selection for LPA and HPA

tropical maize should be based on their physiological performance to be planted in temperate zones to grant higher yield performance.

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DEDICATION

This thesis is dedicated to everyone who keeps advising me to learn and focus forward

To my mother, thank you for your endless prayers

To the sole of my father

To the University of Khartoum

To the Ministry of Higher Education and Scientific Research in Sudan

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LIST OF ABBREVIATIONS AND SYMBOLS

A	Carbon assimilation rate
AA	Accelerated aging
ACCI	African Centre for Crop Improvement
Ci	Intercellular CO2 concentration
Ci/Ca	The ratio of intercellular and atmospheric CO2 and chlorophyll fluorescence parameter
EC	Electrical conductivity
ETR	Electron transport rate
Fv/Fm	Maximum quantum yield of PS II
Gs	Stomatal conductance
GVI	Germination velocity index
Ha	Hectare
HPA	High phytic acid maize
LPA	Low phytic acid maize
Mg	Milligram
MGT	Mean germination time
N	Nitrogen

P	Phosphorus
PA	Phytic acid
PhiPS2	Effective quantum yield of photochemical energy conversion in PSII
qN	Non-photochemical quenching
qP	Photochemical quenching
SA	South Africa
SSA	Sub-Saharan Africa
T	Transpiration rate
T	Tons
TTA	Time (days) to anthesis
TTS	Time (days) to silking
TTT	Time (days) to tasseling

CHAPTER 1

INTRODUCTION

Global food security is a fundamental challenge to the world`s current and future population. Currently, around 840 million people in the world are under-nourished. Due to an increasing global population and changes in food consumption patterns, it is expected that crop production needs to double by 2050 (Bodin et al., 2016). Akumaga et al. (2017) noted that during the past half-century, food productivity in Sub-Saharan Africa (SSA) has lagged behind the rest of the world while population growth has outpaced the rest of the world.

It is well established that the majority of the people in the developing countries depend mainly on cereal grains as their staple food due to limited income and the high prices of animal foods (Notenbaert et al., 2013). Maize is an important staple food crop in Sub-Saharan Africa SSA, covering about 20% of the calorie intake and 13% of the total cultivated land (Folberth et al., 2013). It accounts for 40% of the cereal production in SSA, where more than 80% is used as food (Ekpa et al., 2019). Maize yields in SSA are at the lower end of the global range of yields for decades due to the combination of factors including lower fertilizer application rate (Van Ittersum et al., 2016). It is an important crop for the sustenance and staple crop of many households in SSA where it is consumed as pap in countries such as South Africa, Mozambique and Zimbabwe. Ekpa et al. (2019) estimated that in SSA, consumption of the grain is in the range of 450 g/person/day. Additionally, maize is used as a weaning food for infants as well as for special ceremonies, caring for the sick, aged and pregnant women.

Although maize is considered an important crop, it is also considered as an antinutritional crop because of phytic acid (PA). Phytic acid (myo-inositol 1, 2, 3, 4/5, 6-hexakis [dihydrogen-phosphate]) is a common constituent of seeds (Lehrfeld, 1994), with a significant nutritional role as the principal storage form of phosphorus and metal cations in seeds (Raboy, 2001). It precipitates in the form of phytate salts, binding important mineral cations such as iron, zinc, potassium, calcium, and magnesium (Azeke et al., 2011). Studies have revealed that reducing the PA concentration of staple cereal grains such as maize may contribute to enhancing Zn nutrition of populations consuming these staple foods (Lönnerdal et al., 2011). Egli et al., (2004) and Tamilkumar et al. (2014) mentioned that PA chelates metal ions, especially zinc, iron, and calcium. It complexes metal ions in the gastrointestinal tract that cannot be digested or absorbed in humans because of the absence of intestinal phytase enzymes (Gibson et al., 2010). Phytic acid also complexes endogenously secreted minerals such as zinc and calcium (Guéguen and Pointillart, 2000) and making them unavailable for reabsorption into the body (Gibson et al., 2010). In addition, PA, due to its ability to chelate metal cations and, therefore, to reduce their bioavailability in the digestive apparatus, has long been regarded as an anti-nutrient for monogastric animals. (Vashishth et al., 2017).

Plant breeders in recent times have focused on maize improvement programmes aimed at improving nutritional quality by reducing PA concentration (Ekpa et al., 2018). Phytic acid is a major storage form of phosphate in maize seeds (Raboy, 2007) and it accounts for up to 80% of total phosphorous in seeds (Julia et al., 2018). Generally, 90% of PA is localized in the endosperm and only 10% in the embryo (Dost and Tokul, 2006). PA plays an important role during the early phases of the germination process and a reduction in phytic acid concentration to very low levels may compromise seed quality by affecting seed vigour during germination. This is because, phytic acid undergoes a digestion process and releases phosphorus and cations essential for seedling growth during germination (Azeke et al., 2011; and Bohn et al., 2008). Some researchers have pointed out that there is a limitation of studies that have been published that evaluates genetic variation in phytic acid concentrations and the performance of the diverse inbred lines under various environmental conditions (Queiroz et al., 2011). So therefore, there is a need to evaluate the performance a range of inbred lines with varying concentration of phytic acid for their performance under field condition particularly in arid and semi-arid conditions where the majority of the maize consuming population in SSA is residing.

Seeds utilize PA as a source of inorganic phosphate during germination and thus tend to increase palatability and nutritional value (Azeke et al., 2011). Seed phosphorus (P) content plays a key role in improving seed physiological aspects (Lott et al., 2017) and seedlings metabolism (Mehra et al., 2018). Seed phosphorus reserves are essential for seed germination and seedling development (Lott et al., 2017). Furthermore, phosphorus deficiency in soils is a major problem in cropping systems. Therefore, lowering PA (source of P) would reduce the phosphorus content in the seeds and that might affect seed germination, early seedling growth and development and as results yield (Mustafa et al., 2017).

Besides the antinutritional problem of maize with high PA, SSA faces challenges of water stress. Maize has high water requirements and suffers high yield losses and crop failures under water stress (Basu et al., 2016). Water stress slows crop growth and development resulting in reduced yields (Oury et al., 2016). Salazar et al. (2015) reported that maize have shown that drought stress induces morphological, physiological and biochemical changes. Drought stress also induces changes in photosynthesis, plant height, dry matter production, leaf area and grain yield (Song et al., 2018).

In trying the overcome the problem of food and nutritional security in SSA, researchers have initiated maize improvement programmes aimed at improving maize nutrition by reducing phytic acid concentration (Ekpa et al., 2018). However, these studies have only focused on maize of temperate origin (Raboy et al., 2000; Pilu et al., 2003) with little or no effort that has been made on maize of tropical origin where the problem of malnutrition and drought stress persist. Recently, the African Center for Crop Improvement (ACCI) at the University of KwaZulu-Natal has released low PA synthetic population of tropical origin. However, these varieties have not been tested for their agronomic performance, one aspect of it being seed quality. Maize improvement programs aimed at developing low phytic acid maize varieties to improve grain nutritional quality could potentially have the effect of lowering seed vigour.

Phytic acid is the primary storage form of phosphorus in seeds, representing 50% to over 80% of total phosphorus in mature seeds (Gupta et al., 2015) and a reduction in phytic acid may affect seed vigour as a result of reduced P content. During germination, phytic acid undergoes a digestion process and releases phosphorus and cations essential for seedling growth (Azeke et al., 2011; Bohn et al., 2008 and Lang et al., 2007). Phosphorus stimulates early growth and root formation and growth. This means that reduced P content in seeds as a result of breeding for low phytic acid concentration could negatively impact seed quality and limit crop production. Using low vigour seeds would result in lower percentage germination and seedling emergence speed, initial seedling growth and affect the seedling establishment and optimal plant populations. This could consequently impact dry matter accumulation (yield) and contribute to reduced agricultural productivity and lead to food insecurity.

Therefore, the aim of this study was to evaluate seed quality of low phytic acid maize varieties, with respect to germination and vigor, and their performance under field and water stressed conditions. The specific objectives of the study were to:

- To characterize low phytic tropical maize varieties with respect to germination and vigor.
- To investigate the effect of exogenous phosphorus application on germination, vigor and yield of low phytic acid tropical maize varieties.
- To evaluate field performance of low phytic acid maize varieties under dry conditions.
- To evaluate the performance of low phytic acid maize varieties under water stress conditions.

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CHAPTER 2

LITERATURE REVIEW

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2.1 Importance of maize

The world population continues to increase and it is predicted to reach 9.1 billion by 2050 (Sithole et al., 2016). Consequently, food insecurity is still a major global concern. Global demand for agricultural products is projected to double in the coming decades (Godfray et al., 2010). To meet this demand, we must have to increase the crop yield and production in developing countries, including those in Sub- Saharan Africa (SSA) (Thierfelder et al., 2015; Sithole et al., 2016). Therefore improving food security is a key agenda that is facing governments in SSA (Djurfeldt, 2015).

Maize crop is the third most important grain crop globally after wheat and rice in terms of area and production (Prasanna, 2016; Ladha et al., 2016). Maize crop has extended throughout the world and now has the highest annual production as compared to other cereal crops (Dowswell, 2019). Maize is a high-yielding cereal crop with the largest global production of 2525.7 million tons in 2015 (Afzal et al., 2017). It is grown as a field crop for human food consumption and as field corn for other usages such as feed for animals and biofuels (Serna-Saldivar and Carrillo, 2019). Maize is a major food component for the majority of poor people of the world (Clay, 2013; Deutsch et al., 2018). Maize is also an important main food crop in Sub-Saharan Africa (SSA) (Setimela et al., 2017), the crop contributes about more than 50% of the total calorie consumption and covering 13% of the whole agricultural land (Folberth et al., 2013; Ayalew et al., 2017). In addition, it is a major cereal crop in many African developing countries (Prasanna, 2016). Deutsch et al., (2018) stated that SSA is the region with the lowest maize yields in the world. Many regions in SSA continue to depend on foreign food assistance (Bodin et al 2016; Folberth et al., 2013). Sub-Saharan Africa is the region with greatest food security risk which is expected to increase by 2050 (Van Ittersum et al., 2016). Maize is an important crop for the sustenance of many households in SSA. Ekpa et al. (2019) stated that in SSA, consumption of the grain is in the range of 450 g/person/day. Maize is a staple crop in several countries where it is consumed as *sadza* (Zimbabwe), *nsima* (Malawi), *ugali* (Kenya), *pap* (South Africa). Additionally, irn South Africa maize is used as a weaning food for infants as well as for special ceremonies, caring for the sick, aged and pregnant women (Smuts et al., 2019).

2.2. Maize botanical description and husbandry requirements

Maize, (Zea mays), is an annual grass belong to the family Poaceae, which is commonly known as the grass family. The crop originated in Mexico and Central America (Yathish et al., 2019). It takes one growing season of 100 to 130 days. The plant height can reach 2–3 m with leaves total 8–21 per plant. Leaf-blade width 25-120 mm while Spikelet length 9-14 mm. (Ghorchiani et al., 2018).

Maize is adapted to warm, tropical and sub-tropical regions (Midega et al., 2018). It requires warm soils to develop optimally (Steward et al., 2018). One of the most important necessities for maize growing conditions is the quality of the soil which is required to be fertile and well drained with a pH ranges between 6.0 and 7.0 (Mao et al., 2017; Ahmad, 2017). Most fertile soils may need to be supplemented with nutrients as the plants develop, particularly phosphorus (P) and nitrogen (N) since maize plants are very heavy feeders (Parker et al., 2018). The crop also requires sufficient aeration during the growing period (Willcox et al., 2019). It should be planted where it obtain full sunlight during the day and provided with sufficient moisture (O'Hara, 2020). Therefore, seeds should be planted when the soil has warmed to at least more than 12.7°C (Grassini et al., 2015). To warm up the soil, laying black plastic mulches should be added about one week previous to planting time (Zheng et al., 2019) but this is not feasible for smallholder farmers because of their limited income and resources. Maize seeds should be sown about 2.5 cm depth and 15–20 cm apart allowing 70–80 cm between rows. After two weeks seedlings should be thinned to a final spacing of 20–25 cm when they are approximately 8.0–10.0 cm in height (Cumo, 2013). Required Fertilizers should be applied before the reproductive stage. In general, the recommending harvest time for the crop is 10 or 15 days after the grain has reached physiological maturity (Hallauer et al., 2010; McCann, 2005). Chauhan et al., (2017) reported that the suitable moisture content of maize is 11.8 which is safer for storage.

2.3 Maize production challenges

Although some countries have reached substantial gains in maize production, crop yield on many smallholder farms in African countries is consistently low (Prasanna, 2016). This has been exercarbated by biotic and abiotic stresses such as climate change occurrence which shows that agricultural production will largely be negatively affected (Cairns et al., 2013). Pest and disease, high levels of soil degradation and a decline in soil fertility, insufficient and inappropriate fertilizer application, unreliable rainfall, droughts and climate variability, lack of improved varieties, labor constraints, and in some situations inappropriate tillage practices have also contributed to low yields in Southern Africa (Eisabirye and Rwomushana, 2016 ; Liverpool-Tasie et al., 2017; Thierfelder et al., 2015; Sithole et al., 2016). Reportedly soil degradation level in South Africa is severe and 41% of the cultivated land is highly degraded (Sithole et al., 2016). Crop yields in Africa may fall by 10–20% to 2050 because of global warming and drying (Omoyo et al., 2015). Therefore, low yields in this region are largely associated with drought stress, low soil fertility, weeds, pests, diseases, low input availability, low input use and inappropriate seeds (Cairns et al., 2013). Yield potential is therefore location-specific and depends on solar radiation, temperature, and water supply during the crop growing season (Holzman et al., 2018).

These challenges keep the yield of maize in Sub saharan Africa low which ranges from 1-3 t/ha while the global yield average is around 5 t/ha (Prasanna, 2016; Eisabirye and Rwomushana, 2016). South Africa (SA) is the largest maize producer in the South African region, it exports to other neighboring countries such as Zimbabwe (Mandigora, 2018). Due to the low yield in South Africa, the crop production is also affected as stated by FAO (2019). Wheres the data showed that maize production decreased by 31% and 46% for 2015 and 2018 respectively compared to the year of 2014 where the production was high (Figure 1), but it increased in 2017 by 18% and it decreased again in 2018.

Maize adapting to the tropics has low attention from a breeding program in comparison with its adaptation to its temperate regions (Kim and Ajala, 1996). One of the most important factors affecting the yield is potential varieties because most poor farmers in SSA keep on planting different maize varieties without knowing the real performance of those varieties and adaptability to their environments concerning seed vigour, germination, growth, and yield (Our Badu-Apraku and Fakorede, 2017).

In addition to the above-mentioned challenges based on literature, one of the important problems that maize production is facing, is the nutritive value, because globally people suffer from insufficiencies micronutrients such as iron and zinc (Magallanes-López et al., 2017). Due to the availability of maize breeding agendas that have emphasized improving agronomic traits and have paid relatively little consideration to nutritional values (Ekpa et al., 2018), breeders are now working on developing maize varieties with high nutritional value. Malnourishment is a continual problem, mainly in rural areas in Africa where poor people rely on one staple foods and have limited access to a diverse diet (Bouis et al., 2017). Maize varities with enhanced nutritional quality can alleviate nutritional shortages if they are produced and consumed in sufficient quantities (Garcia-Casal et al., 2018). Therefore, breeders are introducing programs to improve nutritional values such as reducing phytic acid (PA) concentration in different maize varieties.

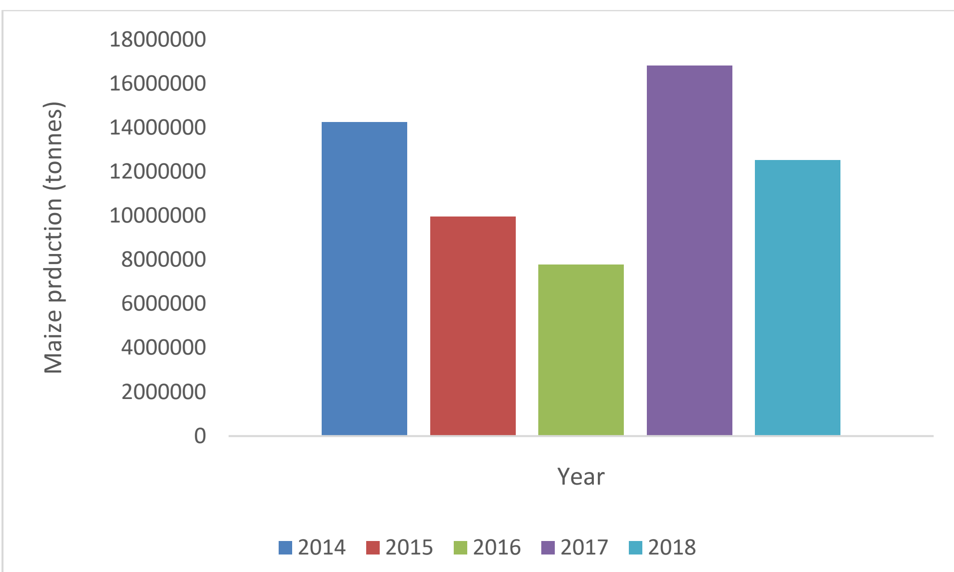

Figure 1. Maize production in South Africa for over 5 years (2014-2018). Source, FAO (2019)

2.4 Phytic acid

2.4.1 Phytic acid (PA) as an anti-nutritional factor in maize

Phytic acid (PA) (*myo*-inositol 1,2,3,4,5,6-hexakisphosphate) is a major storage form of phosphorus in plant seeds (Kaplan et al., 2019; Gupta et al., 2015). It accounts for up to about more than 80% of total phosphorus in seed (Roohani et al., 2013). In humans, phytic acid (PA) inhibiting gut mineral nutrients (such as calcium, manganese, iron, magnesium, zinc) and decreases their absorbability (Bänziger et al., 2000; Azeke et al., 2011). This can cause micronutrient deficiencies and result in malnourishment (Tamilkumar et al., 2014). Phytic acid (Figure 2) is a strong chelator of divalent minerals such as copper, calcium. Generally, approximately 1-2% of the weight of the seed is phytic acid, and sometimes it could reach three to six percent (Bohn et al 2008; Kaplan et al., 2019; Dost and Tokul, 2006; Nadeem et al., 2010). Phytic acid has long been considered as an anti-nutrient because of its strong ability to complex multi-charged metal ions, especially iron, magnesium, zinc. As a consequence, the consumption of great quantities of food containing high phytic acid (PA) levels could produce a deficit in the absorption of some dietary minerals.

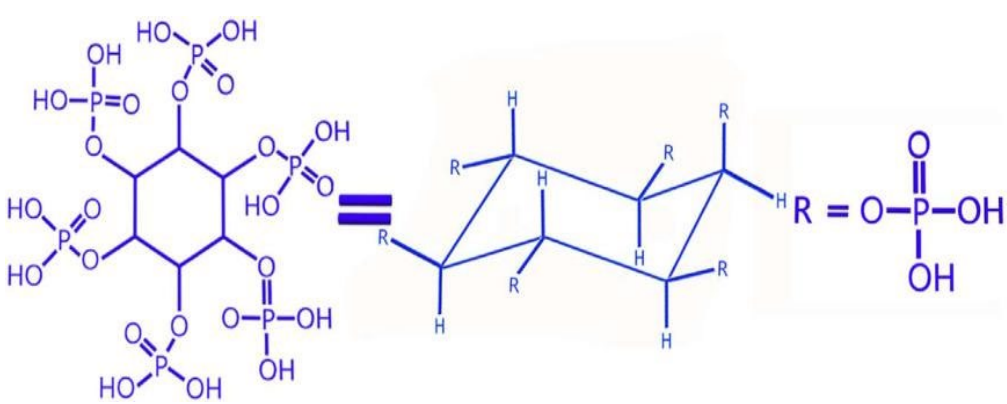

Figure 2. Phytic acid chemical structure. Source, Zhao et al. (2017)

The highest amount of phytic acid among grain crops is found in maize (Deutsch et al., 2018; Kaplan et al., 2019). The anti-oxidative function of phytic acid happens by chelating iron by inhabiting all the available Fe coordination sides, thus inhibiting it (Chen and Li. 2003). In the past few years, many in vitro and in vivo studies have revealed that InsP6, either endogenous or exogenous, also has beneficial effects, such as protection against cancers, heart diseases, diabetes, and renal calculi (Chen and Li, 2003).

The biosynthetic pathway to phytic acid is complex (Raboy, 2003) and can be summarized as consisting of two parts: Myo-inositol (Ins) supply and the subsequent Ins polyphosphate synthesis. The sole synthetic source of the Ins ring is the enzyme Ins (3) P1 synthase (MIPS), that converts Glc-6-P to Ins (3) P1. Phytic acid synthesis may also proceed in part via pathways typically associated with second messenger metabolism that involves phosphatidylinositol (PtdIns) phosphate intermediates and Ins (1, 4, 5) P3 (Loewus and Murthy, 2000).

2.4.2 Effects of reducing phytic acid in crops

There has been considerable progress in plant genetics leading to the identification and successful breeding of grains and legumes that are homozygous for allelic variants at a single gene that alters the phytate concentration of the grain or legume (Raboy, 2020; Kishor et al., 2019; Raboy et al., 2000; Larson et al., 2000; Adams et al., 2002; Hambidgeet al., 2004). In maize, the low phytate alleles have been identified and these can be used to facilitate measurements of the long term effects of dietary reduction on minerals' (zinc and iron) bioavailability in individuals with a high phytate diet (Adams et al., 2002; Naidoo, 2010).

Breeding for low phytic acid (LPA) concentration to improve micronutrient bioavailability is hampered by reduced seed germination and vigour of LPA mutants (Pramitha et al., 2019; Naidoo, 2010). These mutants have substantial decreases in seed phytic acid. However, the mutations block the capability of seed to synthesize phosphorus into phytic acid phosphorus (Pramitha et al., 2020; Jiang et al., 2019; Raboy, 2002; Naidoo, 2010).

An important part of the breeding programme is to include breeding for adequate and stable yield in an LPA background in a wide variety of environments. The use of LPA mutants in plant breeding has been limited due to non-germination of genotypes with homozygous *lpa1* alleles (Raboy, 2000), reduced seed weight of *lpa1-1* (Raboy et al*.,* 2000), and the lower vegetative growth rate and impaired seed development due to the *lpa241* mutation (Piluet al*.,* 2005). These LPA mutants have inferior agronomic and seed viability compared to their wild-type parents, leading to yield reduction (Raboy et al*.,* 2000) due to reduced seed weights and low vegetative growth (Naidoo, 2010). There are no previous germination and vigour studies on the *lpa1-1* mutant lines, however, there are two studies on the *lpa241* mutant which is allelic to *lpa1-1* (Piluet al., 2005) and which show 90% reduction in seed phytic acid (Piluet al., 2003). Studies showed that with LPA mutant (*lpa241*) a 30% decrease in germination rate was observed when compared to the wild type (Piluet al., 2003). Another germination study in Italy of the same LPA mutant line (*lpa241*) was tested and shown to have 72±15% germination under standard conditions which decreased to 45±14% germination under accelerated aging conditions (Doriaet al*.,* 2009).

A negative correlation between yield and phytate was found when comparing 50 different maize lines with a suggestion that the selection of larger kernel size should have a diluted concentration of phytate since 90% of phytate is found within the germ (Lorenz et al., 2007). The *lpa241* mutant has negative affecting embryo development and size, germination rate, seedling growth rate and ear size (Piluet al*.,* 2005). Also, some studies found all LPA mutants showed reduced seed viability (Zhao et al*.,* 2008; Naidoo, 2010). The LPA mutant lines also showed significantly lower field emergence (Zhao et al*.,* 2008; Oltmans et al*.,* 2005; Meis et al*.,* 2003). This could be a result of seed maturation in tropical environments with high temperatures at which the LPA varieties have low-stress tolerance, which results in a reduction of germination and emergence (Raboy, 2007).

Hybrids, varieties, and lines developed using these mutations represent the first generation of plant genetic resources which are useful in studying and possibly reducing problems associated with mineral malnutrition due to high seed phytic acid. Plants having the *lpa* characteristic produce seeds that have normal levels of total P but greatly reduced levels of phytic acid P. These mutations, therefore, do not affect the ability of a plant to take up P and transport it to a developing seed. Instead, *lpa* mutations block the ability of a seed to synthesize P into phytic acid P (Raboy, 2002). Zhao et al., (2008) in their results showed further studies are needed to find out whether this is a common phenomenon of LPA seeds, as such information is important for establishing good agriculture practice particularly for the tropical area where seed viability may be lost rather quickly due to the year-round high temperatures and humidity.

Menkir, (2008) mentioned that the development of an efficient breeding program to increase mineral concentration in maize depends on the presence of genetic variability in this species.

Limited studies have been published concerning the range of genetic variation in phytic acid concentrations among diverse inbred lines adapted to the tropics areas (Queiroz et al., 2011).

Seed viability and vigour are two important characteristics of seeds that regulate their value and usage (Han et al., 2014) of many different factors determine and impact seed germination and vigour such as varieties (genetic background) (Mahajan et al., 2018). Seedling characteristics such as germination percentage and seedling vigour index become the main influence in manipulating performance and crop yield of the LPA maize varieties. Seed germination is a very complex process in low phytic acid plant mutants (Yu et al., 2019). The ISTA defines seed vigour as an index of the level of the physiological deterioration or mechanical integrity of a high germinating seed lot which governs its ability to perform in a wide range of environments". Vigour is a concept that defines several characteristics of the seed such as rate and consistency of germination and growth, tolerance to environmental stresses after planting and retention of performance after storage (Han et al., 2014; Naidoo et al., 2012).

2.5 Phosphorus for plants

Soils in SSA are affected by several nutrient deficiencies including the macronutrients N, P, K, secondary nutrients S, Ca and Mg, as well as the micronutrients Zn, Fe, Cu, Mn, Mo and B (Kihara et al., 2017). Inadequate nutrient availability particularly NPK in soils in these regions not only causes low crop productivity but also the poor nutritional quality of the crops and consequently contributes to malnutrition in the human population (Kihara et al., 2017). The use of fertilizers remains very low in SSA (Liverpool-Tasie et al., 2017). Restricted access and high costs of fertilizers are amongst the major causes of the limited use of fertilizers by smallholder farmers (Taddese, 2019).

Phosphorus (P) is considered as the second major macronutrient after nitrogen N (Ogunsola and Adetunji, 2016) which means that plants need phosphorus in sufficient quantities to complete their lifecycle. Unlike N, P cannot be added to the soil by biological fixation (Betencourt et al., 2012). The P deficiency challenges could partially be relieved by an informed decision on the part of farmers to return to the soil high-quality organic materials that are locally available but are often ignored (Vanlauwe et al., 2014).

The growth and yield of many field crops are improved by the application of phosphorus and also increased the root growth in many crops under water stress condition (Carstensen et al., 2018). Phosphorus deficiency of the soils is a main limiting factor of agricultural production in several tropical regions (Osorio et al., 2017). Root growth depends on the P status of the plant. Root growth and development are critical for early phosphorus (P) uptake by plants since phosphorus is quite unavailable and immobile in several soils (Dwivedi et al., 2016). Phosphorus is required for growth, nucleus formation, photosynthesis, utilization of sugar and starch, cell division and fat and albumen formation (Sadiq et al., 2017; Ali et al., 2019). Significant effects of phosphorus fertilizer application on maize characteristics such as plant height, number of leaves per plant and stem diameter (Sadiq et al., 2017). More studies presented by Dwivedi et al. (2016) and Muhammad et al. (2009) found that phosphorus significantly affected the plant height, ear length, number of grains/cob, 1000-grain weight and grain yield. Phosphorus deficiency has a major influence on plant growth and development. Phosphorus inadequate amount is required for earlier maturity, quick growth and enhances the quality of vegetative growth (Ali et al., 2019). It is involved in enzymatic reactions in the plant. Phosphorus is important for cell division because it is an essential element of nucleoproteins which are involved in the cell reproduction processes (Gul et al., 2015). It is also a component of a chemical essential to the reactions of carbohydrate synthesis and it is important for seed formation and crop maturation (Khan and Singh, 2017).

It has been estimated that 30% of the world's arable soils are deficient in P and require P fertilization to improve yields (MacDonald et al., 2011). For many cropping systems in the tropics, the application of phosphorus from organic and inorganic sources is essential to maximize and sustain high crop yield potential in continuous cultivation systems (Ngetich et al., 2012). Phosphorus deficiency delays flowering by approximately three days to anthesis and six days to silking stage (Ahmad et al., 2019). The deficiency of phosphorus is responsible for small ears in maize due to crooked and missing rows as kernel twist (Ali et al., 2019). In the low phosphorus environment, grain yield was reduced by 44% compared to the mean of the high-P environment (Ahmad et al., 2019). Phosphorus mobilization to grain during the seed filling phase from leaves and stems during grain development depends on variety, soil P level, and environment (Masoni et al., 2007). Phosphorus absorbed by plants is translocated from the roots and leaves to the seeds during seed development, where phytic acid (phytate; myo-inositol 1, 2, 3, 4, 5, 6-hexakisphosphate) is synthesized (Taliman et al., 2019). The application of phosphorus fertilizers enhances the total P and phytate P concentrations significantly (Buerkert et al., 1998; Taliman et al., 2019).

Phosphorus plays a significant part in many physiological characteristics that occur within a developing and maturing plant (Khan and Singh, 2017). Phosphorus (P) is an essential heteroelement in compounds such as ATP, NADPH, nucleic acids, sugar phosphates, and phospholipids, all of which play important roles in photosynthesis (Carstensen et al., 2018). Amujoyegbe et al. (2007) reported that chlorophyll coloration is related to the amount of nutrients absorbed by the plant from the soil. P deficiency influences the balance between the synthesis and catabolism of carbon metabolites. Low Pi levels switch the carbon flow to starch accumulation and reduce CO2 assimilation (Carstensen et al., 2018). Low phosphorus content in maize is associated with low photosynthetic rates (Mahama et al., 2016; Siebers et al., 2017). Phosphorus plays a key role in energy transfer and is essential for photosynthesis and other chemico-physiological processes in plants (Carstensen et al., 2018).

2.6 Effect of water stress on growth and development of maize

Drylands represent 40% of the global earthly surface and offer important ecosystem services (Nawaz and Farooq, 2016; Farooq and Siddique, 2016). While drylands as a whole are estimated to increase in extent and aridity in the coming decade. (Nawaz and Farooq, 2016). In SSA temperature and precipitation forecasts differ by latitude and geographic region (Kotir, 2011). Maize is exposed to several unfavorable environmental conditions that are affecting growth, development, and yield. Among them drought is one of the biggest environmental conditions to agriculture worldwide. The unavailability of water due to continued lack of precipitation or lack of irrigation or a period long enough to deplete the soil and lead to agricultural drought (Nawaz and Farooq, 2016).

2.6.1 Growth

Drought is the one of the important abiotic stress factors that restrict the crop growth and production in favorable condition (Basu et al., 2016). Drought also reduced the uptake of water as well as nutrients to the roots (Zandalinas et al., 2018; Ahmad et al., 2015). In many African developing countries, water stress is the main constraint to maize production and also reduced the quality, growth, and production as a result of poor and great reduction in yield (Rasul, 2016). It is established that a variety of metabolic and physiological processes occurring in plants are reduced by drought (Cotrozzi et al., 2017). It has been stated that the drought stress over vegetative and reproductive phases significantly reduced the relative leaf contents and leaf osmotic potential of plants (Rasul, 2016). Growth, chlorophyll and water contents along with different fluorescence parameters are changed with drought (Ahmad et al., 2015). Under water stress conditions, the reactive oxygen species (ROS) are produced and these ROS cause serious problems inside the plants (Fahad et al., 2017). The plants grow under well-watered conditions performed better however, the poor performance of reducing growth and yield was reported by many scholars under water stress conditions (Steward et al., 2018). The lack of water causes net photosynthesis reduction and decreased shoot and root biomass production in maize crops (Abbas et al., 2018). It was stated that the deficiency of water is the restrictive factor for seed growth having the effects on alteration of its structure (Kaplan et al., 2019; Ali et al., 2010; Zhang et al., 2019). The reaction of the growth of the crop to drought differs significantly at various structural levels depending upon strength and duration of stress as well as plant species and cultivar and the stage of growth (Efeoğlu et al., 2009) therefore, drought affects the percentage of germination and seedling establishment, uniform seedling emergence and their vigorous establishment can ensure good plant population and final yield (Finch-Savage and Bassel, 2015).

The effect of drought stress on morphological traits is drastic and it significantly reduce the expression of most traits. Overall in maize, plant height, ear height, the number of ears per plant and grain yield are reduced during drought stress (Umar et al., 2015). Water stress during vegetative growth is also critical to leaf area development, potential kernel number determination and succeeding grain yield (Pandey et al., 2000).

With regards to relationship between water stress and accumulation of PA in the seeds, Kaplan et al. (2019) reported that water stress had no significant effect on phytic acid content. However, it was observed that there was an increase in the values of resistant starch, amylose and phytic acid level depending on the increase of doses of nitrogen fertilizer but there is no published work talking about the effect of water stress on different levels of phytic acid cultivars.

2.6.2 Flowering

Maize is most sensitive to drought during the flowering phase (Daryanto et al., 2016; Decima Oneto et al., 2017) delay in inflorescence development, flowering time, tassel blasting, decrease in pollen fertility and viability (Kenawy et al., 2018) and abortion of embryos (Oury et al., 2016). It is generally agreed that maize is the most sensitive to stress during the flowering period, when pollen shedding, silking and seed setting take place (Wang et al., 2019). Exposure of maize to water stress could delay the silking stage and the affecting yield because it reduces pollination performance (Hall et al., 1982). Days to 50 % tasselling, days to 50 % silking and anthesis interval increased by 5, 6 and 33 %, respectively, under water stress (Umar et al., 2015). The most sensitive period for yield determination for plants under stress is between 2 weeks before and 2 to 3 weeks after silking (Pandey et al., 2000). Water stress before silking might cause the failure of ear growth and development while stress after pollination results in limitation of kernel number (Oury et al., 2016). Pandey et al., (2000) revealed that the highest yield reductions from stress happened from tassel emergence over the week following silking in maize, the critical yield period placed during flowering. Runge (1968) stated that yields were most sensitive to stress throughout the 2 weeks before silking. The same results stated by (Koch and Ma, 2017). Koch and Ma (2017) concluded that stress before silking could result in reduced kernel number due to the effects of carbohydrate deprivation. It was found that both anthesis and silking of maize varieties were significantly delayed due to water stress and water stress significantly affected the anthesis and silking interval (Molla et al., 2014). The yield potential of maize is, however, reduced by moisture stress that arises just before anthesis (NeSmith and Ritchie, 1992), and during silking and seed filling (Mansouri-Far et al., 2010).

2.6.3 Photsynthesis

Photosynthetic activity and tolerance of high temperatures both affect water use efficiency (Wang et al., 2019). Water scarcity disturbs plant-water relations, photosynthetic gas exchange capacities, cell turgor, source-sink relationships and various metabolic events in plants (Rodrigues et al., 2019). Studies on photosynthesis in maize under different environmental conditions are important tools to attain a more refined selection of resistant cultivars, which can lead to higher yield and production under abiotic stresses (Araus et al., 2018).

Water shortage also affects carbohydrate metabolism, which influences photosynthesis and leaf growth (Kumar et al., 2016). In order to attain maximum rates of dry matter production, the leaf photosynthetic rate must be high throughout the day as well as over the growing season (Mathobo et al., 2017). Overall, as water deficits increase in the soil, leaf water potentials decline, leading to the closure of stomata (Boyle et al., 2016), discernable wilting, and dramatic impairment of many metabolic functions (Zhang et al., 2019). Underwater stress, dehydration of plant tissue causes deterioration in chloroplast structure and this linked with loss in chlorophyll content and this leads to a decrease in photosynthetic activity (Jain et al., 2019).

Numerous physiological features have been described as being reliable pointers for the selection of genotypes or cultivars for drought tolerance e.g., the photochemical activity of photosystem and chlorophyll content (Boyle et al., 2016; Aslam et al., 2006). Drought stress-reducing average yield with 50 % and over. It leads to a reduction in chlorophyll content and changes in fluorescence. Water stress has been revealed to bring about a reduction in leaf chlorophyll levels in maize (Martinez et al., 2018).

Dry matter production of a crop is strongly reliant on its leaf area as well as the rate of leaf photosynthesis (Liu et al., 2015). One of the earliest responses to drought is stomatal closure, Stomatal closure permits plants to limit transpiration, but it also limits CO2 absorption, which leads to a decreased photosynthetic activity (Tombesi et al., 2015). Most of the negative effect of drought is related to the photosynthetic process of the plant. Several studies have shown that the decrease of the photosynthetic activity under drought stress can be associated with stomatal adjustment (Efeoğlu et al., 2009). Nutrient uptake by plants is decreased under drought stress conditions due to reduced transpiration. The dry matter production of a crop is strongly reliant on its leaf area as well as the rate of leaf photosynthesis (Chen et al., 2014).

It is reported that some physiological traits such as stomatal conductance are a major trait that impact yield under drought stress (Khonghintaisong et al., 2018). High stomatal conductance at grain filling stage would be expected as the basic criteria for high grain yield under stressed conditions. It is well-defined that the decrease of relative water content close stomata and also after obstructive stomata will reduce the photosynthesis rate (Keyvan, 2010). Different environmental conditions and water deficits lead to increase stomatal density and reduce the stomatal size, indicating adaptation of crops to drought stress. Soil drying leads to a decrease in stomatal aperture and stomatal conductance (Premachandra et al., 1992). To avoid extreme water loss and physiological damage, plants regulate transpiration by adjusting the stomatal aperture (Van Emmerik et al., 2016). Grain yield was closely and negatively linked with the stomatal conductance of maize hybrids under irrigated conditions (Premachandra et al., 1992). A significant and positive correlation was described between grain yield and stomatal conductance (Tombesi et al., 2015). In the drought environment, moderately high stomatal conductance helps to produce the maximum grain yield (Premachandra et al., 1992). Stomatal closure is the result of hydraulic signaling from the roots to the leaves and an increase in the stress hormone abscisic acid which is transported through xylem flow and regulates stomatal conductance (Van Emmerik et al., 2016). Stomatal conductance has long been identified as a key variable manipulating leaf gas exchange through its regulation of water vapor and CO2 diffusion (Harrison et al., 2019). The imposition of simulated water stress reduced the survival rate and stomatal conductance, while it caused a significant increase in relative cell damage percentage in both water stress-tolerant and water stress-sensitive accessions (Martin-St Paul et al., 2017).

2.6.4 Yield and yield components

Drought is considered to be the major cause of crops yield losses which decreases yield up to 50% depending upon its severity (Potopová et al., 2016). A reduced number of kernels per ear is an immutable component of yield reduction under drought stress. Reduced grain yield under water stress conditions in maize has been recorded by numerous previous studies (Cakir, 2004; Zharfa et al., 2011; Premachandra et al., 1992). A great reduction in grain yield was detected in hybrids under water stress conditions. The reduction of grain yield almost similarly linked with the reduction of kernels per unit area and kernel weight (data not shown) this agree with El-Sabagh et al. (2017).

Therefore, despite the increase in global food production, food insecurity remains a challenge as the nutritional quality of the food is insufficient to meet consumption requirements (Ye et al., 2013). Queiroz et al. (2011) mentioned there is limited results have been published concerning the evaluating of genetic variation in phytic acid concentrations and performance of the diverse inbred lines. This deficiency presents a knowledge gap in our understanding of the role played by phytic acid which this study aims to answer, specifically the consequences of low and high phytic acid maize of the genetic makeup of the crop.

Developing more drought-tolerant crops in a sustainable manner is one means to meet the demand of an increasing human population that will require more food. Improvement in drought tolerance of crops is ultimately measured by an increase in grain yield under water-limiting conditions. (Omoyo et al., 2015). To improve maize yield of low phytic acid maize varieties, it is important to understand crop water use and water stress tolerance levels. In addition, there is a need to study the consequences of reducing phytic acid concentration under drought areas and how these new varieties perform.

2.7 Conclusion

Issues surrounding food and nutritional security remains an issue in SSA. The Food and Agricultural Organization (FAO) of the United Nations defines food security as a condition where people at all times have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for their active and healthy life. Given that most people in SSA still suffer from hunger and chronic diseases associated with food nutrition, it is important that we come with strategies that will have a long lasting solution into the problem. Maize being the staple crop consumed by the millions of people in SSA can be used as a vehicle to solve some of these problems. Breading for maize with high nutrition can help to alleviate some of these problems since most people residing in the region depends on it as a staple crop consumed in large quantities on the daily basis. Although progress has been made in breeding maize with high vitamin content, one of deficient nutrient in many communities in the region, progress in breeding maize with high concentration of Ca, Fe and Zn, the other deficient micronutrients in SSA has been rather slow. One strategy that has been attempted, in other regions, to approach this has been to reduce phytic acid in seeds which chelates these metal ions. However, it has been found that this has some negative consequences in the agronomic performance of these low phytic varieties. These studies however, were conducted with maize of temperate origin with little effort that has been done on the diverse genetic pool of tropical origin where the problems of malnutrition and water stress persist. Therefore, it is therefore important to screen low phytic genetic pool adapted to the SSA conditions and select the most suitable one as a starting point for the main stream breeding programs.

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CHAPTER 3

Characterisation of low phytic acid maize (Zea mays) varieties for seed germination and vigour

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3.1 Abstract

Plant breeders have focused on reducing phytic acid (PA) on maize seeds to improve grain nutritional quality. However, this could possibly have negative effects on seed quality. The objective of this study was to compare the seed quality performance of low phytic acid (LPA) maize seeds of tropical origin with three other varieties (i.e.high phytic acid (HPA) of tropical origin, SC701 (commercial white maize) and LS8520 (commercial yellow maize). Four replicates of 20 seeds per variety were germinated in a paper towel in a randomized complete block design for a standard germination test. Seeds were also subjected to accelerated aging (AA) test. Final germination was significantly higher ($p < 0.05$) in HPA (98.0%); than in SC701 (93.0%) and LPA (90.0%), and the germination percentage for the latter two varieties was not significantly different ($p > 0.05$) from all other varieties. The mean germination time (MGT) was similar in all varieties, however, the germination velocity index (GVI) was higher ($p < 0.05$) in SC701 than in all varieties and similar ($p > 0.05$) in LPA and HPA. Both SC701 and HPA showed higher percentage of germination when they were exposed to the accelerated aging test. It was concluded that LPA showed a fairly similar seed quality parameters than other high performing varieties.

Keywords: Accelerated aging; *lpa (1-1)*; high phytic maize; low phytic acid maize; low phytate maize

3.2 Introduction

Countries in Sub-Saharan Africa (SSA) appear to be struggling to meet the United Nations Sustainable Development Goal 2, which is aimed to "End hunger, achieve food security and improve nutrition and promote sustainable agriculture". This situation has been worsened by global economic conditions, adverse climatic conditions and conflict; in many countries and sometimes the combination of these factors (FAO 2018). The progress to meet this goal is measured against two targets. The first one, Target 2.1, measures progress towards ensuring access to food for all, while the second, Target 2.2, measures progress towards eliminating all forms of malnutrition (FAO 2018). One of the main vehicles to reach Target 2.2 by the agricultural scientific community was and to date is still to breed maize (*Zea mays*) with high nutritional content, in particular, targeting those nutrients that are missing in the diet of many households in the SSA region. Micronutrients ("hidden hunger") such as vitamin A (Pillay et al. 2014), iron (Fe), zinc (Zn) and manganese (Mn) (Berti et al. 2014) are particularly missing in the diet of many communities in the SSA region.

As a result maize being the staple crop for many communities in SSA and also used for animal feeding, a considerable effort has been made, for example, to breed genotypes with high vitamin A content and proteins ("quality protein maize") by the researchers at the University of KwaZulu-Natal in the African Center for Crop Improvement (Pillay 2011; Naidoo et al. 2012). In recent years, the researchers at the University have also released maize tropical lines with low phytic acid (LPA) (*myo*-inositol 1,2,3,4,5,6-hexakisphosphate) to counteract the problem of malnutrition. However, these maize tropical lines have not been tested for their agronomic performance, one aspect of that being seed quality, which is an important basic aspect of crop production (Sithole et al. 2016). Hampton (2002) described seed quality as the standard of excellence that will determine the performance of seeds when sown. The two widely used indicators of seed quality are germination and vigour. Basu (1994) stated that viable seeds are those that are alive and can germinate when exposed to favorable conditions. On the other hand, seed vigour consists of those seed properties that determine the potential for rapid, uniform germination and emergence and development of seedling under a wide range of environmental conditions (Perry 1978; McDonald 1980). Therefore, high quality seeds are the cornerstone for success in sustainable crop production since they provide rapid and even crop stand. Rapid and even crop stand have been associated with high yield since they outcompete and suppress weeds in the early stage of crop establishment. Thus, agronomic studies that seek to evaluate crop performance after a breeding program such as breeding for LPA cannot be substituted.

A high phytic acid (PA) diet causes mineral deficiency and malnutrition (Omosebi et al. 2018). The chelating ability of phytic acid renders it to be an anti-nutritional factor (Singh et al. 2018). Breeders have thus focused on improving the crop to increase its contribution to food and nutritional security (Rivers 2015; Ekpa et al. 2018). In recent years, the focus has been on improvement programmes aimed at improving the nutritional quality of maize by reducing PA (Briat et al. 2015; Gupta 2015). Studies have showed that reducing the PA content of staple cereal grains such as maize may contribute to enhancing Zn nutrition of populations consuming the crop (Lönnerdal et al. 2011). Phytic acid chelates metal ions, especially Zn, Fe, and Ca which are missing in the diet of many communities in the SSA (Gibson et al. 2010; Garcia-Oliveira 2018). Phytate complexes in the gastrointestinal tract cannot be digested or absorbed by humans because of the absence of intestinal phytase enzymes (Gibson et al. 2010; Gibson 2018). Phytate also complexes endogenously secreted minerals such as zinc and calcium (Guéguen and Pointillart 2000; Cangussu 2018), making them unavailable for reabsorption into the body (Gibson et al. 2010). Therefore, mutations that block the phytic acid biosynthesis pathway have been released (Stevenson-Paulik et al. 2005; Glover 2011; Jervis et al. 2015; Redekar et al. 2017). Raboy (2007) and Doria et al. (2009) reported that phosphorus content in phytic acid of seeds is reduced in these mutations by 50% to 66%. However, reducing phytic acid to the lowest amount via breeding programs could be a serious problem and detrimental during seed germination.

Phytic acid is one of the most important biochemical markers that take place during seed development (Le et al. 2007; Kerr et al. 2010; Redekar et al. 2017). It is the primary storage form of P in seeds, representing 50% to 80% of total P in mature seeds (Lin et al. 2005). Therefore, the reduction in PA may affect seed germination and vigour as a result of reduced phosphorus (P) content because during germination, PA undergoes a digestion process and releases P and cations essential for seedling growth (Lang et al. 2007; Bohn et al. 2008; Azeke et al. 2011). Phosphorus stimulates early root formation and growth in plants (Wen et al. 2017). This means that reduced P content in seeds as a result of breeding for LPA content could negatively impact seed quality and limit crop growth, development and as a results yield.

However, studies have presented different results on LPA. For example, in soybean seeds, Raboy and Dickinson (1987) found that reducing PA did not have an effect on seed germination in a comparative study between high (1.0 mg/seed), medium (0.59 mg/seed) and low (0.19 mg/seed) PA concentration. The authors concluded that the studied soybean seeds may contain P reserve far above than that needed for germination and early seedling growth. In addition, although studies of low phytic acid genes have been reported in temperate maize (Raboy et al. 2000; Pilu et al., 2003), the current study was based on the tropical genetic background which were screened for low phytic acid. This is a first report for applied breeding of the trait in the tropical maize. Thus, due to wide genetic variation in maize, it is clear that the effect of reducing PA in maize germination and performance under field conditions remains unknown and controversial.

Therefore, the objective of this study was to evaluate seed quality of Low Phytic Acid (LPA) maize synthetic population of tropical origin with respect to germination and vigour. This was compared to a High Phytic Acid (HPA) synthetic population of the same genetic background, and two commercially grown maize varieties, in South Africa. Secondary to this, the study also evaluated the responses of HPA and LPA varieties subjected to a seed accelerated aging (AA) vigour test. This provides valuable information about storability and field seedling emergence potential.

3.3 Materials and Methods

3.3.1 Germplasm

The seed material used was obtained from the African Centre for Crop Improvement (ACCI) research programme, at the University of KwaZulu-Natal and a local seed company (McDonald Seeds, Pietermaritzburg, South Africa). The study consisted of two maize synthetic populations differing in PA content (ACCI), namely, Low Phytic Acid (LPA) (Figure 1a) and High Phytic Acid (HPA) synthetic populations (Figure 1b). Both LPA and HPA synthetic populations were derived from a tropical second generation (F2) population and were selected biased on their phytic acid (PA) content. The 24 lines with low PA ranging from 1.27 to 32 mg/g were allowed to random mate for two generations to form the LPA synthetic population at the Ukulinga Research Farm, University of KwaZulu-Natal (29°40'05.7"S 30°24'20.9"E), in Pietermaritzburg, South Africa. The other 51 lines, from the same tropical F2 population, with high PA ranging from 43 to 113 mg/g in grain were also recombined to form the HPA synthetic population. These two synthetic populations were compared with two commercial maize varieties (from McDonald Seeds), white maize (SC701) (Figure 1c) and yellow maize LS 8520 R (484) (Figure 1d) which in this study was coded LS8520. All seeds used in this study were produced under identical production conditions and in the same growing season, thereby ensuring that the seeds were of the same physiological age.

Figure 1: Seeds of maize used in the study. (A) Low phytic acid (LPA), (B) High phytic acid (HPA), (C) White commercial maize hybrid (SC701) and (D) Yellow commercial maize hybrid (LS8520).

3.3.2 Laboratory experiments

3.3.2.1 Analysis of phytic acid

Phytic acid content was determined for all varieties according to the method previously described by Aina et al. (2012). Briefly, the test was conducted by preparing 25 seeds from each seedlot. A 2 g of each sample was weighed into a 250 mL conical flask and soaked for three hours in 100 mL of 2% concentrated HCl and then filtered with Whatman No.1 filter paper. The 100 mL of filtrate and 10 mL of distilled water was added in each case to provide proper acidity. Thereafter, 10 mL of 0.3% ammonium thiocyanate solution was added into the solution as an indicator and titrated with standard Iron II Chloride solution containing 0.00195 g Iron/mL with the end point observed to be yellow which persisted for 5 minutes. The percentage of phytic acid was, therefore, calculated using Eq. 1.

$$\text{Phytic acid (mg/g)} = y \times 1.19 \times 100 \qquad (1)$$

Where:

$y = \text{titre value} \times 0.00195$ g

3.3.2.2 Seed electrical conductivity

Four replicates of 30 seeds per genotype were used to access electrical conductivity (EC). It was assessed using a CM 100-2 single cell analyser (Reid and Associates, Durban, South Africa). Seeds were initially weighed before being put into 2 ml wells filled with distilled water. Electrical conductivity of the seeds was then read over a period of 24 hours.

3.3.2.3 Standard germination test

Standard seed germination tests were conducted according to the guidelines set by the International Seed Testing Association (ISTA 2012). Briefly, four replicates of 25 seeds were placed in a moisture double-layered germination paper towel in a randomized complete block design. Seeds were placed equidistantly to reduce the mutual influence and bacterial infection. The germination paper was then rolled and tied at either end using rubber bands and put in a plastic zip-lock bag to prevent any moisture loss. Seeds were then placed in a germination chamber set at 25 ± 1 °C (ISTA, 2012). Germination counts were taken daily for seven days and germination was defined as radicle protrusion of at least 2 mm. On the final day, day seven, final germination was calculated based on normal seedlings according to AOSA (1992). Thereafter, measurements of shoot length, root length and root to shoot ration were taken. Following this, germination indices, namely, germination velocity index (GVI) and mean germination time (MGT) were calculated. GVI indicating the speed of germination was calculated using Eq. 2 in Maguire (1962). MGT, an index used to indicate the average time taken by seed lot to germinate was calculated using Eq. 3, according to Ellis and Roberts (1981) and Hluyako et al. (2017).

$$GVI = G1/N1 + G2/N2 + \ldots + Gn/Nn \quad (2)$$

Where, GVI = germination velocity index

G1, G2…Gn = number of germinated seeds in first, second… last count.

N1, N2…Nn = number of sowing days at the first, second… last count.

$$MGT = \frac{\sum Dn}{\sum n} \quad (3)$$

where:

MGT = mean germination time,

n = the number of seeds which were germinated on day D, and

D = number of days counted from the beginning of germination.

3.3.2.4 Accelerated aging vigour test

Seeds were placed in a plastic chamber box with temperature set at 41 °C and 100% relative humidity (RH) for 48 h and 72 h following the standards set by ISTA (2012). The unaged seeds (0 day) were used as a control. The ageing chamber was a plastic box (8 cm × 8 cm) with a lid which was placed into a wire tray with a 10 cm x 10 cm x 2 cm (length x width x depth) mesh screen and the pore sizes of the mesh screen was 1.89 mm^2. In each accelerated aging box, 40 g of sodium chloride and 100 mL of distilled water was added and a dry screen tray was inserted to prevent splash water on the screen. After each period of accelerated aging treatment, the standard germination test was performed as described in Section 3.3.2.3, above.

3.3.3 Statistical analysis

All data collected were subjected to the analysis of variance (ANOVA) using GenStat® 18th Edition (VSN International, Hemel Hempstead, UK, 2011). Means were compared using Fischer's least significant difference (LSD) test at the 5% level of significance. Multivariate data obtained in this study was further subjected to principal component analysis (PCA) to establish traits that contributed to the observed variation. The PCA was performed using STATISTICA (version 7.0, StatSoft, Southern African Analytics).

3.4 Results

3.4.1 Phytic acid concentration of different maize genotypes

Highly significant ($p < 0.001$) differences were observed across the varieties with respect to PA content (Figure 2). The HPA maize varieties were found to contain 4-folds more PA than LPA varieties whereas, when compared to SC701 and LS8520 it was 2-folds more. On the other hand, significant differences ($p < 0.05$) were found in PA between commercial varieties, SC701 and LS8520 with SC701 having significantly ($p < 0.05$) higher PA than LS8520 varieties.

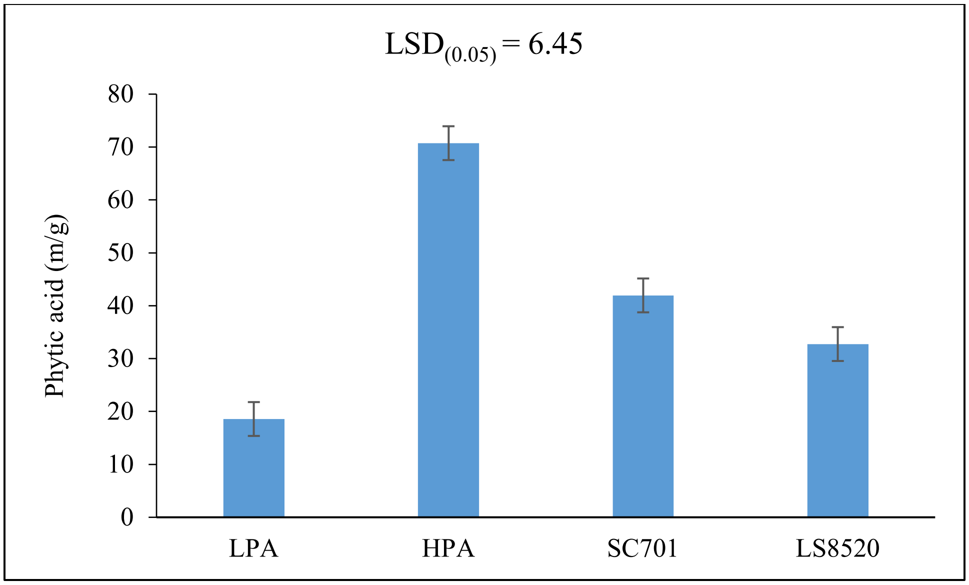

Figure 2: Phytic acid content of different maize seeds. LPA, low phytic acid maize; HPA, high phytic acid maize; SC701, commercially grown white hybrid maize genotype; LS8520, yellow hybrid maize genotype.

3.4.2 Standard germination test

Figure 3 represents seed germination of the four seedlots used in this study. The germination of LPA and HPA seedlot varieties was not significantly different ($p < 0.05$) with time, however, differences were found in final germination with deviation of one seedlot. Similar trends were observed in SC701 and LS8520 seedlots.

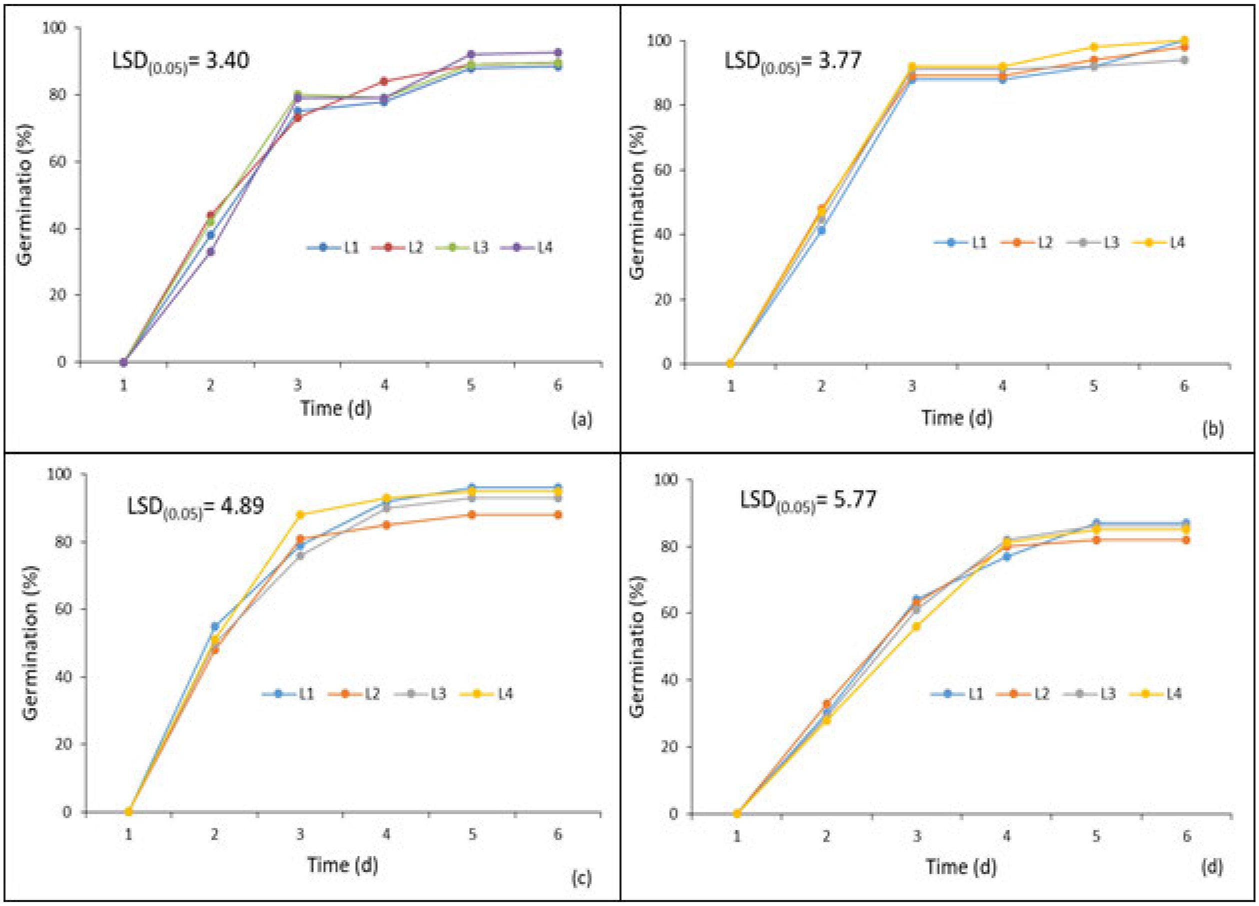

Figure 3: Seed lots daily germination of low phytic acid (LPA) and high phytic acid (HPA) as compared to commercial white hybrid (SC701) and yellow hybrid (LS8520) maize seed lots hybrid varieties. Note: a = LPA, b = HPA, c = SC701 and d = LS8520.

Highly significant differences ($p < 0.001$) were observed in germination among the four tested varieties (Figure 4). The germination percentage of SC701 maize was the highest, more than 40% on the first day of germination count followed HPA, LPA and LS8520 which was 36%, 34% and 26%, respectively. Rapid germination was observed on the second day of germination count with HPA seeds having more than 80% of germination followed by SC701 (80%) and LPA (79%) seeds and the least was observed in LS8520 (60%).

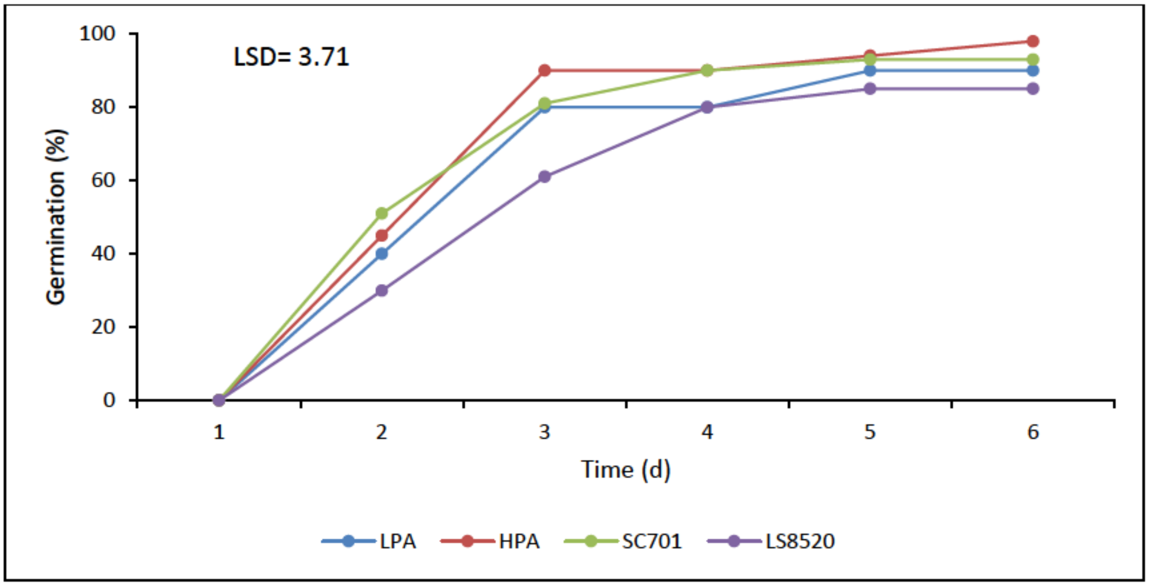

Figure 4: Daily germination of high phytic acid (HPA) and low phytic acid (LPA) maize varieties as compared to commercial white hybrid (SC701) and yellow hybrid (LS8520) maize genotypes.

Highly significant differences ($p < 0.001$) were also found in final germination with the HPA maize showing the highest germination percentage of 98% while the least was observed on LS8520 maize varieties with the final germination of 85% (Table 1). No differences ($p > 0.05$) were observed in all varieties in mean germination time. On the other hand, the GVI of SC701 and HPA maize genotype was significantly higher ($p < 0.05$) than that of LPA and LS8520 varieties. The general trend was that GVI in SC701 > HPA > LPA > LS8520. Contrary to what has been observed in other parameters, the EC of SC701 maize varieties was less than that of HPA which was less than that of LPA and LS8520 maize varieties. Finally, there were no much differences in the root: shoot ratio in all varieties.

Table 1: Seed performance of low phytic acid (LPA) and high phytic acid (HPA) maize lines as compared to commercially grown white hybrid (SC701) and yellow hybrid maize varieties (LS8520).

Genotype	Total germination (%)	MGT	GVI	EC ($\mu S\ g^{-1}$)	Root length (mm)	Shoot length (mm)	Root: shoot ratio
LPA	90.0^{ab}	2.70^{a}	34.6^{ab}	19.0^{b}	12.0^{a}	7.72^{a}	1.57^{ab}
HPA	98.0^{c}	2.86^{a}	38.8^{bc}	11.9^{a}	14.4^{c}	7.77^{a}	1.87^{b}
SC701	93.0^{bc}	2.76^{a}	39.0^{c}	9.94^{a}	13.1^{b}	9.7^{b}	1.36^{a}
LS8520	85.0^{a}	2.96^{a}	30.9^{a}	24.1^{c}	12.3^{ab}	7.70^{a}	1.60^{ab}
CV (%)	3.0	8.3	5.9	26.6	4.0	8.9	11.4
LSD	4.3	0.36	3.23	3.88	0.80	1.12	0.28
p-value	<.001	0.44	<.001	< 0.001	<.001	0.005	0.01

Values within the same column sharing the same letters are not significantly different from each other at $p < 0.05$. Note: MGT = mean germination time, GVI = germination velocity index, EC = electrical conductivity.

3.4.3 Accelerated aging (AA) vigour test

No significant differences ($p > 0.05$) were found in LPA seedloots when exposed to AA vigour test for 48 and 72 hours (Table 2). However, slight deviation ($p > 0.05$) with one seedlot was observed in all other varieties when they were exposed for 48 hours and 72 hours AA vigour test.

Table 2: Final germination percentage of seedlots of different maize varieties exposed to accelerated aging for 48 hours and 72 hours.

Lots	**LPA**		**HPA**		**SC701**		**LS8520**	
	48h	72h	48h	72h	48h	72h	48h	72h
1	70.5[a]	64.3[a]	81.3[ab]	76.8[b]	81.0[b]	73.5[ab]	67.0[ab]	58.8[a]
2	71.6[a]	68.3[a]	81.0[ab]	73.0[a]	77.8[ab]	72.8[ab]	64.3[a]	59.5[ab]
3	73.0[a]	65.8[a]	86.0[b]	74.0[ab]	82.5[b]	76.8[b]	71.5[b]	60.8[ab]
4	71.0[a]	68.8[a]	76.5[a]	74.5[ab]	72.8[a]	70.9[a]	65.0[a]	62.5[b]
CV (%)	3.80	4.00	3.70	1.90	5.00	2.70	3.60	2.60
LSD	4.16	4.14	4.65	2.16	6.00	3.05	3.71	2.41
***p*-value**	0.60	<.002	0.007	0.017	0.019	0.008	0.005	0.027

Values within the same column sharing the same letters are not significantly different from each other at $p < 0.05$. LPA, low phytic acid maize variety; HPA, high phytic acid maize variety; SC701, commercially grown white hybrid maize variety; LS8520, yellow hybrid maize variety

Significant differences ($p < 0.05$) were observed in total germination when seeds were exposed in AA vigour test for 48 hours and 72 hours (Table 3). In both instances the total germination in HPA and SC701 seeds exhibited high germination percentage while in LPA and LS8520 there were no differences ($p > 0.05$) found between these varieties at 48 hours of exposure to AA test while at 72 hours of exposure differences were observed with LS8520 having the lowest germination percentage than all other varieties. Furthermore, the germination percentage was observed to decrease with increase with time of exposure to AA vigour test from 48 hours to 72 hours. Germination percentage was observed to decrease by 4.8% in LPA, 6.5% in LS8520, 6.5% in HPA and 5.1% in SC701. There were no differences in MGT when seeds were exposed for 48 hours in AA vigour test, the differences ($p < 0.05$) were found when seeds were exposed for 72 hours with

HPA significantly ($p < 0.05$) lower MGT than LPA while in all other varieties the differences ($p > 0.05$) were not found. Contrary, the GVI of HPA and SC701, which were similar, was higher than that of LPA and LS8520, which also were similar at 48 hours of exposure to AA test. However, when seeds were exposed for 72 hours in AA test, GVI in HPA > SC701 > LPA > LS8520, respectively (Table 3).

Table 3: Total germination, mean germination time (MGT) and germination velocity index (GVI) of seed AA test exposed for 48 and 72 hours.

Genotype	48h			72h		
	Total germination (%)	MGT	GVI	Total germination (%)	MGT	GVI
LPA	71.6^{a}	4.15^{a}	17.1^{a}	66.8^{b}	5.29^{b}	17.0^{b}
HPA	81.1^{b}	3.63^{a}	27.9^{b}	74.6^{c}	3.89^{a}	24.1^{d}
SC701	78.5^{b}	3.94^{a}	23.4^{ab}	73.4^{c}	4.40^{ab}	21.0^{c}
LS8520	66.9^{a}	4.82^{a}	15.9^{a}	60.4^{a}	4.35^{ab}	13.6^{a}
CV (%)	4.60	8.50	21.1	2.90	13.8	6.20
LSD	5.28	0.52	6.85	3.07	0.95	1.81
p-value	<.001	0.089	0.008	<.001	0.046	<.001

Values within the same column sharing the same letters are not significantly different from each other at $p < 0.05$. LPA, low phytic acid maize variety; HPA, high phytic acid maize variety; SC701, commercially grown white hybrid maize variety; LS8520, yellow hybrid maize variety.

3.4.4 Correlation of traits

A positive relationship between final germination time, MGT and GVI was observed in all varieties (Figure 5). A positive weak relationship between final germination time and EC was also observed in LPA and LS8520 seeds while on the other hand a strong positive relationship was observed in HPA seeds (Figure a & d). The root: shoot (R:S) was also positively correlated to GVI in both HPA and SC701 seeds (Figure b & c).

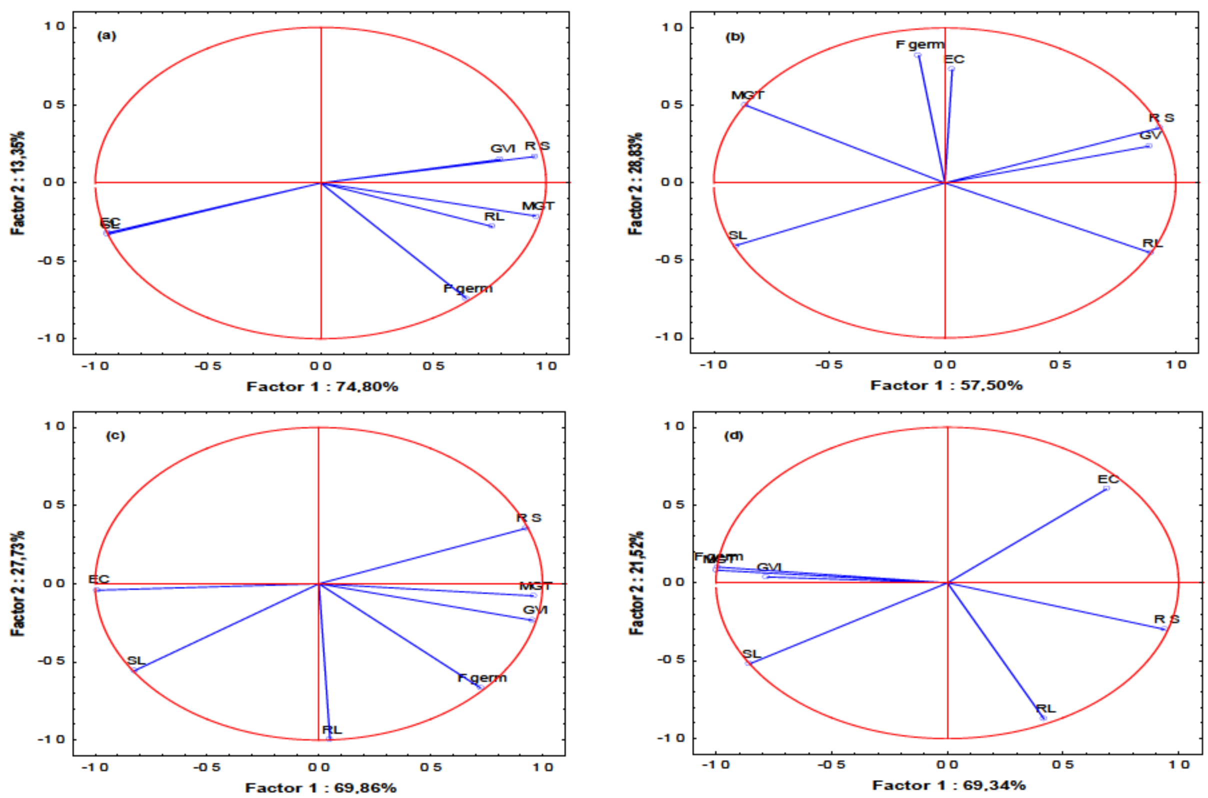

Figure 5: Principal component analysis (PCA) plots of the first two principal components (PCs) depicting relationship amongst seed quality traits and some growth parameters during standard seed germination test. Note: a = LPA, b = HPA, c = SC701 and d = LS8520 maize genotype. GVI = germination velocity index, MGT = mean germination time, F. germ = final germination, EC = electrolyte conductivity, SC = shoot length, RL = root length and R:S = root: shoot ratio.

3.5 Discussion

The objective of this study was to evaluate the seed performance of LPA maize synthetic populations with tropical genetic background as compared to HPA derived from the same population, and two commercially important white and yellow maize hybrid varieties. The results of the standard seed germination test revealed that the final germination percentage of LPA maize was similar ($p > 0.05$) to that of SC701, the high yielding commercial variety (Table 1). It was found to be 8% lower than HPA maize variety, 3% lower than the white maize (SC701) and 5% higher than yellow maize commercial variety, LS8520. On the other hand, the performance of SC701 and HPA varieties was comparable. These results were contrary to what was observed by Pilu et al. (2003) in maize mutant of temperate origin where the authors observed a 30% reduction of the germination rate compared to wild type. These results were also contradictory to those of Naidoo et al. (2012) where a 14% reduction in germination was observed when comparing LPA seeds of temperate origin with normally grown maize varieties. Phytic acid, *myo*-inositol 1,2,3,4,5,6-hexakisphosphate, is a major storage compound of P in plants predominantly accumulating in seeds (about 4-5% dry weight) and pollen (Pilu et al. 2003). In cereals crops such as maize, it is found in the aleurone grain tissue as mixed "phytate", salt of Mg and K although phytates contain other mineral cations such as Zn and Fe. Therefore, during germination, P minerals and *myo*-inositol becomes available to the growing seedlings due to the action of phytases breaking down phytates (Pilu et al. 2003, Azeke et al. 2011). Thus, these results may indicate that the combination of LPA seed lines used in this study produced seed quality parameters or phosphorus (P) reserve required for minimum seed germination that produce commercial acceptable germination and early seedling growth. For certified seed production, the germination percentage needs to be greater than 90%, therefore, the combination of these LPA maize tropical lines may be improved and used for commercial production.

GVI (the vigour test) indicates the speed in which the seeds germinate. In LPA seed lines, GVI was similar to HPA seed lines and LS8520 but significantly lower than in SC701. However, under accelerated aging condition, the final seed germination percentage was significantly reduced in LPA and LS8520 maize varieties than HPA and SC701 varieties. It declined by 18.4%, 16.9%, 14.5% and 18.1% in LPA, HPA, SC701 and LS8520 maize varieties, respectively when these seeds were exposed for 48 hours in AA vigour test. It further decreased by 23.2%, 23.1%, 19.6% and 18.1% in LPA, HPA, SC701 and LS8520 when the seeds were further exposed for 72 hours in AA vigour test. This decline was more pronounced in LS8520, which also showed lower germination, therefore, these results suggest that LPA maize lines and LS8520 are expected to show lower rate of germination while HPA and SC701 are expected to show significant increase in germination under field conditions. The implication of this is competitive advantage on exploitation of resources and the ultimate higher yield in HPA genotype and SC701. Delouche and Baskini (1973) reported that the accelerated aging test method is effective in evaluating vigour and they found that the results were highly correlated with plant growth rate and development including yield. Raboy (2000) on the other hand, reported a 5-15% yield reduction in LPA lines of temperate origin compared to the highest yielding commercial varieties. These results are in line with those of Naidoo et al. (2012) who reported a significant decline in vigour of LPA lines especially when they are under stress condition. The authors concluded that these LPA seeds underscore the challenges that are expected in deploying them in stressful conditions.

The MGT was found to be similar in all varieties. The GVI for LPA was also found to be similar in HPA and LS8520 varieties. However, the EC was found to be higher than that of HPA and SC701. These results indicate the complex interaction of seed quality parameters that determine field performance. This suggests that different measures must be interpreted with care when simulating field performance of a particular seedlot.

3.6 Conclusion

The results of this study indicated that the characteristics of LPA varieties was comparable to those of commercially produces varieties. This study suggest that the combination of LPA lines of tropical origin used in this study was satisfactory to meet the minimum seed quality parameters (with respect to seed germination and vigour) that are required for growth and development of maize.

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CHAPTER 4

Effect of exogenous phosphorus application on seed quality and yield of low phytic acid maize (Zea mays) varieties

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4.1 Abstract

Phytic acid has implications imparting vigour and enrichment of seed mineral reserves. The objective of this study was to investigate the effect of exogenous application of phosphorus on germination and yield of low phytic acid (LPA) maize synthetic populations of tropical compared to high phytic Acid (HPA) synthetic population of the same genetic background, and two commercial hybrids (SC701 and LS8520) and whether this can improve seed performance. The experiment was conducted at the University of KwaZulu-Natal controlled environment facility. The experimental design was a randomized complete block design laid in three replicates. Growth, flowering and yield parameters were measured. The harvested seeds were subjected to a germination test to measure final germination, mean germination time (MGT), germination vigour index (GVI), electrical conductivity (EC) (μS g^{-1}), root length, shoot length and the root-shoot ratio. The results of this study showed that the application of phosphorus improved the growth, flowering and yield of LPA and other varieties as well. The germination MGT, GVI, EC (μS g^{-1}), root length, shoot length and the root-shoot ratio was also improved significantly ($p < 0.05$). These differences between the traits could be helpful for the breeding program and response for LPA tropical variety it recommends to be presented for farmers as technical packages as well.

Keywords: Low phytic acid maize, high phytic maize, seed quality, germination, vigor

4.2 Introduction

Phytic acid: PA (*myo*-inositol 1,2,3,4,5,6-hexakisphosphate or Ins P_6) is a common constituent of seeds (Lehrfeld, 1994), with a significant nutritional role as the principal storage form of phosphorus (P) and metal cations in seeds (Raboy, 2001). PA is a mixture of *myo*-inositol hexakisphosphoric acid salts of cations, which can be extracted from mature seeds (Modi and Asanzi, 2008). Iwai et al. (2012) alluded that, phytic acid has a strong binding affinity to both mono and divalent cations. During the onset of seed embryogenesis, plants remobilize and transport nutrients distributed throughout the vegetative source organs to developing seeds (Martínez-Ballesta et al., 2020). Its accumulation occurs during maturation phase of the seed development, the period of cell expansion and reserve synthesis and accumulation (Lott et al., 1995). Mature seeds contain large amounts of phosphorus (P) in an organic form stored in the form of InsP6, which supports growth during the early stages of seedling establishment (Mandizvo and Odindo, 2019). During germination, phytic acid /phytate is catabolized by hydrolysis reaction catalyzed by phytase, releasing inorganic phosphates (Pi), inositol, and various minerals from the phytate (Bohn et al., 2007).

A novel role in plant seed physiology can be assigned to phytic acid, that is, protection against oxidative stress during the seed's life span. Oxidative stress affects seeds, during the last phase of maturation when seed tissues undergo desiccation accompanied by oxidative stress (Bailly 2004). In maize (*Zea mays*) seeds, Pilu et al. (2005) endowed antioxidant properties of PA against reactive oxygen species, this was consistent with the progressive decrease observed by Dorianet al. (2009) in antioxidant reserves during seed storage and ageing. Therefore, PA can prove to play a major role in maintaining seed quality by protecting embryo viability. There is an increasing interest in the measurement of antioxidants in seeds. This interest is rooted in the cumulative evidence that connects oxidative stress with premature seed aging

PA binds important mineral cations such as Fe, Zn, K, Ca, and Mg which makes them unavailable to animals consuming (Wu et al., 2009; Azeke et al., 2011). Monogastric animals, including humans, lack phytases in their digestive tract and fail to process the phytates present in seeds (Sparvoli and Cominelli, 2015). Phytases are a collective group of enzymes responsible for catalyzing the hydrolysis of PA (Affrifah et al., 2006; Secco et al., 2017). Thus, phytic acid is poorly digested and decreases the nutritional value of the seeds by limiting phosphorus and mineral bioavailability. Poor mineral bioavailability, due to high molar ratios between phytic acid and mineral cations is thought to be one of the most important causes of mineral deficiencies (mainly iron and zinc) in populations whose diet is mostly based on staple crops (Schlemmer et al., 2009; Sparvoli and Cominelli 2015; Garcia-Oliveira et al., 2018). For these reasons, therefore, phytic acid is considered an anti-nutritional factor in humans and monogastric animals (Sparvoli and Cominelli, 2015; Omosebi et al., 2018).

In recent years, there has been a concerted effort to improve the nutritional quality of maize by reducing PA content. Low phytic acid (LPA) cultivars are produced by manipulating the *MIPS* gene through an antisense approach in crops. Moreover, mutants that decrease seed PA content have been isolated and genetically mapped in maize (Shunmugam et al., 2014; Yatou et al., 2018). Sparvoli and Cominelli (2015) reported that these LPA mutations have the potential to alleviate the nutritional problems linked to PA in both humans and animal feeds (Sparvoli and Cominelli, 2015). Therefore, LPA maize may present a better nutrition for human populations that depend on grains as staple foods particularly in SSA (Shunmugam et al., 2014; Yatou et al., 2018).

Reducing phosphorus (P) content in the seeds to improve nutritional quality may affect seed quality and yield. However, the concentration of PA in a given species may vary due to many factors including soil fertility status, climatic condition and cultivar (Horvatic and Balint, 1996). For example, it has been observed that soils with high P concentration produce seeds with high PA content as compared to low P soils. Hence, P nutrition is expected to enhance soluble carbohydrate and hence, *myo*-inositol accumulation. On the other hand, preliminary investigations have shown that LPA maize varieties significantly decrease seed germination and vigour (Pilu et al., 2005; Naidoo et al., 2012) in cultivars of temperate origin. It is therefore hypothesised that increasing the rate of phosphorus application in LPA seeds can improve the germination, vigour and yield of maize. The response of tropical LPA maize to phosphorus fertilizers remains unknown.

Therefore, the objective of this study was to investigate the response of LPA maize varieties of tropical origin to exogenous phosphorus application. Secondary to this, the study evaluated seed quality, with respect to germination and vigour, from the seeds of various treatments produced from different levels of fertilizer application rates of this study.

4.3 Materials and methods

4.3.1 Plant material

Low phytic acid (LPA) and high phytic acid (HPA) seeds were sourced from African Center of Crop Improvement (ACCI). Both LPA and HPA were synthetic populations derived from second generation (F2) which were selected based on PA content. The 24 lines with low phytic acid ranging from 1.27 to 32 mg/g were allowed to randomly mate for two generations to form the LPA synthetic population. The other 51 lines, from the same tropical F2 population, with HPA ranging from 43 to 113 mg/g were recombined to form the HPA synthetic population. The hybrid seeds SC701 and LS8520R (484) which is coded in this study LS8520 were sourced from local seed company (McDonald Seeds, Pietermaritzburg, South Africa). All seeds used in this study were produced under identical production conditions and in the same growing season, thereby ensuring that the seed was of the same physiological age.

4.3.2 Phytic acid analysis assay

PA content was determined for the varieties according to the method previously described by Aina et al. (2012). Briefly, the test was conducted by preparing 25 samples from each variety. A 2 g of each sample was weighed into a 250 ml conical flask and soaked for three hours in 100 ml of 2% concentrated HCl and filtered with Whatman No.1 filter paper. The 100 ml of filtrate and 10 ml of distilled water was added in each case to provide proper acidity. 10 ml of 0.3% ammonium thiocyanate solution were added into the solution as an indicator and titrated with standard iron II chloride solution containing 0.00195 g iron/ml with the endpoint observed to be yellow which persisted for 5 min. The percentage of PA was calculated using Eq. 1.

$$\text{Phytic acid (mg/g)} = y \times 1.19 \times 100 \quad (1)$$

Where: y = titre value × 0.00195 g

4.3.3 Controlled environmental condition

The experiment was conducted at the University of KwaZulu-Natal controlled environmental facility, Pietermaritzburg, South Africa (29°35′S, 30°25′E). The day and night temperatures were 32 and 28 °C, respectively under natural day length. The relative humidity (RH) was maintained at approximately 60% throughout the growing season. The temperature and RH were monitored using a HOBO 2K logger (Onset Computer Corporation, Bourne, USA).

4.3.3.1 Experimental design and trial management

The experiment was laid in as a completely randomized design (CRD) with two treatment factors: varieties (LPA & HPA and hybrid SC701 & LS8520) and phosphorus (P) levels [18 mg/kg (residual), 26 mg/kg (optimum for maize production under selected soil) and 34 mg/kg (high)], replicated three times. Phosphorus levels, derived from superphosphate, was applied immediately before planting. The soil (clay loamy) used in the study was collected from the University of KwaZulu-Natal Research Farm (29°37'S; 30°16'E; 805 m a.s.l) and was analyzed for fertility (Table 1). The soil was fertilized based on recommendations for optimum maize production (data not shown).

Table 1: Physical and chemical properties of soil used in the controlled environmental facility

Soil characteristic	Quantity
Sample density (g/ml)	1.11
N (%)	0.19
P (mg/kg)	18.00
K (mg/kg)	185.50
Ca (mg/kg)	1281.00
Mg (mg/kg)	306.50
Exchangeable acidity (cmol/L)	0.07
Total cations (cmol/L)	9.46
Acid saturation (%)	1.00
pH (KCl)	4.63
Zn (mg/kg)	4.90
Mn (mg/kg)	72.00
Cu (mg/kg)	15.00
Organic carbon (%)	2.10
Clay (%)	35.00

4.3.3.2 Growth parameters and yield

Plant height was measured using tape measure by randomly selecting three plants from each variety. It was measured from the soil to the base of the tassel. Number of leaves were counted for leaves with at least 50% green area up until flowering. Days to tasseling were recorded as the number of days from sowing to 50% of the plant population that had tasseled. Finally, yield components were measured at harvest. The seeds were harvested at their physiological maturity.

4.3.4 Seed quality tests

4.3.4.1 Seed electrical conductivity

Electrical conductivity was assessed using a CM 100-2 single cell analyser (Reid and Associates, Durban, South Africa). Four replicates of 30 seeds per variety were weighed before soaked in 2 ml wells filled with distilled water. Electrical conductivity (EC) of the seeds was recorded over a period of 24 hours.

4.3.4.2 Standard germination test

Four replicates of 25 seeds per variety were placed in a moisture double-layered germination paper towel in a randomized complete block design (ISTA, 2012). The germination paper was rolled and tied at both ends using rubber bands and put in a plastic zip-lock bag to prevent moisture loss. Seeds were placed in a germination chamber set at 25 ± 1 °C (ISTA, 2012). Germination counts were taken daily for seven days and germination was defined as radicle protrusion of at least 2 mm. At the end of the experiment (day 7), final germination was calculated based on normal seedlings according to AOSA (1992). Measurements of root length, shoot length and root to shoot ratio were recorded. Germination indices; (i) germination velocity index (GVI), Eq. 2 (Maguire 1962) and mean germination time (MGT), Eq. 3 (Ellis and Roberts,1981) were computed.

$$GVI = G1/N1 + G2/N2 + \ldots + Gn/Nn \qquad (2)$$

Where:

GVI = germination velocity index

G1, G2…Gn = number of germinated seeds in first, second… last count.

N1, N2…Nn = number of sowing days at the first, second… last count.

$$MGT = \frac{\sum Dn}{\sum n} \quad (3)$$

Where:

MGT = mean germination time,

n = the number of seeds which were germinated on day D, and

D = number of days counted from the beginning of germination.

4.3.4.3 Accelerated aging and vigour test

Seeds were placed in a plastic box with the temperature set at 41 °C and 100% relative humidity (RH) for 48 h and 72 h following the standards set by ISTA (2012). The unaged seeds (0 days) were used as a control. The ageing chamber was a plastic box (8 cm × 8 cm) with a lid which was placed into a wire tray with a 10 cm x 10 cm x 2 cm (length x width x depth) mesh screen and the pore sizes of the mesh screen was 1.89 mm. In each accelerated aging box, 40 g of sodium chloride and 100 ml of distilled water was added and a dry screen tray was inserted to prevent splash water on the screen. After each period of accelerated aging treatment, the standard germination test was performed as described in Section 2.2.1, above.

4.4 Statistical analysis

All data collected were subjected to the analysis of variance (ANOVA) using GenStat® 18th Edition (VSN International, Hemel Hempstead, UK, 2011). Means were compared using Fischer's least significant difference (LSD) at 5% level of significance.

4.5 Results

4.5.1 Phytic acid content

Significant differences ($p < 0.05$) in PA were observed between the varieties (Table 2). The HPA maize varieties recorded the highest ($p < 0.05$) phytic acid content while LPA varieties recorded the least concentration compared to LS8520 and SC701.

Table 2: Phytic acid content of different maize seeds of LPA seeds compared to HPA, SC701 varieties.

Variety	Phytic acid (mg/g)
LPA	18.58^{a}
HPA	70.73^{d}
SC701	41.95^{c}
LS8520	32.75^{b}
CV (%)	28.00
LSD	6.45
p-value	<.001

Note: LPA = low phytic acid, HPA = high phytic acid, SC701 = white maize and LS8520 = yellow maize. Values within the same column sharing the same letter are not significant different at p < 0.05.

Table 3: Growth and yield components of LPA seeds compared to HPA, SC701 and LS8520 seeds under different P concentration.

Variety	**P-level**	**Plant height (cm)**	**Leaf number**	**Days to Tassel**	**Days to Silking**	**Days to Anthesis**	**1000 Grain mass (g)**	**Yield t/ha**
LPA	Residual	215^{a}	15.2^{ab}	65.0^{b}	72.5^{d}	71.5^{d}	205^{a}	1.70^{a}
HPA		221^{abc}	14.1^{a}	64.3^{b}	71.5^{cd}	71.5^{d}	257^{cde}	2.4^{ab}
SC701		225^{bcd}	16.3^{bc}	57.3^{a}	67.7^{bcd}	60.7^{abc}	236^{abc}	2.0^{ab}
LS8520		219^{ab}	16.0^{bc}	57.2^{a}	67.5^{bcd}	61.3^{abc}	214^{ab}	2.03^{ab}
LPA	Optimum	233^{e}	15.7^{bc}	64.1^{b}	66.9^{bc}	66.4^{cd}	233^{abc}	3.0^{abcd}
HPA		228^{cde}	15.0^{ab}	66.2^{b}	64.3^{b}	$66.9.9^{cd}$	282^{e}	2.91^{abc}
SC701		231^{de}	16.8^{c}	56.1^{a}	56.5^{a}	57.5^{ab}	248^{cd}	4.41^{cd}
LS8520		230^{de}	16.1^{bc}	57.0^{a}	54.1^{a}	57.3^{ab}	237^{bc}	3.9^{cd}
LPA	High	228^{cde}	15.5^{abc}	66.1^{b}	66.7^{bc}	64.7^{bcd}	275^{de}	3.23^{bcd}
HPA		234^{e}	15.1^{ab}	63.0^{b}	65.0^{b}	64.5^{bcd}	342^{f}	3.41^{bcd}
SC701		231^{de}	15.9^{bc}	55.3^{a}	54.2^{b}	57.0^{a}	285^{e}	4.48^{d}
LS8520		232^{de}	16.2^{bc}	54.8^{a}	55.9^{a}	56.1^{a}	258^{cde}	4.03^{cd}
$LSD_{(v \times p)}$		4.51	0.88	2.06	3.03	4.26	17.75	0.39
P(v)		0.055	<.001	<.001	<.001	<.001	<.001	<.001
(P)		0.055	<.001	<.001	<.001	<.001	<.001	<.001
(Pxv)		<.001	0.087	0.057	<.001	<.001	<.001	<.001
CV		1.2	3.4	2.0	2.8	4.0	4.1	9.4

Note: LPA = low phytic acid, HPA = high phytic acid, SC701 = white maize and LS8520 = yellow maize. Values within the same column sharing the same letters are not significant different at $p < 0.05$

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4.5.2 Growth parameters and yield

Significant differences ($p < 0.05$) within the treatment fertilizer application rate were observed in plant height, only at the residual level of P where LPA varieties had a significantly reduced plant height than SC701 varieties (Table 3). In this treatment, there were no differences in all other varieties. No significant ($p > 0.05$) differences were found at optimum and high rate of P fertilizer application rate within the treatment in plant height. However, plant height was observed to increase with increase in P application rate from residual to optimum level. In contrast, plant height did not increase ($p > 0.05$) from optimum P fertilizer application rate to higher level of P fertilizer application rate. Further, leaf numbers of HPA varieties at residual P level was found to be significant lower ($p < 0.05$) than that of other varieties. However, there were no significant differences in all other varieties in this treatment (Table 3). At optimum P level SC701 was found to have significant ($p < 0.05$) higher leaf numbers (16.8) than all other varieties. In this treatment, no significant differences ($p > 0.05$) were found in all other varieties, HPA (15.0), LPA (15.7) and LS8520 (16.1). At high rate of P application, no differences ($p > 0.05$) were found within the varieties in leaf numbers. Days to tasseling of commercial varieties (SC701 & LS8520) was significantly lesser that those of LPA and HPA varieties.

P fertilizer application rate did not influence ($p > 0.05$) days to tasseling. On average, days to tasseling of commercial varieties was 56 and that of LPA and HPA was 65. Days to silking and anthesis were influenced by phosphorus treatments within each variety. The most reduction in time to reach the flowering stage (tassel, silking and anthesis) was observed from residual P fertilizer level to optimum P level. Again, commercial varieties were found to take less time to silking and anthesis than low and PA varieties. No significant differences ($p > 0.05$) were found in grain yield at residual P fertilizer application (Table 3). However, yield was found to increase with an increase if P fertilizer application rate. At optimum P fertilizer application rate, no significant ($p > 0.05$) differences were found in grain yield in all varieties. The increase in P fertilizer application rate beyond optimum application did not significantly ($p > 0.05$) increase grain yield.

4.5.3 Standard germination test

Significant differences were not found ($p >0.05$) in seedlots of LPA, HPA and SC701 varieties at residual level of fertilizer P application rate (Table 4). Minor differences ($p > 0.05$) were only found in LS8520 where seedlot 2 was observed to be higher than seedlot 3. At the optimum level of P application, no significant differences ($p > 0.05$) were found between the seedlots of all varieties. At high level of P application, minor differences (most insignificant) were found in between the seedlots of different varieties. With the increase in P application rate, the total germination percentage was also observed to increase from residual to optimum level.

Table 4: Final germination percentage of seedlots of LPA, HPA and commercial hybrids (SC701 and LS8520) in response to P application rate.

Lots	**P Level**	**Total germination (%)**			
		LPA	**HPA**	**SC701**	**LS8520**
1	Residual	92.0^{a}	96.0^{ab}	100^{c}	95.0^{cd}
2		92.0^{a}	96.0^{ab}	100^{c}	96.0^{de}
3		93.0^{ap}	94.8^{ab}	100^{c}	94.0^{bc}
4		91.0^{a}	96.0^{ab}	100^{c}	95.0^{cd}
1	Optimum	96.0^{cd}	95.0^{ab}	100^{c}	97.1^{ef}
2		95.0^{bc}	96.0^{ab}	100^{c}	98.0^{f}
3		96.0^{cd}	95.0^{ab}	100^{c}	98.0^{f}
4		97.0^{cd}	94.0^{a}	100^{c}	97.0^{ef}
1	High	97.0^{cd}	95.0^{ab}	99^{ab}	94.0^{bc}
2		95.0^{bc}	97.0^{b}	99.5^{bc}	93.0^{ab}
3		98^{d}	95.1^{ab}	98.0^{a}	92^{a}
4		97.2^{cd}	97.0^{b}	99.0^{ab}	93.0^{ab}
$LSD_{(lot \times p)}$ *P* (lot) (P) (Pxlot) CV		2.66 0.194 <.001 0.516 2.0	2.35 0.216 0.224 0.461 1.7	0.50 0.196 <.001 0.132 0.4	1.97 0.237 <.001 0.497 1.4

Note: LPA = low phytic acid, HPA = high phytic acid, SC701 = white commercial maize variery and LS8520 = yellow commercial maize variety. Values within the same column sharing the same letters are not significant different at $p < 0.05$

Significant differences ($p < 0.05$) in germination between the varieties grown under different levels of P was observed (Fig. 1). In all P application rates, HPA and SC701 varieties showed high and fast germination rate. The opposite was observed in LS8520 variety while LPA fluctuated. Increased rate of P application was observed to increase the germination rate for LPA, especially the initial stage of germination. Further, significant differences ($p < 0.05$) were found in final germination between LPA and the varieties at residual P fertilizer application rate. LPA maize variety showed lower germination percentage than all varieties at residual P fertilizer application rate while SC701 was found to have higher germination (Table 5). The increase in P fertilizer application rate from residual to optimum level enhanced the germination percentage of LPA by 4%. There were no significant differences ($p< 0.05$) among varieties in their response to P application at optimum P application rate and high P application rate. There were no significant differences ($p < 0.05$) observed in MGT between the varieties in their response to P application (Table 5). Same trend was observed in GVI (Table 5). With increasing phosphorus application from residual P application rate to high P application rate the GVI of LPA increased (5% increase) compared to HPA, SC701 and LS8520 (Table 5).

The results showed significant differences ($p < 0.05$) on electrical conductivity (EC ($\mu S\ g^{-1}$) (Table 5). Differences in EC was only observed at the residual level of P application where LS8520 varieties had significantly higher EC than LPA and HPA varieties. No differences ($p > 0.05$) were found at optimum P level application and high P level application rates within the treatments. The increase in P application rate also did not influence ($p > 0.05$) EC. Significant differences ($p < 0.05$) were also found in the root and shoot length (Table 5). At the residual level of the P application rate, LPA varieties had a significantly ($p < 0.05$) lower root length than HPA and SC701 varieties (Table 5). At optimum P application rate, the root length of LPA varieties improved significantly (*p* > 0.05) and it was similar (*p* > 0.05) to all other varieties. With further increase in P application rate to higher level there was no differences (*p* > 0.05) between LPA, SC701 and LS8520 varieties while HPA varieties had a significantly (p > 0.05) higher root length than LPA and LS8520 varieties. No differences (*p* > 0.05) in shoot length were found at residual level of P application (Table 5). However, the differences (*p* > 0.05) were found at the optimum and higher level of P application rate. At the optimum level of P, HPA varieties were found to have a significant higher shoot length than LPA and SC701 varieties while there were no significant differences between HPA and SC701 varieties (Table 5). At a higher level of P application, similar trends as in optimum were observed. The increase in P application rate was observed to increase the also the shoot length. Finally, significant differences were not found (*p* > 0.05) within and across the treatment in root: shoot ratio (Table 5).

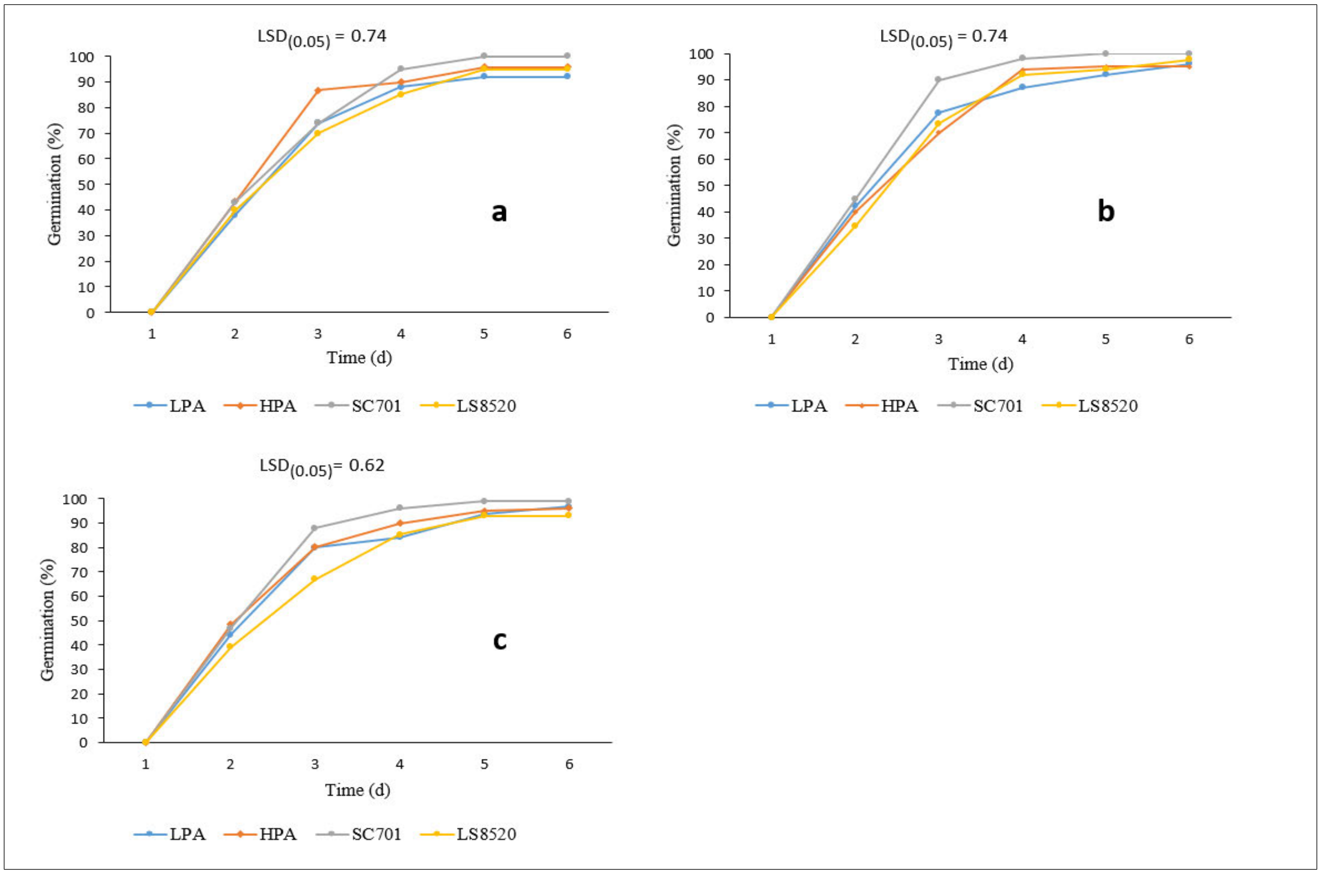

Fig. 1. Daily germination in response to P application rate of low phytic acid (LPA) as compared to HPA (high phytic acid) and two commercial hybrids, SC701 (white maize) and LS8520 (yellow maize). Note: a = reisidual P level, b = optimum P level and c = high P level

Table 5. Seed performance of LPA compared to HPA and commercial hybrids (SC701 and LS8520) in response to P application rate.

Varieties	**P (mg/kg)**	**Final germination.**	**MGT**	**GVI**	**EC (µS g^{-1})**	**Root length**	**Shoot length**	**Root: shoot**
LPA		92.^{a}	2.82^{a}	35.30^{a}	16.67^{bc}	10.6^{a}	7.62^{a}	1.39^{a}
HPA		95.7^{abc}	2.74^{a}	38.39^{abc}	12.50^{ab}	13.5^{bcd}	9.15^{abcd}	1.49^{a}
SC701	18	100^{c}	2.88^{a}	38.23^{abc}	13.17^{abc}	12.8^{bc}	8.80^{abc}	1.45^{a}
LS8520		95.0^{abc}	2.79^{a}	35.20^{a}	17.67^{c}	12.0^{ab}	7.95^{ab}	1.50^{a}
LPA		96.0^{abc}	2.81^{a}	36.40^{abc}	13.0^{abc}	12.5^{abc}	8.45^{ab}	1.49^{a}
HPA		95.0^{abc}	2.61^{a}	36.24^{abc}	10.90^{a}	14.1^{cd}	11.0^{de}	1.28^{a}
SC701		100^{c}	2.49^{a}	39.23^{bc}	10.40^{a}	13.0^{bc}	9.62^{bcd}	1.36^{a}
LS8520	26	97.7^{bc}	2.99^{a}	35.83^{ab}	12.50^{ab}	11.9^{ab}	8.27^{ab}	1.41^{a}
LPA		96.8^{abc}	2.84^{a}	37.18^{abc}	11^{a}	12.8^{bc}	9.70^{bcd}	1.33^{a}
HPA		96.0^{abc}	2.74^{a}	38.53^{abc}	9.80^{a}	15.5^{d}	11.97^{e}	1.29^{a}
SC701	34	99.0^{c}	2.66^{a}	39.66^{c}	9.0^{a}	13.5^{bcd}	10.7^{cde}	1.26^{a}
LS8520		93^{ab}	2.96^{a}	35.13^{a}	10.50^{a}	12.3^{abc}	8.60^{ab}	1.43^{a}
$LSD_{(G×P)}$		3.22	0.30	2.21	2.77	1.23	1.13	0.96
P (G)		<.001	0.035	<.001	0.001	<.001	<.001	0.330
(P)		0.172	0.506	0.268	<.001	<.001	<.001	0.034
(PxG)		0.031	0.278	0.263	0.371	0.128	0.207	0.038
CV		2.0	7.8	4.2	13.4	6.7	8.5	9.9

Note: LPA = low phytic acid, HPA = high phytic acid, SC701 = white commercial maize variery and LS8520 = yellow commercial maize variety. Values within the same column sharing the same letters are not significant different at $p < 0.05$.

4.5.4 Accelerated aging vigour test

There were significant differences ($p < 0.05$) in total germination when seeds were subjected to AA test for 48 hours and 72 hours (Table 6). After 48 hours of exposure to AA test the germination percentage decreased by 14%, 14%, 16% and 20% in LPA, HPA, SC701 and LS8520, respectively at the residual P fertilizer application rate. At optimum P application rate it decreased by 13%, 7.7%, 13% and 14% in LPA, HPA, SC701 and LS8520, respectively and at high rate of P application it decreased by 7.8%, 8%, 9% and 5.4%, respectively in LPA, HPA, SC701 and LS8520. At 48h of exposure to AA test, significant differences were found in total germination between the varieties at residual level of P application (Table 6). The total germination of LPA varieties was similar ($p > 0.05$) to that of HPA and LS8520 varieties. However, at optimum and higher level of P application, there were no significant differences ($p > 0.05$) within the treatments in total germination. The increase in P application rate was observed to improve the total germination percentage in all the varieties. Furthermore, at 72h of exposure to AA test, the total germination of LPA varieties was similar ($p > 0.05$) to that of SC701 and LS8520 at residual P level of application. In contrast, at optimum and higher level of P application rates, there were no significant differences ($p > 0.05$) within these treatments in total germination. Again as in 48h of exposure to AA test, there was an improved germination percentage with the increase in P application rate.

There were differences ($p < 0.05$) in MGT when seeds were exposed for 48 and 72 hours in the AA vigour test. In both instances the MGT decreased with an increase in the application rate of P. However, the results indicated that the MGT increased by increasing the time of exposure to AA from 48 hours to 72 hours. At the residual level, LPA had a significantly higher MGT but at high rate of P application the differences were not significant ($p > 0.05$) between the varieties. There were no significant ($p > 0.05$) differences in GVI observed between the varieties at both optimum and high P fertilizer application rates. The differences were only observed at the residual P fertilizer application rate (Table 6) where SC701 was observed to have higher GVI than other varieties.

Table 6: Total germination, mean germination time (MGT) and germination velocity index (GVI) of seed AA test exposed for 48 and 72 hours under different P concentration.

Variety	**P mg/kg**	48h			72h		
		Germination (%)	**MGT**	**GVI**	**Total germination (%)**	**MGT**	**GVI**
LPA	18	78.0^{ab}	3.38^{ef}	22.0^{ab}	74.0^{ab}	4.27^{e}	21.5^{bc}
HPA		82.0^{bc}	3.20^{cd}	25.0^{bc}	77.0^{efd}	3.77^{cd}	22.5^{cd}
SC701		84.0^{cde}	2.98^{ab}	24.2^{c}	79.0^{bcdef}	3.86^{de}	23.2^{cde}
LS8520		75.0^{a}	3.50^{f}	20.2^{a}	70.4^{a}	3.84^{d}	18.5^{a}
LPA	26	83.0^{bcd}	3.20^{cd}	26.2^{cd}	77.8^{bcd}	3.65^{cd}	23.4^{cde}
HPA		87.3^{cdef}	3.0^{a}	27.0^{cd}	78.0^{bcde}	3.54^{bcd}	23.4^{cde}
SC701		87.0^{cdef}	3.05^{bc}	27.5^{cd}	82.0^{cdef}	3.12^{ab}	24.4^{de5}
LS8520		84.0^{cde}	3.30^{de}	25.7^{cd}	76.0^{abc}	3.42^{abc}	19.9^{ab}
LPA	34	89.0^{ef}	2.90^{ab}	28.2^{cd}	84.6^{ef}	3.02^{a}	25.1^{e}
HPA		88.0^{def}	2.85^{a}	27.9^{cd}	83.0^{def}	3.41^{abc}	24.3^{de}
SC701		90.0^{f}	2.86^{a}	28.9^{d}	85.5^{ef}	3.03^{a}	25.6^{e}
LS8520		87.6^{cdef}	2.96^{a}	28.8^{d}	83.4^{def}	3.18^{ab}	23.8^{cde}
$LSD_{(v \times p)}$		3.48	0.101	2.12	3.86	0.24	1.46
P (v)		<.001	<.001	<.001	<.001	<.001	<.001
(P)		<.001	<.001	<.001	<.001	<.001	<.001
(Pxv)		0.036	<.001	<.001	0.162	<.001	0.019
CV		2.9	2.3	5.6	3.4	4.8	4.5

Note: LPA = low phytic acid, HPA = high phytic acid, SC701 = white maize and LS8520 = yellow maize. Values within the same column sharing the same letters are not significant different at $p < 0.05$

4.6 Discussion

The objective of this study was to investigate the response of low phytic maize varieties of tropical origin to exogenous phosphorus application and to evaluated seed quality, with respect to germination and vigour, from the seeds produced from different levels of fertilizer application rates of this study. The results of this study showed a significant lower plant height in low phytic varieties at the residual level of P application rate while at an optimum and higher level, there were no significant differences ($p > 0.05$) in plant height (Table 3). With the increase in P application rate, plant height of low phytic acid varieties also increased significantly from residual to the optimum rate of P application. Leaf numbers on the other hand were found to be significantly lower ($p < 0.05$) in high phytic acid varieties at the residual level of P application rate than all other varieties (Table 3). With the increase in P application from residual (18 mg/kg) to optimum (26 mg/kg) and high level (34 mg/kg) there were no significant differences in plant leaf numbers within the treatments except at the optimum level where SC701 varieties were having significantly more leaves than all other varieties. However, with the increase, in fertilizer application rate from residual to optimum, there was no significant in increase in leaf number in all the varieties. These results indicated a comparable and/ similar performance between low and high phytic acid with regards to these two growth parameters although one commercial variety, SC701, seemed to outperform the other varieties. The results further indicated that applying the optimum amount of P improved the performance of all the varieties.

The decline in days to tasselling, silking and anthesis stages (Table 3) might be due to the reason that P enhances the growth of the crop and thus providing adequate nutrients increased the life cycle of the plants. These results agree with Ali et al. (2019) and Ye et al. (2019) however, contradict with those by (Amanullah, 2015) who reported delay of maize silking due to P application. Liu et al., (2018) stated that, although flowering time and period is genetically determined it can be also affected by soil nutritional status.

For 1000 grain mass and yield (Table 3) these results agreed with Usandivaras et al. (2018), and Piergiovanni et al. (2017) and Nadeem et al., (2012) they reported that P deficiency affects grain filling. Seed mass is one of the yield components, it can be revealed that applying phosphorus affecting yield this finding supported by Imran et al. (2016) who reported growth and yield of maize genotypes were increased by the application of P treatments compared with control. Phosphorus application could enhance photosynthesis due to its role in increasing cell division and increasing leaf growth. Therefore seed P reserves can sustain the maximal growth of maize seedlings for several weeks after germination and the yield.

The findings from this study on the standard seed germination test indicate that the final germination of LPA improved with increasing P application (Table 5). Hrdličková et al. (2011) and El-Waraky. (2014) also stated that phosphorus application enhances seed germination. Phosphorus mobilization of P from soils and translocation to the plants and finally influenced total P content in seeds. P helps seeds to germinate with vigorous growth because seed phosphorus reserves were rapidly mobilized during germination and compensate the low P in LPA seeds translocated to emerging root and shoot tissues. P is an element that is part of many organic compounds building cell structures as well as stimulating growth. This indicates that total P content in seeds was highly correlated with PA, the main form of storage P and related to metabolic functions in seeds P.

Electrical conductivity results showed loss of cell membrane organization. Selective permeability, can stimulate the growth of pathogenic microorganisms and impair seed germination and seedling emergence with the consideration that P enhances and forms cellular membrane in LPA as well as in HPA, SC071 and LS8520 varieties (Table 5). These results align with Amjad et al. (2004) who is stated that, electrical conductivity of seed leachates decrease with the increasing rate of applied phosphorus although he mentioned that some studies don't agree with his findings.

The observed results in this study which were showing decreeing in MGT and increasing in GVI increased was due to P fertilizer these results are supported by Seyyedi et al. (2015). P mobilizes to improve germination aspects and therefore, P could compensate for the role of reduced P in the seed that has been reduced by breeders to improve nutritional quality.

Regarding root length and shoot length, it was observed that increasing the P from 18 mg/kg to 34 mg/kg influenced the LPA variety the most compared to other varieties (HPA, SC071 and LS8520). These findings are supported by White and Veneklaas, (2012). P allows seedlings to establish faster and ultimately produce plants with higher yields. Subjecting the studied varieties to exogenous phosphorous helps to improve seed quality of maize which contain low and high phytic acid concentration.

4.7 Conclusion

The results indicated that yield and germination of LPA improved and responded positively to P application. Therefore, that could compensate for the reduced phytic acid in tropical maize and the earliest released varieties produced by breeders to improve the nutritional quality of maize. Increasing the dose of P application improved vegetative growth and yield of LPA tropical maize. The harvested seeds of HPA tropical maize, SC701 and LS 8520 also improved in germination MGT, GVI, EC and root length, and shoot length and that reflecting responding of these varieties to P application. At 18 mg/kg LPA maintained lower yield although at the same P treatment level it was recorded to have high comparable germination. Therefore, more studies with respect to physiologic performance are recommended with emphasis on photosynthesis and water use efficiency. Since this is first reported for applied breeding of the trait in the tropical maize it is good to have such information about phosphorus application. The study can also recommend that these findings should be added to the maize technique packages especially for the farmers who planting tropical maize in SSA where they face seed quality challenges. These differences between the LPA & HPA tropical maize, SC701 and LS8520 and their response to P application could be useful for breeding programs.

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CHAPTER 5

Performance of low phytic acid maize (Zea mays L.) tropical lines under dryland field condition

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5.1 Abstract

Phytic acid (PA) is a major storage form of phosphorus in maize seeds and its reduction can negatively affect on seed germinability, crop growth, development, and yield. The objective of this study was, therefore to assess the performance of low-PA (LPA) maize cultivars in terms of growth and yield under field conditions in a dryland ecosystem. Specifically, a field experiment was conducted at the University of KwaZulu-Natal Research Farm to compare some growth and yield traits among LPA, high PA (HPA) and two commercial (SC701 and LS8520) maize cultivars. The experiment was laid out as a randomized complete block design (RCBD) with three replications over two seasons (2015/2016 and 2016/2017). The studied parameters were plant height (cm), number of leaves per plant, chlorophyll content, time to reach reproductive stage (tasselling, silking and anthesis), phytic acid content, myo-inositol and yield &yield components (ear length (cm), ear weight (g), 1000 seed mass (g) and yield (t/ha)). The results showed highly significant differences ($p \leq 0.001$) in all under-studied growth and yield traits among the cultivars. The results of this study revealed that the SC701 variety outperformed the other three cultivars in growth and yield. It was concluded that the LPA maize performed lower under field conditions therefore, there is a need to conduct more experiments to demonstrate the performance of LPA under field conditions in response to phosphorus application and water stress.

Key words: Phytic acid; LPA; Growth; Yield

5.2 Introduction

Worldwide, maize is a valuable cereal crop that is cultivated in nearly more than 160 countries (Abebe et al., 2016). It is the third most grown cereal crop after rice and wheat (Ladha et al., 2016), and is the main staple food in sub-Saharan Africa (SSA) (Nuccio et al., 2015). However, maize yield in SSA is threatened by a number of biological and environmental factors, among which soil fertility is the main constraint of the crop production in SSA (Musokwa et al., 2019). In particular, in SSA most of the maize producers are smallholder farmers who have limited access to fertilizers (Azevedo et al., 2015). The most limiting fertilizers for maize production are N and P fertilizers (Zhihui et al., 2016). The reduction in maize production due to P deficiency could worsen when seeds with low phytic acid (LPA) are grown. The PA is the storage form of P in the seeds that accounts for up to 80% of total P (Roohani et al., 2013).

On the other hand, PA is considered an anti-nutrient because of its strong ability to complex multi-charged metal ions, especially Zn, Ca and Fe (Nadeem et al., 2010; Kaplan et al., 2019). As a consequence, the consumption of enormous quantities of food that contain high PA could result in inadequate absorption of some dietary minerals. Therefore, in recent years, plant breeders have established maize improvement programs aimed at improving the crop nutritional quality by reducing seed PA content (Ekpa et al., 2018). However, the newly developed maize lines and cultivars of LPA in the seeds could have a relatively lower yield. This is because when seeds of LPA are grown, the amount of PA that undergoes digestion to release P and other cations essential for seedling growth and crop establishment is not sufficient (Azeke et al., 2011).

Moreover, Lott et al., (2017) mentioned that PA plays a key role as a source of the seed myo-inositol, phosphate, and cations. Myo-inositol is a precursor for the production of stachyose and verbascose and storage carbohydrates enzymes that are associated with membrane synthesis during temperature stress by maintaining cell wall integrity in seeds and vegetative tissues (Peterbauer et al., 2001). The integrity of the cell membrane minimizes the loss of cations during seed germination. Also, PA controls the inorganic phosphate homeostasis in both germinating seeds and developing seedlings (Raboy, 1997). On the other hand, hydrolysis of PA during germination is accompanying by the release of inositol, phosphate, and cations, which are mobilized to the growing seedlings to support anabolic metabolism (Hegeman et al., 2001). Modi and Asanzi (2008) stated that high myo-inositol concentrations may be used to indicate poor phytate synthesis associated with poor seed germination and vigour. In this context, Raboy (2000) developed new maize mutants (lpa1-1) with reduced seed PA concentration of 1.1 mg/g to improve the bio-availability of P, Fe, and Zn during seed germination and plant growth and development. However, their study found that the yield of the newly developed mutants was relatively lower than that of commercial maize cultivars of HPA concentrations. In other studies, Naidoo et al. (2012) and Modi and Asanzi (2008) compared the performance of a maize cultivar of high amounts of lysine and tryptophan amino acids with a cultivar of HPA, high mineral phosphorus and lower levels of myo-inositol. These studies found that the cultivar of higher amino acids concentration had higher germination percentage and more vigorous seedlings as opposed to the HPA maize cultivar. This finding needs further investigation as to whether maize cultivars of HPA could affect seeds germination and crop yield in comparison with the commercially grown ones. In this regard, the newly produced maize inbred lines and cultivars of different PA concentrations like the ones produced by the University of KwaZulu-Natal's (UKZN) African Centre for Crop Improvement

(ACCI) breeding program should be tested for their germination rate and productivity. In specific, the present study aimed to test the newly produced ACCI's two genetically synthetic maize populations of LPA and HPA and compare them with the commercial tropical maize cultivars that are commonly known white and yellow maize for their germination, growth, yield and its components.

5.3 Materials and methods

5.3.1 Plant materials

The planting materials of this study consisted of four maize cultivars viz., LPA, HPA, white maize (SC701) and yellow maize (LS8520R (484)). The LPA and HPA cultivars were sourced from the University of KwaZulu-Natal's ACCI breeding program and selected on the basis of their PA concentration. The LPA and HPA seeds used in this study were produced by randomly crossing 24 inbred lines from each population for two generations to produce LPA and HPA synthetic populations. The PA concentration ranged from 1.27 to 32 mg/g and 43 to 113 mg/g in the LPA and HPA varieties, respectively. On the other hand, the other two white (SC701) and yellow (LS 8520 R (484), which is herein named (LS 8520) commercial maize cultivars were obtained from a local seed company (i.e. McDonald Seeds, Pietermaritzburg, South Africa). To ensure that the plant materials of the same physiological age, all seeds for each variety were harvested from maize crops that were grown under the same conditions and growing season.

5.3.2 Experimental site and field layout

A field experiment was conducted at the University of KwaZulu- in Pietermaritzburg South Africa for two consecutive seasons (i.e. 2015/2016 and 2016/2017), Ukulinga Research farm (29°37' S 30°16' E) under dryland conditions. The mean monthly rainfall and temperature at the Ukulinga Research Farm during the experimental periods are presented in Table 1. The experiment was laid out in a randomized complete block design (RCBD) with three replicates. Each replicate consisted of four experimental units (plots) which were randomly assigned to one of the four cultivars. The plots which were 16 m^2 each consisted of four rows with a space of 0.7 m between them.

Table 1: Mean monthly rainfall and temperature received during the experimental periods for the two consecutive growing seasons (2015/2016 and2016/2017).

Month	2015/2016		2016/2017	
	Rainfall (mm)	Temperature (ºC)	Rainfall (mm)	Temperature (ºC)
December	3.72	22.61	1.08	22.39
January	4.00	22.32	2.43	22.09
February	2.47	23.48	4.11	22.66
March	1.65	22.68	0.90	22.19
April	0.36	21.19	1.14	19.59

5.3.3 Agronomic practices

The experimental field was disc plowed, disc harrowed, leveled, ridged at 70 cm space and divided into plots of the same area as previously mentioned. Three seeds for each understudied maize variety were sown in holes at the western side of the ridges, 20 cm apart and then thinned to one plant after two weeks thereafter. The sowing dates were 05/12/2015 and 10/12/2016. Fertilizer application was performed based on soil analysis recommendations of 20 kg phosphorus (P) per hectare in form of tri-superphosphate at planting and 200 kg nitrogen (N) per hectare in form of urea (46% N) to split into two doses, the first dose immediately after planting and the second dose after four weeks of crop establishment. Weeding was done manually after14 days of sowing and 30 days.

5.3.4 Data collection

5.3.4.1 Growth parameters

Five plants were randomly selected from each variety for each replicate to measure plant height (cm), number of leaves per plant and chlorophyll content index. Plant height was measured using a measuring tape, while the number of leaves per plant was physically counted. On the other hand, the chlorophyll content was estimated on fully exposed and fully matured leaf using a handheld chlorophyll meter devise (CCM-200 plus) that measures chlorophyll content index. These parameters, with an exception of chlorophyll content, were measured after 30 days from planting and every 30 days thereafter till the harvesting date (i.e. at 120 days). The measurements of each parameter were averaged across the five randomly selected plant samples during each sampling occasion.

5.3.4.2 Reproductive stage

Following an expert knowledge, three crop reproductive indicators were estimated: (1) time (days) to tasseling (TTT), which was counted as the number of days from sowing to when 50% of the plants had tasseled, (2) time (days) to silking (TTS), which was counted as the number of days from sowing to when 50% of the plants reached the silking stage, and (3) time (days) to anthesis (TTA), which was counted as the number of days from sowing to when 50% of the plants reached the anthesis stage.

5.3.4.3 Phytic acid and myo-inositol concentrations (mg g $^{-1}$)

Five plants from each variety for each replicate were randomly selected and tagged to weekly measure phytic acid and myo-inositol concentrations (mg g $^{-1}$) during seed development that is between 86 and 135 days after planting. Therefore, from each plant 20 seeds were harvested weekly dried and ground to prepare three samples for each parameter. The phytic acid concentration was estimated following the procedure described by Aina et al. (2012). Firstly, 2.0 g from each sample was weighed and added to a 250 mL. Secondly, 100 mL HCl of 2% concentration was utilized to douse the samples for three hours and then sifted with a Whitman. 1 paper. Thirdly, 50 cm^3 of the filtrate and 10 cm^3 of distilled water were added to each sample. Fourthly, 10 mL of 0.3% ammonium thiocyanate solution was added into the solution of each sample and titrated with the standard Iron II Chloride (0.00195 g Iron/mL) with yellow color observed as titration endpoint. Fifthly, phytic acid was calculated using Eq. 1.

Phytic acid % = $y \times 1.19 \times 100$ ……………………………………………………………. (1)

Where:

y = titre value × 0.00195 g

With regards to myo-inositol measurements, briefly the seed samples were freezed dried to prepare samples of 50 mg each. The samples were ground and dissolved in 5 mL of 80% ethanol, and centrifuged (11500 g) after heating them for 15 minutes at 80°C. Following the protocol described by Modi and Asanzi, (2008), Myo-inositol was estimated using co-chromatography with a standard (Sigma, St. Louis, MO).

5.3.4.5 Yield and yield components

The same five randomly selected plants were used to measure four yield components at harvesting time. These yield components were maize ear length (cm), ear weight (g), 1000 seed mass (g) and yield (t ha^{-1}).

5.3.4.6 Statistical analysis

Analysis of variance (ANOVA) was performed on the data using GenStat® 18^{th} Edition (VSN International, Hemel Hempstead, UK, 2011). Means were then separated using Fischer's least significant difference (LSD) test at the 5% level of significance.

5.4. Results

5.4.1 Growth parameters

5.4.1.1 Plant height (cm)

In general, the results indicated significantly ($p \leq 0.05$) shorter LPA plants during all sampling dates (Table 2), except when the crop was two months old in the first season (2015/2016). While in the second season (2016/2017), the results showed no significant differences ($p \geq 0.05$) in plant height among the understudied cultivars during 30 and 60 days sampling occasions, however, when the crop was four months old where SC701 and LS8520 recorded significantly ($p \leq 0.05$) taller plants (201.7 cm) and (213.4 cm) , respectively (Table 2).

Table 2: Plant height (cm) of LPA compared to HPA and commercial hybrids (SC701 and LS8520) under dryland field condition.

Variety	**Season**	**Plant height (cm)**			
		30	**60**	**90**	**120**
LPA		52.15^{abc}	105.0^{b}	154^{ab}	206.3^{abc}
HPA	Season 2015/2016	52.29^{abc}	94.44^{ab}	160.5^{bc}	210.2^{abcd}
SC701		57.33^{c}	102.07^{ab}	164.0^{c}	221.0^{d}
LS8520		56.71^{bc}	91.17^{ab}	157^{bc}	202.0^{ab}
LPA		46.85^{a}	85.61^{a}	146^{a}	198.0^{a}
HPA	Season 2015/2016	51.87^{abc}	90.93^{ab}	157.9^{bc}	198.3^{a}
SC701		51.12^{abc}	93.43^{ab}	157.6^{bc}	217.7^{cd}
LS8520		50.12^{ab}	93.07^{ab}	152.3^{ab}	213.4^{bcd}
$LSD_{(V\times S)}$		4.29	10.93	9.19	13.63
P(V)		0.303	0.413	0.022	0.002
(S)		<.001	0.005	0.020	0.145
(VxS)		0.014	0.111	0.495	0.942
CV		4.70	6.70	3.40	3.80

Note: HPA = high phytic acid, SC701 = white maize and LS8520 = yellow maize. Values within the same column sharing the same letters are not significant different at $p < 0.05$

5.4.1.2 Number of leaves per plant

There were significant differences ($p < 0.05$) among the cultivars on number of leaves per plant (Table 3). In both seasons the results indicated that the LPA variety consistently had a smaller number of leaves per plant at all sampling occasions in the two seasons (Table 3). Among the other three cultivars, namely, HPA, SC701 and LS8520, SC701consistently recorded a higher number of leaves followed by HPA and LS8520, respectively at all sampling periods in the two seasons, except when the crop was 60 days of age (Table 2).

Table 3: Number of leaves of LPA compared to HPA and commercial hybrids (SC701 and LS8520) under dryland field condition

Variety	**Season**	**Number of leaves/plant**			
		30	**60**	**90**	**120**
LPA		6.66^{a}	9.6^{a}	11.8^{b}	13.3^{b}
HPA	Season 2015/2016	7.87^{c}	10.7^{c}	12.9^{d}	14.4^{d}
SC701		8.66^{d}	11.7^{d}	13.4^{e}	14.9^{e}
LS8520		7.40^{b}	10.5^{cd}	12.4^{c}	13.8^{c}
LPA		9.33^{e}	9.3^{a}	11.3^{a}	12.6^{a}
HPA	Season 2015/2016	10.7^{g}	11.8^{d}	12.5^{cd}	13.4^{b}
SC701		11.8^{h}	10.73^{c}	13.4^{e}	15.3^{f}
LS8520		10.0^{f}	10.0^{b}	11.8^{b}	12.8^{c}
$LSD_{(V\times S)}$		0.44	0.38	0.41	0.27
P(V)		<.001	<.001	<.001	<.001
(S)		<.001	0.87	0.002	<.001
(VxS)		0.212	0.86	0.107	<.001
CV		2.80	2.10	1.90	1.10

Note: HPA = high phytic acid, SC701 = white maize and LS8520 = yellow maize. Values within the same column sharing the same letters are not significant different at $p < 0.05$

5.4.1.3 Chlorophyll content

Like for the other growth parameters, the LPA variety recorded significantly ($p \leq 0.05$) lower chlorophyll content as compared to the other three cultivars at the two crop ages (30 and 60 days) in the two seasons (Table 4). Likewise, the SC701 variety had significantly ($p \leq 0.05$) highest chlorophyll content in the two seasons followed by HPA and LS8520, respectively. Nonetheless, the differences among HPA and LS8520 were not significant ($p \geq 0.05$) at 60 days period in both seasons (2015/2016) and (2016/2017).

Table 4 Chlorophyll content of LPA compared to HPA and commercial hybrids (SC701 and LS8520) under dryland field condition.

Variety	**Season**	**Chlorophyll content over days**	
		30	**60**
LPA		33.7^{a}	28.6^{a}
HPA	Season 2015/2016	47.1^{c}	37.5^{b}
SC701		57.8^{d}	44.6^{c}
LS8520		41.1^{b}	33.9^{b}
LPA		35.8^{a}	27.0^{a}
HPA	Season 2015/2016	48.1^{c}	37.6^{b}
SC701		55.2^{d}	42.0^{c}
LS8520		42.4^{b}	34.3^{b}
$LSD_{(V\times S)}$		4.47	3.78
P(V)		<.001	<.001
(S)		0.683	0.318
(VxS)		0.415	0.574
CV		5.70	6.10

Note: HPA = high phytic acid, SC701 = white maize and LS8520 = yellow maize. Values within the same column sharing the same letter are not significant different at $p < 0.05$.

5.4.2 Reproductive stage: time (days) to tassel, silking and anthesis

Table (5) shows that the LPA variety revealed significantly ($p \leq 0.05$) higher number of days to all studied reproductive stages in the two seasons. Regarding the other three cultivars, the HPA variety recorded significantly ($p \leq 0.05$) fewer number of days to the reproductive stages as opposed to SC701 and LS8520 cultivars in both seasons, notwithstanding the difference in days to tassel in the second season (2016/2017) between HPA and SC701 was not significant ($p \geq 0.05$). Furthermore, the differences in days to silking and anthesis stages between SC701 and LS8520 were not significant in both seasons, except for anthesis in the first season.

Table 5: Time to Tassel, Silking and Anthesis (days) of LPA compared to HPA and commercial hybrids (SC701 and LS8520) under dryland field condition.

Variety	**Season**	**Time to Tassel, Silking and Anthesis (days)**		
		Tassel	**Silking**	**Anthesis**
LPA	Season 2015/2016	64.0^{d}	71.2^{d}	69.8^{d}
HPA		52.6^{a}	60.0^{a}	58.6^{a}
SC701		56.0^{b}	66.4^{c}	65.7^{c}
LS8520		59.0^{c}	65.3^{bc}	63.3^{b}
LPA		63.0^{d}	70.5^{d}	69.8^{d}
HPA	Season 2015/2016	52.6^{a}	59.8^{a}	56.93^{a}
SC701		55.3^{ab}	63.9^{b}	61.8^{b}
LS8520		59.0^{c}	65.1^{b}	62.9^{b}
$LSD_{(V\times S)}$		2.73	1.67	2.14
P(V)		<.001	<.001	<.001
(S)		0.528	0.043	0.009
(VxS)		0.928	0.167	0.068
CV		3.70	1.50	2.0

Note: HPA = high phytic acid, SC701 = white maize and LS8520 = yellow maize. Values within the same column sharing the same letter are not significant different at $p < 0.05$.

5.4.3 Phytic acid content (mg g $^{-1}$)

Generally, PA concentration increased as seeds were developing in all studied maize cultivars (Table 6). The LPA variety yielded significantly ($p \leq 0.05$) lower PA compared to the HPA and SC701 cultivars during all sampling occasions (Table 6). At 135 days (fully seed maturity), the LPA variety recorded lower PA by 82%, 62% and 65% than HPA, SC701 and LS8520, respectively. Likewise, the HPA variety produced significantly ($p \leq 0.05$) higher PA in comparison to SC701. But no significant differences ($p \leq 0.05$) recorded in PA between SC701 and LS8520 when the crop was older than 100 days. Moreover, the results showed relatively higher CV%, ranged between 11.3% and 24.1% that indicating dispersed PA estimates during all sampling periods.

Table 6: Phytic acid content (mg g $^{-}$1) of LPA compared to HPA and commercial hybrids (SC701 and LS8520) under dryland field condition during seed development.

Variety	**Phytic acid over days after planting during seed development mg g $^{-1}$**							
	86	**93**	**100**	**107**	**114**	**121**	**128**	**135**
LPA	1.73^{a}	3.70^{a}	8.20^{a}	9.00^{a}	11.60^{a}	12^{a}	14.94^{a}	18.42^{a}
HPA	15.88^{c}	21.06^{d}	35.16^{c}	51.24^{c}	53.30^{c}	71.85^{c}	93.13^{c}	102.54^{c}
SC701	9.79^{b}	13.68^{c}	35.16^{d}	36.73^{b}	38.56^{b}	44.13^{b}	46.50^{b}	48.73^{b}
LS8520	4.96^{a}	8.83^{b}	15.16^{b}	31.40^{b}	33.50^{b}	35.60^{b}	37.64^{b}	52.77^{b}
CV%	24.1	15.5	11.3	17.0	16.0	11.6	17.8	16.5
LSD	2.59	2.46	2.97	7.33	7.40	6.36	11.47	12.33
P-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Note: HPA = high phytic acid, SC701 = white maize and LS8520 = yellow maize. Values within the same column sharing the same letter are not significant different at $p < 0.05$.

5.4.4 Myo-inositol (mg g $^{-1}$)

Unlike the PA, myo-inositol decreased as seeds were developing in all studied maize cultivars. The LPA variety recorded significantly ($p \leq 0.05$) higher myo-inositol as opposed to the HPA variety during all sampling times (Table 7), except at the first sampling time. There was no consistent trend in the differences among the LPA, SC701 and LS8520 cultivars. Also, during almost all sampling times the HPA variety recorded significantly lower myo-inositol compared to SC701 and LS8520.

Table 7: Myo-inositol content (mg g $^{-1}$) of LPA compared to HPA and commercial hybrids (SC701 and LS8520) under dryland field condition during seed development.

Variety	**myo-inositol over days after planting during seed development mg g $^{-1}$**							
	86	**93**	**100**	**107**	**114**	**121**	**128**	**135**
LPA	1.38^{b}	1.26^{b}	1.26^{c}	1.16^{d}	1.06^{c}	0.98^{d}	0.94^{d}	0.85^{c}
HPA	0.58^{a}	0.47^{a}	0.44^{a}	0.42^{a}	0.40^{a}	0.37^{a}	0.27^{a}	0.19^{a}
SC701	0.65^{a}	0.59^{a}	0.58^{b}	0.56^{b}	0.53^{b}	0.48^{b}	0.41^{b}	0.39^{b}
LS8520	1.26^{b}	1.19^{b}	1.18^{c}	1.04^{c}	0.98^{c}	0.91^{c}	0.83^{c}	0.77^{c}
CV%	6.90	8.80	8.20	12.2	9.50	5.20	6.50	15.9
LSD	0.09	0.10	0.09	0.12	0.09	0.04	0.05	0.11
P-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Note: HPA = high phytic acid, SC701 = white maize and LS8520 = yellow maize. Values within the same column sharing the same letter are not significant different at $p < 0.05$.

5.4.5 Yield and yield components

The study found that the LPA variety underperformed all other understudied cultivars in terms of yield and yield components (Table 8) in the two seasons. The yield of LPA was lower than HPA, SC701 and LS8520 by 28%, 52% and 40%, and 28%, 57% and 45% in the first (2015/2016) and second (2016/2017) seasons, respectively. On the other hand, the SC701 variety outperformed the other three cultivars in yield and yield components in both seasons, but the significant differences ($p \leq 0.05$) were mostly observed when it was compared with the LPA and LS8520 (Table 8). Furthermore, the yield and yield components were estimated with reasonably good precision as measured by the low CV% that ranged between 4.20% and 7.0% for all yield components and yield as shown in Table (8).

Table 8. Yield ($t\ ha^{-1}$) and yield components (ear length (cm), ear weight (g), 1000 seed mass (g)) of LPA compared to HPA and commercial hybrids (SC701 and LS8520) under dryland field condition.

Variety	Season	Ear length (cm), ear weight (g), 1000 seed mass (g) and yield ($t\ ha^{-1}$)			
		Ear length (cm)	**Ear weight (g)**	**1000 seed mass (g)**	**yield (t/ha)**
LPA		9.60^{a}	136^{a}	189.6^{a}	1.73^{a}
HPA	Season	14.8^{de}	229^{cd}	220.7^{b}	2.4^{b}
SC701	2015/2016	13.3^{c}	222^{c}	249.3^{c}	3.6^{d}
LS8520		11.1^{b}	185^{b}	190^{a}	2.86^{c}
LPA		9.33^{a}	134^{a}	190^{a}	1.6^{a}
HPA	Season	15.37^{e}	246^{d}	209^{b}	2.2^{b}
SC701	2015/2016	13.8^{cd}	235^{cd}	240^{c}	3.7^{d}
LS8520		11.5^{b}	177^{b}	191.3^{a}	2.9^{c}
$LSD_{(V \times S)}$		1.493	22.67	15.29	0.30
P(V)		<.001	<.001	<.001	<.001
(S)		0.446	0.372	0.199	0.937
(VxS)		0.840	0.341	0.506	0.394
CV		7.0	6.70	4.20	6.70

Note: HPA = high phytic acid, SC701 = white maize and LS8520 = yellow maize. Values within the same column sharing the same letter are not significant different at $p < 0.05$.

5.5 Discussion

The objective of this study was to evaluate and predict the growth and yield of low phytic acid tropical maize under drylands field conditions compared to HPA tropical maize and two commercial maize varieties (SC701 and LS8520). The differences between the cultivars in plant height (Table 2) can be attributed to the amount of PA concentration in the seeds (P source) and the genetic variation of these cultivars. P is an essential element or nutrient and it is the main component of nucleoprotein. It is vital to cells, especially during cell division and reproduction. Similar findings were made by Khan et al. (2014) were LPA cultivars of temperate origin underperformed when compared to commercial varieties. During the grain filling stage, phosphorus stored in the form of phytic acid and then regularly recycled to support the subsequent growth of the plant and developing grains, especially under P deficiency (Wang and Ning, 2019). Therefore the height of LPA was shorter when compared to HPA, SC701 and LS8520 in both seasons. This is in line with the findings of Asanzi (2006) on the normal maize where the author found higher plant height than quality protein maize, the maize that has been breed to produce high proteins. Phytic acid within the seed is usually hydrolyzed by the activity of phytase enzyme thereby releasing phosphorus which helps to improve root growth and the overall plant growth (Masood et al., 2011).

The number of leaves per plant was affected significantly by the variety (Table 3). The observed results from the two seasons (Table 3) on leaf number may be due to the amount of rain which was reported to be lower in the second season. From the result, it can be stated that LPA tropical maize produced a lower number of leaves due to its response to accumulated phosphorus in the seeds. Asanzi, (2006) reported in their study that quality protein maize had a lower number of leaves. The findings in this study are supported by (Cakmak, 2008; Shunmugam et al., 2014; Lemmens et al., 2018and) where they stated that endogenous phytase enzymes break down phytate during seed germination and release its phosphorus, myo-inositol, and mineral contents for the use of the growing seedling and plant growth generally. Plant growth and the number of leaves are usually affected by the reduction in P contents of seeds (Plénet et al., 2000; Julia et al., 2018).

The chlorophyll content is an essential indicator of crop growth. Chlorophyll content within the studied cultivars (Table 4) can be associated with the availability of PA content in the seeds. The HPA tropical maize obtained the highest means values compare to LPA, and these findings agreed with the earlier reports of Zhihui et al. (2016) as they stated P that comes from phytic acid in the seeds improved the chlorophyll content of leaves, growth, and metabolism of plants thereby exhibiting a significant positive correlation with grain yield. P deficient plants usually contain lower chlorophyll content and this eventually leads to poor yield (Ca et al., 2012). Therefore, LPA tropical maize with a low concentration of seed stored PA is likely deficient in elemental phosphorus. This shows that phosphorus deficiency affects the growth of LPA tropical maize through its effect on LPA tropical maize chlorophyll content Plénet et al., (2000).

In addition to the above mentioned in regards to the performance of tropical maize, the study revealed by Betran et al. (2003) stated tropical maize able to tolerate drought in addition enhanced growth and development of tropical maize can be achieved if maize grown based on proper sustainable farming systems.

The obtained differences (Table 5) can be attributed to the amount of phytic acid within the seeds whereas, the cultivars with higher phytic acid concentration (HPA, SC701) reached the reproductive stage earlier (Tassel, Silking and Anthesis) in both seasons (Table 6). During these stages, the inner seed P enhanced seed germination and seedling growth. This is in agreement with the earlier findings of Chen et al. (2019) where rapid growth and flowering of certain plant cultivars were attributed to P content in their seeds. Simillarly, Sangoi and Salvador, (1998) reported a strong positive correlation between filling period duration and final yield. Furthermore supported studies reported by White et al. (2011) mentioned that tropical maize flower later than temperate maize when they both exposed to drought stress.

In the obtained results (Table 6) the differences in PA concentrations between the cultivars could be attributed to the genetic diversity between these cultivars since LPA and HPA were derived from the scene tropical population and were selected based on their PA concentration. Both cultivars were allowed to random mate for generations to form the LPA and HPA genetic synthetic population. Phytic acid concentration is considered one of the most important factors that assist in the differentiation of varieties of the same crop Raboy et al. (2000). Phytate accumulation in seeds generally occurs about the same time food reserves are built up (Lott et al., 2017). During seed filling, although the PA acid content varies among the four varieties interestingly, the PA concentration in all the varieties increased with seed development and maturation. This agreed with the findings of Gupta et al. (2015) where phytate rapidly accumulated in seeds during the ripening period.

The obtained results for myo-inositol (Table 7) during seed formation and development correlates with phytate accumulation supported reported by Raboy, (1997). The reduction in myo-inositol concentration during seed maturation indicates that myo-inositol plays a vital role in the regulation and synthesis of phytic acid (Modi, 2002). LPA with lower PA (Table 7), displayed significantly higher myo-inositol concentrations compared with HPA, SC701 and LS8520. This further elucidates the myo-inositol utilization efficiency of LPA and suggest that LPA is comparatively a poor variety in myo-inositol utility. The higher amount of PA in HPA and normal maize (LS8520) agreed with the study of Modi and Asanzi, (2008) where the normal maize which had high PA concentration had a low concentration of myo-inositol compared with quality protein maize.

The differences in yield and yield component (Table 8) are in line with White et al. (2011) as mentioned yield of tropical maize affected by drought stress however, crossing and combining the temperate maize with tropical maize together improves the yield of tropical maize under dry conditions. The differences could also be related to either the gene background of these cultivars and this agreed with Sebetha et al. (2015) or to the PA content since the phosphorus in seeds plays a principal role in establishing crops. Hence, it is expected that the growth of LPA tropical maize will be affected by a low amount of PA which in turn influences crop yield. This finding is supported by the findings of Naidoo et al., (2012) when they compared normal maize with quality protein maize. The low concentration of PA influenced the ear length and weight, 1000 grain mass and yield of LPA compared to other varieties (Table 8). The increase in the number of grains per cob ultimately leads to a direct effect on grain yield (Masood et al., 2011). Guttieri et al., (2006) reported that the grain yield of LPA varieties was reduced to 20 -24% in the high yield environment. Still, more studies should be conducted because all LPA mutant lines showed varying degrees of reduction in grain yield and seed viability, but the negative effects on yield could be improved by further breeding (Zhao et al., 2008). Therefore, the high yield in normal maize (SC701) and HPA and LS8520 compared to LPA could also be related to vigour of their seeds.

5.6 Conclusion

From the conducted experiment, the results showed that there were significant differences in all targeted parameters especially the yield and yield components for the four different cultivars. More studies should be conducted to address the consequences of reducing PA concentration based on seed vigour tests besides the performance of LPA tropical maize varieties under drought stress, also a different regime of fertilizer studies need to be conducted. Tropical maize could be a recommended variety for farmers in dryland areas with normal maize varieties but the varieties of tropical maize should be selected based on their PA concentration. The differences between the studied cultivars could be beneficial for breeding programs to improve the yield of low phytic varieties acid as they improve the nutritional value.

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CHAPTER 6

Photosynthetic efficiency and yield responses of low and high phytic acid maize (Zea mays) tropical lines to deficit irrigation

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6.1 Abstract

Low phytic acid (LPA) maize varieties have been recently developed to improve the nutritional quality of maize by reducing the concentration of PA in seed grain thus reducing the binding of nutritionally important minerals while improving the bio availability of phosphorus. Although the development of these varieties has brought so many benefits in terms of increasing nutritional quality of maize grain, the agronomic performance with regard to their response to water stress is still unknown. This is essential because these lines were developed in tropical with adequate rainfall. An experimental study was conducted in a controlled environment, at the University of KwaZulu-Natal, South Africa, to evaluate the effects of deficit irrigation on photosynthetic efficiency and yield performance of LPA in comparison with high phytic acid (HPA) and a maize hybrid (SC701). The study was laid out in a complete randomised designed arranged in a factorial structure. The experiment consists of two factors (variety and water stress level) at three levels and each treatment was replicated 3 times. The results from this study revealed significant ($P< 0.001$) differences among the varieties for both normal and water stress conditions with regard leaf gas exchange, chlorophyll fluorescence parameters and yield and yield components. LPA maize varieties recorded higher values of stomatal conductance (*gs*) and transpiration rate (*T*) compared to HPA and SC701 for both normal and water stress conditions. The maximum value of (0.4 mmol m^2s^{-1}) was recorded at flowering stage and the value of (2.9 mmol H_2O m^2s^{-1}) at seedling stage for *gs* and *T*, respectively. However, HPA showed a significantly ($P< 0.001$) higher value of photosynthetic rate (*A*) than LPA and SC701 for all the water stress treatments. The highest value (73.3 µmol CO_2 m^2s^{-1}) was recorded at flowering stage and LPA recorded the lowest value (24.67 µmol CO_2 m^2s^{-1}) at grain filling. Similar pattern was observed for intercellular carbon dioxide (C_i) and the ratio of internal carbon dioxide to atmospheric carbon dioxide (C_i/C_a). Significant (P<

0.05) differences were also observed on leaf chlorophyll fluorescence parameters except for effective quantum efficiency of photosystem II photochemistry (ΦPSII). LPA showed higher values of (Fv/Fm) with a maximum value of (0.7250) relative to HPA (0.4253) and SC701 (0.4463) and these varieties maintained a similar pattern under both normal and water stress conditions. However, HPA maize varieties showed increased values of photochemical quenching (qP), non-photochemical quenching (qN) and electron transport rate (ETR). With regard to yield, HPA showed increased performance in terms of overall yield and seed weight, and this suggest that HPA variety are less sensitive to water stress than LPA maize varieties. Therefore, the results from this study have proven that LPA maize varieties are sensitive to limited conditions and further research under a wide range of environmental conditions is required.

Keywords: Low phytic acid; Maize varieties; Water stress; photosynthetic efficiency

6.2 Introduction

Maize (*Zea mays* L.) is the third largest cereal crop and the major staple food for an estimated 50% of the population in Sub-Saharan Africa (SSA). The crop provides about 50% of the nutritional requirements and is considered the main source of food for people living in this region (FAO, 2010). However, several issues regarding the grain's nutritional quality have been a major concern which influenced the need for breeding of high-quality maize genotypes. This include the development of high-quality protein maize (QPM) and Low phytic acid: LPA (hexa and penta phosphate) maize genotypes, to increase their nutritional benefits (Shawa, 2019). Phytic acid, which is a dominant form of P storage in seeds is known to be responsible for binding nutritionally important minerals such as Za, Ca and Fe thus affecting their bioavailability in gastro intestinal tract of humans and monogastric animals (Aydemir et al., 2006; Hambidge et al., 2011). The lack of phytase enzymes in most of the digestive systems of these animals is known to be the main contributing factor for the excretion of PA by these animals resulting to high phosphorous pollution in the environment.

One approach used to curb the release of too much P in the environment is to improve its bioavailability during digestion. This can be achieved by the development of low phytic cereal grains genotypes using molecular genetics (Raboy et al., 2000). Several studies have shown that substituting normal phytic acid cereal grains (maize, barley and rice), with low phytic acid type as an animal feed resulted in reduced levels of phosphorus derived from poultry and swine excreta (Ertl et al., 1998; Raboy et al 2000). In addition, the accumulation of Ca and P in bones and blood of chickens fed with low phytic acid grains were reported to be higher than those in normal grains (Hill et al., 2009). The increased in mineral availability could be attributed to reduced chelation caused by low phytic acid content in the feed grains (Perera et al., 2018).

Despite ongoing breeding efforts to develop LPA acid cultivars, the performance of these genotypes under diverse environments with limited resources is not well documented, and their effect on agronomic, and physiological characteristics need to be studied further. This will encourage the widespread production of these genotypes and their adoption in a wide range of environments. Water stress is one of the most critical factors limiting maize growth and development for most cereal crops, and South Africa is among the countries that faces inadequate rainfall with most of their agricultural activities depending on irrigation (Sithole and Modi, 2015). Approximately 25% of the cultivated maize area is reported to be under water sress , and the effect of water stress has been known to limit plant growth through its influence on morphological, physiological and biochemical processes (Setter et al., 2001; Daryanto et al., 2016). Maize as crop is reported to have high irrigation requirements and its performance is affected by water stress at a cellular level and whole-plant level (Rymaszewski et al. 2017; Ghorchiani et al. 2018). Results by Salazar et al. (2015) and Song et al.(2018) have shown that water stress induces morphological, physiological and biochemical changes, including changes to photosynthesis, plant height, dry matter production, leaf area and grain yield.

Generally plants develop physiological mechanisms to regulate tissue turgor and stomatal conductance to maximize photosynthetic efficiency (Mashilo et al., 2017). In a study conducted by Engineer et al., (2016), a low stomatal conductance was reported in immature leaves than in fully mature leaves. In constrast, Assis et al. (2019) reported that stomatal conductance of mature leaves was similar to immature leaves during the beginning of water stress, signifying the role of stomatal closure on improving plant water use efficiency (WUE) and productivity under water-stressed conditions (Leakey et al., 2019). Hence, parameters like stomatal conductance, transpiration rate, photosynthetic rate and intercellular CO2 concentration (leaf gas exchange) are used as important indices for the selection of drought tolerance closely related varieties (Ma'arup et al., 2019; Avramova et al., 2019).

In addition to leaf gaseous exchange, chlorophyll fluorescence of the leaves is also used as a direct indicator for photosynthetic activity for both light and dark leaves (Hailemichael et al., 2016). It is an essential factor used to monitor the status of photosynthetic apparatus, hence the analysis of chlorophyll fluorescence parameters such as Fv/Fm (maximum quantum yield of PS II photochemistry), qP (photochemical quenching) and qn (non-photochemical quenching) are considered important for the evaluation of drought tolerance in crops as reported by Kalaji et al. (2016), Szafranska et al. (2017), Matsuoka et al. (2018). The objective of this study was to evaluate the photosynthetic efficiency of LPA and HPA tropical maize cultivars grown under water - stressed conditions.

6.3 Materials and Methods

6.3.1 Planting material

The seed material used was obtained from the African Centre for Crop Improvement (ACCI) research programme, at the University of KwaZulu-Natal and a local seed company (McDonald Seeds, Pietermaritzburg, South Africa). The study consisted of two maize synthetic populations differing in phytic acid (PA) content, namely, low phytic acid (LPA) and high phytic acid (HPA) synthetic populations. Both LPA and HPA synthetic populations were derived from a tropical second generation (F2) population and were selected on the basis of their phytic acid (PA) content. These two synthetic populations were compared with a commercial white maize variety sourced from McDonald Seeds (SC701), which was used as a control for this experiment.

6.3.2 Experimental design and crop establishment

The experiment was conducted at the University of KwaZulu-Natal controlled environment facility, Pietermaritzburg, South Africa (29°40'05.7"S 30°24'20.9"E). The day and night temperatures were 32 and 28 °C, respectively under natural day length. The relative humidity (RH) was maintained at approximately 60% throughout the growing season. The temperature and RH were monitored electronically using a HOBO 2K logger (Onset Computer Corporation, Bourne, USA). The experiment was laid out in a complete randomized design arranged in a factorial structure with two treatment factors: variety at 3 levels [tropical maize varieties (LPA & HPA) and commercial maize (SC701)] and water regimes at 3 levels [30%, 50% and 100% crop water requirement (ETc)]. The treatments were replicated three times resulting to 27 experimental units. Maize seeds were planted in 25 L pots filled with 20 kg soil, collected from University Research Farm. Soil fertility test were done and the soil was fertilized based on recommendations for optimum maize production (Table.1).

Table 1. Analytical results of the Chemical and physical characteristics of the soil

Soil characteristic	**Quantity**
Sample density (g ml-1)	1.11
N(%)	0.19
P (mg kg^{-1})	18.00
K (mg kg^{-1})	185.5
Ca (mg kg^{-1})	1281
Mg (mg kg^{-1})	306.5
Exchangeable acidity (cmol L^{-1})	0.07
Total cations (cmol L^{-1})	9.46
Acid saturation (%)	1.00
pH (KCl)	4.63
Zn (mg kg^{-1})	4.90
Mn (mg kg^{-1})	72.0
Cu (mg kg^{-1})	15.0
Organic carbon (%)	2.10
Clay (%)	35.00

The crop water requirement was calculated as described by (Allen et al., 1998) using reference evapotranspiration (ETo) and crop factors recommended for cucurbits as described by Allen et al. (1998).

6.3.3 Data collection

6.3.3.1 Growth and yield parameters

Plant growth and yield parameters were measured at 30 days after planting and the following parameters were taken: Plant height (cm) was measured from the base of the plant to the top of the plant; the number of leaves per plant was counted as a fully expanded green leaf; chlorophyll content (SPAD unit) the chlorophyll content meter model (CCM-200 plus) was used for measuring. Time (days) to [tasseling (TTT), silking (TTS) and anthesis (TTA)], 1000 seed mass (g) and yield (t/ha).

6.3.3.2 Leaf gaseous exchange and chlorophyll fluorescence parameters

Leaf gaseous exchange; [carbon assimilation rate (*A*), stomatal conductance (*gs*), transpiration rate (*T*), intercellular CO2 concentration (*Ci*), the ratio of intercellular and atmospheric CO2 (Ci/Ca) concentrations] and chlorophyll fluorescence parameter; [maximum quantum yield of PS II photochemistry (Fv/Fm), photochemical quenching (qP), non-photochemical quenching (qN), electron transport rate (ETR) and PhiPS2 (effective quantum yield of photochemical energy conversion in PSII)] was measured using LI-6400 XT Portable Photosynthesis System (Licor Bioscience, Inc. Lincoln, Nebraska, USA) fitted with an infrared gas analyser attached to a leaf chamber fluorometer (LCF) (6400-40B, 2 cm^2 leaf area, Licor Bioscience, Inc. Lincoln, Nebraska, USA). Regarding the temperature of the leaf, it was adjusted at 25 °C with external leaf CO2 concentration (C_a) and fixed at 400 mmol m^{-2} s^{-1} while, the artificial saturating photosynthetic active radiation (PAR) fixed at 1000 mmol m^{-2} s^{-1}. The fourth leaf from the top was selected to measure Leaf gas exchange and chlorophyll fluorescence parameters [photosynthetic rate (A). Data were collected morning time between 8h00 and 11h00 on the clear, sunny days according to Mashilo et al. (2017).

6.3.4 Statistical analyses

Data collected from measured variables were subjected to the two-way analysis of variance (ANOVA) using Genstat version 18 (VSN International, Hemel Hempstead, UK). Treatment means were compared using Fischer's Least Significant Difference (LSD) test at 5% level of significance.

6.4 Results

6.4.1 Effect of varieties, water regime and Variety x water regime interaction on leaf gas exchange and chlorophyll fluorescence parameters

Significant differences were observed among the varieties, water stress treatments and their interactions for all the leaf gas exchange and some of the chlorophyll fluorescence parameters (Table 2). Several chlorophyll fluorescence parameters were not significantly ($P < 0.05$) affected by the interaction between variety and water stress. A non-significant variety x water interaction was observed for the maximum quantum yield of PS II photochemistry (Fv/Fm), non-photochemical quenching (qN) and effective quantum yield of photochemical energy conversion in PSII (ΦPSII), suggesting that the chlorophyll fluorescence was not influenced by both non-stressed and drought-stress conditions for all the varieties (Table 2). Only electron transfer (ET) was significantly affected by variety and water stress. A significant variety x water regime interaction was observed for stomatal conductance, transpiration rate, photosynthetic rate, intercellular carbon dioxide (C_i) and the ratio of intercellular and atmospheric CO_2 (C_i/C_a) as well as chlorophyll fluorescence parameters such as electron transfer rate (ETR). However, the effects of variety and water stress was influenced to a lesser extent by the growth stage of the plant. A significant interaction was observed at an early growth stage (vegetative growth) of the plant for qN and qP with no significant interaction during the reproductive stage. This significant ($P < 0.05$) interaction between the variety x water stress for these parameters suggests a differential response of varieties under water-stressed and non-stressed conditions (Table 2).

Table 2: Analysis of variance with mean squares and significant tests of leaf gas exchange and chlorophyll fluorescence parameters of 3 maize varieties tested under water-stressed and non-stressed conditions.

			leaf gas	**exchange**	**parameters**			
Growth stage	Source of variation	df	gs	T	A	Ci	Ci/Ca	WUE_i
Seedling	Variety	2	0.027***	2.055***	435.4***	12297***	9.495***	596.6***
	Water Regime	2	0.018***	1.074***	270.8***	25101***	12.024***	159.28***
	Variety x Water regime	4	0.004***	0.156***	5.444**	10057**	0.217***	6.85^{ns}
	Residual	18	0.0003	0.033	1.509	2596	0.20	20.29
Vegetative	Variety	2	0.009***	3.599***	421.22***	36037***	5.906***	1482.9***
	Water Regime	2	0.006***	1.792***	190.12***	53460***	4.993***	423.7***
	Variety x Water regime	4	0.001^{ns}	0.193*	8.056***	50745**	0.876***	116.8***
	Residual	18	0.0004	0.063	1.110	10772	0.165	14.85
Flowering	Variety	2	0.022***	2.747***	767.3***	22445***	5.386***	5521.4***
	Water Regime	2	0.019***	4.836***	1136***	24839***	5.453***	208.0**
	Variety x Water regime	4	0.002**	0.148*	23.26**	20231***	0.530***	59.35^{ns}
	Residual	18	0.0003	0.043	5.889	2473	0.1063	36.95
Grain filling	Variety	2	0.044***	2.840***	774.8***	1694***	5.839***	7647.2***
	Water Regime	2	0.057***	3.704***	1558***	2285***	2.731***	422.7***
	Variety x Water regime	4	0.009***	0.113*	18.7**	6765*	0.082***	98.97***
	Residual	18	0.0003	0.038	4.78	1999	0.008	6.75

Chlorophyll fluorescence

Growth stage	Source of variation	df	Fv_Fm	ΦPSII	qN	qP	ETR
Seedling	Variety	2	0.071***	0.031*	1.209***	1982ns	4.4E+09***
	Water Regime	2	0.148***	0.071***	2.361***	1956ns	2.7E+10***
	Variety x Water regime	4	0.002ns	0.004ns	0.213***	1989ns	1.9E+07ns
	Residual	18	0.002	0.006	0.014	1990	1.8E+07
Vegetative	Variety	2	0.061***	0.012ns	0.493***	0.145***	4.8E+09***
	Water Regime	2	0.258***	0.060***	0.567***	0.338***	1.0E+10***
	Variety x Water regime	4	0.006ns	0.012ns	0.025*	0.010***	2.9E+09***
	Residual	18	0.004	0.008	0.008	0.002	2.7E+08
Flowering	Variety	2	0.062***	0.006***	0.222***	0.290***	3.4E+08***
	Water Regime	2	0.101***	0.005***	0.127***	0.472***	6.0E+08***
	Variety x Water regime	4	0.001ns	0.0002ns	0.011ns	0.006ns	2.2E+07**
	Residual	18	0.002	0.0003	0.005	0.004	5.5E+06
Grain filling	Variety	2	0.009***	0.015ns	0.391***	1291ns	88182***
	Water Regime	2	0.026***	0.017ns	0.448***	1264ns	12506***
	Variety x Water regime	4	0.001ns	0.015ns	0.018ns	1292ns	35361***
	Residual	18	0.001	0.011	0.043	1292	48449.0

gs; stomatal conductance; T, transpiration rate, A; net CO2 assimilation rate; A/Ci, CO2 assimilation rate/intercellular CO2 concentration; Ci, intercellular CO2 concentration; Ci/Ca, ratio of intercellular and atmospheric CO2; Fv'/Fm', maximum quantum efficiency of photosystem II photochemistry; ΦPSII, the effective quantum efficiency of PSII photochemistry; qP, photochemical quenching; qN, non-photochemical quenching; ETR, electron transport rate; * Significant at the 0.05 probability level. ** Significant at the 0.01 probability level, *** Significant at the 0.001, ns non-significant difference. df, degrees of freedom

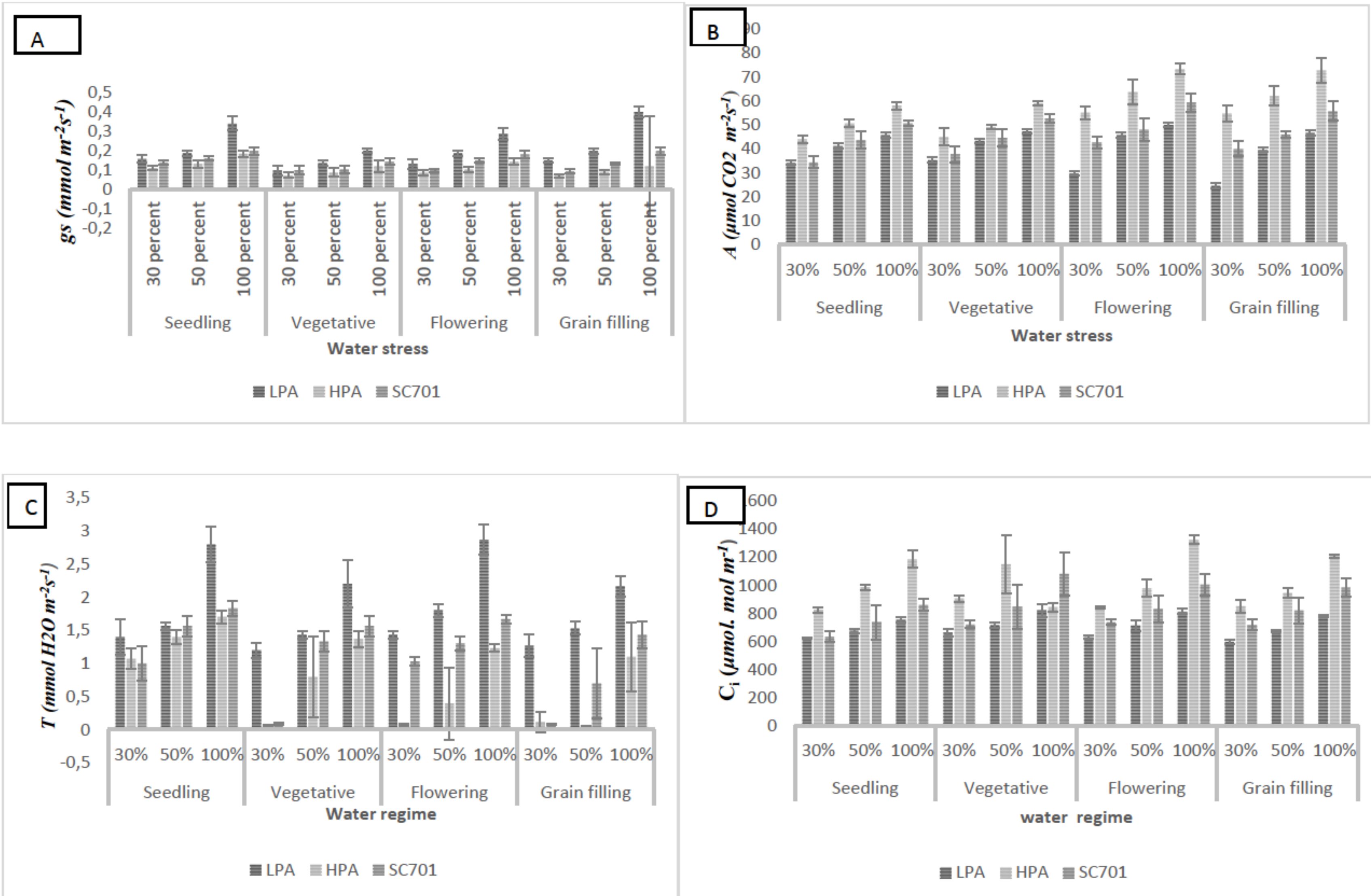
6.4.2 Effect of water stress on leaf gas exchange parameters for different maize varieties

Changes in leaf gas exchange parameters in response to water stress among the maize varieties are shown in Fig. 1A–E. Under non-stressed condition, LPA maize variety maintained higher stomatal conductance (*g*s) values of up to 0.4 mmol m^2s^{-1}, relative to SC701 and HPA which had significantly lower conductance (> 0.07 mmol m^2s^{-1}) (Fig. 1A). The conductance values varied with the growth stages of the plants, however, both the highest and the lowest value was recorded during grain filling. All the varieties maintained a similar pattern for all the water stress level for all the growth stages with LPA achieving the highest stomatal followed by SC701 and lastly HPA. The rate of stomatal conductance increased during the early stages of growth (seedling), declined shortly during the vegetative and flowering stage and later peaked up during grain filling. However, decreasing the crop water requirement to 30% reduced the stomatal conductance of HPA, and SC701 by 32- 63 % and 30- 53%, respectively, for all the growth stages. These values were considerably lower compared to the low phytic acid maize varieties which recorded a reduction of 52- 63%. Similarly, the different varieties showed a similar trend when the crop water requirement was reduced to 50%, however the level of reduction was relatively lower compared to the 30% ETc (Fig 1 A).

Genotypic variation was also observed with respect to photosynthetic rates (*A*) among the cultivars exposed to both water- stressed and non-stressed conditions. High phytic acid maize varieties recorded the highest rate of photosynthesis (58.00 - 73.33 µmol CO_2 m^2s^{-1}) compared to SC701 and LPA which recorded the photosynthetic rate of 50.00 -63.67 µmol CO_2 m^2s^{-1} and 24.67–35.33 µmol CO_2 m^2s^{-1}, respectively across the different growth stages of the crop (Fig 1 B). Decreasing the crop water requirement (ETc) to 30 % reduced the photosynthetic rate of maize varieties, with the LPA being the most affected. The level of reduction was reported to range between 25 -47%, 24-25% and 28-32 % for LPA, HPA and SC701, respectively. Unlike stomatal conductance, the photosynthetic rate of the varieties increased steadily in relation to the growth stages, with higher values observed as the plants reached maturity and lower values being observed at the early growth stages of the plants for all the varieties. With regards to transpiration rate (*T)* under non-stressed condition, LPA variety expressed a high transpiration which ranged between 2.2 – 2.9 mmol H_2O m^2s^{-1}, 1.4 – 1.8 mmol H_2O m^2s^{-1} and 1.1 – 1.7 H_2O m^2s^{-1} for LPA, SC701 and HPA respectively for all the growth stages (Fig 1 C). Reducing the crop water requirement to 30 and 50% reduced the rate of transpiration for all the varieties with the highest reduction level being observed in HPA (37 -99% and 18- 96%), SC701 (38- 95% and 15- 51%) and LPA (42 -50 % and 29 -37%) respectively. The reduction in *T* was significant and considerably lower during the early growth stages (month 1) for all the water stress level, and increased drastically as the plant continued to grow (month 2- month 4) except for LPA which showed a fluctuating trend across the growth stages.

Generally, the mean leaf stomatal conductance of varieties followed a similar trend as the transpiration rates. The reduction in stomatal conductance due to water stress was probably responsible for the decline in transpiration rate particularly during month 2 and 4. Intercellular CO_2 concentration increased significantly in response to non –stressed and water stress conditions. The highest values were observed in HPA with a maximum of 1574.7 µmol. mol m^{-1} followed by SC701 (1081 µmol. mol m^{-1}) and lastly LPA (821.3 µmol. mol m^{-1}), which recorded the lowest Ci for both non -stressed and water stress conditions (Fig 1 D). This explained the increase in the photosynthetic rate for both HPA and SC701 despite the reduction in stomatal conductance and transpiration rate. Highly significant ($P < 0.001$) values with respect to the ratio of the intercellular and atmospheric CO_2 (C_i/C_a) were observed for HPA and SC701 relative to LPA (Fig. 1E). Like all the other leaf gas exchange parameters, the ratio of intercellular to atmospheric CO2 concentration (Ci/Ca) was significantly higher ($P < 0.05$) under non-stressed than water stress conditions. Overall, HPA exhibited significantly ($P < 0.05$) higher C_i/C_a for both non-stressed and water stress condition with the maximum value of 5.463 for all the growth stages. However, reducing the crop water requirement to 30% and 50% percent did not affect ($P > 0.05$) the C_i/C_a of LPA and SC701 except for the grain filling stage, where significant ($P < 0.001$) differences were observed for all the maize varieties (Fig. 1E).

Significant ($P< 0.001$) differences were observed among the maize varieties for intrinsic water efficiency (WUE_i) under both water stress and non-stressed treatments across all the growth stages (Table 2). The interaction between variety and water stress level were significantly different, however, non-significant differences were also observed during seedling and flowering stage. Subjecting the maize varieties to water stress significantly ($P< 0.05$) reduced WUEi for all the maize varieties. HPA maintained significantly higher values of $WUE_{i,}$ with the maximum value of 90.83 (CO_2) m^{-2} (H_2O) and the minimum value of (34.18 (CO_2) m^{-2} (H_2O) for both water stress and non-stressed conditions respectively (Fig 1 F). The highest value was recorded at 30% ETc and the lowest value was recorded at 100% ETc for all the growth stages except for the grain filling stage. LPA exhibited a significantly ($P< 0.05$) lower rate of the WUE_i for all the crop water requirement levels. Unlike HPA, LPA variety recorded the highest WUE_i value was achieved at 50% ETc for all the growth stages except for the seedling stage, where the highest WUE_i was observe at 30% ETc (Fig 1F).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Seedling
Vegetative
Flowering
Grain filling
Water stress
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B
A (µmol CO2 m-2s-1)
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SC701
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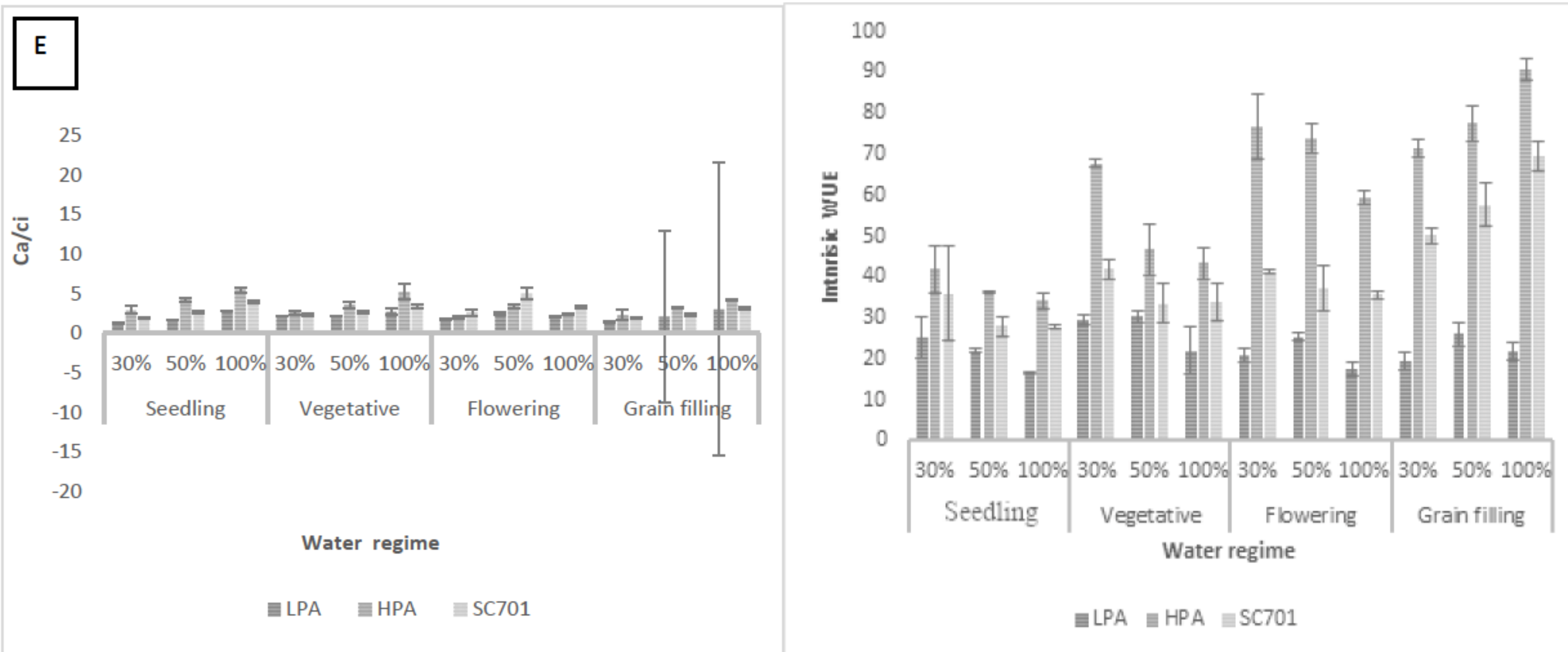


Figure (1A-F): Effect of water stress on stomatal conductance (A), photosynthetic rate (B) transpiration rate (C), intercellular carbon dioxide (D), ratio of atmospheric carbon dioxide and internal carbon dioxide (E) and intrinsic water use efficiency (F) of maize varieties. Data presented as means ± SE (n = 108).

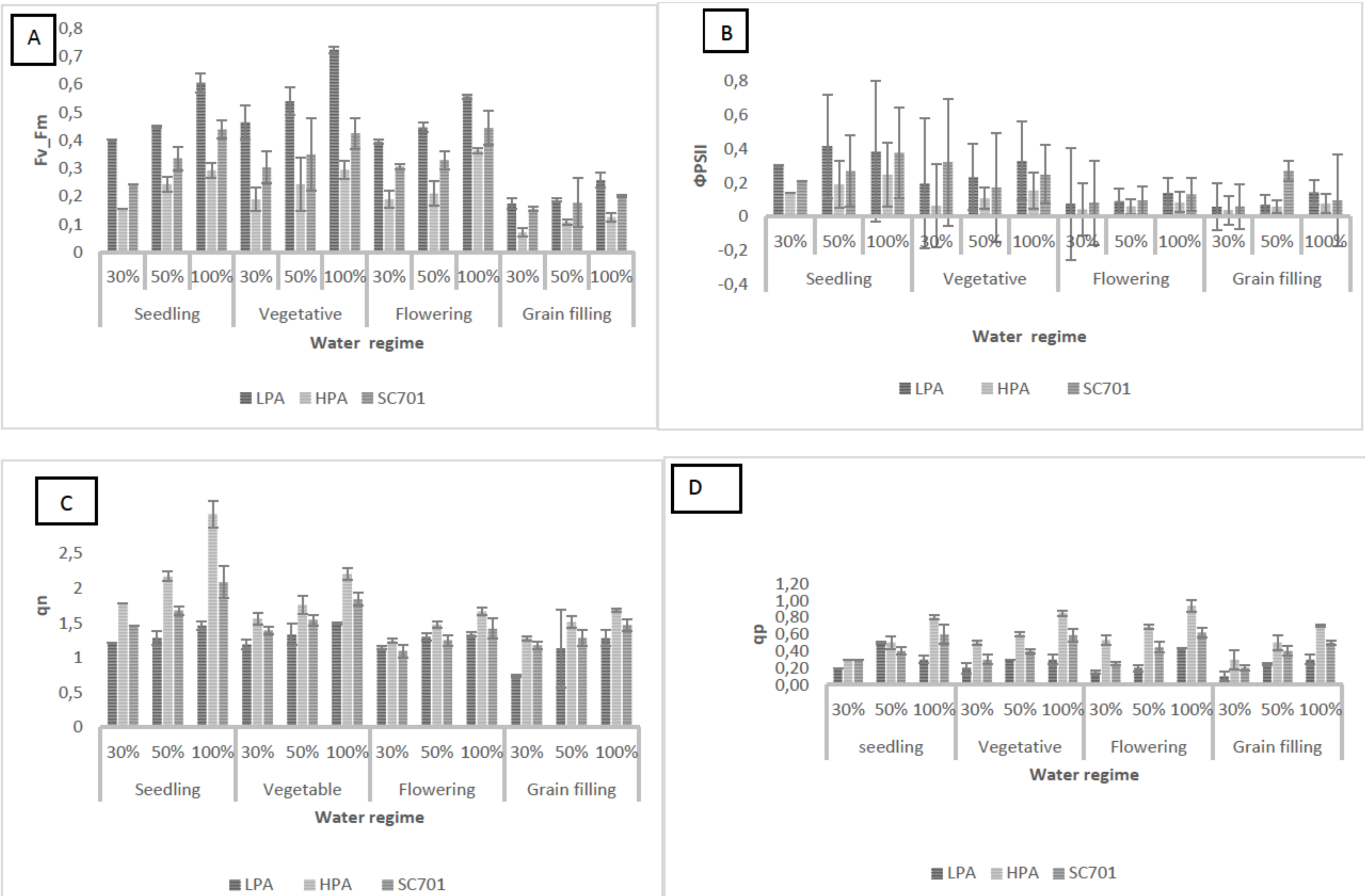
6.4.3 Changes in chlorophyll (fluorescence photosynthetic efficiency) of maize varieties in response to water stress.

The effect of drought stress on maize varieties in relation to chlorophyll fluorescence is summarized in Fig. 2A–E. The maximum quantum efficiency of PSII photochemistry (Fv/Fm) showed significant differences ($P < 0.05$) among the tested varieties under non-stressed and stressed conditions (Fig. 2A). However, the interaction between varieties and water stress levels were non- significant ($P < 0.05$). HPA and SC701 had a significantly ($P < 0.05$) reduced chlorophyll fluorescence with the minimum Fv'/Fm' values of 0.0717 and 0.1553, respectively; whereas, LPA maize varieties showed relatively higher Fv'/Fm' values (0.7250) (Fig. 2A) for all the growth stages. The lowest and the highest Fv'/Fm' was recorded at grain filling and vegetative stage, respectively.

The effective quantum efficiency of photosystem II photochemistry (ΦPSII) showed non-significant difference ($P < 0.05$) among the tested varieties under both non-stressed and water-stress conditions (Fig. 2B). However, significant differences with regard to variety and water stress level at grain filling were reported. LPA and SC701 exhibited significantly higher ΦPSII values of 0.1407 and 0.1303 respectively, compared to HPA under non-stressed condition. A similar trend was observed for the quantum yield of ΦPSII under water stress condition for all the water stress levels (ETc 30% and 50%). Significant ($P< 0.001$) differences were reported on photochemical quenching (qP) among the maize varieties. However, non- significant (P< 0.05) differences were also observed during the early growth stages (seedling stage) and at grain filling (Fig 2C). The highest qP was recorded on HPA with the maximum value of 0.9360 and the lowest value (0.1520) was recorded on LPA during seedling stage.

Non-photochemical quenching (qN) was significantly increased by water stress for all the maize varieties across all the growth stages (Fig. 2D). Varying genotypic responses with respect to qN were observed under both non-stressed and water stress conditions except for all the growth stages. However, there were no significant ($P < 0.05$) differences observed on the effect of variety and water stress condition during the flowering and grain filling stage of the maize varieties. HPA achieved the highest qN with the maximum value of 0.3063 reported during the seedling stage. LPA reported the lowest qN at a minimum value of 0.740 during grain filling stage.

Highly significant ($P < 0.001$) differences were reported with regard to electron transport rate (ETR). HPA achieved the highest ETR with the maximum value of 160098 μ mol e−m^2 s^{-1}, compared to LPA and SC701 which reported the maximum value 51406 and 82941 μ mol e−m^2 s^{-1} respectively for all the growth stages (Fig 2E). Reducing the crop water requirement by 30 and 50% reduced ETR significantly with LPA reporting the highest reduction of 82. 24 and 42.41%, respectively. HPA reported the reduction of 30.69 and 13.29% after the plants were exposed under 30 and 50%, whereas, SC701 showed the reduction of 49.6 and 22.17%, respectively.


A
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Seedling Vegetative Flowering Grain filling
Water regime
LPA HPA SC701
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Seedling Vegetative Flowering Grain filling
Water regime
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Seedling Vegetable Flowering Grain filling
Water regime
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seedling Vegetative Flowering Grain filling
Water regime
LPA HPA SC701

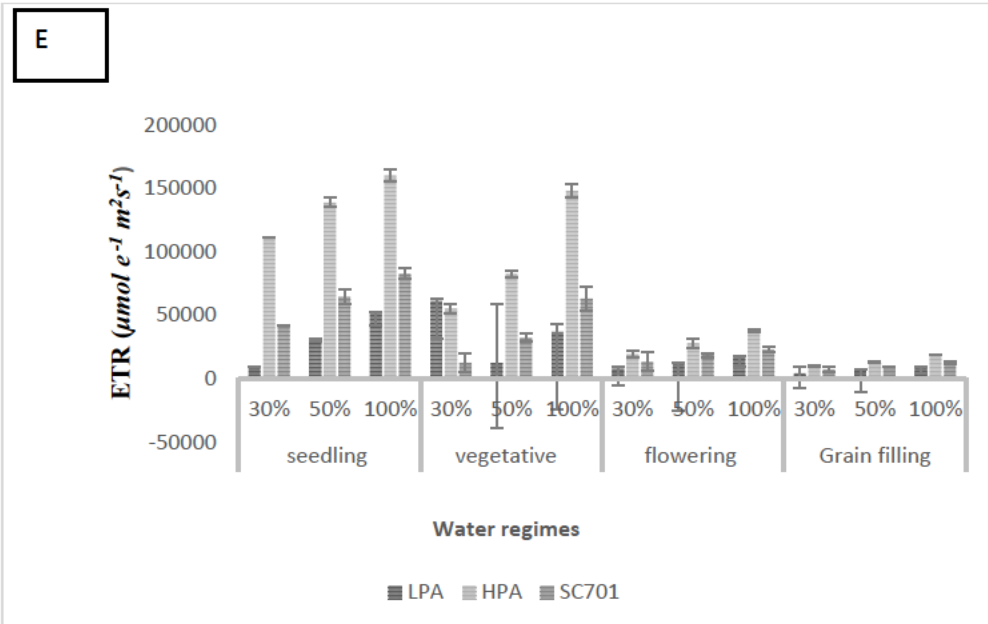

Figure (2A-E): Effect of water stress on Maximum quantum efficiency of PSII photochemistry (A), effective quantum efficiency of photosystem II photochemistry (B) photochemical quenching (C), Non-photochemical quenching (D) and electron transport rate (E) of maize varieties. Data presented as means ± SE (n = 108).

6.4.4 Effect of water stress on growth and yield performance of maize varieties

Significant differences ($p < 0.05$) in plant height were observed among the varieties under different levels of water stress for all the growth stages (Table 3). Reducing the ETc from 100% down to 30% affected the plant height negatively for all the varieties. LPA reported the lowest plant height relative to the HPA and SC701 variety. SC701 reported the highest plant height for all the water stress levels, with the maximum value of 233.7 cm, however, no significant ($p < 0.05$) differences were observed between HPA and SC701 across all the growth stages. Reducing the crop water requirements slightly decreased the height of the plants suggesting that the differences among the plants was not influenced by water stress levels but genotypic variation. Contrary to plant height, no significant differences ($p < 0.05$) were observed among the varieties on the number of leaves per plant for all the water stress levels. However, the results showed a similar trend for all the growth stages except for the vegetative growth stage where SC701 recorded a greater number of leaves compared to the other treatments (Table 3).

Regarding leaf chlorophyll content, the results reported significant differences ($p < 0.05$) among the studied varieties. Exposing the varieties to different levels of water stress negatively influenced the chlorophyll content of the plants (Table 3). The SPAD values varied according to the growth stage of the plants with the lowest value was recorded during the seedling stage and the highest value at flowering stage for all the varieties. However, a decline in chlorophyll content was observed during grain filling. Significant differences ($P < 0.05$) among the varieties were also observed for the number of days to flowering (tasseling, silking and anthesis) (Table 3). Generally, imposing water stress was shown to delay the process of flowering for all the studied varieties. Reducing ETc from 100% to 30% delayed days to Tasseling by 3, 8 and 6 days for LPA, HPA and

SC701, respectively. The same trend was observed on days to silking and anthesis (Table 2.2). In terms of grain yield, significant ($P < 0.05$) differences were reported among the varieties under different levels of water stress (Table 2.2). Reducing the crop water requirement from 100% to 30% ETc decreased the 1000 seed mass by 37%, 24 % and 32% for LPA, HPA and SC701 respectively (Table 3). The most effect of water stress was observed on LPA, followed by SC701 and HPA was the least affected. HPA and SC701reported the highest yield for all the stress levels except for 100% where HPA was significantly higher than SC701 and LPA (Table 2.2).

Table 3. effect of water stress on Plant height (cm), number of leaves, chlorophyll content, 1000 grain mass and Yield (t/ha). SE= seedling stage, VE= vegetative stage, FL= flowering stage and GF= grain filling.

Variety	Irrigation regimes (ETc %)	Plant height (cm)				Number of leaves / plants				Chlorophyll content				Time (days) to Tassel, Silking and Anthesis (days)			1000 Grain mass (g)	Yield t/ha
		Growth stage				Growth stage				Growth stage								
		SE	VE	RE	GF	SE	VE	FL	GF	SE	VE	FL	GF	Tasseling	Silking	Anthesis		
LPA	30 %	44.0^{ab}	72.3^{a}	140.0^{a}	190.3^{a}	7^{a}	9^{a}	11^{a}	11^{a}	20.3^{a}	31.2^{a}	44.5^{b}	39.1^{bc}	68.0^{d}	70.0^{c}	69.7^{e}	120.3^{a}	1.17^{a}
HPA		52.3^{c}	84.3^{b}	153.0^{bcd}	222.3^{c}	7^{a}	9^{a}	11^{a}	12^{a}	30.5^{d}	33.5^{ab}	36.0^{a}	32.7^{a}	64.7^{cd}	68.0^{bc}	66.7^{cde}	182.0^{cde}	1.57^{bc}
SC701		41.0^{a}	99.7^{de}	165.0^{e}	233.7^{d}	7^{a}	12^{b}	14.^{a}	14^{a}	32.3^{de}	36.7^{bc}	47.3^{bc}	40.1^{bcd}	65.7^{d}	68.3^{bc}	68.0^{de}	164.0^{bc}	1.43^{b}
LPA	50 %	45.7^{ab}	72.7^{a}	143.7^{ab}	196.3^{a}	7^{a}	1O^{a}	12^{a}	12^{a}	23.8^{b}	34.7b	44.7^{b}	36.9^{ab}	66.3^{d}	68.0^{bc}	68.7^{de}	151.3^{b}	1.39^{b}
HPA		52.0^{c}	95.0^{cd}	158.7^{de}	228.7^{cd}	7^{a}	10^{a}	12^{a}	12^{a}	33.8^{ef}	36.2^{bc}	43.7^{b} SE	40.8^{bcd}	62.0^{c}	66.3^{b}	64.3^{bc}	199.0^{ef}	1.87^{d}
SC701		53.3^{cd}	84.0^{b}	148.3^{abc}	208.3^{b}	7^{a}	10^{a}	13^{a}	13^{a}	37.3^{g}	42.8^{d}	50.6^{c}	43.2^{cd}	61.7^{bc}	66.0^{b}	66.0^{bcd}	179.0^{cd}	1.73^{cd}
LPA	100 %	52.3^{C}	91.0^{c}	157.7^{cde}	198.3^{ab}	7^{a}	10^{a}	12^{a}	12.0^{a}	27.2^{c}	39.2^{c}	46.8^{bc}	37.1^{ab}	65.0^{cd}	67.7^{bc}	66.7^{cde}	191.7^{de}	1.77^{d}
HPA		43.0^{ab}	99.7^{de}	165.0^{e}	233.7^{d}	7^{a}	12^{b}	14^{a}	14^{a}	35.8^{fg}	38.5^{c}	47.5^{bc}	38.0^{b}	56.0^{a}	62.0^{a}	59.0^{a}	238.3^{g}	3.93^{f}
SC701		57.7^{d}	101^{e}	166.7^{e}	224.0^{cd}	7^{a}	12^{b}	14^{a}	14^{a}	41.8^{h}	46.9^{e}	56.3^{d}	44.2^{d}	58.3^{ab}	65.0^{ab}	63.3^{b}	210.3^{f}	3.58^{e}
CV		3.3	2.2	2.3	1.9	8.8	5.4	7.4	7.4	3.3	3.0	3.5	4.5	1.9	1.8	1.7	3.5	5.5
LSD		2.78	3.31	5.94	6.75	0.99	0.930. 0.001	1.58	1.58	1.78	1.94	2.78	3.03	2.03	2.06	1.89	10.80	0.192
P< 0.05		0.001	0.001	0.021	0.021	0.977		0.306	0.306	0.038	0.011	<.001	0.001	0.004	0.053	0.022	0.035	<.001

6.5 Discussion

The current study determined the effects of water restriction on leaf gas exchange and chlorophyll fluorescence parameters in LPA and HPA maize varieties. The results from the study provide a clear evidence on differences among the tested varieties with regard to plant water relations and their response to water deficit. It is well established that water deficit reduces plant growth and productivity by decreasing leaf area, photosynthetic rate, transpiration rate, stomatal conductance and chlorophyll fluorescence for maize cultivars (Liu et al 2015; Jabeen et al., 2008). This is in agreement with our current findings that maize varieties subjected to water stress showed reduced photosynthetic activity, chlorophyll fluorescence and growth for all the different growth stages. In the present study, photosynthetic rate (*A*) declined severely in all the maize varieties under water limited conditions. The decline in photosynthetic rate is mainly due to lower stomatal conductance *gs* which reduces the ratio of carbon dioxide and oxygen in plant leaves and eventually inhibits photosynthesis (Gururani et al., 2015).

Similar results were found by Liu et al. (2016), who reported that low stomatal conductance reduced both photosynthetic and transpiration rate of winter wheat varieties grown under drought conditions. Among the various responses of plants under limited water conditions, stomatal closure is the earliest response of the plant preventing the leaf from losing excess water. Water deficit leads to a reduction in stomatal conductance along with the photosynthetic rate, and this is attributed to the closure of stomatal pores which inhibits the intake of CO_2 hence the reduction in carbon assimilation rate (Pelleschi et al., 1997). Furthermore, stomatal closure is known to decrease photosynthesis because the photosynthetic apparatus is resistant to dehydration in both

C_3 and C_4 plants (Pelleschi et al., 1997). Transpiration rate decreased in all the maize varieties due to drought stress and this can also be attributed to the closure of stomata. Plants are known to compensate the effect of water stress by closing their stomata to avoid further loss through transpiration.

In terms of the varieties, LPA maize variety obtained high stomatal conductance and transpiration rate for all the water stress levels whereas lower levels were reported on high phytic acids maize variety (Fig 1A and C). However, HPA varieties reported a high A, C_i and C_i/C_a irrespective of the lower stomatal conductance. This results corresponds with the assumption made by Liu et al. (2016), who suggested that low *gs* would slightly reduce the rate of photosynthesis and significantly decrease transpiration due to the linear relationship that exist between stomatal conductance and transpiration under both water stressed and non -stressed conditions. Values of Intrinsic water use efficiency (WUEi) calculate as *A/ T* were significantly different among the maize varieties under both water stress and non-stressed conditions.

High values of WUE_i were reported in 50% ETc for all the varieties and the lowest were reported in 30 % ETc, suggesting that sufficient soil water content increased the water use efficiency of these three maize varieties. LPA recorded significantly higher values of WUE_i for all the water stress level except at grain filling where HPA obtained the highest values. The ability of the maize varieties to increase water use efficiency under limited water conditions is critical for the plant’s survival. The results for the current study contrast with the findings of Jabeen et al. (2008) who found that drought tolerant maize varieties achieved high values of WUE_i under limited water conditions. However, subjecting the plants to mild stress (50% ETc) increased the water use efficiency of the maize varieties indicating that the low phytic acid maize varieties are sensitive to water deficit and can only tolerate moderate water stress.

Chlorophyll fluorescence is described as electromagnetic radiation emitted by the chlorophyll in plants and its analysis is used to measure the maximum quantum efficiency of photosystem II (Fv/Fm), The effective quantum efficiency of photosystem II photochemistry (ΦPSII), photochemical quenching, non -photochemical quenching, electron transfer rate (ETR), etc. The present study reported significant ($P < 0.05$) differences among the varieties under both water stress and non-stressed conditions for Fv/Fm in all the growth stages (Fig 2A). However, the interaction between the variety and water stress treatment was not significantly different for all the growth stages. Among the varieties tested, LPA achieved the highest value of Fv/Fm in all the irrigation treatments whereas HPA reported the lowest value.

Subjecting the varieties to 30% ETc decreased the Fv/Fm for all the varieties and a similar trend was observed for all the growth stages. Fv/Fm is known to be a sensitive indicator of the plant's photosynthetic efficiency and a maximum value of approximately 0.83 is reported to be achieved by healthy samples (Badr and Bruggemann, 2020). However, this statement contradicts our current findings in which the maximum of 0.73 was recorded by the tested varieties, which suggest that the plants were undergoing stress. Exposing plants under biotic and abiotic stress is reported to decrease the maximum quantum efficiency of photosystem II by reducing its capacity for photochemical quenching through the process of photo inhibition, which occurs when the level of photo damage above the level of repair in photosystem II (Murata et al., 2012).

Reducing the crop water requirement to 30% and 50% significantly affected the photochemical quenching (qP) of the maize varieties for all the growth stages. HPA maize varieties reported the highest qP than the LPA maize variety, and this increased linearly with the increase in the level of water stress imposed for the tested varieties. Unlike HPA maize variety, LPA reported the lowest qP and the highest value was recorded at 50% ETc with 30% and 100% achieving the lowest. This results are in agreement with the findings by De Souza et al. (2013), who reported similar results for drought tolerant maize varieties. This is because drought stress reduces the opening of reaction centers of photosystem II and drought tolerant plants tend to have low photochemical quenching. Non- photochemical quenching measures the energy that is emitted as heat in photosystem II (Lawlor and Cornic, 2002).

HPA showed higher values under limited irrigation (Fig 2C.), suggesting that this variety is efficient at reducing increased excitation energy through dissipation thereby preventing the leaves from photo-damage (Mashilo et al., 2018). ETR is used as an important mechanism for protection against photo-inhibition (Singh and Raja, 2011; Yi et al., 2016). Electron transport rate declined due to irrigation water limitation for LPA and SC701, while it increased in HPA maize varieties (Fig 2E.). According to Maxwell and Johnson (2000), reduced stomatal conductance decreases the rate of consumption of ATP and NADPH for CO_2 assimilation, which could result in reduced electron transport rate.

Chlorophyll which is a photosynthetic pigment, is responsible for the absorption of light and plays an important role in the process of photosynthesis in plants. Drought stress is reported to speed up the depletion of chlorophyll in plants and this is used to measure the severity of drought stress

(Sun et al., 2015). The current findings demonstrated a positive interaction between variety and water stress level for chlorophyll content for all the growth stages. HPA reported the higher SPAD values compared to LPA and SC701 for both water stressed and non-stressed conditions, and the chlorophyll content increased linearly with the level of water stress. The highest chlorophyll content was observed at 100% ETc during flowering stage for all the varieties. Limited water conditions reduced the greenness of the plants for all the varieties and HPA exhibited a relatively higher value as compared to the other varieties which explains the increase in *A* for HPA when the plants were exposed to 30% and 50% ETc.

Drought resistant varieties are known to maintain a low chlorophyll content when are exposed to severe drought and this is regarded as an adaptive mechanism used by these plants to reduce photo-oxidative damage which results in photo-inhibition due to excess light excitation energy (Aranjuelo et al., 2011). These findings correspond with the findings made by Chen et al. (2016) who reported lower chlorophyll content of maize lines planted under drought stress environment. Water stress also reduced plant height for all the varieties, and HPA reported the highest plant height compared to LPA and SC701 (Table 3). Irrigation deficit reduced the plant height of the maize varieties which is common when the plants are exposed to waters stress, cell growth is affected as a result of reduced cell turgor (Ghooshchi et al., 2008).

Limited water conditions affected yield and yield components of all the varieties (Table 3). Among the varieties HPA and SC701 performed better than the LPA under both limited water conditions and sufficient water supply. Grain yields under 100% ETc were significantly different among the varieties and the yield ranged from 3.93 t/ha to 1.77 t/ha for HPA and LPA. Limited water conditions significantly reduced grain yield of all the varieties and HPA recorded lower yield reduction than the LPA. Generally, the yield reduction as affected by water stress was smaller in the maize varieties that had high yield under well-watered conditions. These results agree with the results of Seghatoleslami et al. (2008), who reported that high yielding maize had greater yield than lower yielding varieties under both optimal and suboptimal water availability. This is partly due to high kernel weight (1000 seed mass) and increased translocation of photo assimilates to the grain as indicated by the high photosynthetic rate of these verities.

6.6 Conclusion

The present study demonstrated the effect of irrigation deficit on photosynthetic efficiency and chlorophyll fluorescence of high phytic and low phytic acid maize varieties. Results from this study indicated that HPA maize varieties showed increased performance with regard to photosynthetic performance and yield in general, under both normal and water stressed conditions. Reducing the level of crop water requirements (ETc) decreased the overall performance of these varieties and LPA showed to be the most sensitive to water stress, this was also shown by the increased in the stomatal conductance and transpiration rate of LPA verities. However, a considerable improvement on the overall performance was observed when the ETc was increased to 50 % for low phytic maize varieties and this can be used in areas where irrigation water is limited. Although the breeding of low phytic maize variety seem to be an ideal strategy to improve availability of nutrients in humans and mono-gastric animals as well as mitigating the negative impact of phosphorus, it has some limitations on physiological and yield performance. It is time that the impact of water stress receives the scientific attention it deserves, particularly because these varieties were bred in tropical areas where rainfall is sufficient to sustain growth. The effect of water stress should be further studied in areas with limited rainfall under field conditions.

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CHAPTER 7

General discussion and conclusions

Severe food insecurity at the global level has been rising in recent years; more than 800 million people (10% of the world population) are exposed to food insecurity (Lentz et al., 2019). Stability in availability and accessibility of food for the population is essential for the wellbeing and health of all humankind (Mbow et al., 2019; D'Amelio et al., 2016). To meet future food demand, the supply of food has to increase. Crop production in Africa must be projected to face a population increasing in the future (Onyutha 2018). This situation has been worsened by global economic conditions, adverse climatic conditions and conflict, in many countries and sometimes the combination (FAO 2018). Cereals are the main staple crops in terms of calorie intake in SSA and widely cultivated crops and are of great importance to the SSA people's food security and livelihoods.

Maize (*Zea mays*) has many desirable qualities making it a significant food source for humans (Anami et al., 2009). Maize is one of the most important crops globally, particularly in SSA as one of the main foods. Tropical maize refers to corn (*Zea mays* L.) varieties that are widely grown in tropical and sub-tropical regions, including Sub-Saharan Africa and Central and Latin America (Anami et al., 2009). In sub-Saharan Africa, tropical maize has been customarily the main staple of the diet and its consumption exceeds its production. The whole grain, either mature or immature, can be roasted or cooked and served as food (Anami et al., 2009).

Therefore, due to the importance of maize, the agricultural scientific community, besides introducing new varieties, is also working to improve maize nutritional quality through breeding programs, mainly targeting those nutrients that are missing in the diet of many households in the SSA region to address hidden hunger. Plant breeders have focused on reducing phytic acid on maize seeds to improve grain nutritional quality that is because phytic acid quickly precipitates in the form of phytate salts, binding essential mineral cations such as iron, zinc, potassium, calcium, and magnesium and make them unavailable for the human diet. However, reducing PA is associated with reducing phosphorus (P) because phytic acid is a primary storage form of phosphorus (P) in seeds. This has a negative effect on seed quality (germination and vigour) and yield.

Therefore, this study aimed to assess seed quality and yield of recently produced varieties of low phytic acid tropical maize compared with commercially cultivated varieties. The study investigated the characterization of low phytic acid tropical maize varieties for seed germination and vigour compared to high phytic acid maize and normal maize (white and yellow). The study also examined the seed performance of low phytic acid, high phytic acid and normal maize (white and yellow) to increase P application rates. Additionally, the study evaluated and compared the growth and yield of low phytic acid tropical maize, high phytic acid tropical maize and normal maize (white and yellow) under dry conditions. Furthermore, the study investigated the growth, physiological and yield performance of LPA tropical maize in response to water stress compare to HPA tropical maize and normal white maize.

In chapter three, the study's objective was to assess seed performance of LPA maize synthetic populations with tropical genetic background compared to HPA derived from the same population and two commercial varieties. The results showed that there were differences between the varieties with regards to PA content. The LPA recorded the lowest value compare to HPA, SC701 and LS8520. Significant differences were found in germination among the four tested varieties. The highest percentage of germination was observed in HPA, followed by SC701, LPA and LS8520, respectively. Contradictory results were revealed by Pilu et al. (2003) and Naidoo et al. (2012)

when they investigated the effect of reducing PA on seed performance. These results show that the combination of LPA seed lines used in this study produced seed quality parameters or phosphorus (P) reserve required for minimum seed germination that produces commercially acceptable germination and early seedling growth.

Under the standard germination test, the results showed no differences detected among varieties concerning mean germination time (MGT). The germination vigour index (GVI) was significantly higher in SC701 and HPA than in LPA and LS8520. Therefore, because of low GVI in LPA and LS8520, the results propose that LPA maize varieties and LS8520 are expected to show a lower germination rate while HPA and SC701 are expected to show a significant increase in germination under field conditions. There were no much differences in the root: shoot ratio in all varieties.

When seeds were subjected to accelerated aging (AA), the germination percentage was reduced in all varieties. The germination decreased with an increase in time of exposure to AA from 48 hours to 72 hours. In both cases, HPA and SC701 recorded higher germination than LPA and LS8520. LPA obtained significantly higher MGT than HPA at 72 hours of AA. The GVI of HPA and SC701 was similar, but at 72, HPA was higher, followed by SC701, LPA and LS8520, respectively. Therefore, the GVI of LPA followed the same trend in both standard germination and AA vigour tests. Delouche and Baskini (1973) reported that the accelerated aging test method is useful in evaluating vigor. These results indicate the complex interaction of seed quality parameters that determine field performance. This proposes that different measures must be interpreted with care when simulating the field performance of LPA seeds. The study also suggests that suspecting LPA to different tests and environmental conditions will fully understand the growth and yield prediction. Therefore, since the GVI of LPA remains lower than HPA and SC701, this creates a future research gap on performance assessments of LPA under field conditions besides the performance under water stress conditions.

Chapter four examined the response of LPA maize to exogenous P application on seed germination, vigour and yield compared to HPA maize and two commercial grown maize hybrids. The results revealed significant differences ($p < 0.05$) recorded among the varieties in response to exogenous P application. Plant height, leaf number of LPA improved with increasing P application rate as well as other varieties. In terms of days to reach the flowering stage, P application was found to decrease significantly ($p < 0.05$) time to silking and anthesis in all studied varieties. LS8520 recorded minimum reduction time. With increasing, P fertilizer to 1000 grain mass and LPA yield improved significantly by 25% and 90% for both parameters, respectively.

In the same study, harvested seeds were subjected to standard germination tests and an accelerated aging (AA) test. At the standard germination test, the results showed that, at the maximum P application rate (34 mg/kg), LPA's total germination was comparable with HPA and SC701. Phosphorous application enhanced electrical (EC) conductivity of all varieties to be reduced significantly. Root length and shoot length increased significantly ($p < 0.05$) in LPA after applying exogenous P. The results revealed significant differences ($p < 0.05$) between the varieties when the seeds were subjected to 48 and 72 hours of AA test. In both times, the germination, GVI increased with time while MGT showing contrary results. At 48 hours, AA seeds showed better performance than the 72 hours AA. In this research, compared to the results in chapter two, the results indicated that P improved germination and seed vigour. Besides that, the results confirmed that the germination of LPA was high and comparable to other varieties that met requirements for germination. Although the germination of LPA was high, its yield remained low unless it is treated with P. That drives the authors in this research to further investigate the physiological aspects of LPA varieties to determine the exact reason behind that, although it is germinated well.

In chapter five, the objective of the study was to evaluate and predict the growth and yield of LPA maize under dry field conditions compared to HPA maize and two commercial maize varieties (SC701 and LS8520). Unfortunately, although LPA's germination was comparable with other varieties in previous research chapters, its yield is still low unless fertilized with P. Therefore, this chapter investigated more about LPA's performance under field conditions, specifically under dry areas where rain is a limiting factor. The results revealed significant differences ($p < 0.05$) significant differences were observed among the varieties. On growth parameters, LPA showed significantly shorter plant height; lower accumulation leaves number and chlorophyll content. White et al., (2011) was noted the growth and yield of tropical maize was affected under teprate regiens. The results also indicated that LPA was performing lower in terms of time to reach flowering. The performance of LPA during growth and development affected the LPA yield and yield components. Generally, the yield of both LPA and HPA tropical maize was affected by dry conditions compared to SC701 and LS8520 commercial maize. However, LPA achieved the lowest yield. LPA's lower performance and yield could be an adaptation to dry condtions or the amount of phytic acid (P source). This study suggests that further investigation of tropical maize should be conducted based on physiological aspects, particularly the photosynthesis and chlorophyll fluorescence under water stress.

Chapter six's objective was to evaluate the photosynthetic efficiency and yield of low phytic acid (LPA) and high phytic acid (HPA) tropical maize varietys grown under water-stressed conditions. Therefore the study investigated the impact of reducing PA content on physiological (leaf gas exchange and chlorophyll fluorescence parameters) and yield performance of LPA compared to

HPA and hybrid commercial maize. Overall the targeted parameters of the studied varieties showed significant differences in their responses to water deficit.

LPA registered significantly the highest stomatal conductance, followed by SC701 and HPA, respectively. The rate of stomatal conductance increased during seedling growth and seed filing opposite result was deducted during vegetative and flowering phases in all varieties. Although it is obtained higher stomatal conductance, the LPA also shows lower photosynthetic rates (*A*) while the highest rate recorded by HPA and SC701. The photosynthetic rates in all varieties increased as the plant developed, unlike the stomatal conductance trend. The transpiration rate (*T*) of LPA was higher than HPA and SC01 in stressed and non-stressed conditions. The reduction in stomatal conductance due to water stress was possibly responsible for the decline in transpiration rate. Intercellular CO_2 and the ratio of intercellular and atmospheric CO_2 (C_i/C_a) followed the same trend of Photosynthetic rate as it increased significantly in response to non –stressed and water stress conditions.

The results revealed that there were the increase in photosynthesis rate for both HPA and SC701 despite the reduction in stomatal conductance and transpiration rate. These findings agreed with studies reported by Liu et al., (2016) and Jabeen et al., (2008), where they approved that drought stress affected maize growth and yield by decreasing photosynthetic rate, transpiration rate, stomatal conductance and chlorophyll fluorescence. The study showed highest values of WUE_i in 50 % ETc for all the varieties, while the lowest was registered in 30% ETc. LPA was reported to have significantly higher values of WUE_i for all the water stress levels except at grain filling, where HPA achieved the highest values. These findings contradict Jabeen et al. (2008), where they stated that water deficit enhances WUE_i of maize. It can be stated that both LPA and HPA tolerate only moderate stress in terms of $WUE_{i.}$

There were significant differences between the studied varieties chlorophyll fluorescence parameters. In terms of the interaction between the water stress and the varieties, there were no significant differences in chlorophyll fluorescence parameters except non-photochemical quenching (qn). LPA had significantly maximum quantum efficiency of PSII photochemistry (Fv/Fm) followed by SC701 and HPA, respectively. The effective quantum efficiency of photosystem II photochemistry (ΦPSII) was comparable between the tested varieties. The highest qn was recorded on HPA at the seedling stage and the lowest value recorded by LPA during grain filling phase Fv/Fm is reported to be a sensitive sign of the plant's photosynthetic efficiency. The highest qP was recorded on HPA with the maximum value of 0.9360 and the lowest value (0.1520) was recorded on LPA during the seedling stage. Water stress reduces the opening of reaction centers of photosystem II; therefore, over the drought period, plants reduce the photochemical quenching. HPA achieved the highest ETR with the maximum value of the electron transport rate (ETR) compare to SC701 and LPA. LPA reported a higher reduction in ETR as irrigation regimes (ETc%) reduced to 50% and 30%. Other studies reported by Maxwell and Johnson (2000) stated that reduced stomatal conductance decreases the rate of ATP and NADPH consumption for CO_2 assimilation, which could result in reduced electron transport rate. Generally, all chlorophyll fluorescence parameters start increasing at early growth and decrease towards grain filing.

Application of the water's stress regimes affected the plant height negatively. The lowest value of plant height recorded by LPA. No significant differences recorded on the number of leaves per plant except at vegetative growth the SC701 showed significantly higher value at 30% ETc. The differences in plant height attributed to these varieties genetic background because, at harvest time, the plant height was not affected significantly by reducing applied water. This finding applies to the number of leaves as well. Contrary results stated by Anami et al. (2009) mentioned that drought stress impacts the growth cycle of maize. The chlorophyll content of the studied varieties is affected negatively by increasing water stress applications. Chlorophyll increase towards flowering and decreases towards maturity. At seed filling, the chlorophyll of LPA was comparable, although it is had the lowest values. Plants reduce chlorophyll content to reduce photo-oxidative damage to adapt to drought stress (Aranjuelo et al., 2011). The study revealed a delay in flowering (tasselling, anthesis and silking) in all studied varieties, supported results reported by Al-Naggar et al., (2016). Therefore, water stress reduces the rate of fertilization and decreases kernel set and yield as final. Significant differences were reported on 1000 seed mass and grain yield among the varieties under different water stress regimes. The varieties that reported higher photosynthetic rate enhanced 1000 seed mass and yield at final.

Breeding programmes produced low phytic maize varieties to improve the availability of nutrients in humans and mono-gastric animals and mitigate phosphorus's negative impact. The new low PA has some affection for physiological and yield performance. Exceptionally because these varieties were bred in tropical areas where rainfall is sufficient to sustain growth, but the effect of water stress should be further studied in areas with limited rainfall under field conditions.

Generally the study indicated that the seed quality performance of LPA varieties was comparable to those of commercially produced varieties. This study suggests that the combination of LPA lines of tropical origin used in this study was satisfactory to meet the minimum seed quality parameters (with respect to seed germination and vigour) required for maize's growth and development. Although the low PA meets the minimum requirement for seed germination but did not meet the requirement for physiological aspects, as shown in the water stress chapter that could be a reason for the low yield.

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