SELECTION OF IBM POPULATION INBRED LINES WITH IMPROVED ROOT ARCHITECTURAL TRAITS AND STEM DIAMETER THAT CAN ENABLE THEM TO TOLERATE NITROGEN AND PHOSPHORUS STRESSES

BY

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DECLARATION

“I, Shaku Manchidi Melda do hereby declare that this research report submitted to the University of Limpopo for the degree of MSc in Agriculture (Agronomy) has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution and that all material contained herein has been duly acknowledged”.

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Signature: Ms M.M Shaku Date:

DEDICATION

I would like to dedicate this work:

To my late sister Manaso Reshokwetswe Tladi even though she did not live long enough to see my success, she remains my inspiration, may her soul rest in peace.

To my father Molope Shaku and mother Phahlakwena Shaku, who worked so hard to train me from childhood as they did a lot so that I can get this far, to my beloved aunt Mahlako Tladi and my two brothers Machiri Shaku and Seporo Tladi.

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ABSTRACT

Two experiments were conducted at Ukulima Root Biology Centre, Waterberg district in Limpopo Province (24°32'58.1"S, 28°06'21.1"E) during 2013-2014 cropping season to select IBM population inbred lines with improved root whorl and stem diameter that can enable them to tolerate nitrogen and phosphorus stress. The experiments were laid out in a Split plot format based on a randomized complete block design with four replicates. The main plot factors were: nitrogen levels (low and high) and phosphorus (low and high), in the respective trials and maize inbred lines (MO345, MO034, MO001, MO199, MO031 and MO196) were in the sub plots. Traits investigated included shoot morphological traits (plant height, leaf area per plant, chlorophyll content, stem diameter, number of leaves), root architectural traits (Whorl angles, root area, average root density, number of adventitious roots, number basal roots, average lateral root length, lateral branching frequency, root top angle, root bottom angle, distance to the first lateral root) and dry biomass.

Results showed morphological traits, root architectural traits and biomass were affected by nitrogen fertilizer. Those traits were greater under high nitrogen level. On the other hand nitrogen had no influence on stem diameter size variation and whorl distribution. Plant height, number of leaves and dry biomass were significantly different among the inbred lines. The interaction of inbred and nitrogen fertilizer level had significant effect on leaf width and leaf area per plant. The lowest leaf width was recorded on inbred MO345 under low nitrogen level, while the highest value was recorded on inbred MO345 under high nitrogen level. Inbred MO031 and MO199 had highest values of leaf area per plant under high nitrogen level and inbred MO345 had the lowest value under low nitrogen level. Inbred lines planted under high nitrogen level had relative advantage in leaf growth over inbred lines planted under low nitrogen level. The study showed that nitrogen fertilizers have positive effect on some root architectural traits and growth parameters of maize. Maximum leaf area was obtained by inbred MO031 and MO199 under high nitrogen level. Thus, in order to enhance leaf growth and physiological traits, the use of either MO031 or MO199 is recommended under high nitrogen level while any of these inbred lines MO001, MO034 or MO199 can be used under low nitrogen production as they are highly tolerant to low soil

nitrogen. Morphological and root architectural traits correlated positively with dry biomass in both low and high N level.

Results from the phosphorus split plot showed that only projected root area was affected by phosphorus level. Chlorophyll content, plant height, 1st whorl angle, 4th whorl angle, root top angle, root bottom angle, average lateral root length and lateral branching frequency differed significantly among the inbred lines. The interaction effect of phosphorus and inbred on root top angle and average lateral root length was significant. Inbred MO199, inbred MO034 and MO031 recorded the shallowest angles under low and high phosphorus level respectively, while inbred MO345 recorded the steeper root top angle at 54.44° under high phosphorus level. Thus to enhance P uptake, inbred MO199 is a potential candidate on low P soils. To improve water and N acquisition efficiency inbred MO345 with high phosphorus level can be used, therefore MO345 with high phosphorus can be recommended for water scarce areas such as Limpopo province. Inbred MO199 had the longest lateral roots of 251.46 mm under lower P level and significantly longer than inbred MO199 and MO001 both at lower phosphorus level. Inbred MO345 (182.88 mm) and MO001 (179.22 mm) were highly tolerant to the low P conditions as the two had shorter lateral roots, a trait vital for uptake of P. Inbred MO199 (251.46 mm) had the longest lateral roots under low P conditions showing higher tolerance to low P conditions. There were positive and significant correlations between dry biomass and morphological traits and root architectural traits on both low and high phosphorus levels. A strongly negative correlation was however observed between biomass and 2nd whorl angle on high phosphorus level. The high significant correlations indicate that selection of high yielding inbreds may be useful based on phosphorus level and biomass.

This study showed that several traits have potential under low N and P levels, hence they can be used as selection criteria for inbred lines with improved nutrient use efficiency.

Keywords: Maize inbred lines, fertilizer, growth, correlation, root angles

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of the study

Maize (*Zea mays*) is a grass belonging to the Poaceae family. It can be grown in areas with 400-600 mm precipitation per annum. According to Stephen *et al*. (2006), maize is an important cereal in the world, which ranked first in seed yield production. In South Africa, it is considered as a staple food, mostly used for breakfast cereals, thick porridge (pap), and snacks. Maize productivity has progressively increased as the food demand gets higher. Global food insecurity remains an alarming challenge in this 21st century (Funk and Brown, 2009), and it has been predicted that by 2050 the rate of food production needs to be increased by at least 50% to meet requirements of 9.5 billion people (World Bank 2016). Currently, of the seven billion people now living on earth, one billion are food insecure which accelerates the need to improve food production (Godfray *et al*., 2010), particularly in the third world.

There is a need to enhance land use efficiency. York *et al*. (2014) and Lynch (1998) indicated that one of ways to enhance land use productivity is to optimize plant nutrient-use efficiency especially in developing countries where there is little use of nitrogen (N) and phosphorus (P) fertilizers. However, fertilizers are the greatest contributors to environmental pollution as less than half of the applied N is acquired by plants and most of the remaining N becomes a source of environmental pollution (Tilman *et al*., 2002).

One of the challenges faced in maize production today is the reliance on the use of expensive and scarce inorganic fertilizers (Du Plessis, 2003). Erratic environmental changes also play very important role in the need for new varieties (Derby *et al*., 2004). The developments of maize populations suited to the current challenges are becoming limited. However, Derby *et al*. (2005) suggested that production can be improved by economization of the cost of production by adjusting inputs through proper management.

Nitrogen plays an important role in improving crop vegetative growth especially in protein synthesis (Jones, 2003), while phosphorus improves photosynthesis (Griffith,

2010), transfer of genetic information, metabolism (Daka, 2013) and root formation (Vessey, 2004). Both N and P, as macro nutrients, are therefore essential for crop growth and development. Most soils lack these nutrients as they tend to be unavailable for plant usage, making supplementation with the correct amount of fertilizers vital (Eltelib *et al.,* 2006).

Lynch (2007) indicated that the development of maize genotypes with improved nutrient acquisition is an important goal in both commercial and subsistence agriculture in order to aid in reducing fertilizer requirements. Over the years, crop yield improvement has been achieved through breeding efforts or cultural improvements in which N and P played important roles (Mollier and Pellerin, 1999). Several reports have shown markedly improved maize yield following application of nitrogenous fertilizers (Caitt, 2005; Veen, 2007). Phosphorus fertilization leads to an increase in root length and biomass of a wide range of species under variable experimental conditions (Rosolem *et al.,* 1994).

Clark (1983) indicated that there is a significant genetic variation in nutrient uptake, accumulation and use in maize. Nevertheless, the complexity of factors controlling nutrients (N and P) in the plant soil system and the difficulty in defining optimal phenotypes for specific environments tend to make selection criteria difficult (Robinson, 1989). Chun *et al*. (2005) reported that plants generally respond to nutrient deficiency by increasing the total length of both axial and lateral roots. One of the major advantages of crown roots is to supply plants with water during drought stress by absorbing water from deeper soil layers (Araki *et al*., 2000). There is therefore a need to develop maize varieties with good root whorl development and rigid stems to adapt to the current moisture and nutrient stresses, and to improve and sustain production.

1.2 Problem statement

Most South African soils lack N and P or both nutrients tend to be unavailable for plant usage (Eltelib *et al*., 2006). This situation is exacerbated by the current erratic climatic conditions which result in poor nutrient cycling. Maize is highly dependent on high soil fertility, particularly the presence of both N and P, for it to attain high yield (Veen, 2007). Replenishment of soil nutrients and increase in crop yield can be achieved through the use of inorganic fertilizers but this solution is limited in application because

of the dynamics and heterogeneity of the African agro-ecosystems in terms of biophysical and socio-economic gradients (Ikerra *et al*., 2007). Increased soil acidity, nutrient imbalance and soil degradation have been associated with the use of inorganic fertilizers (Kang and Juo, 1980). Hence the need for development of maize varieties from available germplasm that are able to withstand the current challenges of moisture and nutrient stresses.

1.3. Motivation of the study

Maize is an important crop in South Africa for human food and animal feed (Du Plessis, 2003). Its crop residues can also be used as mulch or as organic manure for soil fertility amelioration and moisture conservation (Rattan, 1995). Maize also has many industrial uses (Du Plessis, 2003). The high cost of nitrogenous and phosphoric fertilizers makes it difficult for farmers to apply them in adequate amounts to enhance growth and development of maize (Below, 2002). While maize productivity relies mainly on the use of fertilizers (Du Plessis, 2003), nutrient tolerant varieties can also be used to enhance productivity. Investigating root and plant morphology, as well as plant plasticity when faced with environmental changes, is relevant for a clear understanding of the nutritional use efficiency of plants while it also helps in the selection of genotypes that are more tolerant to abiotic stresses (Ruta *et al*., 2010). This study was set to select IBM population inbred lines with improved root whorl and stem diameter that can enable maize plants to be tolerant to nutrient stress for improved performance and productivity under given abiotic stress conditions.

1.4 Aim

The aim of the study was to select IBM population inbred lines with improved root whorl and stem diameter that can enable them to tolerate N and P stresses.

1.5 The objectives of the study were to:

i) determine the effect of N application on whorl distribution and stem diameter size variation of various maize inbred lines,

ii) determine the effect of P application on whorl distribution and stem diameter size variation of various maize inbred lines,

iii) assess the relationship between morphological and root architectural traits with biomass production under N and P stresses,

iv) determine the morphological and root architectural traits that can be used to identify superior performing inbred lines for nutrient stress under field conditions, and

v) identify superior maize performing lines in morphological, phenological and root architectural traits under nitrogen and phosphorus stress.

1.6 Hypotheses of the study were:

i) nitrogen application has no effect on whorl distribution and stem diameter size variation of various maize inbred lines,

ii) phosphorus application has no effect on whorl distribution and stem diameter size variation of various maize inbred lines,

iii) the is no relation between morphological and root architectural traits with biomass production under nitrogen and phosphorus stress,

iv) morphological and root architectural traits cannot be used to identify superior performing inbred lines for nitrogen or phosphorus stress under field conditions, and

v) Maize variety does not influence morphological, phenological and root architectural traits under nitrogen and phosphorus stress.

CHAPTER 2

LITERATURE REVIEW

2.1 Water scarcity

Globally, water scarcity remains an alarming problem particularly in the developing world where rain-fed agriculture is an important economic activity. Eighty percent of the total physical agricultural practices are under rain-fed agriculture and they generate 62 % of the world staple food (Bhattacharya, 2008). In sub-Saharan Africa 93 % of the cultivated land is rain-fed (FAO, 2002).

About 13 % of South Africa's surface area can be used for crop production while only 22 % of the total arable land can be considered as high potential arable land. Slightly more than 1.3 million ha of land is under irrigation. Water scarcity is one of the major causes of yield loss in agricultural production in the country (DoA, 2007). South Africa is generally a semi-arid country with about two third of the country receiving less than 500 mm mean annual precipitation (DoA, 2007). In South Africa, agriculture is one of the driving forces for economic growth; while low agricultural productivity is a major source of poverty, food insecurity, and malnutrition (Sharma, 2006). As of July 2014 the estimated population of South African was 53,139 528 (Worldometers, 2012), and this population is expected to grow to 82 million by 2035 (Goldblatt, 2010). More than a million households directly depend on agriculture for their livelihood therefore their food production or food import should double to feed the expanding population. Furthermore, there is a need to increase food production using the same fewer natural resources (Goldblatt, 2010).

2.2 The effect of climate change on maize production

The current changes in climate severely affect agriculture as it is expected that the frequency of drought will increase and higher spatial variability in rainfall will have negative effect on farming which is already on marginal lands (Durand, 2006). This was confirmed by the national maize production which has a 30% coefficient of variation (Du Toit *et al*., 1999). Maize has annual increase in demand by 3% as it is considered as one of the staple foods in South Africa. Maize is the largest locally produced crop and also in the Southern African Development Community (SADC) region. South Africa is considered as the main regional producer of the crop (Durand,

2006). Maize contributes 70% of grain production and covers 60 % of arable land in South Africa (Durand, 2006; Benhin, 2006). More than 9000 commercial farmers are responsible for the major part of the crop while the rest is produced by small-scale farmers (SAFS, 2008). North West province, Free State, Mpumalanga High veld and KwaZulu-Natal Midlands are the main maize producing areas in the country. The country consumes about 8 million tons of maize per year and the surplus is exported to other countries (SAFS, 2008). One major challenge is that 60% of maize is produced on drier areas of the country (Durand, 2006). Therefore, the adverse climatic changes in South Africa have potential to destabilize food security in the whole SADC region. For example, IPCC (2007) predicted that temperature is expected to increase by 1.5 to 3°C by 2050 in Africa and will continue to increase beyond this time.

BFAP (2007) indicated that maize is a hardy crop which is adaptable to harsh environmental conditions. However, its yield can still be detrimentally affected by a drier or warmer climate and lower rainfall. Since 1982, South Africa only had its largest production of maize of about 14.3 million tons in 2013/2014 season (Mahlangu, 2015). However, due to severe drought condition in 2016 the official estimate for maize production (from both commercial and non-commercial) stands at approximately 7.7 million tonnes, which is 27% less than 2015 weather affected output (FAO, 2016). This strongly indicates that investment in efficient crop water use will be very important (Kiker, 2000).

2.3 Nutrient use efficiency

Nutrient-use efficiency (NUE) can be described in many ways with emphasis on the different components of soil and plant systems (Good *et al*., 2004) or it can be based on economic returns to fertilizer use (Moose and Below, 2009). In cereals crops, nutrient-use can be expressed as the ratio of nutrient supplied to the grain yield. However, a more reasonable recent definition that is being adopted by researchers describes NUE as the ability of a genotype to produce biomass or grains when supplemented with a certain nutrient (Whang *et al*., 2010). Hence, NUE is a collective term that is composed of the acquisition efficiency (AE) and the internal utilization efficiency (UtE). Acquisition efficiency can be described as the ability of a genotype to acquire or uptake specific nutrient from the soil while the ability of this genotype to produce biomass or grain using the absorbed nutrient refers to UtE (DoVale *et al*., 2012). Therefore, an increase in AE and UtE can be used to achieve a greater N- and

P-use efficiency, which is designated NUE and PUE, respectively (Chen *et al*., 2009). On comparison of maize grain yield and N fertilizer usage globally, maize nutrient use efficiency ranges from 25-50% (Tilman *et al*., 2002). This indicates that in maize production more than half of the applied fertilizers is lost to the environment hence highlighting the need to enhance nutrient use efficiency (Moose and Below, 2009). To increase crop utilization efficiency, several biochemical and physiological traits are involved (DoVale *et al*., 2012). For example, due to the fact P availability is unevenly distributed throughout the soil profile both morphological and root architectural traits are important in P acquisition (Whang *et al*., 2010). According to Lynch and Brown (2001), the root architecture (the space configuration of the root system) determines the extent to which nutrients are explored through the soil profile. Hence the genetic makeup of the root architecture may suffer changes as a result of seeking to adapt to adverse conditions (DoVale *et al*., 2012). One clear example is the increase in root system depth and lateral root formation under low moisture stress. Hammer *et al*. (2009) indicated that majority of roots may exude organic acids into the rhizosphere or increase their root hairs in the upper root layers to provide an increase in nutrient acquisition under low P availability conditions. Therefore, variation in genotypes in the extension of lateral roots and thrust can lead to the selection of more genotypes that are nutrient efficient (DoVale *et al*., 2012).

2.5 Problems of low soil fertility in agricultural production

Soil plays a very important role in maintaining crop growth and development as it functions as a medium for eco-biological, chemical and physical processes (Omotayo and Chukwuka, 2009). Hence, there is a need to invest in soil health by managing resources to obtain optimum productivity (Omotayo and Chukwuka, 2009). The main goal of sound soil management is the creation of a healthy soil environment in which the status of nutrients is balanced in such a way that the fertility of the soil is maintained over time (Omotayo and Chukwuka, 2009).

There is a great variability in terms of African soil fertility and how these soils respond to inputs (AGRA, 2007). Due to the fragile nature of most soil resources in Africa, the soils are low in nutrient levels and have high tendency to lose nutrients (Juo and Wilding, 1996). Most of the cultivated soils also show signs of nutrient imbalances and multiple nutrient deficiencies (Mokwunye *et al*., 1996). Productivity is threatened by soil nutrient depletion and degradation, which are two important factors that have been

identified as the main contributors to decrease in crop yield as well as food production per capital in sub Saharan Africa (Henao and Baanante, 2006). Furthermore, low soil fertility and a combination of weeds, insect pests and diseases also contribute to low capital food production in Africa. Hence traditional practices are broken down resulting in government giving priority to commercial farmers than small-scale farmers (Sanchez, 2002). Majority of small-scale farmers' practices remove large quantities of nutrients from the soil without replacing it with either chemical fertilizers or manure (Sanchez, 2002). Over the last 30 years, the average depletion rate in 37 African countries was 22 kg of N, 2.5 kg of P, and 15 kg of potassium (K) per hectare of cultivated land which is equivalent to U.S. $4 billion in fertilizer annually (Sanchez, 2002).

Currently, emphasis is on developing more efficient crop cultivars for sustainable agricultural production. However soils with depleted plant nutrients make it difficult to realize the potential of genetically improved crops (SPIA, 2001). SPIA (2001) indicated that in the last 38 years, the rate of adoption of improved varieties has been similar in Asia, Latin America, the Middle East, and Sub-Saharan Africa. However, those varieties account for only 28% increase in yield in Africa while for the other regions, increase in crop yield ranges from 66 to 88% (SPIA, 2001). Traditionally, the problem of nutrient depletion can be solved by the use of inorganic fertilizers. Nevertheless, the input cost in agricultural production, fuelled by the increasing cost of fertilizers, tend to make inorganic fertilizer application very difficult especially by the small scale farming sector (Rengel and Damon, 2008).

2.5 The use of fertilizers in cropping systems

Worldwide N deficiency is one of the limiting factors in maize production (Ladha *et al*., 2005). Worku *et al*. (2007) found that less than 20 kg N ha^{-1} is applied to fields of smallholder farmers in developing countries such as those in sub-Saharan Africa. This is due to high fertilizer cost in developing countries. Elsewhere, farmers use N fertilizers intensively to maintain optimum yield (Tilman *et al*., 2002). The effectiveness of phosphorus source is controlled by both chemical and physical properties of soil, soil type, rate of application, method of application, climatic conditions and the crop species grown (Mokwunye and Biational, 2002). Over the years, there was high demand of fertilizers mainly to improve crop production, thus to increase economic returns e.g.: phosphorus was used in the form of di-ammonium phosphate, triple super

phosphate and Minjingu rock phosphate (Negassa *et al*., 2005). The use of fertilizers leads to increased crop yield and recovery of applied elements, excessive use of fertilizers can results in environmental hazards while adequate application still remain a challenge for most farmers (Sanchez *et al*., 1997). Therefore efficient fertilization and adequate application rate is vital within a growing season to ensure that plants attain maturity (Lelei *et al*., 2006).

2.5.1 Negative impact of fertilizers

High usage of fertilizers in developed countries results in abundance of inorganic nutrients in the ecosystem that may lead to disturbance of normal bio-geographic nutrient cycling (Chietera and Chardon, 2014). For example, N and P runoff into water systems from agricultural fields cause eutrophication and hypoxic zones (Robertson and Vitousek, 2009). Nitrate may also contaminate surface water or be leached out of the soil profile to contaminate underground water systems; such water has serious health risks, such as methemoglobinemia and N-nitroso-induced cancers (UNEP and WHRC, 2007).

Several agricultural activities are the contributors of emission of nitrous oxides which contributes to ozone layer depletion and global warming (Sutton *et al*., 2011). Furthermore, the production of nitrogenous fertilizers through Haber process requires high amount of energy from fossil fuels. Since the process requires the use of fossil fuel, a relative amount of nitrogen oxide is also released (Saengwilai *et al*., 2014). Kant *et al*. (2011) estimated that an increase of 1% in crop use efficiency of fertilizers could save more than $1 billion (U.S.) annually throughout the world. Therefore the environment and the economy would significantly benefit from even a minor improvement in nitrogen use efficiency (Saengwilai *et al*., 2014).

2.5.2 Soil nitrogen

Saengwilai *et al*. (2014) stated that the availability of N in the soil relies on the balance between the rate of mineralization, nitrification and de-nitrification. Factors such as soil composition, microbial activity, soil temperature and soil water status determine these processes (Miller and Cramer, 2004). Plants absorb nitrogen from the soil in the form of ammonium and nitrate; however nitrate is highly soluble in water thus mobile in soil (Barber, 1995). At the beginning of a growing season, mineralization of organic matter or application of N fertilizers followed by precipitation and irrigation can result in

additional nitrate that may exceed the capacity of the seedlings to acquire N and resulting in leaching of N below the root zone (Saengwilai *et al*., 2014).

Lynch (2013) proposed that N acquisition could positively benefit from the increase in speed of root exploration of deeper soil layers. Lambers *et al*., (2002) emphasized that there are substantial structural investments and metabolic expenditures of the root system which can exceed half of daily photosynthesis. Therefore in water and nutrient deficient environments, it is important to take full consideration of the cost and the benefits of root systems for identification of root traits that help improve crop production (Lynch, 2007).

2.6 Crop adaptation

Maize is produced throughout South Africa. It is the most important grain crop contributing almost 8.0 million tons per year and is planted on almost 3.1 million ha of land. Maize requires warm weather for effective growth and development hence a temperature of greater than 16°C is needed. A minimum requirement of 450 to 600 mm of precipitation is required per year.

According to Du Plessis (2003), high production of maize can be achieved on soil with good effective depth, good drainage, favourable morphological properties, good water holding capacity, sufficient, balanced amount of plant nutrients and chemical properties. N, P and potassium (K) are the most essential macro-nutrients which are required in large quantities for effective maize growth and development (Du Plessis, 2003). During flowering, the assimilation of N, P and K peaks, by maturity a single maize plant can uptake a total of 8.7 g of N, 5.1 g of P, and 4.0 g of K, while each ton of grain produced removes 15- 18 kg of N, 2.5 - 3 kg of P and 3 - 4 kg of K from the soil (Du Plessis, 2003). Maize kernels contain 84% carbohydrates, 10.2% protein, 4.5% fats and mineral content of 1.3% (Du Plessis, 2003).

2.7 Macro nutrient: Nitrogen

Soil N occurs in organic and inorganic forms (Tisdale *et al*., 1985). Inorganic N is in the form of ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) ions, nitric oxide (NO) and elemental nitrogen (N). Plants only absorb N in the form of ammonium and nitrate through the root by ammonia and nitrate transporters and plants grow optimally when both are present in the soil.

2.7.1 Role of N.

Nitrogen plays an important role in genetic variation of plants as it is a component of both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (Wiesler, 1998). Nitrogen is also a component of adenosine di-phosphate (ADP) and adenosine tri-phosphate (ATP) which are important in distribution of energy in plants. Nitrogen contributes about 1-4% of dry matter of the plants and it is important for plant growth. When N is not at an optimum, growth rate decreases (Brady, 1984). It facilitates the utilization of P, K and other elements in plants, and the availability of those nutrients in the soil cannot be efficiently realised by plants under N stress (Anonymous, 2000). Availability of N is vital throughout the maize growing season for optimum growth. According to Jones (2003), N is a structural component of the chlorophyll which is involved in the process of photosynthesis. It plays a significant role in protein synthesis as it biologically combines with C, H, O and S to form amino acids, which are the building blocks of proteins (Uchida, 2000). Amino acids also aid in plant growth and development as they are used in formation of protoplasm which is a site for cell division (Uchida, 2000). All plant enzymes are made up of protein hence N is required for all plant enzymatic reactions; and it is also a necessary component in many vitamins such as Vitamin B2, Vitamin B3, Vitamin B5, Vitamin B6 and Vitamin B7 (Uchida, 2000). In leafy vegetables, the quality of dry matter is improved and in grain crops protein is improved (Uchida, 2000).

2.7.2. Nitrogen deficiency

In most soils, N is reserved for plant growth and development. However, plants tend to require high amount of N that often exceeds the reserved N; hence, most crops positively respond to additional N either in the form of animal manure or inorganic (chemical) fertilizers such as Urea. Common causes of N deficiency are lack of available N in the soil or inhibited absorption of N and other nutrients due to imbalance of pH in the soil (Holtkamp Greenhouses, Inc, 1999). Plants deficient N often show chlorosis with spindly stalks beginning from older leaves to new leaves, the growth of such plants is reduced and the leaves are small (Sawyer, 2004). The symptoms of N deficiency include an appearance of V-shaped chlorosis which starts from the tip and progresses down to the midrib towards the leaf base (Sawyer, 2004). Maize ear and grain development can also be inhibited by N deficiency. It was found that at low N stress yield reduction is mainly caused by increased number of kernel abortions

(Below, 2002). Singletary and Below (1990) indicated that it appears that kernel development and productivity are directly affected by N metabolism in kernels since an increase in the capacity to synthesize protein and utilize sugar from biosynthesis of starch can be achieved through the provision of N to developing maize kernels. According to Sawyer (2004), areas with cold or saturated soil; dry soil, especially after mid-season; large amounts of low-nitrogen residue; sandy soil, inadequate fertilization; leaching from heavy rainfall; and flooded or ponded soil when the temperature rises are conditions mostly prone to the deficiency.

2.7.3 Physiological response of plants to N deficiency

Funk (2013) stated that in both N- and P-deficient soils, species that are found in the ecosystem tend to have morphological and physiological traits that enhance N and P acquisition. Plants can increase their total root length, increase root length (specific), increasing root longevity and stimulation of microbial decomposers through rhizodeposition or allocating carbon to mycorrhizae in response to the deficiency. Maize responds to low N level by increasing root to shoot ratio resulting in more assimilates being allocated from shoot to roots. Generally at low N supply, axial and the lateral root elongation is enhanced (Tian *et al*., 2005). Lateral root elongation is inhibited at extreme low N supply (Guo *et al*. 2005a). Guo *et al*. (2005b) indicated that in this type of situation a local supply of nitrate can cause significant increases of lateral root elongation. Hence in many arable lands where there is extreme heterogeneous distribution of N (variability in availability), the changes in root morphology may represent a combination of the above mentioned responses that makes it difficult to distinguish between the two (Mi *et al*., 2009). Lynch (2013) proposed an ideotype for superior N and water acquisition in maize called Steep, Cheap, and Deep (SCD), which integrates root architectural, anatomical, and physiological traits to increase rooting depth and, therefore, the capture of N in the leaching environments.

2.8 Macro nutrient: Phosphorus

Phosphorus is an important plant macro nutrient, making up about 0.2% of a plant's dry weight. After N, P is the second most frequently limiting macro nutrient for plant growth (Diskowski and Hofmann, 2005). Phosphorus is taken up by plants in the form of $H_2PO_4^-$, PO_4^{3-} and HPO_4^{2-} depending on the level of pH in the soil. Soil pH less than 5.5 may reduce the availability of phosphorus in the soil solution by 30 % or more,

while an increase in soil pH (greater than 7.2) lead to decrease in relative proportion of H_2PO^{4-} and the proportion of HPO_4^{2-} increases (Daka, 2013).

2.8.1 Role of phosphorus

Griffiths (2010) indicated that P is an essential element in the storage of sun's energy during photosynthesis and plant growth. Ninety five percent of the dry weight of plant is made up of carbohydrate, while the production of carbohydrate relies on the rate of photosynthesis. Therefore, increasing photosynthesis would improve the overall crop yield (Baker and Ort, 1992). Hence, the shortage of inorganic phosphate in the chloroplast could result in reduced photosynthesis. Phosphorus is a component of the complex nucleic acid structures (DNA and RNA) of plants, which regulate protein synthesis and also in phospholipids that form all cell membranes (Daka, 2013). Phosphorus is therefore important in cell division and the development of new tissues. Phosphorus is also associated with complex energy transformations in the plant (ATP and ADP). Vessey (2004) stated that P assists in developing vigorous seedlings and also promotes root growth. Phosphorus is involved in enzymatic reactions in the plant and is also important for seed and fruit formation and crop maturation. Phosphorus hastens the ripening of fruits thus counteracting the effect of excess N application to the soil (Daka, 2013). It helps to strengthen the skeletal structure of the plant thereby preventing lodging as such it will improve stem development of maize (Daka, 2013). Phosphorus is also essential for growth, cell division, root lengthening, seeds and fruit development as well as early ripening (Daka, 2013). It is part of several compounds, oils, and amino acids (FAO/FPN, 2006).

2.8.2 Phosphorus deficiency

According to Barber (1984), P is relatively unavailable and immobile in many soils hence root growth and development are important for the uptake of P. However, within the plant, P is readily mobile in both xylem and phloem tissues (Daka, 2013). When maize plant is exposed to P stress, P from old tissues (leaves and stem) is translocated to young tissues (shoot tip, root tip, expanding leaves and later the developing seed (Daka, 2013). The deficiency of P is usually visible in young maize plants displaying dark green with reddish purplish leaf tips including in older leaf margins; but usually the newly emerging leaves will not show any discoloration (Sawyer, 2004). The deficiency symptoms of P include retarded growth, poor root development and delayed maturity (FAO/FPN, 2006). The visual signs of deficiency are stunted growth, limited

root development, poor seed/fruit development and delayed maturity. The symptoms usually start from old leaves to new leaves; however when plants grow one meter or taller, the deficiency symptoms nearly always disappear. According to Sawyer (2004), maize hybrids tend to vary in terms of visual symptoms for P-deficiency; some hybrids tend to show purple colours at early stages of growth even though there are enough P nutrients while other hybrids do not show the symptoms even if there is inadequate P which can lead to severe negative impact on yield. However, Van Straaten (2002) emphasized that leaves and stems show purpling discolouration in severe cases. Phosphorus deficiencies are mostly found on inherently P-deficient soils and in soil systems where nutrients are removed and not adequately replaced. Conditions which promote soil P deficiency include cold soils that are either too wet or too dry for applied P to be efficiently used by plants roots, compacted soils and plant roots which are injured by either insects, herbicides or fertilizers (Sawyer, 2004).

2.8.3 Physiological response of plants to phosphorus stress

Plants respond to P deficiency in different ways, these include improving P acquisition and internal P recycling. The mechanisms of responses include intensified secretion of acid phosphatase (Bozzo *et al*., 2006), increased production of transcription factors (Li *et al*., 2010) and also altered root morphology (Zhang *et al.,* 2009). Some plant physiologists have also suggested that P has several impacts on photosynthesis: it can affect transfer of photons across the thylakoid membranes (Wissuwa *et al*., 2005), it results in inactivation of several pivotal enzymes involved in the Calvin cycle (Jacob and Lawlor, 1992) and the feedback of photosynthesis is inhibited across the thylakoid membranes through a reduction in electron transfer (Preiss, 1984). There are several hormones and organic acid syntheses that are regulated mainly to promote absorption and immobilisation of inorganic phosphate (Yao, 2011). Lan *et al*. (2012) found that during P deficiency, *Arabidopsis thaliana* responded by alteration of the composition of lipid membranes and the activity of the alternative glycolysis pathway to increase internal P utilization efficiently. According to Li *et al*. (2008), when plants are under P starvation, accumulation of defence or stress related proteins such as superoxide dismutase (SOD), heat shock proteins (HSP) and proteins involved in the ubiquitin/26S proteasome pathway are initiated as a mechanism to prevent stress.

Furthermore, Laegreid *et al*. (1999) emphasized that for efficient plant growth and development mineral nutrients such as N, P, K and sulphur (S) are required in large

amounts. Therefore deficiency of any of those nutrients can negatively affect plant metabolism which then results in poor yield, reduced nutritional quality and taste, as well as the ability to resist pests and pathogens in crops.

2.9 Description of the maize root system and its function

The root system is vital to maintaining the productivity of plants under environmental stress (Lynch 1995). The maize root system consists of two main components which are the embryonic and post embryonic. The embryonic is classified into two different classes, which are the primary root and the scutellar where variable number of seminal roots are formed. The roots that are formed consecutively on the shoot nodes and lateral roots in the pericycle of all root classes, are classified as post embryonic roots. The crown roots are those shoot borne or nodal roots that are formed below ground whereas the brace roots are those roots that are formed above ground (Hochholdinger, 2009). The primary roots and seminal roots are mainly responsible for early stages of growth. They help to establish the seedling after germination while the nodal and crown roots are mainly responsible for the uptake of soil resources at the later stages of growth. Most of the root system is made up of the nodal and specifically the crown roots (Figure 2.1) (Hoppe *et al*., 1986).

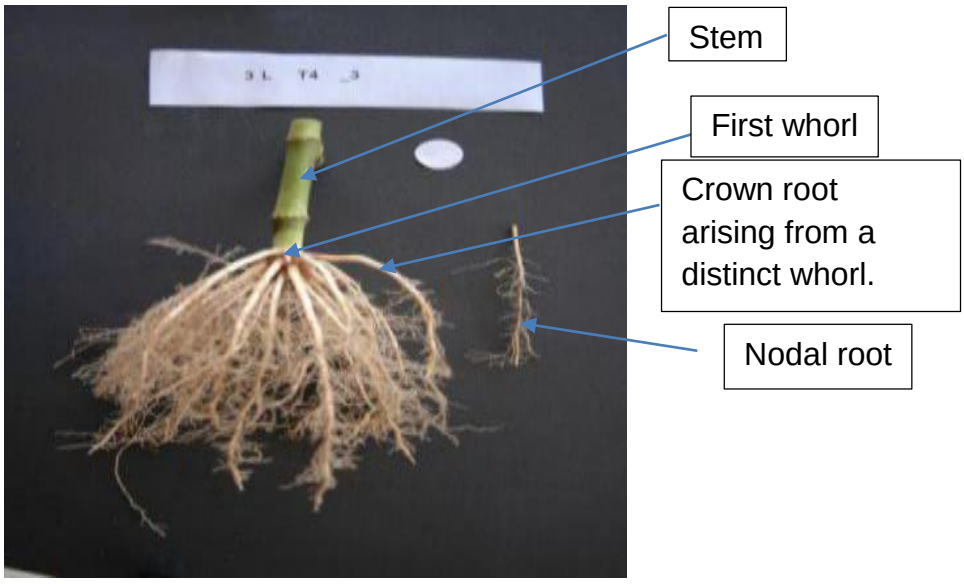


Figure 2.1 Maize root crown.

Lynch (2013) proposed the Steep, Cheap, and Deep (SCD) ideotype, this ideotype accentuate crown root number as one of the traits which enhance nutrient use efficiency in plants. The crown root number is a collective trait that refers to number of

belowground nodal whorls and the number of roots per whorl (Lynch, 2013). Hochholdinger *et al*. (2004) emphasized the significance of crown roots during vegetative growth after the first few weeks and also during reproductive stage indicating that they are dominant in resource acquisition. According to Trachsel *et al*. (2011), the number of crown roots ranges from 5 to 50 under fertile soil conditions; but the roots at lower range may be too widely spread to adequately explore the soil. However, there is a high risk of damage by herbivores, pathogens and loss of some roots on infertile soil resulting in fewer crown roots to support the plant in terms of nutrients, water and anchorage. Crown roots at higher ranges could lead to high competition within the crown roots for nutrients and water resulting in considerable metabolic cost for plant. Hence, the proposed SCD ideotype in maize emphasizes the need for optimal crown roots number to enhance N acquisition.

Saengwilai *et al*. (2014b) indicated that under low N conditions where there are limited resources for root growth, a dispersed root system can be vital in capturing nitrate, as nitrate is mobile. In this case, the best crown root number tends towards the lower end of phenotypic variation that could help develop genotypes make resource more available for the development of longer and deeper roots unlike having greater crown roots number (Saengwilai *et al*., 2014b). According to Lynch (2013), maize genotypes with fewer crown root number could explore soils at greater depth, ensure a greater N uptake, growth, development and also yield than genotypes with a larger number of crown roots in low N soils. Naturally, P is largely found in the top soil (Lynch, 1995). Bonser *et al*. (1996) reported that low P availability increases the shallowness of basal roots, especially in P-efficient genotypes in common bean. Furthermore, the basal root shallowness was correlated with yield performance in low P tropical soil among a set of unrelated genotypes and among the recombination inbred lines (Liao *et al.,* 2001). Ge *et al*., (2000) indicated that based on a geometric modelling, the shallow basal roots system suffers less inter-root competition for top soil P than deeper root system. These observed results are in agreement with efficient top soil foraging regulated by basal roots for enhanced P efficiency hypothesis as reported by Lynch and Brown (2001).

Contrarily, Rubio *et al*. (2003) reported that shallow root system did not have any positive competitive advantage on common bean when P was evenly distributed in the soil. However root architectural traits encouraging foraging in common bean (Lynch

and Brown, 2001) and maize genotypes (Zhu *et al.* 2005a) lead to best growth when P was restricted to the top soil. Where resources are highly limited, adjustments of the root system were experienced when roots were not organized to enhance resource acquisition in common bean (Lynch and Ho, 2005). Phenes responsible for resource exploration can affect the root density (number or length of roots in a volume) by increasing of the axial roots, lateral branching, or root hairs and modification of the rhizosphere. For example the rhyzosphere can be modified by releasing protons, organic acids, and by exudation of enzymes that release P from organic compounds to decrease the pH (Lambers *et al*., 2006). Depending on the spatial scale, resources exploration and exploitation can be affected by Mycorrhizal symbioses. Mycorrhizal fungi have the ability to increase soil exploration by the growth of their hyphae, and exchange P and carbon with their host plant (Harley, 1989).

According to York *et al*. (2013), resource acquisition phenes do not only differ in terms of foraging strategies but also vary in the way they influence plant metabolism while the mechanism through which the phenes are used is affected by the metabolism. The relative costs and benefits of the root phene are greatly influenced by their usefulness in soil resource acquisition (Lynch and Ho, 2005). Several economic currencies are used to estimate the benefits/costs relationship such as carbon (C), N and P (Lynch and Beebe, 1995). Root metabolic demand is one of their major costs while metabolic cost can be divided into the cost to maintain and construct the roots (Chapin III *et al*., 1987). The volume of the root strongly influences the construction of the roots. The volume of root is relative to the length and diameter therefore, the phenes that determine elongation rate, branching, number of roots formed, and root diameters will influence the construction costs. For construction and maintenance roots require C minerals and nutrients. An adaptive trait for nutrient acquisition where phenes alter their root metabolic demand by decreasing their root diameter in order to increase root length has been proposed as adaptive trait for nutrient acquisition (Lynch and Brown, 2008). The conversion of living cortical tissue to air space through programmed cell death by root cortical aerenchyma can also help lower the respiration of root segments (Fan *et al*., 2003) and additionally enhance mobilizing nutrients for other uses (Postma and Lynch, 2010).

Hence breeders need to select genotypes with root architectural traits adapted to the targeted environmental conditions for effective improvement of plant productivity or

performance (Trachsel *et al*., 2011). However, the basic qualitative genetics studies require rapid, accurate and robust phenotyping protocols but the evaluation of the root architecture directly in soil is difficult. Therefore, the use of shevelomics was proposed to allow rapid evaluation of root architecture phenotypes that thrive under marginal environmental conditions for effective water and nutrient acquisition (Trachsel *et al*., 2011).

Walter *et al*. (2009) suggested that there are complex interactions between plants and extrinsic and intrinsic abiotic and biotic soil factors as well as the dominating environmental conditions that artificial system fail to mimic. There are various environmental conditions which roots and shoots are exposed to in the field especially regarding temperature, which is also the most important root development regulator (Hund, 2010). Controlled conditions lead to highly artificial conditions for the root system due to typically simulated shoot conditions exposure.
Compared to field conditions when plants are grown in a small container the root system is shielded from the atmospheric environment in a completely different way. Hence in investigations under controlled conditions there is high risk of artifacts of root growth or of root shoot interaction, when the aim is mainly to simulate field conditions.

In both agricultural systems and natural ecosystem, the root system plays a significant effect in resource acquisition, plant interactions, and nutrient cycling (Lynch, 1995). Crop improvement can be achieved through the identification of root phenes and understanding their utilization in terms of resource acquisition phene-based or ideotype breeding (York *et al*., 2014). However, in maize root system the outer whorl are the youngest roots, which occlude the older roots in the interior making it difficult to study the variation of phenes within the maize root system. For example, when only one line may be reported when the root phenes are different (Picard *et al*., 1985) or measurements may be incomplete of only a few nodes for many lines (Guingo *et al*. 1998).

2.10 Work done on research problem

Karasu *et al*. (2009) reported that application rates of 0, 150, 300 and 450 kg N ha^{-1} did not have significant difference on plant height, first ear height, stem diameter, the number of leaf and ear per plant, ear percentage in green herbage, except forage and dry matter yield of maize. The study however reported that application rate of 300 kg

N ha^{-1} was suitable for high forage and dry matter yield; with the highest obtained forage cultivar yields of between 81457 and 92913 kg ha^{-1}. Rubio *et al*. (2003) reported the lack of competitive advantage in terms of nutrient uptake on shallow rooted common bean genotypes contrary to similar study in maize (Zhu *et al*., 2005a) Genotypes having root architectural traits that enhance top soil foraging grew best. Trachsel *et al*. (2011) reported that the shallow root angles of the first arm of the brace roots originated from the first whorl and brace roots which grew from above ground.

Miiller *et al*. (2003) reported that P stimulated adventitious rooting in two P-efficient common bean genotypes while adventitious rooting was not stimulated on two inefficient genotypes. Furthermore, adventitious roots had greater length per unit biomass than other roots types in P-stressed soil (Miller *et al*., 2003). On both low and high N level, adventitious roots seem to have less construction cost than basal roots (Miller *et al*., 2003). Miiller *et al*. (2003) further indicated that low soil-P level tend to reduce the lateral root density on adventitious roots than basal roots. A study by DoVale *et al*. (2013) on 15 maize inbred lines revealed that both the shoot and root morphology traits have an association with the acquisition efficiency of both N and P in the entire evaluated environment. Specifically, the results indicated that there was a significant and positive relationship between lateral root length and axil root length on both the high and low N and P soil conditions. On both low and high N and P a significant and positive relation was observed on acquisition efficiency with axil root length (P=0.01) and lateral root length (P=0.01). In both experiment utilization efficiency had no significant correlation with any of the morphological traits in both levels. However, the utilization efficiency negatively correlated with acquisition efficiency in low soil-P conditions. Shoot dry weight was also reported to have a strong significant correlation with morphological traits and the acquisition efficiency in both low and high level of N and P experiments, except for lateral root length in high P level (DoVale *et al*., 2013).

2.11 Work not yet done on the problem

Most of the studies relate root development to drought stress. Most studies were focused on brace roots, number of roots, branching density, number of roots, and not on whorl and stem development and other morphological traits. The limited reports on phenotypical traits suggest the need for investigation of these traits to broaden the

knowledge on intermated B73 × Mo17 (IBM) inbred population of maize response to nutrient stress under South African climatic conditions.

2.12 Addressing the gaps

To understand how nutrient stress affect root whorl development and the size of the stem diameter, a series of experiments needs to be conducted under both N and P stress conditions. Various morphological and root architectural parameters need to be measured and analysed. In relation to nutrient status soil analysis also needs to be taken into consideration as it plays a vital role in plant growth and development.

CHAPTER 3

METHODOLOGY AND ANALYTICAL PROCEDURES

3.1 Description of study site

Two comparison experiments were conducted at the Ukulima Root Biology Centre (24°32'58.1"S, 28°06'21.1"E and 1237 m above sea level) in Waterberg district, Limpopo Province during the 2013-2014 cropping season. The climate of the area is considered as tropical with a dry season, receiving less than 1000 mm of precipitation annually and with mild temperatures that range from 14°C to 30°C and the soil is sandy to sandy clay loam (Baker, 2011). The farm is part of the bushveld ecoregion and primarily comprised of grassland and dry deciduous forests (Baker, 2011). The average temperature between planting and sampling was 22°C, total rainfall was 340 mm with an average relative humidity of 70%, while the soil type was clovelly loamy sand (York and Lynch, 2015).

3.2 Plant material

Seeds of maize from the Intermated B73 and Mo17 (IBM) population were obtained from Dr Shawn Kaeppler (University of Wisconsin, Madison, USA). Lee *et al*. (2002) reported that genetic resolution was improved resulting in nearly fourfold increase in the genetic map distance because additional opportunities for recombination were provided during the multiple generation of the intermating process. Ten inbreds derived from IBM population were grown under low and high nitrogen (56 kg N $ha^{-1}$and 243 kg N ha^{-1}) and phosphorus levels (0 kg P ha^{-1} and 56 kg P ha^{-1}) respectively, and Pioneer hybrid 60MK was used as a surrounding border crop. The tested inbreds were lines MO007, MO364, MO199, MO034, MO196, MO248, MO165, MO031, MO345 and MO001. Furthermore six lines with high vigorous growth were selected for root architectural traits and biomass data: MO345, MO034, MO001, MO199, MO031 and MO196. Standard agronomical practices for maize consisting of weeding, pest and diseases management were implemented up to harvest stage.

3.3 Research design

3.3.1 Experiment 1: Nitrogen

The experiment was conducted in split plot arrangement based on randomized complete block design (RCBD) with four replicates. The main plot factor was N and the subplot factor was IBM inbred lines. A jab planter was used to open rows for manual planting. The field received P and potassium (K) as determined by soil test. Within the high and low N, split plot IBM inbred lines were randomly assigned to plots. Each split plot comprised of 3 rows of 4.5 m length with inter-row spacing at 76 cm and in-row spacing at 20 cm. The seeds were planted by hand on the 26th November 2013 into the rows marked by planter to accommodate a density of 65790 plant ha^{-1}. The test site generally had soil low in N (Table 4.1), at planting 23 kg N ha^{-1} was applied to all the blocks through centre pivot fertigation, and additional 23 kg N ha^{-1} was applied to the high N blocks using granular urea (46%N). On the third week after planting the high N split plots received additional 46 kg ha^{-1} of N using granular urea (46%N). The high N split plot received 46 kg N ha^{-1} using granular urea every three weeks to reach to total of 243 kg N ha^{-1}. The low N split plots received 10 kg N ha^{-1} 8 weeks after planting, furthermore an additional 23 kg N ha^{-1} was applied on the 10th week to reach to total of 56 kg N ha^{-1} using granular urea (46%N). A uniform application rate of 3 kg ha^{-1} of secondary nutrients (Ca and Mg) using dolomite (34% Ca and 18% Mg) as well as 21 kg K ha^{-1} using muriate of potash (0-0-60 NPK) were applied at seven weeks after planting across all plots. Additional of 3 kg ha^{-1} of secondary nutrients (Ca and Mg) were applied at the 10th week using dolomite (34% Ca and 18% Mg) to reach to a total of 6 kg ha^{-1}. The experiment was irrigated through the use of centre pivot system; with a total of 584.2 mm of irrigation was applied throughout the season. Rainfall data were collected during the growing season from weather station installed at the farm.

3.3.2 Experiment 2: Phosphorus

The experiment was conducted in split plot arrangement based on randomized complete block design (RCBD) with four, replicates. The main plot factor was phosphorus and the subplot factor was the IBM inbred lines. A jab planter was used to open rows for manual planting. The test site generally had soil high in P (Figure 5.1), the experiment was carried out by applying two levels of phosphorus as main

plots consisting of 0 kg P ha^{-1} and 56 kg P ha^{-1} from single super phosphate (10.5%P) at planting. Within the high and low P split plots IBM inbred lines were randomly assigned to sub plots. Each sub plot comprised of 3 rows of 4.5 m length with inter-rows spacing at 76 cm and in-row spacing at 20 cm. The seeds were manually planted by hand on the 27th November 2013 into the rows marked by planter to accommodate a density of 65790 plants per ha^{-1}. A uniform application rate of 24 kg N ha^{-1} was applied to the experiment using granular urea (46%N) 11 days after planting. Potassium at 42 Kg ha^{-1} using muriate of potash (0-0-60 NPK) and 3 kg ha^{-1} of secondary nutrients (Ca and Mg) using dolomite (34% Ca and 18% Mg) together with an additional 50 kg N ha^{-1} using granular urea (46%N) was applied 39 days after planting. Additional 23 kg N ha^{-1} was subsequently applied using granular urea (46%N) through drip fertigation every three weeks to reach to a total of 120 kg N ha^{-1}. Additional application of 3 kg ha^{-1} of secondary nutrients (Ca and Mg) using dolomite (34% Ca and 18% Mg) was applied 10 weeks after planting to reach a total of 6 kg ha^{-1}. The experiment was irrigated through the use of drip irrigation system; a total of 584.2 mm of irrigation was applied throughout the season. Rainfall data were collected during the growing season from weather station installed at the farm.

3.4. Data collection

Soil data

Soil sampling was conducted randomly (Pennock *at al*., 2006) on each replicate by taking soil cores using a steel soil corer lined with a plastic tube (60 cm depth and 42 mm diameter) at the beginning of the experiment, and in each plot two replicates were taken after harvesting (Figure 3.1).

Figure 3.1 Procedure for collection (left) and sectioning (right) of soil cores.

Analysis for both macro and micro nutrients, pH and bulk density was conducted by the Omnia fertilizers soil analysis laboratory, Bryanston, South Africa for the first experiment on the 14 January 2014 (Table 4.1). Ammonium acetate (NH_AOAC) extraction method was used to determine cation content of the soil (Kitsopoulas, 1999) and P content with P-Bray P1 method (Bray and Kurtz, 1945). Sulphur was determined using calcium phosphate ($Ca(H_2PO4)_2$) extraction method (Wrenshall and Mackibbin,1935). Effective cation exchange capacity was calculated based on summation of extracted cation: K^+, Na^+, Mg^{2+} and $Ca2^+$. Analysis for soil available P was conducted at Ukulima Root Biology Centre for the second experiment using Bray 1 method before planting (Figure 5.1). At harvesting the soil was analysed for pH, total N using Kjeldahl method (Bremner and Mulvaney, 1982), available P using Bray P1 method (Bray and Kurtz, 1945) and soil organic carbon using Walkley-Black method (Walkley and Black, 1934) at the University of Limpopo Soil Science laboratory (Nitrogen experiment: Table 4.2 and Phosphorus experiment: Table 5.1).

Morphological data

Data were collected from three plants per plot at tasseling. The number of leaves per plant was counted manually, stem diameter was measured using an AmiPro T74615 Electronic calliper at the first node, while leaf area and plant heights were measured using a ruler (Figure 3.2). Length and width of the third leaf from the top were measured to determine leaf area. The leaf area was calculated by the non-destructive measurement of length x width method using the relation: Leaf area = 0.75 (length x

width) where 0.75 is a constant (Saxena and Singh, 1965). This area was then multiplied by the number of leaves to determine the total leaf area per plant. Chlorophyll content of the flag leaf was measured using a chlorophyll meter (Spad-502779 23062; Konica Minolta sensing. Inc., Japan) as shown in figure 3.2.

Figure 3.2 Sampling procedures for morphological traits.

Biomass data

To determine above ground biomass three adjacent plants which were randomly selected for morphological traits were cut at ground level at harvest. A pruning shear was used to cut the maize on the second node to separate the crown roots from the stover. The stover was then oven dried for 72 hours at a temperature of 60°C and weighed with a weighing balance.

Root architecture data

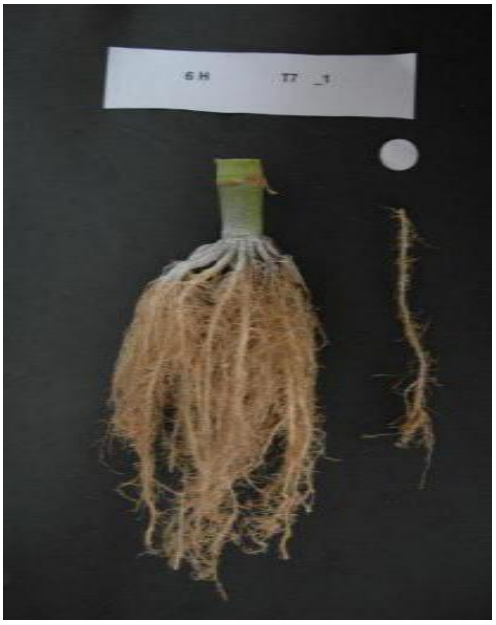
Phenotyping for root architecture was carried out during the tasselling stage of plants by using two representative plants per plot for each experiment. Roots were carefully harvested by applying a shovelomics technique (Trachsel *et al*., 2011). The crown roots were kept in large plastic bins immersed in water in a 5°C cold room until they were imaged and root whorls were counted within two weeks. The crowns were imaged using a digital Nikon PL000 coolpix P60000 camera attached to a frame with the camera mounted facing down from a height of 50 cm. The crowns were placed on a black background; a round small white paper of 3 cm was included as a scale in every image with printed sample labels (Figure 3.3). A representative nodal root was

removed from the side of the crown not facing the camera from the second whorl and placed at the side of the crown root in such a way that both crown root and representative nodal root were in a frame of the image (Figure 3.4). The images were evaluated using Digital imaging of root traits (DIRT) software (Bucksch *et al*., 2014). Measured traits include: projected root area, average root density, root top angle, root bottom angle, number of adventitious roots, number of basal roots, tap root diameter, average lateral root length, lateral branching frequency and distance to the first lateral root. The number of whorls formed was counted manually. The whorl angle was measured by displaying roots on a 180° protractor sketch board where the stem was at 0° and the angle on both sides will be measured and their average determines whorl angles (Figure 3.3).

Figure 3.3 Sampling procedures for assessment of root architectural traits.

HIGH NITROGEN LEVEL

LOW NITROGEN LEVEL

A 

B 

HIGH PHOSPHORUS LEVEL

LOW PHOSPHORUS LEVEL

C 

D 

Figure 3.4 Root crown image on low and high nitrogen and phosphorus respectively.

3.5 Data analysis

The data collected were subjected to analysis of variance (Appendix 51) using statistics software, STATISTIX 10.0 package. Treatment means were separated using Tukey's multiple range tests at a probability level of 5%. Simple Pearson correlations were conducted among various components to determine the relationship of the root traits with dry biomass.

Chapter 4

Nitrogen experiment

4 Results and discussion

4.1 Results

4.1.1 Soil analysis results 8 weeks after planting.

The soil properties of the soil at 8 weeks after planting of the experiment are shown in Table 4.1. The soil was sandy with a bulk density of 1362.30 and 1373.50 $kg.m^{-3}$ from low and high nitrogen level, respectively. Total nitrogen was 8.10 and 13.10 mg. kg^{-1} on low and high nitrogen, respectively. Soil pH value for both low (6.29) and high (6.54) nitrogen level indicate slight acidity when measured in water (H_2O) but showed very strong acidity for both low (4.73) and high (4.49) nitrogen trial when measured in KCl indicating the need for lime so as to correct for the potential negative impact on root growth. Soil P and K levels were interpreted based on Shober *et al*. (2013) soil interpretation procedure. The available soil phosphorus was low on the high nitrogen level while medium on the low nitrogen level and in both nitrogen levels sulphur was low. The exchangeable cations: (K^+) was at an optimum level, Ca^{2+} was at excessively high, Mg^{2+} was at a medium level while the Na^+ was at a low level in both high and low nitrogen level, respectively; with a CEC of 1.46 and 1.41 $cmol_c.kg^{-1}$ on low and high nitrogen level, respectively. The observed low soil fertility could be as a result of continuous cropping of land with little nutrient returns.

Table 4.1 Soil analysis for nitrogen experiment at the beginning of the experiment

Parameter measured	Soil nitrogen level			
	Low		High	
	Concentration	Standard deviation	Concentration	Standard deviation
Total N ($mg.kg^{-1}$)	8.10	2.47	13.10	5.88
Bulk density($kg.m^{-3}$)	1362.30	40.29	1373.50	78.64
pH (KCl)	4.73	0.65	4.49	0.48
pH (Water)	6.29	1.49	6.54	1.45
S ($mg.kg^{-1}$)	26.10	27.79	39.20	26.65
P ($mg.kg^{-1}$)	28.00	27.20	16.70	19.43
K ($mg.kg^{-1}$)	58.60	7.07	58.50	8.78
Ca ($mg.kg^{-1}$)	155.40	55.81	137.80	32.77
Mg ($mg.kg^{-1}$)	40.40	10.44	39.10	7.34
Na ($mg.kg^{-1}$)	15.20	4.87	18.60	5.10
ECEC ($cmol_c.kg^{-1}$)	1.46	0.26	1.41	0.16
Ca/Mg	2.31	0.45	2.13	0.22
Mg/K	2.84	2.20	2.18	0.45
(Ca+Mg)/K	7.20	1.87	6.80	1.69

* ECEC = calculated from the sum of the cations (K^+, Ca^{2+}, Mg^{2+} and N^+ $cmol_c.kg^{-1}$)

4.1.2 Soil analysis results after harvesting.

The chemical properties of the soil after harvesting are shown in Table 4.2. The experiment had reserved acidic soils, reserved acidity is the acidity which is absorbed on the surfaces of soil and organic particles, this portion of acidity accounts to 99% of the total acidity (Bast *et al*., 2011). The top soil depth (0-20 cm) of the high nitrogenous split plot had very strong acidic soil with a pH of 4.85, while the lower soil depth (20-40 cm) and the top soil depth (0-40 cm) of low nitrogenous split plot had slightly acid soils with pH ranging from 6.18 to 6.40 respectively. Soil depth (40-60 cm) on high nitrogenous split plot had soils which were moderate in acidity while the low nitrogen split plot has soils which were strongly acidic, with pH of 5.66 and 5.49, respectively. The pH measurement (KCl) indicated that both low and high nitrogenous split plots had soils which were highly acidic, the top soil depth (0-40 cm) of the high nitrogenous

split plot had strongly acidic soils with pH less than 5.63 while the top soil on low nitrogenous split plots were moderate in acidity. In both low and high nitrogen split plots the lower soil depth (40-60 cm) had soils which were extremely acidic with pH of 4.41 and 4.48, respectively. Both split plots had high concentration of phosphorus on the top soil depth (0-20 cm) while the lower soil depth (20-60 cm) had a medium soil phosphorus content. The recorded soil organic carbon and organic matter showed low contents in both plots. Carbon ranged from 1.36 g/100g on low nitrogen split plots to 1.92 g/100g on high nitrogenous split plots with the top soil depth (0-20 cm) recording highest values of soil organic carbon in both low and high nitrogen splits plots.

Table 4.2 Soil analysis for nitrogenous split plots at harvesting.

Nutrient level	Low			High		
Soil depth	0-20	20-40	40-60	0-20	20-40	40-60
pH (KCl)	5.56	5.18	4.48	5.55	5.63	4.41
pH (water)	6.40	6.18	5.49	4.85	6.41	5.66
Organic carbon (g/100g soil)	1.91	1.36	1.17	1.92	1.14	1.51
Organic matter (%)	3.28	2.34	2.01	3.30	1.96	2.60
Phosphorus ($mg.kg^{-1}$)	45.17	24.85	26.23	51.89	38.40	30.88

4.1.3 The effect of nitrogen on growth attributes of maize inbred lines.

4.1.3.1 Leaf length

There was a significant difference between leaf length per plant ($P \leq 0.05$) obtained from low and high nitrogen treatments with high nitrogen level resulting in plants with longer leaves that averaged 56.45 cm while plants planted on low nitrogen level had shorter leaves that averaged 39.46 cm (Table 4.3). Leaf length was not significantly different among the inbred lines ($P \geq 0.05$); and ranged from 44.48 cm per plant for inbred MO196 to 52.28 cm per plant for inbred MO001 (Table 4.3).

4.1.3.2 Leaf width

Interaction of nitrogen and inbred was significant ($P \leq 0.05$) on leaf width, the lowest leaf width was 5.62 cm recorded on inbred MO345 under low nitrogen level, while the highest value recorded was 8.96 cm on inbred MO345 under high nitrogen level (Table 4.4). There were significant differences in leaf width per plant ($P \leq 0.01$) between low

and high nitrogen with high nitrogen level resulting in plants with broader leaves which averaged 7.96 cm while plants on low nitrogen level averaged 5.99 cm (Table 4.3). Leaf width showed no significant differences among the inbred lines (P≥ 0.05), leaf width ranged from 6.19 cm per plant for inbred MO196 to 7.29 cm per plant for inbred MO345 (Table 4.3).

4.1.3.3 Leaf area per plant

Interaction of nitrogen and inbred was not significant (P≤ 0.05) on leaf area, leaf area ranged from 1644.60 cm^2 per plant for inbred MO345 under low nitrogen level to 4295.20 cm^2 per plant for inbred MO031 under high nitrogen level. There were significant differences in leaf area per plant (P≤ 0.01) between low and high nitrogen levels with high nitrogen level resulting in larger leaf area per plant, with plants averaging 3728.80 cm^2 while plants planted on low nitrogen level had smaller leaf area per plant that averaged at 1904.10 cm^2 (Table 4.3). Leaf area was not significantly different among the inbred lines (P≥ 0.05); and ranged from 2323.20 cm^2 per plant for inbred MO196 to 3122.10 cm^2 per plant for MO031 (Table 4.3).

4.1.3.4. Chlorophyll content

Interaction of nitrogen and inbred was not significant (P≥ 0.05) on chlorophyll content. Chlorophyll content ranged from 26.725 mg/g per plant for inbred MO345 under low nitrogen level to 46.075 mg/g per plant for inbred MO001 under high nitrogen level. There were significant differences in chlorophyll content (P≤ 0.05) between the low and high nitrogen levels with high nitrogen level resulting in higher chlorophyll content per plant, with plants averaging 40.94 mg/g while plants planted on low nitrogen level had lower chlorophyll content per plant averaged at 30.575 mg/g (Table 4.3). Chlorophyll content was not significantly different among the inbred lines (P≥ 0.05); and ranged from 31.212 mg/g per plant for inbred MO345 to 39.863 mg/g per plant for inbred MO001 (Table 4.3).

4.1.3.5 Plant height

Interaction of nitrogen and inbred was not significant (P≥ 0.05) on plant height. Plant height ranged from 86.75 cm per plant for Inbred MO001 under low nitrogen level to 136.10 cm per plant for inbred MO199 under high nitrogen level. Plant height was not significantly different between low and high nitrogen levels (P≥ 0.05), both levels had tall plants, with plants averaging 124.28 cm on high nitrogen level while plants planted

on low nitrogen level averaged at 96 cm in height (Table 4.3). Plant height showed significant differences among the inbred lines (P≤ 0.05). Inbred MO031 recorded the tallest plants at 118.33 cm in height while Inbred MO001 recorded shortest plants at 99.37 cm in height (Table 4.3).

4.1.3.6 Stem diameter

Interaction of nitrogen and inbred was not significant (P≥ 0.05) on stem diameter. Stem diameter ranged from 11.89 mm per plant for inbred MO034 under low level to 36.47 mm per plant for inbred MO031 under high nitrogen level. Stem diameter was not significantly different between low and high nitrogen levels (P≥ 0.05), both levels had thin plants, with plants averaging 20.18 mm on high nitrogen level while plants planted on low nitrogen level averaged at 13.33 mm in diameter (Table 4.3). Stem diameter was not significantly different among the inbred lines (P≥ 0.05); and ranged from diameter ranged from 13.63 mm per plant for inbred MO034 to 25.65 mm per plant for inbred MO031 (Table 4.3).

4.1.3.7 Number of leaves

Interaction of nitrogen and inbred was not significant (P≥ 0.05) on number of leaves. Number of leaves ranged from 10 leaves per plant for inbred MO001 under low nitrogen level to 11 leaves per plant for inbred MO031 under high nitrogen level. Number of leaves was not significantly different between low and high nitrogen levels (P≥ 0.05), with plants averaging 10.79 leaves on high nitrogen level while plants planted on low nitrogen level averaged at 10.62 leaves (Table 4.3). Number of leaves was significantly different between the inbred lines (P≤ 0.01) with Inbred MO031 recording the highest number of leaves of 11 leaves per plant while Inbred MO001 recorded lowest leaf number of 10 leaves per plant (Table 4.3).

Table 4.3 Influence of nitrogen level and inbred on plant height, leaf length, leaf width, leaf area per plant, average leaf area, chlorophyll content, stem diameter and number of leaves of selected maize IBM inbred lines.

Nitrogen level		Plant height (cm)	Leaf length (cm)	leaf width (cm)	Leaf area per plant (cm^2)	Chlorophyll content (mg/ g)	Stem diameter (mm)	Number of leaves
High		124.28[a]	56.45[a]	7.95[a]	3728.80[a]	40.94[a]	20.18[a]	10.79[a]
Low		96.03[a]	39.46[b]	5.99[b]	1904.10[b]	30.57[b]	13.33[a]	10.62[a]
P value		0.0540	0.0261	0.0026	0.0029	0.0201	0.1224	0.5515
Significance		ns	*	**	**	*	ns	ns
Turkey HSD		29.161	13.157	0.6692	646.580	7.2787	10.221	0.7931
CV%		28.81	29.86	10.44	24.99	22.15	66.37	8.06
Maize inbred	MO031	118.33[a]	51.72[a]	6.81[a]	3122.10[a]	35.02[a]	25.65[a]	11.33[a]
	MO199	115.79[ab]	46.64[a]	7.20[a]	3075.30[a]	35.73[a]	14.46[a]	11.16[ab]
	MO196	112.92[ab]	44.48[a]	6.19[a]	2323.20[a]	37.64[a]	15.64[a]	10.83[ab]
	MO034	109.87[ab]	45.07[a]	7.06[a]	2694.10[a]	35.10[a]	13.63[a]	10.54[ab]
	MO345	104.63[ab]	47.55[a]	7.29[a]	2698.60[a]	31.21[a]	15.03[a]	10.21[b]
	MO001	99.37[b]	52.28[a]	7.28[a]	2985.40[a]	39.86[a]	16.12[a]	10.16[b]
P value		0.0207	0.0560	0.0751	0.1512	0.1596	0.2199	0.0056
Significance		*	ns	ns	ns	ns	ns	**
Turkey HSD		17.208	9.139	1.210	984.220	9.497	15.623	1.030

Means in the same column followed by the same letters are not significantly different at P ≤ 0.05. * = significant at P ≤ 0.05, ** = significant at P ≤ 0.01, ns = not significant at P ≤ 0.05.

Table 4.4 Interactive effect of nitrogen level and inbred on plant leaf width of selected maize IBM inbred lines.

Nitrogen level	Inbred	Leaf width (cm)
High	MO345	8.96[a]
High	MO199	8.40[ab]
High	MO034	8.04[abc]
High	MO001	8.04 [abc]
High	MO031	7.86[abcd]
High	MO196	6.42[bcde]
Low	MO001	6.52 [bcde]
Low	MO034	6.09[cde]
Low	MO199	6.01 [cde]
Low	MO196	5.97 [cde]
Low	MO031	5.75[de]
Low	MO345	5.62[e]
P value		0.0293
Significance		*
Turkey HSD (means for the same level of nitrogen)		1.9886
Turkey HSD (means for different level of nitrogen)		2.2402
CV%		11.40

Means in the same column followed by the same letters are not significantly different at P ≤ 0.05, * = significant at P ≤ 0.05, ns = not significant at P ≤ 0.05. .

4.1.4 The effect of nitrogen on root architectural traits of maize inbred lines.

4.1.4.1 First whorl angle

Interaction of nitrogen and inbred was not significant (P≥ 0.05) on first whorl angle, First whorl angle ranged from 54.38° per plant for inbred MO031 under low nitrogen level to 65.63° per plant for inbred MO345 under high nitrogen level. First whorl angle was not significantly different between low and high nitrogen levels (P≥ 0.05), with plants averaging 60.67° on high nitrogen level while plants planted on low nitrogen level averaged at 60.83°. First whorl angle was not significantly different among the inbred lines (P≥ 0.05); and ranged from 57.25° per plant for inbred MO031 to 62.39° per plant for MO034.

4.1.4.2 Second whorl angle

Interactive effect of nitrogen and inbred was not significant (P≥ 0.05) on second whorl angle. Second whorl angle ranged from 59.50° per plant for inbred MO345 under high nitrogen level to 70.25° per plant for inbred MO001 under low nitrogen level. Second whorl angle was not significantly different between low and high nitrogen levels (P≥ 0.05), with plants averaging 65.66° on high nitrogen level while plants planted on low nitrogen level averaged at 64.13° in angle. Second whorl angle was not significantly different among the inbred lines (P≥ 0.05); and ranged from 60.94° per plant for inbred MO199 to 68.69° per plant for MO001.

4.1.4.3 Third whorl angle

Interaction of nitrogen and inbred was not significant (P≥ 0.05) on third whorl angle, Third whorl angle ranged from 64.63° per plant for inbred MO199 under high nitrogen level to 75.88° per plant for inbred MO031 under low nitrogen level. Third whorl angle was not significantly different between low and high nitrogen levels (P≥ 0.05), with plants averaging 68.74° on high nitrogen level while plants planted on low nitrogen level averaged at 67.63° in angle. Third whorl angle was not significantly different among the inbred lines (P≥ 0.05); and ranged from 65.94° per plant for inbred MO199 to 72.31° per plant for inbred MO031.

4.1.4.4. Fourth whorl angle

Interaction of nitrogen and inbred was not significant (P≥ 0.05) on fourth whorl angle. Fourth whorl angle ranged from 59.54° per plant for inbred MO034 under low nitrogen

level to 71.75° per plant for inbred MO345 under high nitrogen level. Fourth whorl angle was not significantly different between low and high nitrogen levels (P≥ 0.05), with plants averaging 66.29° on high nitrogen level while plants planted on low nitrogen level averaged at 62.88° in angle. Fourth whorl angle was not significantly different among the inbred lines (P≥ 0.05); and ranged from 61.83° per plant for inbred MO034 to 69.06° per plant for MO031.

4.1.4.5 Fifth whorl angle

Interaction of nitrogen and inbred was not significant (P≥ 0.05) on fifth whorl angle, fifth whorl angle ranged from 45.63° per plant for inbred MO345 under low nitrogen level to 70.25° per plant for inbred MO196 under high nitrogen level. Fifth whorl angle was not significantly different between low and high nitrogen levels (P≥ 0.05), with plants averaging 65.67° on high nitrogen level while plants planted on low nitrogen level averaged at 60.35° in angle. Fifth whorl angle was not significantly different among the inbred lines (P≥ 0.05); and ranged from 54.50° per plant for inbred MO345 to 66.44° per plant for MO031.

4.1.4.6 Sixth whorl angle

Interaction of nitrogen and inbred was not significant (P≥ 0.05) on 6^{th} whorl angle. 6^{th} whorl angle ranged from 33.75° per plant for inbred MO345 under low nitrogen level to 70.00° per plant for inbred MO034 under high nitrogen level. Sixth whorl angle was not significantly different between low and high nitrogen levels (P≥ 0.05), with plants averaging 55.89° on high nitrogen level while plants planted on low nitrogen level averaged at 47.56° in angle. Sixth whorl angle was not significantly different among the inbred lines (P≥ 0.05); and ranged from 38.31° per plant for inbred MO345 to 62.06° per plant for MO031.

4.1.4.7 Number of Root whorls

Interaction of nitrogen and inbred was not significant (P≥ 0.05) on number of whorls, number of whorls ranged from 5.25 per plant for inbred MO345 under low nitrogen level to 6.03 per whorls per plant for inbred MO034 under low nitrogen level. Number of Root whorls was not significantly different between low and high nitrogen levels (P≥ 0.05), both levels had few whorls, with plants averaging 5.92 on high nitrogen level while plants planted on low nitrogen level averaged at 5.88 in whorls. Number of whorls

was not significantly different among the inbred lines (P≥ 0.05); and ranged from 5.63 per plant for inbred MO345 to 6.02 per plant for MO034.

4.1.4.8 Projected root area

There were significant differences in projected root area (P≤ 0.05) between low and high nitrogen levels with high nitrogen level resulting in larger projected root area per plant, with plants averaging 19965.00 mm^2 while plants planted on low nitrogen level had smaller projected root area that averaged at 14238.00 mm^2 (Table 4.5). Projected root area was not significantly different among the inbred lines (P≥ 0.05); and ranged from 14528.00 m^2 for inbred MO196 to 18994.00 mm^2 for inbred MO034 (Table 4.5).

4.1.4.9. Root top angle

Interaction of nitrogen and inbred was not significant (P≥ 0.05) on root top angle, the recorded roots formed steeper and shallow angles. Root top angle ranged from 4.95° per plant for inbred MO199 under low nitrogen level to 44.40° per plant for inbred MO034 under high nitrogen level. There were significant differences in root top angle (P≤ 0.05) between low and high nitrogen levels with high nitrogen level resulting in steeper root top angle per plant, with plants averaging 28.41° while plants planted on low nitrogen level had shallower root top angle per plant that averaged at 15.70° (Table 4.5). Root top angle was not significantly different among the inbred lines (P≥ 0.05); and ranged from 14.73° per plant for inbred MO199 to 29.24° per plant for inbred MO031 (Table 4.5).

4.1.4.10. Root bottom angle

Interaction of nitrogen and inbred was not significant (P≥ 0.05) on root bottom angle, the recorded roots had shallow and steeper angles. Root bottom angle ranged from 19.58° per plant for inbred MO031 under high nitrogen level to 39.91° per plant for inbred MO199 under low nitrogen level. Root bottom angle was not significantly different between low and high nitrogen levels (P≥ 0.05), with plants averaging 31.66° on high nitrogen level while plants planted on low nitrogen level averaged at 23.74° in angle. Root bottom angle was not significantly different among the inbred lines (P≥ 0.05); and ranged from 22.56° per plant for Inbred MO031 to 35.50° per plant for inbred MO199.

4.1.4.11. Average root density

Interaction of nitrogen and inbred was not significant (P≥ 0.05) on average root density, however the results indicated that the roots were denser. Average root density ranged from 3.06 mm per plant for inbred MO199 under low nitrogen level to 5.15 mm per plant for inbred MO034 under low nitrogen level. Average root density was not significantly different between low and high nitrogen levels (P≥ 0.05), both levels had denser roots, with plants averaging 3.92 mm on high nitrogen level while plants planted on low nitrogen level averaged at 3.84 mm. Average root density was not significantly different among the inbred lines (P≥ 0.05); and ranged from 4.59 mm per plant for inbred MO034 to 3.19 mm per plant for inbred MO199.

4.1.4.12 Number of adventitious roots

Interaction of nitrogen and inbred was not significant (P≥ 0.05) on number of adventitious roots, number of adventitious roots ranged from 1.37 roots per plant for inbred MO031 under high nitrogen level to 4.62 roots per plant for inbred MO034 under high nitrogen level. Number of adventitious roots was not significantly different between low and high nitrogen levels (P≥ 0.05), both levels had few adventitious roots, with plants averaging 3.20 on high nitrogen level while plants planted on low nitrogen level averaged at 2.47 in roots. Number of adventitious roots was not significantly different among the inbred lines (P≥ 0.05); and ranged from 1.87 roots per plant for inbred MO031 to 3.68 roots per plant for inbred MO034.

4.1.4.13 Number of basal roots

Interaction of nitrogen and inbred was not significant (P≥ 0.05) on number of basal roots, the recorded roots had fewer basal roots. Number of basal roots ranged from 4.37 roots per plant for inbred MO196 under low nitrogen level to 9.00 roots per plant for inbred MO345 under high nitrogen level. There were significant differences in number of basal roots per plant (P≤ 0.05) between low and high nitrogen levels with high nitrogen level resulting in greater number of basal roots per plant, with plants averaging 7.77 while plants planted on low nitrogen level had smaller number of basal roots per plant that averaged at 5.26 (Table 4.5). Number of basal roots was not significantly different among the inbred lines (P≥ 0.05); and ranged from 5.73 roots per plant for inbred MO199 to 7.00 roots per plant roots for inbred MO345 (Table 4.5).

4.1.4.14. Tap root diameter

Interaction of nitrogen and inbred was not significant (P≥ 0.05) on tap root diameter. Taproot diameter ranged from 1.10 mm per plant for inbred MO001 under low nitrogen level to 1.34 mm per plant for inbred MO034 under high nitrogen level. Tap root diameter was not significantly different between low and high nitrogen levels (P≥ 0.05), with plants averaging 1.24 mm on high nitrogen level while plants planted on low nitrogen level averaged at 1.15 mm in diameter. Tap root diameter was not significantly different among the inbred lines (P≥ 0.05); and ranged from 1.14 mm per plant for inbred MO196 to 1.29 mm per plant for inbred MO034.

4.1.4.15 Average lateral root length

Interactive effect of nitrogen and inbred was not significant (P≥ 0.05) on average lateral root length. Average lateral root length ranged from 170.45 mm per plant for inbred MO345 under low nitrogen level to 219.86 mm per plant for inbred MO199 under high nitrogen level. Average lateral root length was not significantly different between low and high nitrogen levels (P≥ 0.05), with plants averaging 207.82 mm on high nitrogen level while plants planted on low nitrogen level averaged at 187.94 mm in length. Average lateral root length was not significantly different among the inbred lines (P≥ 0.05); and ranged from 188.16 mm per plant for inbred MO001 to 210.06 mm per plant for inbred MO199.

4.1.4.16 Lateral branching frequency

Interaction of nitrogen and inbred was not significant (P≥ 0.05) on lateral branching frequency, Lateral branching frequency ranged from 9.19 mm^{-1} per plant for inbred MO345 under high nitrogen level to 17.89 lateral roots mm^{-1} per plant for inbred MO345 under nitrogen level. Lateral branching frequency was not significantly different between low and high nitrogen levels (P≥ 0.05), with plants averaging 13.44 mm^{-1} on high nitrogen level while plants planted on low nitrogen level averaged at 11.46 mm^{-1}. Lateral branching frequency was not significantly different among the inbred lines (P≥ 0.05); and ranged from 10.41 mm^{-1} per plant for inbred MO196 to 16.69 lateral roots mm^{-1} per plant for inbred MO345.

4.1.4.17 Distance to the first lateral root

Interaction of nitrogen and inbred was not significant (P≥ 0.05) on distance to the first lateral root, the recorded distances were short. The distance to the first lateral root

ranged from 0.49 mm per plant for inbred MO345 under high nitrogen level to 2.52 mm per plant for inbred MO196 low nitrogen level. Distance to the first lateral root was not significantly different between low and high nitrogen levels (P≥ 0.05), with plants averaging 1.55 mm on high nitrogen level while plants planted on low nitrogen level averaged at 1.28 mm in distance. The distance to the first lateral root was not significantly different among the inbred lines (P≥ 0.05); and ranged from 0.81 mm per plant for inbred MO345 to 1.89 mm per plant for inbred MO196.

4.1.5 The effect of nitrogen on dry biomass

There were significant differences in dry biomass per plant (P≤ 0.05) between low and high nitrogen levels with high nitrogen level resulting in larger dry biomass per plant, with plants averaging 289.84 kg/ha^{-1} while plants planted on low nitrogen level had smaller biomass per plant that averaged at 136.67 kg ha^{-1} (Table 4.5). There was a significant difference in plant dry biomass among the inbred lines (P≤ 0.01), with the highest biomass of 295.11 kg ha^{-1} recorded on inbred MO031 while 158.45 kg per ha^{-1} was the lowest biomass recorded on inbred MO196 (Table 4.5).

Table 4.5 Influence of nitrogen level and inbred on projected root area, root top angle, number of basal roots and plant dry biomass of selected maize IBM inbred lines.

Nitrogen level		Projected root area (mm^2)	Root top angle(°)	Number of basal roots	Dry biomass (Kg/ha^{-1})
High		19965.00[a]	28.41[a]	7.77[a]	290[a]
Low		14238.00[b]	15.70[b]	5.26[b]	137[b]
P value		0.0481	0.0319	0.0316	0.0373
Significant difference		*	*	*	*
Turkey HSD		5638.000	10.629	2.088	136.270
CV%		35.88	52.44	34.86	69.55
Maize inbred	MO034	18994.00[a]	28.98 [a]	6.18 [a]	189[ab]
	MO031	18281.00 [a]	29.24 [a]	6.93 [a]	295[a]
	MO001	17108.00 [a]	24.99 [a]	6.87 [a]	207[ab]
	MO345	16875.00 [a]	19.62 [a]	7.00 [a]	207[ab]
	MO199	16823.00 [a]	14.73 [a]	5.73 [a]	223[ab]
	MO196	14528.00 [a]	14.75 [a]	6.37 [a]	159[b]
P value		0.2974	0.5589	0.8101	0.0142
Significance		ns	ns	ns	*
Turkey HSD		5817.00	10.63	3.24	105.99

Means in the same column followed by the same letters are not significantly different at P ≤ 0.05. * = significant at P ≤ 0.05, ns = not significant at P ≤ 0.05.

4.1.6 Correlation analysis for measured parameters

Simple person correlations were conducted among various components to determine the relationship of the traits with dry biomass.

4.1.6.1 Correlation between biomass and morphological traits on nitrogen experiment.

There were positive and significant correlations (P≤ 0.05) between dry biomass and selected plant morphological traits (stem diameter (R^2 = 0.485***), plant height (R^2 = 0.381**), leaf area per plant (R^2 = 0.354**) and leaf length (R^2 = 0.267*)) only under high nitrogen level (Table 4.6). Similarly, positive and significant correlations (P≤ 0.05) between dry biomass and morphological traits (stem diameter (R^2 = 0.605***), plant height (R^2 = 0.424**) and leaf length (R^2 = 0.189*) were obtained under low nitrogen level. However there were no significant (P≤ 0.05) correlations between dry biomass and other measured morphological traits (number of leaves, chlorophyll content, leaf width and leaf area per plant) at low nitrogen level (Table 4.6).

Table 4.6 Association of morphological traits with dry biomass (kg/ha^{-1}) of all maize lines for low and high nitrogen levels

Morphological trait	Nitrogen level			
	Low		High	
	r	P value	R	P value
Plant height	0.651	0.001**	0.617	0.002**
Stem diameter	0.778	0.000***	0.677	0.000***
Chlorophyll content	0.129	0.550 ^{ns}	-0.161	0.464 ^{ns}
Leaf width	0.244	0.250 ^{ns}	0.419	0.047 ^{ns}
Leaf length	0.435	0.034*	0.517	0.011 *
Number of leaves	-0.041	0.850 ^{ns}	0.159	0.469^{ns}
Leaf area per plant	0.320	0.127 ^{ns}	0.595	0.003**

r= Pearson correlation coefficient, * = significant at 0.05, **= significant at P ≤ 0.01, *** = significant at P ≤ 0.001, ns = not significant at P ≤ 0.05.

4.1.6.2 Correlation between biomass and root architectural traits under low and high nitrogen level.

There were positive and significant correlations (P≤ 0.05) between dry biomass and root architectural traits of the projected root area (R^2 = 0.664**), tap root diameter (R^2

= 0.196**) and average lateral root length (R^2 = 0.263**) under low nitrogen level. However there was no significant correlation between dry biomass with any of the 1st to 6th whorl angles, number of whorls, average root density, root top angle, root bottom angle, number of adventurous roots, number basal roots, tap root diameter, average lateral root length, lateral branching frequency) at both high and low nitrogen level (Table 4.7).

Table 4.7 Association of root architectural traits with dry biomass (kg/ha^{-1}) of all maize lines for data under low and high nitrogen levels

Root architecture traits	Nitrogen level			
	Low		High	
	r	P-value	r	P-value
1st whorl angle	-0.316	0.133ns	-0.039	0.855ns
2nd whorl angle	-0.335	0.110ns	0.093	0.665ns
3rd whorl angle	-0.015	0.946ns	-0.067	0.755ns
4th whorl angle	0.177	0.409ns	-0.098	0.650ns
5th whorl angle	0.311	0.139ns	-0.136	0.526ns
6th whorl angle	0.271	0.201ns	0.094	0.661ns
Number of whorls	0.262	0.216ns	0.187	0.383ns
Projected root area	0.815	0.000**	0.348	0.096ns
Average root density	0.328	0.118ns	-0.073	0.733ns
Root top angle	0.243	0.253ns	0.109	0.613ns
Root bottom angle	-0.251	0.238ns	-0.310	0.140ns
Number of adventurous roots	0.121	0.574ns	-0.341	0.104ns
Number basal roots	0.243	0.253ns	-0.287	0.173ns
Tap root diameter	0.443	0.030**	0.226	0.290ns
Average lateral root length	0.513	0.010**	0.050	0.818ns
Lateral branching frequency	-0.333	0.111ns	-0.386	0.062ns

r= Pearson correlation coefficient, **= significant at P ≤ 0.01, *** = significant at P ≤ 0.001, ns = not significant at P ≤ 0.05.

4.2 Discussion

4.2.1 The effect of nitrogen on growth attributes and root architectural traits of maize inbred lines.

There were no significant differences between low and high nitrogen levels on whorl distribution and variation in stem diameter. The non-significant difference in the whorl distribution and stem diameter sizes among inbred line from low and high N levels suggests that there was no variation in N-use efficiency under low and high nitrogen on those traits.

However results obtained from this study indicate that application of high level of N improves plant and root growth as well on dry biomass of maize. This was evident in the maximum values in plant height, leaf length, leaf width, average leaf area, leaf area per plant and chlorophyll content produced under high nitrogen level. The increase in plant and root growth is therefore attributed to the increased available N due to high N fertilizer application rate. Gudu *et al*. (2005) also reported similar results. Crop response to N fertilizer was expected from the initial soil analysis results. Both high and low nitrogen level had soils which are slightly acidic with pH (KCl) showing that these soils will be strongly acidic. Acidic soils tend to make P and N unavailable for plant usage through P fixation and slowing down of nitrification rates respectively (Gudu *et al*., 2005). Therefore plants grown under low nitrogen level had reduced growth probably because of reduced N and P for growth.

Plant height

N level × inbred interaction was not statistically significant, the tallest plants were obtained from inbred MO199 at higher N level. Initially, the available total nitrogen was 8.10 and 13.10 mg. kg^{-1} on low and high nitrogen levels, respectively, the observed slight variation in height of the plants also conforms to the initial soil results which also showed a slight variation in N level in both high N and low N level. The higher rainfall during vegetative growth stage coupled with the N topdressing might have increased N uptake by plants and more assimilates partitioned to the maize stems resulting in taller maize plants. Higher values in plant height is a result of the positive benefits of nitrogenous fertilizers on improving vegetative growth as nitrogen enhances length of internode length hence plants grow taller (Kaur *et al*., 2012). Plant height was not significantly affected by nitrogen level with averaged plant height of 96.00 and 124.30

cm obtained under low and high nitrogen level, respectively. The lack of response was not expected since initial soil N was low. However higher N level gave plants which were 29.42% taller than those under lower N level. Similarly, Karasu *et al*. (2009) also indicated that nitrogen has no significant effect on plant height of maize. However results from this study contradict with the findings of Eltelib *et al*. (2006) who reported that addition of N fertilizer significantly increased maize plant height on both N levels their results suggest that the applied nitrogen significantly increased plant growth and N use efficiency. Inbred MO031 was tallest at 118.33 cm and significantly taller than inbred MO001. These results therefore suggest that plant height can be useful criterion for selecting maize inbreds.

Number of leaves

The interaction between N level and inbred was not statistically significant, the highest number of leaves was obtained on inbred MO031 under high N level. The similarity might be due to similarity in nitrogen use efficiency or uptake by the inbred lines. These results therefore suggest that nitrogen level does not influence leaf number in maize. However, leaf number varied among the inbred lines indicating that this could be a useful parameter for selecting maize inbreds. The number of leaves was not significantly influenced by nitrogen level, plants planted under high and low nitrogen level averaged 10.62 and 10.79, respectively. Higher N level had plants with 1.60% higher leaf number than those under the lower N level. These results agrees with the findings of Eltelib *et al.,* (2006) who reported that increasing nitrogen level had no significant effect on the number of leaves per plant. Genetic factors that control leaf formation might have been responsible for the observed results as the planted inbreds belong to the same population. It can be said that the number of leaves of inbreds belonging to the same population barely differ at the different nitrogen levels. Number of leaves was significantly different among the inbred lines, Inbred MO031 had highest leaf number per plant at 11.33 and significantly higher than inbred MO001 (10.16) and MO345 (10.21). From number of leaves perspective, inbred MO001 and MO345 would be considered for selection for fewer leave number while inbred MO031 can be selected for greater leave numbers.

Chlorophyll content

The interaction between N level and inbred was not statistically significant. Therefore chlorophyll content cannot be used to identify superior maize performing lines under

nitrogen stress. High N level resulted in significant increase in chlorophyll content (P≤ 0.05). High N level had plants with 33, 92% higher in chlorophyll content than plants planted under lower N level. The high chlorophyll content under high N level suggests that the applied N was effectively utilized resulting in higher potential for photosynthesis. According to Ali (2014), the higher leaf width under high nitrogen level turn to results in an increase chlorophyll content leading to a relative increase in crop growth and development. In the present study high nitrogen level had plants with greater leaf width and chlorophyll content. The results conform to Eghball and Power (1999) who indicated that application of nitrogenous fertilizers helps plants to maintain their chlorophyll content for a long time, facilitates the reduction of leaf senescence and also enhances plant ability to supply nitrogen and photo assimilates to seeds and growing points hence enhance crop growth as well as yield. Chlorophyll content had no significant variation among inbred lines. Therefore this suggests that chlorophyll is unlikely going to be useful basis for selection of maize inbreds.

Stem diameter

N level × inbred interaction was not statistically significant, plants with thickest stem diameter were recorded on inbred MO031 at 36.47 mm under high nitrogen level. These results indicate that stem diameter is a trait which cannot be used to identify superior performing inbred lines under N stress condition in field condition, as inbreds from the same population are unlikely to vary in stem diameter. Stem diameter was not significantly different among low and high nitrogen levels, both levels had thin plants, with plants averaging 20.18 mm on high nitrogen level while plants planted on low nitrogen level averaged at 13.33 mm in diameter. High N level resulted plants with 51.39% greater stem diameter than plants planted under low N level. Similarly Karasu *et al.* (2009) reported that N levels had no influence on the stem diameter of maize. These results disagree with the findings of Eltelib *et al*. (2006) who reported that nitrogen significantly increased stem diameter. However there was high CV% therefore for future studies should increase number of plants sampled per plot and the number of replications to improve precision. However the thicker stems in this study can be explained by the efficiency of the applied nitrogenous fertilizer and the essential role played by nitrogen in improving growth. Furthermore, there was no significant variation in stem diameter among maize inbreds. This may be due stem diameter being a genetically controlled trait.

Leaf length

High nitrogen level resulted in a significant increase in leaf length. Maize plants with higher N level had 43.06% greater leaf length than those with lower N level. Results from this study agrees with those of Haghighi *et al*. (2011) who reported that addition of nitrogen up to a level of 250 kg/ha increased leaf length on flue-cured tobacco (Coker 347). The improved leaf length implies an increased leaf area, which may result in increased photosynthetic area, which could lead to higher growth and development. There was no significant variation among the inbreds on leaf length, however all inbred lines had longer leaves. The greater leaf lengths in all inbreds suggest the potential of all inbreds to increase leaf length if well fertilized.

Leaf width

The interaction between N level and inbred was not significant. Inbred MO345 surpassed all other inbreds with a leaf width of 8.96 cm under high nitrogen level, while under low nitrogen level the same inbred had the lowest leaf width. Therefore in inbred MO345 would not be considered for selection under low N levels. However with high N level inbred MO345 can give maximum leaf growth. Leaf width was significantly affected by nitrogen levels resulting in plants with broader leaves than those under low N level. Higher N level had plants with 32.72% greater leaf width than those from the lower N level. Similarly Haghighi *et al*., (2011) who reported that increasing nitrogen application rate resulted in an increase in leaf width of flue-cured tobacco (Coker 347). The increase in leaf width and length signifies that nitrogen stimulated the biosynthesis and export of cytokinin hormones form roots to leaves and that causes an increase in cell division hence an increase in leaf growth and development (Haghighi *et al.*, 2011). Higher leaf width suggests a significant increase in leaf area that could eventually lead to higher growth rate and yield. Therefore farmers could benefit from the increased leave growth by increasing their nitrogen application rate. There was no significant variation among the inbreds on leaf width. This suggests that leaf width is unlikely going to be useful basis for selection of maize inbreds.

Leaf area

N level × inbred interaction was not significant, inbreds planted on high nitrogen level had higher leaf area than inbreds planted on low nitrogen level. Increased in leaf area at high N level might also be due to increased leaf expansion rate as a result of increased cell division and cell expansion, which further enhance phothosynthate

formation hence higher leaf area per maize plant (Amanullah *et al*., 2009). High nitrogen level significantly increased leaf area and had plants with 95.79% greater in leaf area than the lower N level. Nitrogen enhances vegetative growth, and aids the expansion of leaves as well as their development. The observed increases in leaf width and length under high nitrogen level contribute to the greater leaf area. Jones (2003) also related greater leaf area in plants to increases in leaf length and leaf width. The results from the present study are in agreement with previous studies which indicated that high application rates of nitrogenous fertilizers enhances leaf growth during vegetative growth stage and hence aids in maintenance of the leaf area during the growth period (Cox, *et al*., 1993 and Onasanya, *et al*., 2009). The interception and utilization of solar radiation by crop canopies is influenced by the area of the leaf which further influences the accumulation of dry matter and eventually the grain yield (Kaur *et al*., 2012). Squire *et al*. (1987) concluded that application of nitrogenous fertilizers leads to an increase in the rate of leaf expansion which further results in an increase in interception of daily solar radiation by the canopy, resulting in net positive benefit of increasing the rate of photosynthesis. Earlier there were some speculations indicating that during morphogenesis, N taken up by maize during early growth stages is invested mainly in the production of other plant structures (formation and expansion of leaves and stems) rather than chlorophyll (Argenta *et al*., 2004). Investing in such structures has net positive benefits such as enhancement of light interception which results in an increase in grain yield and crop use efficiency (Marschner, 1995). There was no significant variation among the inbreds on leaf area. Therefore this suggests that leaf area is unlikely going to be useful basis for selection of maize inbreds.

Projected root area

Application of high nitrogen influenced root growth as evident in the higher projected root area at high N level. Higher N level had plants that had marginally (0.22%) greater root area than plants planted under lower N level. These results are in conformity with the indication that the total amount of roots of plants grown under low N grown are less as compared to plants planted on higher normal nitrogen N fertilization (Bänziger *et al*., 2000). Similarly, Lynch *et al*. (2012) reported that increasing nitrogen supply has the benefit of increasing root growth. Root area had no significant variation among the inbreds. The root area ranged from 18994.00 mm^{-2} for inbred MO034 to 14528.00 mm^{-}

^{2} for inbred MO196. All inbreds had fairly pronounced root area, therefore they can all be selected for good root system which can effectively take up nutrients.

Number of basal roots

The interaction between N level and inbred also showed no significant variation. This suggests all inbreds showed capacity to increase basal roots in response to N level. However, plants with the highest number of basal roots were obtained from inbred MO345 under high nitrogen level. High N level significantly increased the number of basal roots and higher N level had plants had 47.72% greater number of basal roots than plants planted under lower N level. The greater number of basal roots suggests high number of root whorls. Whorls emerge along the base of the hypocotyl and basal roots develop from each whorl (Miguel *et al*., 2013). The number of basal roots showed no significant differences among the inbred lines.

Root top angle

The interaction between inbred and N level was not significant. Plants with highest root top angle were recorded on inbred MO034 under high nitrogen level. This is however unexpected since deeper rooting would be expected under low N level and as these results seem to suggest that root top angle is not necessarily a trait that can be used for selection. Earlier study based on a qualitative dynamic crop growth model suggested that an increase in maize yield in the mid-western USA was mainly influenced by the root angle of the primary roots systems (Hammer *et al*., 2009). The positive increase in root and plant growth with increase in N level clearly justifies the significance of application of nitrogenous fertilizers to enhance growth. N plays a significant role in plants as it is a structural component of protoplasm, enzymes and chlorophyll which is involved in photosynthesis. Furthermore, it acts as a catalyst in various physiological processes. The enhanced growth is due to the fact that N help speed up cell division and also assimilation of photons during photosynthesis hence higher growth rate as well as improved yields (Agber and Ali, 2012). The enhanced root growth has the net benefits of enhancing soil exploration to deeper soil horizons. High N level resulted in a significant increase in root top angle. Higher N level had plants which had 55.47% greater root top angle than plants planted under lower N level. Although there was no significant difference in root top angle among the inbreds lines, the recorded root top angles showed that roots had both shallower and steeper

angles. The angles ranged from 14.74° recorded on inbred MO199 to 29.98° recorded on inbred MO031. Shallow root angles are vital for the uptake of phosphorus while the steeper rooted genotypes would be suited for uptake of N from deeper soil horizons as well as water uptake under dry soil conditions (Lynch *et al*., 2012). Therefore inbreds MO199, MO196 and MO345 could be selected to enhance P uptake while inbreds MO001, MO031 and MO034 could be considered for selection to enhance N uptake and water use efficiency.

Biomass

The results from this study indicate that high N gave more promising dry biomass than plants which were planted at low N level. Higher N level had 52.55% greater biomass than the lower N level. This result was expected since parameters such as leaf length, leaf width, leaf area per plant as well as the chlorophyll content increased significantly with high nitrogen level, which led to a net increase in biomass. The higher biomass produced under high nitrogen splits may be as a result of extended growth phase, as nitrogen enhances production and translocation of more photo assimilates hence longer growth period (Amanullah *et al*., 2009). These results conform to previous findings of Mariga *et al*. (2000) who reported that reported that N application up to the tassel initiation stage significantly increased biomass yield in maize significantly. The results suggest that the applied N in high N split doses significantly increased plant growth and development in this study. Inbred MO031 had the greatest biomass at 295 kg ha^{-1} which was only significantly higher than inbred MO196 at 159 kg ha^{-1}. From biomass perspective, only inbred MO196 would not be considered for selection.

4.2.2 Correlations

One of the objectives of this study was to evaluate if particularly root architecture and morphological traits directly relate to dry biomass production both under low and high nitrogen levels. For this, traits measured at different nitrogen levels were related to the dry biomass (Table 4.6 and 4.7).

4.2.2.1 Correlation between biomass and morphological, root architectural traits on nitrogen experiment

The hypothesis of homogeneous variation in relationships between morphological and root architectural traits with biomass production under nitrogen stress was rejected as there was high variation in the relationships. There were positive and significant

correlations between dry biomass and morphological traits (stem diameter, plant height, leaf length and leaf area per plant) on high nitrogen level. Coefficient of multiple determinations revealed stem diameter, plant height, leaf length and leaf area per plant had strong influence on biomass explaining 45.8%, 38.1%, 26.7%, and 35.4% in total treatment variation on high nitrogen level respectively. This means that plant biomass was dependent on stem diameter, plant height and leaf area per plant on high nitrogen level.

There were also positive and significant correlations between dry biomass and morphological traits (stem diameter, plant height and leaf length) on low nitrogen level. While on low nitrogen level stem diameter, plant height and leaf length contributed 60.5%, 42.40% and 18.9% in total treatment variation. Furthermore, biomass positively correlated with several root architectural traits (projected root area, tap root diameter and average lateral root length) at low nitrogen level. Projected root area, tap root diameter and average lateral root length contributed 66.4%, 19.6% and 26.3% in total treatment variation, respectively. This suggests that biomass was dependent on stem diameter, plant height, leaf length, projected root area, tap root diameter and average lateral root length. The positive correlation between biomass and several root architectural traits (projected root area, tap root diameter and average lateral root length) under low N level suggests that those traits perform well under low nitrogen conditions which could result in possible greater low nitrogen tolerance. Results from this study are consistent with the findings of DoVale *et al*. (2013) who reported that shoot dry weight had strong significant correlation with morphological traits and acquisition efficiency in low and high level of both N and P high experiments. The high significant positive correlations indicate that selection of high yielding inbred may be successful based on biomass. The results show some similarities with (Ali, 2014; Carpici and Celik, 2010; Derera *et al*., 2008). More traits correlated with biomass of plants grown under low nitrogen soil than in high nitrogen soil, especially the root architectural traits. This high degree of variance in relationship of traits under low and high nitrogen level suggests that the relationships are not allometric i.e. not integrally related to the size of the plant (Niklas, 2004). There was no variation in relationships of biomass with plant height and stem diameter under low and high N levels signifying that those relationships are allometric. Long lateral roots were recently demonstrated to increase nitrate acquisition (Postma *et al*., 2014). Earlier, similar results were

reported by Tian *et al*., (2005), Lynch (2013) and York (2014). In this study, a positive relationship between biomass and lateral root length existed for plants grown only under low N soil. There was no variation in lateral root length and biomass further suggesting that they were not related under high N level. The present study was conducted on a sandy soil, which is highly prone to leaching; hence, long lateral roots were beneficial in enhancing N uptake.

5. Conclusions and recommendations

This study showed that nitrogen application had no significant effect on whorl distribution and stem diameter size variation of various IBM maize inbred lines. The results confirmed that the response of whorl distribution and stem diameter in maize to nitrogen application depended on population group which the inbred line belonged to, this implies that inbreds belonging to the same family have slight chance of being different. There were high variations in the relationships between biomass production under low and high nitrogen level. Biomass production depended on morphological traits as well as root architectural traits. Several traits showed potential to be the basis for selection to enhance nutrient uptake, this indicates that some morphological and root architectural traits can be used to identify superior performing inbred lines for nitrogen stress under field conditions. Furthermore, this study showed that high nitrogen fertilizers have positive effect on some root architectural traits and growth parameters of maize. Inbred MO345 had the highest leaf width at high nitrogen level, therefore to enhance leaf area and plant photosynthetic rate inbred MO345 can be used with high nitrogen rate. Inbreds MO001, MO034 and MO199 had greater leaf width under low N level, thus making them adaptable to low N soil conditions. Inbreds MO001, MO034 and MO199 can be can be selected for improved leaf growth in low N soil. These inbred lines can therefore be recommended to be used in the development of local maize germplasm.

Chapter 5

Phosphorus experiment

5 Results and discussion

5.1 Results

5.1.1 Soil analysis at the beginning of the experiment

Available soil phosphorus was determined and the results are shown in Figure 5.1. Phosphorus availability was the highest at 0-10 cm, lowest at 40-50 cm and intermediate in the 10-30 cm depth and generally low in the deeper soil horizon (40-60 cm).

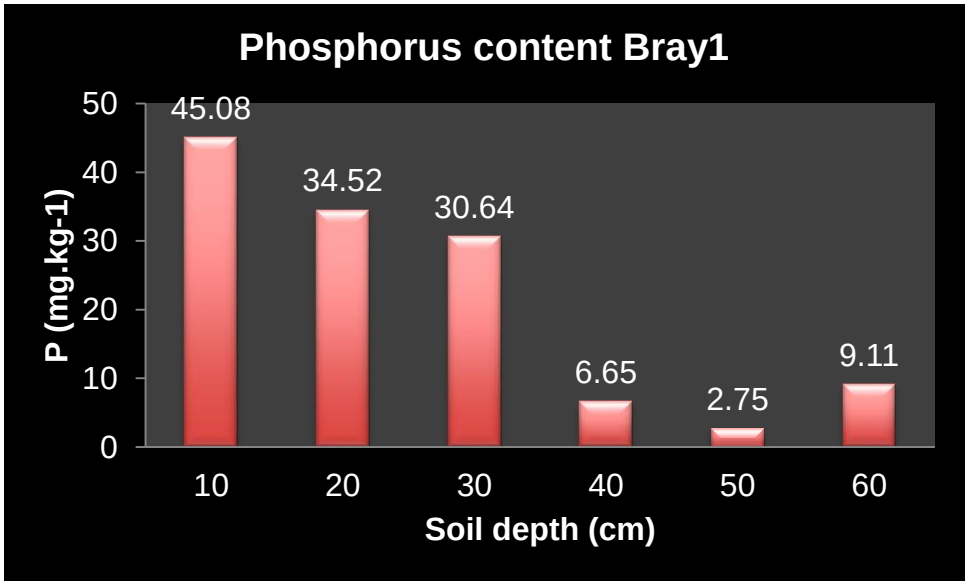


Figure 5.1 Available soil phosphorus before planting on phosphorus experiment

5.1.2 Soil analysis at harvesting

The chemical properties of the soil at harvesting are shown in (Table 5.1). The high phosphorus split plot had top soil depth (0-20 cm) which was slightly acidic, while the lower soil depth (20-60 cm) as well as the soil depth (0-60 cm) on low phosphorus split plot had neutral soils with pH (H_2O) ranging from 6.12 to 7.71. The pH measurement (KCl) showed variation within the profiles, the top soil (0-20 cm) of high phosphorus split plot and the deeper soil depth (20-40 and 40-60 cm) of the low phosphorus split plot, the soil depth had moderate acidic soils with pH from 5.56 to 5.90 respectively.

However, the lower soil depth (20-40 cm and 40-60 cm) of high phosphorus split plot and the top soil depth (0-20 cm) of low phosphorus split plot had soils which were slightly acidic, with pH ranging from 6.27-6.37 respectively. The high phosphorus split plot had top soil depth (0-20 cm) with medium concentration of phosphorus. However, the lower soil depth (20-60 cm) as well as the all the soil depth (0-60 cm) on low phosphorus split plot had a relative low phosphorus content ranging from 6.86 -17.13 $mg.kg^{-1}$. Soil P was classified based on Shober *et al*. (2013) soil interpretation procedure. The level of organic carbon ranged from moderate to very low level. The moderate organic carbon level was recorded in the top soil depth (0-20 cm) of the high phosphorus split plot, while soil depth (20-40 cm) of high phosphorus split plot as well as the top soil depth (0-20 cm and 20-40 cm) of the low phosphorus split plot recorded the lowest organic carbon, with carbon ranging from 0.60-0.63 g/100g. In both high and low P split plots the deeper soil depth (40-60 cm) had a very low organic carbon; the available soil carbon was 0.47 and 0.44 g/100g on high and low P split plots respectively. The organic matter was very low in both high and low P split plots ranging from 1.03 to 1.89% respectively.

Table 5.1 Soil analysis for phosphorus split plots at harvesting.

Nutrient level	Low			High		
Soil depth	0-20	20-40	40-60	0-20	20-40	40-60
pH (KCl)	6.27	5.70	5.56	5.59	6.37	5.97
pH(water)	6.93	6.73	6.71	6.38	7.12	6.90
Organic carbon (g/100g soil)	0.63	0.63	0.44	1.10	0.60	0.47
Organic matter %	1.08	1.08	0.76	1.89	1.03	0.81
Soil phosphorus ($mg.kg^{-1}$)	6.86	8.57	8.85	27.53	17.13	10.77

5.1.3 The effect of phosphorus on growth attributes of maize inbred lines.

5.1.3.1 Chlorophyll content

Chlorophyll content was not significantly different between low and high phosphorus levels ($P \geq 0.05$), with plants averaging 35.16 mg/g on high phosphorus level while plants planted on low phosphorus level averaged at 31.12 mg/g in chlorophyll content (Table 5.2). Chlorophyll content showed significant differences among the inbred lines

(P≤ 0.01), Inbred MO196 recorded the highest chlorophyll content of 39.35 mg/g per plant while the lowest chlorophyll content of 27.614 mg/g per plant (Table 5.2) was recorded on inbred MO196.

5.1.3.2 Plant height

Interactive effect of phosphorus and inbred was not significant (P≥ 0.05) on plant height. Plant height ranged from 82.92 cm for inbred MO034 under low phosphorus level to 127.23 cm for in bred MO196 under high phosphorus level. Plant height was not significantly different between low and high phosphorus levels (P≥ 0.05), both levels had tall plants, with plants averaging 116.24 cm on high phosphorus level while plants planted on low phosphorus level had an average of 103.79 cm in height (Table 5.2). Plant height showed significant differences among the inbred lines (P≤ 0.01). Inbred MO196 recorded the tallest plants at 123.92 cm while Inbred MO034 recorded shorter plants at 87.67 cm (Table 5.2).

5.1.3.3 Number of leaves

Interactive effect of phosphorus and inbred was not significant (P≥ 0.05) on number of leaves. Number of leaves ranged from 10 leaves per plant for inbred MO034 under low phosphorus level to 12 leaves per plant for inbred MO199 under high phosphorus level. Number of leaves was not significantly different between low and high phosphorus levels (P≥ 0.05), both levels had plants with relatively few leaves, with plants averaging 11 leaves on high phosphorus level whereas plants planted on low phosphorus level averaged at 10 leaves. Number of leaves was not significantly different among the inbred lines (P≥ 0.05); and ranged from 10 leaves per plant for MO345 to 12 leaves per plant for Inbred MO199.

5.1.3.4 Leaf length

Interactive effect of phosphorus and inbred was not significant (P≥ 0.05) on leaf length. Leaf length ranged from 42.06 cm for inbred MO034 under low phosphorus level to 52.91 cm for inbred MO345 under high phosphorus level. Leaf length was not significantly different between low and high phosphorus levels (P≥ 0.05), Leaves averaged 48.83 cm on high phosphorus level while plants on low phosphorus level had leaves averaging 45.58 cm in length. Leaf length was not significantly different among the inbred lines (P≥ 0.05); and ranged from 44.37 cm for inbred MO199 to 49.79 cm for inbred MO001.

5.1.3.5 Leaf width

Interactive effect of phosphorus and inbred was not significant (P≥ 0.05) on leaf width. Leaf width ranged from 5.68 cm for inbred MO196 under low phosphorus level to 7.48 cm for inbred MO034 under high phosphorus level. Leaf width was not significantly different between low and high phosphorus levels (P≥ 0.05), both levels had broad leaves, with plants averaging 6.67 cm on high phosphorus level while plants planted on low phosphorus level averaged at 6.39 cm in width. Leaf width showed significant differences among the inbred lines (P≤ 0.05). Inbred MO345 recorded the broadest leaves at 6.99 cm while inbred MO196 recorded narrowest leaves at 5.85 (Table 5.2).

5.1.3.6 Leaf area per plant

Interactive effect of phosphorus and inbred was not significant (P≥ 0.05) on leaf area. Leaf area ranged from 1863.90 cm^2 per plant for inbred MO034 under low phosphorus level to 3120.90 cm^2 per plant for inbred MO034 under high phosphorus level. Leaf area per plant was not significantly different between low and high phosphorus levels (P≥ 0.05), with plants averaging 2700.90 cm^2 on high phosphorus level while plants planted on low phosphorus level averaged at 2276.60 cm^2 in leaf area per plant. Leaf area was not significantly different among the inbred lines (P≥ 0.05); and ranged from 2182.80 cm^2 per plant for inbred MO196 to 2606.40 cm^2 per plant for inbred MO345.

5.1.3.7 Stem diameter

Interactive effect of phosphorus and inbred was not significant (P≥ 0.05) on stem diameter. Stem diameter ranged from 13.95 mm per plant for inbred MO199 under low phosphorus level to 18.19 mm per plant for inbred MO345 under high phosphorus level. Stem diameter was not significantly different between low and high phosphorus levels (P≥ 0.05), both levels had fairly thick stems, with plants averaging 17.02 mm on high phosphorus level while plants planted on low phosphorus level averaged at 15.31 mm in diameter. Stem diameter was not significantly different among the inbred lines (P≥ 0.05); and ranged from 15.04 mm per plant for inbred MO199 to 16.79 mm per plant for inbred MO345..

Table 5.2 The influence of phosphorus level and inbred on chlorophyll content, plant height and leaf width of selected maize IBM inbred lines

Phosphorus level		Chlorophyll content (mg/g)	Plant height (cm)	Leaf width (cm)
High		35.16[a]	116.24[a]	6.67[a]
Low		31.12[a]	103.79[a]	6.39[a]
P value		0.0675	0.1212	0.4348
Significant difference		ns	ns	ns
CV%		15.07	18.26	16.86
Maize inbred	MO196	39.35 [a]	123.92 [a]	5.85 [b]
	MO001	36.24 [ab]	97.58 [bc]	6.49 [ab]
	MO199	34.50 [abc]	121.58 [a]	6.65 [ab]
	MO345	32.51 [abc]	114.33 [ab]	6.99[a]
	MO031	28.65 [bc]	115.00[ab]	6.42[ab]
	MO034	27.61 [c]	87.67 [c]	6.77[ab]
P value		0.0007	0.0003	0.0352
Significant difference		***	***	*
Turkey HSD		7.9789	23.809	1.0046

Means in the same column followed by the same letters are not significantly different at P ≤ 0.05, * = significant at P ≤ 0.05, *** = significant at P ≤ 0.001, ns = not significant.

5.1.4 The effect of phosphorus level on root architectural traits of maize.

5.1.4.1 First whorl angle

Interactive effect of phosphorus and inbred was not significant (P≥ 0.05) on first whorl angle. First whorl angle ranged from 57.50° per plant for inbred MO199 under low phosphorus level to 68.13° per plant for inbred MO034 under high phosphorus level. First whorl angle was not significantly different between low and high phosphorus levels (P≥ 0.05), with plants averaging 61.69° on high phosphorus level while plants planted on low phosphorus level averaged at 64.16° in angle (Table 5.3). First whorl angle showed significant differences among the inbred lines (P≤ 0.05). Inbred MO034 recorded the steepest angle at 67.87° per plant while Inbred MO199 recorded shallowest angle at 58.18° per plant (Table 5.3).

5.1.3.2 Second whorl angle

Interactive effect of phosphorus level and inbred was not significant (P≥ 0.05) on second whorl angle. Second whorl angle ranged from 62.06° per plant on inbred MO001 under high phosphorus level to 70.87° per plant MO034 under high phosphorus level. Second whorl angles were not significantly different between low and high phosphorus levels (P≥ 0.05), with plants averaging 67.83° on high phosphorus level while plants planted on low phosphorus level averaged at 65.62° in whorl angle. Second whorl angle was not significantly different among the inbred lines (P≥ 0.05); and ranged from 64.01° per plant for inbred MO001 to 70.00° per plant for MO034.

5.1.4.3 Third Whorl angle

Interactive effect of phosphorus level and inbred was not significant (P≥ 0.05) on third whorl angle, 3rd whorl angle ranged from 63.00° per plant for inbred M0345 under high phosphorus level to 74.38° per plant for inbred MO034 under phosphorus level. Third whorl angles were not significantly different between low and high phosphorus levels (P≥ 0.05), with plants averaging 65.48° on high phosphorus level while plants planted on low phosphorus level averaged at 69.48° in whorl angle. Third whorl angles were not significantly different among the inbred lines (P≥ 0.05); and ranged from 64.06° per plant for inbred MO345 to 71.56° per plant for MO034.

5.1.4.4 Fourth whorl angle

Interactive effect of phosphorus level and inbred was not significant (P≥ 0.05) on fourth whorl angle. Fourth whorl angles ranged from 57.87° per plant for inbred MO001 under low phosphorus level to 71.75° for inbred MO345 under low phosphorus level. Fourth whorl angles were not significantly different between low and high phosphorus levels (P≥ 0.05), with plants averaging 66.87° on high phosphorus level while plants planted on low phosphorus level averaged at 66.38° in whorl angle (Table 5.3). Fourth whorl angles differed significantly among the inbred lines (P≤ 0.05). Inbred MO345 recorded the steepest angle at 70.68° while inbreds MO199 and inbred MO001 had the shallowest angles at 61.68º and 61.82° per respectively (Table 5.3).

5.1.4.5 Fifth whorl angle

Interactive effect of phosphorus and inbred was not significant (P≥ 0.05) on fifth whorl angle. Fifth whorl angle ranged from 45.13° for inbred MO199 under low phosphorus level to 79.75° under low phosphorus level. Fifth whorl angles were not significantly different between low and high phosphorus levels (P≥ 0.05), with plants averaging 69.04° on high phosphorus level while plants planted on low phosphorus level averaged at 60.39° in whorl angle. There were no significant differences among the inbred lines (P≥ 0.05). The shallowest angle was recorded on inbred MO345 at 75.25° and the steepest angle was recorded on inbred MO199 at 54.50.

5.1.4.6 Sixth whorl angle

Interactive effect of phosphorus level and inbred was not significant (P≥ 0.05) on sixth whorl angle. Sixth whorl angle ranged from 15.00° for inbred MO345 under low phosphorus level to 69.38° for inbred MO034 under high phosphorus level. Sixth whorl angles were not significantly different between low and high phosphorus levels (P≥ 0.05), with plants averaging 48.08° on high phosphorus level while plants planted on low phosphorus level averaged at 36.12° in angle. Sixth whorl angles were not significantly different among the inbred lines (P≥ 0.05); and ranged from 15.63° for inbred MO345 to 63.13° for inbred MO034.

5.1.4.7 Number of root whorls

The interactive effect of phosphorus level and inbred was not significant (P≥ 0.05) on number of root whorls. Number of root whorls ranged from 5.00 whorls per plant for inbred MO031 under low phosphorus level to 6.00 whorls per plant for inbred MO034

under high phosphorus level. Numbers of root whorls were not significantly different between low and high phosphorus levels (P≥ 0.05), with plants averaging 5.70 on high phosphorus level while plants planted on low phosphorus level averaged at 5.50 whorls. The numbers of whorls were not significantly different among the inbred lines (P≥ 0.05); and ranged from 5.00 whorls per plant for inbred MO345 to 6.00 whorls per plant for inbred MO034.

5.1.4.8 Projected root area

Interactive effect of phosphorus level and inbred was not significant (P≥ 0.05) on projected root area. Projected root area ranged from 16071.00 mm^2 per plant for inbred MO001 under low phosphorus level to 22204.00 mm^2 per plant for inbred MO199 under high phosphorus level. There were significant differences in projected root area per plant (P≤ 0.05) between low and high phosphorus levels with high phosphorus level resulting in larger projected root area per plant, with plants averaging 20169.00 mm^2 while plants planted on low phosphorus level had smaller root area per plant that averaged at 18396.00 mm^2 (Table 5.3). Projected root areas were not significantly different among the inbred lines (P≥ 0.05); and ranged from 16552.00 mm^2 per plant for inbred MO034 to 21517.00 mm^2 per plant for inbred MO199 (Table 5.3).

5.1.4.9 Average root density

Interactive effect of phosphorus level and inbred was not significant (P≥ 0.05) on average root density on average root density, average root density ranged from 3.29 mm per plant for inbred MO196 under low phosphorus level to 6.54 mm per plant for inbred MO196 under high phosphorus level. Average root densities were not significantly different between low and high phosphorus levels (P≥ 0.05), with plants averaging 4.47 mm on high phosphorus level while plants planted on low phosphorus level averaged at 3.55 mm in root density. Average root densities were not significantly different among the inbred lines (P≥ 0.05); and ranged from 3.54 mm per plant for inbred MO199 to 4.92 mm per plant for inbred MO196.

5.1.4.10 Root top angle

Interactive effect of phosphorus level and inbred was not significant (P≥ 0.05) on root top angle. Root top angle ranged from 12.05° per plant for inbred MO199 under low phosphorus level to 54.44° per plant for inbred MO345 under high phosphorus level

(Table 5.4). Root top angles were not significantly different between low and high phosphorus levels (P≥ 0.05), with plants averaging 27.95° on high phosphorus level while plants planted on low phosphorus level averaged at 27.51° in angle (Table 5.3). Root top angles showed significant differences among the inbred lines (P≤ 0.01). Inbred MO345 recorded the steepest angle at 44.57° while inbred MO034 recorded the shallower angle at 18.12° (Table 5.3).

5.1.4.11 Root bottom angle

Interactive effect of phosphorus level and inbred on root bottom angle was not significant (P≥ 0.05), root bottom angle ranged from 14.28° per plant for inbred MO001 under high phosphorus level to 31.65° per plant for inbred MO199 under low phosphorus level. Root bottom angles were not significantly different between low and high phosphorus levels (P≥ 0.05), with plants averaging 23.94° on high phosphorus level while plants planted on low phosphorus level averaged at 21.56° in angle (Table 5.3). Root bottom angles showed significant differences among the inbred lines (P≤ 0.05). Inbred MO199 recorded the steepest angle at 31.42° while inbred MO031 recorded the shallower angle at 16.97° per plant (Table 5.3).

5.1.4.12 Number of adventitious roots

Interactive effect of phosphorus level and inbred was not significant (P≥ 0.05) on number of adventitious roots, number of adventitious roots ranged from 1.70 per plant for inbred MO196 under low phosphorus level to 3.41 per plant for inbred MO196 under high phosphorus level. Number of adventitious roots were not significantly different between low and high phosphorus levels (P≥ 0.05), with plants averaging 2.00 on high phosphorus level while plants planted on low phosphorus level averaged at 3.00 roots per plant. Numbers of adventitious roots were not significantly different among the inbred lines (P≥ 0.05); and ranged from 2.14 per plant for inbred MO031 to 3.29 per plant for inbred MO001.

5.1.4.13 Number of basal roots

Interactive effect of phosphorus level and inbred was not significant (P≥ 0.05) on number of basal roots, number of basal roots ranged from 5.08 per plant for inbred MO199 under high phosphorus level to 7.91 per plant for inbred MO001 under high phosphorus level. Numbers of basal roots were not significantly different between low and high phosphorus levels (P≥ 0.05), both levels had few basal roots, with plants

averaging 6.17 on high phosphorus level while an average of 6.1 was found on plants planted under low phosphorus level. Numbers of basal roots were not significantly different among the inbred lines (P≥ 0.05); and ranged from 5.12 per plant for inbred MO199 to 7.00 per plant for inbred MO001.

5.1.4.14 Tap root diameter

Interaction of phosphorus and inbred was not significant (P≥ 0.05) on tap root diameter. Tap root diameter ranged from 1.19 mm per plant for inbred MO031 under low phosphorus level to 1.53 mm per plant for inbred MO196 under low phosphorus level. Tap root diameters were not significantly different between low and high phosphorus levels (P≥ 0.05), with plants averaging 1.36 mm on high phosphorus level while plants planted on low phosphorus level averaged at 1.34 mm in diameter. Tap root diameters were not significantly different among the inbred lines (P≥ 0.05); and ranged from 1.26 mm per plant for inbred MO031 to 1.51 mm per plant for inbred MO196.

5.1.4.15 Average lateral root length

There was significant interaction of inbred and phosphorus level on average lateral root length (P≤ 0.05). Inbred MO199 recorded the longest lateral root of 251.46 mm per plant under low phosphorus level while inbred MO001 recorded the shorted lateral root length of 179.22 mm per plant under low phosphorus level (Table 5.4). Average lateral root lengths were not significantly different between low and high phosphorus levels (P≥ 0.05), both levels had long lateral roots, with plants averaging 214.34 mm on high phosphorus level while plants planted on low phosphorus level averaged at 204.64 mm in length (Table 5.3). Average lateral root lengths differed significantly among the inbred lines (P≤ 0.01). Inbred MO199 recorded the longest lateral roots at 242.54 mm while inbred MO001 had the shortest lateral roots of 193.55 mm in length (Table 5.3).

5.1.4.16 Lateral branching frequency

Interaction of phosphorus and inbred was not significant (P≥ 0.05) on lateral branching frequency. Lateral branching frequency ranged from 8.61 mm per plant for inbred MO034 under low phosphorus level to 21.25 mm per plant for inbred MO345 under low phosphorus level. Lateral branching frequencies were not significantly different between low and high phosphorus levels (P≥ 0.05), with plants averaging 15.49 mm

on high phosphorus level while plants planted on low phosphorus level averaged at 15.87 mm in branching frequency. Lateral branching frequencies were not significantly different among the inbred lines (P≥ 0.05); and ranged from 9.30 mm per plant for inbred MO034 to 20.38 mm per plant for inbred MO345.

5.1.4.17 Distance to the first lateral root

Interaction effect of phosphorus and inbred was not significant (P≥ 0.05) on distance to the first lateral root, distance to the first lateral root ranged from 0.66 mm per plant for inbred MO001 under high phosphorus level to 4.52 mm per plant for inbred MO199 under high phosphorus level. Distances to the first lateral root were not significantly different between low and high phosphorus levels (P≥ 0.05), with plants averaging 2.04 mm on high phosphorus level while plants planted on low phosphorus level averaged at 1.92 mm in distance. Distances to the first lateral root were not significantly different among the inbred lines (P≥ 0.05); and ranged from 1.26 mm per plant for inbred MO001 to 3.00 mm per plant for inbred MO199.

5.1.5 The influence of phosphorus on dry biomass production of selected maize IBM inbred lines.

Interaction of phosphorus level and inbred was not significant (P≥ 0.05) on biomass, Biomass ranged from 113.3 kg per ha^{-1} for inbred MO345 under low phosphorus level to 377.5 kg per ha^{-1} for inbred MO345 under high phosphorus level. Dry biomass was not significantly different between low and high phosphorus levels (P≥ 0.05), with plants averaging 259.9 kg per ha^{-1} on high phosphorus level while plants planted on low phosphorus level averaged at 167.9 kg per ha^{-1}. Biomass was not significantly different among the inbred lines (P≥ 0.05); and ranged from 181.8 kg per ha^{-1} for inbred MO199 to 245.4 kg per ha^{-1} for inbred MO345.

Table 5.3 Influence of phosphorus level and inbred on whorl angles, projected root area, root top angle, root bottom angle, average lateral root length, lateral branching frequency and average lateral root angle of selected maize IBM inbred lines.

Phosphorus level		1st whorl angle (°)	4th whorl angle (°)	PRA (mm^2)	RTA (°)	RBA (°)	AVLL (mm)	LBF (mm)
High		64.16[a]	66.38[a]	20169.00[a]	27.95[a]	23.94[a]	214.34[a]	15.49[a]
Low		61.69[a]	63.87[a]	18396.00[b]	27.51[a]	21.56 [a]	204.60[a]	15.87[a]
P value		0.2021	0.1981	0.0241	0.9123	0.2866	0.4244	0.8933
Significant difference		ns	ns	*	ns	ns	ns	ns
Turkey HSD		4.8346	4.4809	1331.90	11.682	5.8627	33.615	11.007
CV%		8.36	8.09	7.52	45.85	22.757	17.47	57.73
Maize inbred	MO034	67.87[a]	66.56 [ab]	16552.00[a]	18.12[a]	18.89[ab]	199.73[b]	9.30[b]
	MO196	63.87[ab]	66.37 [ab]	20280.00[a]	29.46[ab]	25.62[ab]	214.22[ab]	18.10[ab]
	MO345	63.06 [ab]	70.68 [a]	20000.00[a]	44.57[a]	24.74[ab]	209.89[ab]	20.38[a]
	MO031	62.43 [ab]	63.62 [ab]	19489.00[a]	29.49[ab]	16.97[b]	196.89[b]	14.97[ab]
	MO001	62.14 [ab]	61.82[b]	17855.00[a]	25.56[ab]	18.86[ab]	193.55[b]	16.96[ab]
	MO199	58.18[b]	61.68[b]	21517.00[a]	19.17[b]	31.42[a]	242.54[a]	14.39[ab]
P value		0.0448	0.028	0.0770	0.0055	0.0146	0.0021	0.0788
Significant difference		*	*	ns	**	*	**	ns
Turkey HSD		8.309	8.6455	5158.50	20.237	12.782	34.376	11.007

Means in the same column followed by the same letters are not significantly different at P ≥ 0.05, * = significant at P ≤ 0.05, ** = significant at P ≤ 0.01, ns = not significant, PRA: Projected root area, RBA: Root top angle, RBA: Root bottom angle, AVLL: Average lateral root length and LBF: lateral branching frequency.

Table 5.4 Interactive effect of P level × inbred on root top angle and average lateral root length of selected maize IBM inbred lines.

Phosphorus Level	Inbred	Root top angle (°)	Average lateral length (mm)
Low	MO031	38.476 ab	197.35 ab
Low	MO345	34.708 ab	182.88 b
Low	MO196	32.025 ab	212.62 ab
Low	MO001	26.616 ab	179.22 b
Low	MO034	21.207 ab	204.05 ab
Low	MO199	12.047 b	251.46 a
High	MO196	26.913 ab	215.82 ab
High	MO345	54.438 a	236.89 ab
High	MO199	26.296 ab	233.62 ab
High	MO001	24.511 ab	207.87 ab
High	MO031	20.512 b	196.43 ab
High	MO034	15.046 b	190.46 ab
P value		0.078	0.039
Significant difference		ns	*
Turkey HSD (means for the same level of phosphorus		33.259	5.012
Turkey HSD (means for different level of phosphorus)		38.063	6.418
CV%		47.98	10.98

Means in the same column followed by the same letters are not significantly different at P ≥ 0.05, * = significant at P ≤ 0.05, ns = not significant P ≥ 0.05.

5.1.6 Correlation analysis

Simple person correlations were conducted among various components to determine the relationship of the traits with dry biomass.

5.1.6.1 Correlation between biomass and morphological traits on phosphorus experiment

There was a positive and significant correlation (P≤ 0.05) between dry biomass and selected morphological traits (stem diameter ($R^2 = 0.397^{***}$), leaf length ($R^2 = 0.436^{***}$), leaf width ($R^2 = 0.348^{***}$), leaf area per plant ($R^2 = 0.476^{***}$)) only under high phosphorus level (Table 5.5). However there was no significant (P≤ 0.05) correlation between dry biomass and other measured morphological traits (number of leaves, chlorophyll content and plant height) at high phosphorus level (Table 5.5). Similarly, positive and significant correlations (P≤ 0.05) between dry biomass and morphological traits (stem diameter ($R^2 = 0.252^{**}$), plant height ($R^2 = 0.374^{***}$)) were obtained under low phosphorus level. However there were no significant (P≥ 0.05) correlation between dry biomass and other measured morphological traits (Number of leaves, chlorophyll content, leaf length, leaf width and leaf area per plant) at low phosphorus level (Table 5.5).

Table 5.5 Association between morphological traits and dry biomass (kg/ha^{-1}) of all maize lines for low and high phosphorus level.

Morphological trait	Phosphorus level			
	Low		High	
	r	P value	r	P value
Chlorophyll content	0.0842	0.690 ^{ns}	0.320	0.120 ^{ns}
Stem diameter	0.5018	0.010**	0.630	0.000***
Plant height	0.6119	0.000***	0.290	0.170 ^{ns}
Number of leaves	0.0687	0.740 ^{ns}	0.060	0.770 ^{ns}
Leaf length	0.3021	0.150 ^{ns}	0.660	0.000***
Leaf width	0.2192	0.300 ^{ns}	0.590	0.000***
Leaf area per plant	0.2958	0.160 ^{ns}	0.670	0.000***

r= Pearson correlation coefficient, * = significant at P ≤ 0.05, ** = significant at P ≤ 0.01, *** = significant at P ≤ 0.001, ns = not significant P ≥ 0.05.

5.1.6.2 Correlation between biomass and root architectural traits under low and high phosphorus level.

There was a negative and significant correlation (P≤ 0.05) between dry biomass and 2nd whorl angle (R^2 = 0.436**). Furthermore there was a positive and significant correlation between dry biomass and lateral branching frequency (R^2 = 0.292**) under high phosphorus level. However, there was no significant correlation (P≥ 0.05) between dry biomass with several root architectural traits (1st whorl angle, 3rd whorl angle, 4th whorl angle, 5th whorl angle, 6th whorl angle, number of whorls, projected root area, tap root diameter, average lateral root length, projected root area, average root density, root top angle, root bottom angle, number of adventitious roots, number basal roots, tap root diameter, average lateral root length and lateral branching frequency) under high and low phosphorus (Table 5.6).

Table 5.6 Association of root architectural traits with dry biomass (kg/ha^{-1}) of all maize lines for low and high phosphorus level.

Root architectural trait	Phosphorus level			
	Low		High	
	r	P value	r	P value
1st whorl angle	-0.380	0.070 ns	-0.370	0.070ns
2nd whorl angle	-0.100	0.650 ns	-0.660	0.000**
3rd whorl angle	0.160	0.460 ns	-0.370	0.070 ns
4th whorl angle	-0.240	0.260 ns	-0.110	0.610 ns
5th whorl angle	0.010	0.980 ns	-0.290	0.160 ns
6th whorl angle	-0.050	0.820 ns	0.110	0.620 ns
Number of whorls	0.150	0.480 ns	0.120	0.590 ns
Projected root area	0.280	0.180 ns	0.260	0.220 ns
Average root density	0.000	1.000 ns	-0.290	0.170 ns
Root top angle	0.140	0.510 ns	0.370	0.080 ns
Root bottom angle	-0.260	0.220 ns	-0.270	0.200 ns
Number of adventitious roots	0.040	0.840 ns	-0.100	0.650 ns
Number of basal roots	0.280	0.180 ns	0.110	0.600 ns
Tap root diameter	-0.070	0.760 ns	-0.010	0.970 ns
Average lateral root length	0.150	0.480 ns	0.350	0.090 ns
Lateral branching frequency	-0.360	0.080 ns	0.540	0.010**
Distance to the first lateral root	0.010	0.950 ns	0.360	0.090 ns

r= Pearson correlation coefficient, * = significant at P ≤ 0.05, ** = significant at P ≤ 0.01, *** = significant at P ≤ 0.001, ns = not significant P ≥ 0.05.

5.2 Discussion

The results from this study support the hypothesis that phosphorus has no effect on whorl distribution and stem diameter size variation of various maize inbred lines, as evidenced by the similarities of all morphological traits and several root architectural traits on both low and high phosphorus level. Those similarities meant that there was no significant variation in phosphorus use efficiency among all the morphological traits and several root architectural traits; hence application of phosphorus had no benefit on the vegetative growth of maize. Crop response to P fertilizer was expected from the initial soil analysis results. P is a highly immobile macro nutrient this makes it more readily available for plant usage. Therefore phosphorus had no significant effect on growth attributes probably because of the high level of phosphorus on the top soil profile (0-10 cm). These results are in agreement with Eltelib *et al*. (2006) who reported that phosphorus application has no significant effect on growth attributes of maize. However crop response to P fertilizer was unexpected from the soil analysis results at harvesting, the top soil of the high phosphorus split had top soil profile which was slightly acidic, under acidic condition many minerals dissolve resulting in an increase concentration of metal to toxic levels and hence growth and development is inhibited under those conditions (Gudu *et al*., 2005). Nutrient availability is also affected by pH level both phosphorus and molybdenum are less available in acid soils (Gudu *et al*., 2005). Calcium and Magnesium deficiency is also reported on acid soil. Therefore slight decrease in growth was expected on the high P split plot in response to the slight acidic condition on the top soil. However, phosphorus increased root area significantly, suggesting that adequate application of P fertilizer can lead to increased root growth which eventually leads to improved growth and development, as well as high yield

5.2.1 The effect of phosphorus on growth attributes of maize inbred lines.

Chlorophyll content

Chlorophyll content was not significantly affected by phosphorus level, plants planted on high and low phosphorus levels averaged 35.16 and 31.12 mg/g respectively. Similarly, uniformity in chlorophyll concentration on all phosphorus treatments on soya bean was reported by (Rotaru *et al*., 2015). However, higher P level had plants that were12.95% greater in chlorophyll content than plants under lower P level. These results suggest that plants planted on high phosphorus have greater chances for

improved root system, photosynthesis rate and overall growth and development; therefore farmers should consider increasing P application rate to enhance growth and development. Chlorophyll content varied significantly among the inbred lines with inbred MO196 significantly higher in chlorophyll content, at 39.35 mg/g, than inbred lines MO034 and MO031. Therefore, inbred lines MO196 and MO031 would not be considered for selection due to their low chlorophyll content. These results suggest that leaf chlorophyll content can be a useful selection criterion for maize inbreds.

Plant height

P level by inbred interaction was not statistically significant, the tallest plants were obtained from inbred MO196 at higher P level. This suggests that inbred MO196 has the potential to grow taller under high phosphorus level. Plant height was not significantly affected by phosphorus level; high phosphorus level of 56 kg P per ha^{-1} had plants with the average height of 116.24 cm as compared to low phosphorus (0 kg P per ha^{-1}) at 103.79 cm. The higher P level had plants 11.99% taller than the lower P level. Crop response to P fertilizer was not expected from the initial soil analysis results as the top soil was high in P. These results contradict with Masood *et al*., (2011) who reported that increasing phosphorus level increased plant height of maize. Phosphorus enhances root growth which affects the overall plant growth and development; therefore it is possible that a further increase in phosphorus application rate might trigger increase in production. The slightly increased height was probably due to better root system and improved nutrient uptake than the low P level (Masood *et al*., 2011). Therefore farmers could consider increasing their P application rate to above 56 kg P per ha^{-1} for better growth and development, if costs allow. Plant height varied significantly among the inbred lines, inbred lines MO199 and MO196 were significantly taller at 121.58 cm and 123.92 cm, respectively. These two were significantly taller than inbred lines MO001 and MO034. Therefore in this case inbred lines MO001 and MO034 can be considered for selection of short inbred lines whereas inbred lines MO199 and MO196 can be considered for selection of taller inbred lines. These results show that plant height can be a useful criterion for selecting maize inbred lines.

Leaf width

Phosphorus level by inbred interaction had no significant effect on leaf width ($P \geq 0.05$). The width ranged from 5.68 cm for inbred MO196 under low phosphorus level to 7.48

cm for inbred MO034 under high phosphorus level. These results therefore suggest that phosphorus level does not influence leaf width in maize, it is more of a genetic trait. However, leaf width varied among the inbred lines indicating that this could be a useful parameter for selecting maize inbred lines. Leaf width was not significantly affected by P level, plants planted under low and high P level averaged 6.39 to 6.67 cm respectively. These results contradicts with Piri (2012) who reported that application of phosphorus significantly affected leaf width with an application rate of 200 kg P ha^{-1} giving the greatest leaf width of 5.33 cm in sorghum. Studied inbred lines gave broad leaves regardless of the P level signifying that they have potential to produce greater leaf area. Therefore this suggests that farmers can benefit from increased leaf growth if adequate phosphorus is supplied. Inbred MO345 had the broadest leaves with leaf width of 118.33 cm and was significantly broader than inbred MO196. The results suggest that inbred MO345 would be the best inbred to be selected for broader leaves.

Projected root area

The interaction of P level and inbred was not statistically significant for projected root area, plants with the greatest root area were obtained on inbred MO199 under high phosphorus level. Therefore in this perspective projected root area cannot be used to identify superior performing inbred lines for P stress. However, farmers should apply P adequately to improve root growth. The results showed that phosphorus significantly increased root area ($P \leq 0.05$). Higher P level had plants 9.63% greater in root area than the lower P level. The observed greater root area indicates that plants planted under high phosphorus level have potential to have increased root length or growth rates, effective water transportation (Hund *et al*., 2009), and increased root penetration index in soil (Chandra *et al*., 2001) and moreover plants become more resistant to lodging (Ennos, 1991) as P in the soil enhances such traits. Results from this study agrees with the findings of Hajabbasi and Schumacher (1994) who reported that addition of P increased roots relative growth rate in maize (cultivar CM37). There was no significant variation among the inbred lines in terms of projected root area, root area ranged from 16552.00 mm^2 for inbred MO034 to 21517.00 mm^2 for inbred MO199.

First whorl angle

The interaction between P level and inbred was not statistically significant, however plants with the greatest first whorl angle were obtained on inbred MO034 under high phosphorus level. This means that to enhance growth in the first whorl angle it can be considered to select inbred MO034 with the use of high phosphorus level. These results show that first whorl angle can be useful criterion for selecting maize inbred lines with improved nutrient uptake efficiency. First whorl angle was not significantly affected by P level; plants planted under high and low P level averaged 61.69° and 64.16° respectively. However higher P level had plants which are 4.00% greater in whorl angle than the lower P level. This meant that the applied levels of P had no influence on the orientation of the 1st whorl angle. Inbred MO034 was significantly greater in 1st whorl angle at 67.87° and significantly greater than inbred MO199. These results indicate that in considering selection whorl angle inbred MO199 would be selected for shallowest angle while inbred MO034 can be the best selection for steeper 1st whorl angled plants.

Fourth whorl angle

The interaction between P level by inbred was not statistically significant, the fourth whorl angle ranged from 57.87° per plant for inbred MO001 under low phosphorus level to 71.75° per plant for inbred MO345 under low phosphorus level. Fourth whorl angle was not significantly affected by P level. Plants planted under high and low P level averaged 66.38° and 63.87° respectively. However higher P level had plants which are 3.93% greater whorl angle than the lower P level. The results seem to suggest that P had no influence on the fourth whorl angle however both levels had shallow whorl angles. Inbred MO345 was significantly greater in 4th whorl angle at 70.68° and significantly greater than inbred lines MO199 and MO001. In this perspective inbred line MO345 can be considered for steepest whorl angle while inbred lines MO199 and MO001 can be considered for shallow angle. MO196 and MO031 had average angles. The shallow roots have wide root system hence they have potential to uptake more water as soon as rain falls especially in areas where there is low rainfall and also the root system can enhance P uptake as it is immobile and is mostly found in the top soil. The steeper rooted inbred lines can have the potential to uptake highly mobile nutrients such as N from deeper soil horizons. Results from this study agree with the findings of York and Lynch (2015) who reported

that nodal root growth angles were steeper in the younger whorl (the outer whorl angles) in some genotypes of maize (IBM population). However for the same inbred lines whorl angles were almost the same in older whorls. Often times studies of single maize genotypes are extrapolated to species level. In this study substantial variation for fourth whorl angle was demonstrated, this shows that almost any measured root architectural trait may vary, so general extrapolation on species level are not necessary. This result shows that to predict the overall growth pattern among the whorls, all the whorls (outer and inner whorls) need to be measured.

Root top angle

P level by inbred was statistically not significant, Inbred MO345 was significantly steeper at 54.44° under high P level and significantly steeper than inbred lines MO034, MO031 and MO199. Shallower angles were recorded on inbred lines MO034 (20.51°) and MO031 (15.045°) both under high phosphorus level and also on inbred MO199 (12.05°) under low nitrogen level. A shallow root system has more potential in the uptake and utilization of phosphorus from the soil and also improvement of water use uptake in drier soils, generally P is found in the top soil, so a shallow root system can make it easier to uptake this nutrient. On the other hand, a steeper root system has more potential to uptake soil N and water from deeper soil horizons especially in drought conditions. Inbred MO199 was shallow rooted under low P conditions indicating that this inbred was more adapted to low phosphorus availability than inbred lines which were planted on higher phosphorus level. Therefore on low P stress inbred MO199 can be recommended. Both inbred lines MO034 and MO031 had shallow root top angles under high P level therefore these inbred lines can also be considered in selection for improved top soil P foraging. The steepest root top angle obtained from inbred MO345 indicates that the inbred has potential of enhancing the uptake of nitrogen from the soil, therefore it can be recommended on leaching soils or in high rainfall areas where the applied N is likely to leach from the top soil profile. Results from this study are consistent with previous reports of the importance of top soil foraging for absorption of phosphorus in maize (Zhu *et al*., 2005) and common bean (Lynch and Brown 2001). Based on a qualitative dynamic crop growth model it was concluded that an increase in maize yield in the mid-western USA is mainly influenced by the root angle of the primary roots systems (Hammer *et al*., 2009). These results therefore suggest that root top angle is a useful parameter in selecting inbred lines

with improved nutrient use efficiency. In this study measurement of this trait indicated that high and low phosphorus level enhanced distribution of root top angle which can also have a potential in increasing crop yield. Both high and low phosphorus levels gave shallower and steeper roots angles. Phosphorus level had no significant effect on the root top angle. Plants planted under high and low P level averaged 27.95° and 27.51°, respectively. Results from this study agrees with the findings of Ho *et al.* (2004) who reported that the range of basal root angles among a population of recombinant inbred lines (RILs) of common bean was similar on low and high phosphorus availability. This result was unexpected, as it seems to suggest that root top angle may not be a necessary trait for adaptation to low P environments. Inbred MO345 was significantly steeper at 44.57° and significantly steeper than inbred MO034 and MO199. This result indicates that for root top angle inbred MO034 and MO199 could be selected for root shallowness, while inbred lines MO345, MO031, MO196 and MO001 could be selected for root steepness.

Root bottom angle

The interaction between P level by inbred was statistically significant. Tested inbred lines formed steeper and shallow bottom root angle. The steepest (31.65°) root bottom angle was obtained from inbred MO199 at lower P level while the shallowest angle (14.28°) was obtained on inbred MO001 at high P level. These results seem to suggest that the root bottom angle plasticity may not be necessary for adaptation to low phosphorus environments. Root bottom was not significantly affected by P levels. Plants planted under high and low P level averaged 23.94° and 21.56° respectively. These results suggest that P availability has no influence on the orientation of the bottom root angle. Inbred MO199 was significantly steeper at 31.43° and significantly steeper than inbred MO031. Therefore from a root bottom angle perspective, inbred MO001 could be considered for selection for shallowest bottom root angle, while inbred MO199 can be considered for steepest root bottom angle. All other inbred lines had average root bottom angle. Selection can be made depending on what is needed.

Average lateral root length

P level by inbred was statistically significant. Inbred MO199 was significantly longer in lateral roots at 251.46 mm under lower P level and significantly longer than inbred MO199 and MO001 both at lower phosphorus level. This means that inbred MO199 was highly tolerant to the low P conditions hence it altered its root system to enhance

top soil foraging of nutrients. Zhang *et al*., (2009) also emphasized that that plants respond to phosphorus deficiency by altering their root morphology to enhance acquisition and internal phosphorus recycling. Long rooted lateral roots are vital for optimum acquisition of nitrate from the soil while short lateral roots are vital for optimum phosphorus acquisition (Postma *et al*., 2014). In this study inbred MO345 (182.88 mm) and MO001 (179.22 mm) had shorter lateral roots, a trait vital for enhancing P uptake, hence they can be considered for enhancing P uptake. Inbred MO199 (251.46 mm) had the longest lateral roots under low P conditions showing higher tolerance to low P condition. Inbred MO199 and other inbred lines had long lateral roots under low P level showing potential to be selected for inbred lines with potential to enhanced N uptake under low P condition. Average lateral root length was not significantly affected by P levels. Plants planted under high and low P level averaged 214.34 mm and 204.60 mm, respectively. Crop response to P fertilizer was expected from the initial soil analysis results as the top soil was high in P. These results contradict findings of Basirat *et al*. (2011) who reported that limiting P supply led to increase in root length, root volume and root density on tomatoes. In this study the similarity in root length in both low and high phosphorus suggest that the planted inbred lines have the ability to elongate at reduced phosphorus cost that would allow them to acquire more P from the soil. Inbred MO199 had significantly longer lateral roots of 242.54 mm than inbred lines MO001, MO034 and MO031. Therefore inbred lines MO199, MO345 and MO196 could be selected for long lateral roots, while inbred lines MO001, MO034 and MO031 could be selected for shorter lateral roots.

Lateral branching frequency

Interaction between P level by inbred was not statistically significant, plants with greatest branching frequency were obtained from inbred MO345 at lower P level. Signifying that inbred MO345 with low P level have the potential to greater number of lateral roots elongated on parent roots, this is an important trait to improve P use efficiency. These results show that lateral branching frequency merits consideration as a selection criteria for inbred lines with greater crop N use efficiency. Selection can be based on whether there is a need for reduced or higher branching frequency required. Lateral branching frequency was not significantly affected by P level. Plants planted under high and low P level averaged 15.49 mm and 15.87 mm respectively. These results suggest that P availability has no influence on lateral branching

frequency. Inbred MO345 was significantly higher in lateral branching frequency at 20.38 mm and significantly higher than inbred MO034. Zhan and Lynch (2015) associated reduced branching frequency with improvement of N uptake in low N soil. Therefore inbred MO034 can only be considered for selection for reduced branching frequency while inbred MO345 can be considered for higher branching frequency which can also have potential to improve P uptake. Inbred lines MO001, MO199, MO031 and MO196 all had optimal branching frequency hence they have potential to improve both N and P use efficiency.

Dry biomass

The interaction between P level and inbred was not statistically significant. The highest dry biomass (377.5 kg per ha^{-1}) was obtained on inbred MO345 in plots under high P level as compared to inbred MO345 which was planted on the low P level where the biomass was lowest (113.3 kg per ha-1). These results signify that farmers can enhance their biomass by adequate application of P during planting. However biomass cannot be used to identify superior maize performing lines under phosphorus stress. Therefore, with regard to biomass, the hypothesis that states that superior maize performing inbred lines are overlooked in morphological and root architectural traits under phosphorus stress is accepted. Data pertaining to biomass revealed that P levels had no influence on dry biomass of maize, the highest biomass (259.94 kg ha^{-1}) was obtained in plots with P applied at the rate of 56 kg P per ha^{-1} and the lowest (167.92 kg per ha^{-1}) biomass was obtained on the low P plot (0 kg P per ha^{-1}). These results were expected since all morphological traits were not affected by the application P, while these traits account for major part of the total biomass per plant. These results are in conformity with the findings of Eltelib *et al*., (2006) who reported that P level had no significant effect on growth attributes of maize. However dry biomass was slightly greater under high P level. The slightly greater biomass on high P level was due to the fact that root growth of maize was best at high P level which resulted in greater biomass on high P level due to proficient photosynthesis and nutrient uptake as well as other physiological function at this level. Biomass showed no significant difference among the inbred lines. Signifying that biomass is unlikely going to be useful trait for selection of maize inbred lines.

5.2.2 Correlations

One of the objectives of this study was to evaluate if particularly root architecture and morphological traits directly relate to dry biomass production under low and high P level. For this, traits were measured at different phosphorus levels and related to the dry biomass respectively (Table 5.5 and 5.6), correlations are discussed below.

5.2.2.1 Correlation between biomass and morphological traits, root architectural traits on phosphorus experiment.

The results from this study contradict with the hypothesis that there is no relation between morphological and root architectural traits and biomass production under phosphorus stress. There were positive and significant correlations between dry biomass and selected morphological traits (stem diameter, plant height) under low phosphorus level. Coefficients of multiple determination revealed that stem diameter and plant height had influence on biomass explaining 25.2% and 37.40% in total treatment variation on low phosphorus level, respectively. This meant that plant biomass was partially dependent on stem diameter and plant height under low phosphorus level. The positive correlation between biomass and stem diameter as well as plant height under low phosphorus suggest that those traits perform better under Low P conditions resulting in greater Low P tolerance.

There were positive and significant correlations between dry biomass and several traits (stem diameter, leaf length, leaf width and leaf area per plant, lateral branching frequency) under high P level. Coefficients of multiple determination revealed that stem diameter, leaf length, leaf width, leaf area per plant and lateral branching frequency had strong influence on biomass explaining 39.7%, 43.6%, 34.8% 47.6% and 29.20% in total treatment variation on high phosphorus level, respectively. There was a negative and significant correlation between biomass with 2^{nd} whorl angle, explaining 43.6% in total treatment variation. This meant that plant biomass was dependent on stem diameter, leaf length, leaf width, leaf area per plant, lateral branching frequency, 2^{nd} whorl angle and average nodal root diameter on high P soil. The significant negative correlation between biomass and 2^{nd} whorl angle meant that an increase 2^{nd} whorl angle due to high phosphorus level resulted in a decrease in biomass in high phosphorus soil. Therefore 2^{nd} whorl angle could be indirectly used as criteria selection of inbred lines that have greater P use efficiency.

Results from this study are in agreement with the findings of DoVale *et al*. (2013) who reported that shoot dry weight had a strong significant correlation with morphological traits in both high and low P experimental plots. However, there was no variation in relationships of biomass with stem diameter in low and high P level signifying that the relationships are allometric. Several traits also differed in their relationships with biomass in low and high P soil level, this suggests that the relationships are not allometric (Niklas, 2004). Recently reduced lateral root branching frequency was demonstrated to improve N uptake from the soil (Zhan and Lynch, 2015). In this study a positive relationship between lateral branching frequency and biomass existed for plants grown under high P level, but not for plants grown under low P soil level, suggesting that the relationship in high phosphorus level was allometric because there was neither relationship nor variation between lateral branching frequency and biomass in low phosphorus level. The results conform to Postma *et al*. (2014) who reported that denser branches (greater than 9 branches cm^{-1}) with shorter lateral roots improves P acquisition from the soil. The high significant positive correlations indicate that selection of high yielding inbreds may be useful based on phosphorus level and biomass.

6. Conclusions and recommendations

This study showed that phosphorus has no effect on whorl distribution and stem diameter size among maize inbred lines. The results confirmed that phosphorus availability indeed is important for enhancement of root growth in maize, this implies the need for appropriate management of P in maize production. There was high variation in relationships between shoot morphological and root architectural traits with biomass production under phosphorus stress. The inbred lines tested differed significantly in shoot morphological and root architectural traits as well as in biomass production, making it necessary to evaluate several inbreds before inclusion in germplasm development program. The results showed that inbred and phosphorus availability can modulate root shallowness and lateral root growth in maize which is vital for adaptation to low phosphorus soils. Inbred MO199 had shallow root top angle at low phosphorus level, thus making it adaptable to low soil P conditions. Inbred MO345 recorded the steepest root top angle under high phosphorus level. To enhance N and water acquisition efficiency it can be suggested to use inbred MO345 together with high phosphorus level. Inbred MO345 can be recommended for water scarce

areas such as Limpopo province, as well as areas which are prone to leaching (high rainfall areas) where N is applied on the top soil but plants cannot use it effectively as it is leached to lower horizons. This inbred can therefore be recommended to be used in the development of local maize germplasm. Future studies can use higher P rates than in the current study, particularly in locations of extremely low P levels as are obtained in many areas of Limpopo.

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APPENDICES

Analysis of variance (ANOVA) tables for nitrogen experiment

Appendix 1 Plant height.

Source	DF	SS	MS	F	P
Replication	3	940.60	313.52		
Nitrogen	1	9576.80	9576.75	9.51	0.0540
Error Replication*Nitrogen	3	3021.90	1007.29		
Inbred	5	2025.20	405.04	3.16	0.0207
Nitrogen*Inbred	5	582.90	116.57	0.91	0.4875
Error Replication*Nitrogen*Inbred	30	3841.20	128.04		
Total	47	19988.40			
Grand Mean	110.15				
CV (Replication*Nitrogen)	28.81				
CV (Replication*Nitrogen*Inbred	10.27				

Appendix 2 Leaf length.

Source	DF	SS	MS	F	P
Replication	3	567.01	189.00		
Nitrogen	1	3466.81	3466.81	16.91	0.0261
Error Replication*Nitrogen	3	615.18	205.06		
Inbred	5	441.24	88.25	2.44	0.0568
Nitrogen*Inbred	5	355.64	71.13	1.97	0.1122
Error Replication*Nitrogen*Inbred	30	1083.47	36.12		
Total	47	6529.34			
Grand Mean	47.95				
CV (Replication*Nitrogen)	29.86				
CV (Replication*Nitrogen*Inbred)	12.53				

Appendix 3 Leaf width.

Source	DF	SS	MS	F	P
Replication	3	3.62	1.20		
Nitrogen	1	46.18	46.17	87.06	0.0026
Error Replication*Nitrogen	3	1.59	0.53		
Inbred	5	7.12	1.42	2.25	0.0751
Nitrogen*Inbred	5	9.22	1.84	2.91	0.0293
Error Replication*Nitrogen*Inbred	30	18.99	0.63		
Total	47	86.72			
Grand Mean	6.97				
CV (Replication*Nitrogen)	10.44				
CV (Replication*Nitrogen*Inbred)	11.4				

Appendix 4 Stem diameter.

Source	DF	SS	MS	F	P
Replication	3	261.03	87.01		
Nitrogen	1	564.19	564.19	4.56	0.1224
Error Replication*Nitrogen	3	371.25	123.75		
Inbred	5	790.74	158.15	1.50	0.2199
Nitrogen*Inbred	5	554.61	110.92	1.05	0.4066
Error Replication*Nitrogen*Inbred	30	3166.21	105.54		
Total	47	5708.03			
Grand Mean	16.76				
CV (Replication*Nitrogen)	66.37				
CV (Replication*Nitrogen*Inbred)	61.29				

Appendix 5 Chlorophyll content.

Source	DF	SS	MS	F	P
Replication	3	141.54	47.18		
Nitrogen	1	1290.65	1290.65	20.57	0.0201
Error Replication*Nitrogen	3	188.27	62.76		
Inbred	5	336.10	67.22	1.72	0.1596
Nitrogen*Inbred	5	71.87	14.37	0.37	0.8661
Error Replication*Nitrogen*Inbred	30	1169.95	39		
Total	47	3198.37			
Grand Mean	35.76				
CV (Replication*Nitrogen)	22.15				
CV (Replication*Nitrogen*Inbred)	17.46				

Appendix.6 Number of leaves.

Source	DF	SS	MS	F	P
Replication	3	2.59	0.86		
Nitrogen	1	0.33	0.33	0.45	0.5515
Error Replication*Nitrogen	3	2.24	0.75		
Inbred	5	9.49	1.90	4.13	0.0056
Nitrogen*Inbred	5	1.03	0.21	0.45	0.8107
Error Replication*Nitrogen*Inbred	30	13.77	0.46		
Total	47	29.45			
Grand Mean	10.70				
CV (Replication*Nitrogen)	8.06				
CV (Replication*Nitrogen*Inbred)	6.33				

Appendix 7 Leaf area per plant

Source	DF	SS	MS	F	P
Replication	3	4732680.00	1577560.00		
Nitrogen	1	39960000.00	39960000.00	80.69	0.0029
Error Replication*Nitrogen	3	1485606.00	495202.00		
Inbred	5	3688645.00	737729.00	1.76	0.1512
Nitrogen*Inbred	5	3977236.00	795447.00	1.90	0.1241
Error Replication*Nitrogen*Inbred	30	12570000.00	418838.00		
Total	47	66410000.00			
Grand Mean	2816.40				
CV (Replication*Nitrogen)	24.99				
CV (Replication*Nitrogen*Inbred)	22.98				

Appendix 8 First whorl angle.

Source	DF	SS	MS	F	P
Replication	3	1506.35	502.12		
Nitrogen	1	0.52	0.52	0.00	0.9687
Error Replication *Nitrogen	3	863.1	287.70		
Inbred	5	134.35	26.87	0.30	0.9068
Nitrogen*Inbred	5	306.35	61.27	0.69	0.6331
Error Replication *Nitrogen*Inbred	30	2654.29	88.48		
Total	47	5464.98			
Grand Mean		60.729			
CV (Replication*Nitrogen)		27.93			
CV (Replication*Nitrogen*Inbred)		15.49			

Appendix 9 Second whorl angle.

Source	DF	SS	MS	F	P
Replication	3	303.93	101.31		
Nitrogen	1	26.26	26.26	11.03	0.045
Error Replication*Nitrogen	3	7.14	2.38		
Inbred	5	325.28	65.06	1.65	0.1762
Nitrogen*Inbred	5	164.53	32.91	0.84	0.5342
Error	30	1179.74			
Replication*Nitrogen*Inbred			39.33		
Total	47	2006.87			
Grand Mean		64.865			
CV (Replication *Nitrogen)		2.38			
CV (Replication *Nitrogen*Inbred)		9.67			

Appendix 10 Third whorl angle

Source	DF	SS	MS	F	P
Replication	3	10.94	3.65		
Nitrogen	1	11.02	11.02	0.68	0.4693
Error Replication *Nitrogen	3	48.44	16.15		
Inbred	5	197.10	39.42	1.49	0.2241
Nitrogen*Inbred	5	141.10	28.22	1.06	0.4002
Error Replication *Nitrogen*Inbred	30	796.38	26.55		
Total	47	1204.98			
Grand Mean		68.10			
CV (Replication *Nitrogen)		5.90			
CV (Replication *Nitrogen*Inbred)		7.57			

Appendix 11 Fourth whorl angle.

Source	DF	SS	MS	F	P
Replication	3	156.52	52.17		
Nitrogen	1	136.69	136.69	0.93	0.4066
Error Replication *Nitrogen	3	442.19	147.40		
Inbred	5	321.10	64.22	1.01	0.4316
Nitrogen*Inbred	5	223.81	44.76	0.7	0.6271
Error Replication *Nitrogen*Inbred	30	1916.17	63.87		
Total	47	3196.48			
Grand Mean		64.60			
CV (Replication *Nitrogen)		18.79			
CV (Replication *Nitrogen*Inbred)		12.37			

Appendix 12 Fifth whorl angle.

Source	DF	SS	MS	F	P
Replication	3	2606.3	868.76		
Nitrogen	1	396.70	396.75	1.27	0.3417
Error Replication *Nitrogen	3	937.00	312.32		
Inbred	5	841.50	168.31	0.98	0.4461
Nitrogen*Inbred	5	586.50	117.30	0.68	0.6399
Error Replication *Nitrogen*Inbred	30	5152.90	171.76		
Total	47	10520.90			
Grand Mean		62.79			
CV (Replication *Nitrogen)		28.14			
CV (Replication *Nitrogen*Inbred)		20.87			

Appendix 13 Sixth whorl angle.

Source	DF	SS	MS	F	P
Replication	3	2789.10	929.71		
Nitrogen	1	713.00	713.02	0.77	0.4438
Error Replication *Nitrogen	3	2763.60	921.20		
Inbred	5	3272.60	654.52	1.13	0.3667
Nitrogen*Inbred	5	294.00	58.81	0.10	0.9911
Error Replication *Nitrogen*Inbred	30	17398.50	579.95		
Total	47	27230.90			
Grand Mean		52.04			
CV (Replication *Nitrogen)		58.32			
CV (Replication *Nitrogen*Inbred)		46.27			

Appendix 14 Number of whorls.

Source	DF	SS	MS	F	P
Replication	3	0.83	0.28		
Nitrogen	1	1.33	1.33	3.43	0.1612
Error Replication *Nitrogen	3	1.17	0.39		
Inbred	5	3.67	0.73	1.07	0.3949
Nitrogen*Inbred	5	3.17	0.63	0.93	0.4775
Error Replication *Nitrogen*Inbred	30	20.50	0.68		
Total	47	30.67			
Grand Mean		6.33			
CV (Replication *Nitrogen)		9.85			
CV (Replication *Nitrogen*Inbred)		13.05			

Appendix 15 Projected root area.

Source	DF	SS	MS	F	P
Replication	3	63770000.00	21260000.00		
Nitrogen	1	393500000.00	393500000.00	10.45	0.0481
Error Replication *Nitrogen	3	113000000.00	37650000.00		
Inbred	5	93810000.00	18760000.00	1.28	0.2974
Nitrogen*Inbred	5	208900000.00	41780000.00	2.86	0.0317
Error Replication *Nitrogen*Inbred	30	438900000.00	14630000.00		
Total	47	1312000000.00			
Grand Mean	17101.00				
CV (Replication*Nitrogen)	35.88				
CV (Replication*Nitrogen*Inbred)	22.37				

Appendix 16 Average root density

Source	DF	SS	MS	F	P
Replication	3	3.57	1.19		
Nitrogen	1	0.07	0.07	0.02	0.9082
Error Replication *Nitrogen	3	13.67	4.56		
Inbred	5	8.91	1.78	1.95	0.1156
Nitrogen*Inbred	5	5.29	1.06	1.16	0.3526
Error Replication *Nitrogen*Inbred	30	27.43	0.91		
Total	47	58.94			
Grand Mean	3.88				
CV (Replication*Nitrogen)	54.98				
CV (Replication*Nitrogen*Inbred)	24.63				

Appendix 17 Root top angle.

Source	DF	SS	MS	F	P
Replication	3	1969.60	656.54		
Nitrogen	1	1939.30	1939.27	14.49	0.0319
Error Replication *Nitrogen	3	401.40	133.82		
Inbred	5	1768.90	353.78	0.80	0.5589
Nitrogen*Inbred	5	1436.30	287.26	0.65	0.6643
Error Replication *Nitrogen*Inbred	30	13275.50	442.52		
Total	47	20791.00			
Grand Mean	22.06				
CV (Replication*Nitrogen)	52.44				
CV (Replication*Nitrogen*Inbred)	95.37				

Appendix 18 Root bottom angle.

Source	DF	SS	MS	F	P
Replication	3	224.11	74.70		
Nitrogen	1	752.85	752.85	4.05	0.1375
Error Replication *Nitrogen	3	557.21	185.74		
Inbred	5	911.27	182.25	2.47	0.0547
Nitrogen*Inbred	5	254.13	50.83	0.69	0.6355
Error Replication *Nitrogen*Inbred	30	2212.86	73.76		
Total	47	4912.43			
Grand Mean	27.70				
CV (Replication*Nitrogen)	49.2				
CV (Replication*Nitrogen*Inbred)	31				

Appendix 19 Number of adventitious roots.

Source	DF	SS	MS	F	P
Replication	3	5.06	1.69		
Nitrogen	1	6.38	6.38	0.95	0.4011
Error Replication *Nitrogen	3	20.10	6.70		
Inbred	5	14.30	2.86	1.61	0.1887
Nitrogen*Inbred	5	11.84	2.37	1.33	0.2784
Error Replication *Nitrogen*Inbred	30	53.41	1.78		
Total	47	111.08			
Grand Mean	2.84				
CV (Replication*Nitrogen)	91.02				
CV (Replication*Nitrogen*Inbred)	46.92				

Appendix 20 Number of basal roots.

Source	DF	SS	MS	F	P
Replication	3	9.43	3.14		
Nitrogen	1	75.25	75.25	14.57	0.0316
Error Replication *Nitrogen	3	15.49	5.16		
Inbred	5	10.20	2.04	0.45	0.8101
Nitrogen*Inbred	5	27.49	5.50	1.21	0.3274
Error Replication *Nitrogen*Inbred	30	136.04	4.53		
Total	47	273.89			
Grand Mean	6.52				
CV(Replication*Nitrogen)	34.86				
CV(Replication*Nitrogen*Inbred)	32.67				

Appendix 21 Tap root diameter.

Source	DF	SS	MS	F	P
Replication	3	0.26	0.09		
Nitrogen	1	0.10	0.10	1.20	0.3527
Error Replication *Nitrogen	3	0.24	0.08		
Inbred	5	0.11	0.02	0.85	0.5264
Nitrogen*Inbred	5	0.03	0.01	0.21	0.9538
Error Replication *Nitrogen*Inbred	30	0.79	0.03		
Total	47	1.52			
Grand Mean	1.20				
CV(Replication*Nitrogen)	23.51				
CV(Replication*Nitrogen*Inbred)	13.48				

Appendix 22 Average lateral root length.

Source	DF	SS	MS	F	P
Replication	3	3210.20	1070.05		
Nitrogen	1	4744.40	4744.39	6.02	0.0914
Error Replication *Nitrogen	3	2365.50	788.51		
Inbred	5	2982.90	596.58	0.64	0.6729
Inbred*Nitrogen	5	4331.90	866.38	0.93	0.4782
Error Replication *Nitrogen*Inbred	30	28080.10	936.00		
Total	47	45714.90			
Grand Mean	197.88				
CV (Replication*Nitrogen)	14.19				
CV (Replication*Nitrogen*Inbred)	15.46				

Appendix 23 Lateral branching frequency.

Source	DF	SS	MS	F	P
Replication	3	159.94	53.32		
Nitrogen	1	47.33	47.33	1.50	0.3084
Error Replication *Nitrogen	3	94.82	31.61		
Inbred	5	265.26	53.05	1.78	0.1481
Nitrogen*Inbred	5	26.26	5.25	0.18	0.9696
Error Replication *Nitrogen*Inbred	30	896.27	29.88		
Total	47	1489.88			
Grand Mean	12.46				
CV (Replication*Nitrogen)	45.13				
CV (Replication*Nitrogen*Inbred)	43.88				

Appendix 24 Distance to the first lateral root.

Source	DF	SS	MS	F	P
Replication	3	3.20	1.07		
Nitrogen	1	0.92	0.92	0.60	0.4954
Error Replication *Nitrogen	3	4.62	1.54		
Inbred	5	5.31	1.06	0.75	0.5901
Nitrogen*Inbred	5	12.63	2.53	1.79	0.1449
Error Replication *Nitrogen*Inbred	30	42.31	1.41		
Total	47	69.00			
Grand Mean	1.4189				
CV (Replication*Nitrogen)	87.46				
CV (Replication*Nitrogen*Inbred)	83.7				

Appendix 25 Dry biomass (above ground).

Source	DF	SS	MS	F	P
Replication	3	7213.00	2404.00		
Nitrogen	1	228044.00	228044.00	12.80	0.0373
Error Replication *Nitrogen	3	53449.00	17816.00		
Inbred	5	67663.00	13533.00	3.44	0.0142
Nitrogen*Inbred	5	36623.00	7325.00	1.86	0.1309
Error Replication *Nitrogen*Inbred	30	118031.00	3934.00		
Total	47	511023.00			
Grand Mean	191.93				
CV (Replication*Nitrogen)	69.55				
CV (Replication*Nitrogen*Inbred)	32.68				

Analysis of variance (ANOVA) tables for phosphorus experiment

Appendix 26 Chlorophyll content.

Source	DF	SS	MS	F	P
Replication	3	229.94	76.65		
Phosphorus	1	196.47	196.47	7.87	0.0675
Error Replication *Phosphorus	3	74.85	24.95		
Inbred	5	808.81	161.76	5.88	0.0007
Phosphorus*Inbred	5	253.46	50.69	1.84	0.1348
Error Replication *Phosphorus*Inbred	30	825.80	27.53		
Total	47	2389.33			
Grand Mean	33.14				
CV (Replication*Phosphorus)	15.07				
CV (Replication* Phosphorus*Inbred)	15.83				

Appendix 27 Plant height.

Source	DF	SS	MS	F	P
Replication	3	1429.80	476.60		
Phosphorus	1	1858.80	1858.79	4.61	0.1212
Error Replication *Phosphorus	3	1210.80	403.59		
Inbred	5	8195.10	1639.02	6.69	0.0003
Phosphorus*Inbred	5	930.60	186.13	0.76	0.5861
Error Replication *Inbred*Phosphorus	30	7352.80	245.09		
Total	47	20977.90			
Grand Mean	110.01				
CV (Replication*Phosphorus)	18.26				
CV (Replication*Phosphorus*Inbred)	14.23				

Appendix 28 Number of leaves.

Source	DF	SS	MS	F	P
Replication	3	1.73	0.58		
Phosphorus	1	2.52	2.52	4.84	0.1152
Error Replication *Phosphorus	3	1.56	0.52		
Inbred	5	11.10	2.22	1.91	0.1229
Phosphorus*Inbred	5	5.10	1.02	0.88	0.5089
Error Replication *phosphorus*Inbred	30	34.96	1.17		
Total	47	56.98			
Grand Mean	10.65				
CV (Replication*Phosphorus)	6.78				
CV (Replication*Phosphorus*Inbred)	10.14				

Appendix.29 Leaf length.

Source	DF	SS	MS	F	P
Replication	3	1035.73	345.25		
Phosphorus	1	126.50	126.50	1.44	0.3159
Error Replication *Phosphorus	3	263.06	87.69		
Inbred	5	176.50	35.30	1.87	0.1295
Phosphorus*Inbred	5	200.44	40.09	2.12	0.09
Error Replication *Phosphorus*Inbred	30	566.54	18.89		
Total	47	2368.77			
Grand Mean	47.205				
CV (Replication*Phosphorus)	19.84				
CV (Replication*Phosphorus*Inbred)	9.21				

Appendix 30 Leaf width.

Source	DF	SS	MS	F	P
Replication	3	7.48	2.49		
Phosphorus	1	0.98	0.98	0.81	0.4348
Error Replication *Phosphorus	3	3.64	1.21		
Inbred	5	6.07	1.21	2.78	0.0352
Phosphorus*Inbred	5	3.53	0.71	1.62	0.1859
Error Replication *Phosphorus*Inbred	30	13.09	0.44		
Total	47	34.79			
Grand Mean	6.53				
CV (Replication*Phosphorus)	16.86				
CV (Replication*Phosphorus*Inbred)	10.11				

Appendix 31 Leaf area per plant.

Source	DF	SS	MS	F	P
Replication	3	8091699.00	2697233.00		
Phosphorus	1	2160653.00	2160653.00	1.77	0.2751
Error Replication *Phosphorus	3	3655435.00	1218478.00		
Inbred	5	965223.00	193045.00	0.98	0.448
Phosphorus*Inbred	5	2319435.00	463887.00	2.35	0.0652
Error Replication *Phosphorus*Inbred	30	5929602.00	197653.00		
Total	47	23120000.00			
Grand Mean	2488.8				
CV (Replication*Phosphorus)	44.35				
CV (Replication*Phosphorus*Inbred)	17.86				

Appendix 32 Stem diameter.

Source	DF	SS	MS	F	P
Replication	3	32.34	10.78		
Phosphorus	1	34.90	34.90	2.88	0.188
Error Replication *Phosphorus	3	36.31	12.10		
Inbred	5	21.32	4.26	0.96	0.4558
Phosphorus*Inbred	5	9.11	1.82	0.41	0.8369
Error Replication *Phosphorus*Inbred	30	132.84	4.43		
Total	47	266.82			
Grand Mean	16.17				
CV (Replication*Phosphorus)	21.51				
CV (Replication*Phosphorus*Inbred)	13.01				

Appendix 33 Dry biomass (above ground).

Source	DF	SS	MS	F	P
Replication	3	115706.00	38568.60		
Phosphorus	1	82321.00	82321.40	4.01	0.1391
Error Replication *Phosphorus	3	61659.00	20553.00		
Inbred	5	26589.00	5317.90	0.93	0.4763
Phosphorus*Inbred	5	96895.00	19379.10	3.38	0.0153
Error Replication *Phosphorus*Inbred	30	171788.00	5726.30		
Total	47	554959.00			
Grand Mean	192.54				
CV (Replication*Phosphorus)	74.46				
CV (Replication*Phosphorus*Inbred	39.3				

Appendix 34 Projected root area.

Source	DF	SS	MS	F	P
Replication	3	38800000.00	13000000.00		
Phosphorus	1	37700000.00	37700000.00	17.96	0.0241
Error Replication *Phosphorus	3	6304205.00	2101402.00		
Inbred	5	128000000.00	25700000.00	2.23	0.077
Phosphorus*Inbred	5	27400000.00	5486111.00	0.48	0.7906
Error Replication *Phosphorus*Inbred	30	345000000.00	11500000.00		
Total	47	584000000.00			
Grand Mean		19282.00			
CV (Replication*Phosphorus)		7.52			
CV (Replication*Phosphorus*Inbred)		17.59			

Appendix 35 Average root density.

Source	DF	SS	MS	F	P
Replication	3	22.08	7.36		
Phosphorus	1	10.20	10.20	2.79	0.1935
Error Replication *Phosphorus	3	10.97	3.66		
Inbred	5	10.32	2.06	1.42	0.2464
Phosphorus*Inbred	5	13.18	2.64	1.81	0.1408
Error Replication *Phosphorus*Inbred	30	43.66	1.46		
Total	47	110.41			
Grand Mean		4.01			
CV (Replication*Phosphorus)		47.65			
CV (Replication*Phosphorus*Inbred)		30.06			

Appendix 36 Root top angle.

Source	DF	SS	MS	F	P
Replication	3	3796	1265.34		
Phosphorus	1	2.30	2.32	0.01	0.9123
Error Replication *Phosphorus	3	485.00	161.66		
Inbred	5	3679.90	735.98	4.16	0.0055
Phosphorus*Inbred	5	1964.70	392.94	2.22	0.0784
Error Replication *Phosphorus*Inbred	30	5312.50	177.08		
Total	47	15240.40			
Grand Mean		27.73			
CV (Replication*Phosphorus)		45.85			
CV (Replication*Phosphorus*Inbred)		47.98			

Appendix 37 Root bottom angle.

Source	DF	SS	MS	F	P
Replication	3	128.74	42.91		
Phosphorus	1	68.05	68.05	1.67	0.2866
Error Replication *Phosphorus	3	122.14	40.71		
Inbred	5	1206.39	241.28	3.42	0.0146
Phosphorus*Inbred	5	239.61	47.92	0.68	0.6432
Error Replication *Phosphorus*Inbred	30	2119.20	70.64		
Total	47	3884.13			
Grand Mean		22.75			
CV (Replication*Phosphorus)		28.04			
CV (Replication*Phosphorus*Inbred)		36.93			

Appendix 3.38 Number of adventitious roots.

Source	DF	SS	MS	F	P
Replication	3	14.47	4.82		
Phosphorus	1	2.41	2.41	1.97	0.2549
Error Replication *Phosphorus	3	3.67	1.22		
Inbred	5	5.76	1.15	0.51	0.7638
Phosphorus*Inbred	5	6.56	1.31	0.59	0.711
Error Replication *Phosphorus*Inbred	30	67.25	2.24		
Total	47	100.11			
Grand Mean		2.61			
CV (Replication*Phosphorus)		42.32			
CV (Replication*Phosphorus*Inbred)		57.30			

Appendix 39 Number of basal roots.

Source	DF	SS	MS	F	P
Replication	3	8.73	2.91		
Phosphorus	1	0.04	0.04	0.02	0.9061
Error Replication*Phosphorus	3	6.76	2.25		
Inbred	5	20.83	4.17	1.70	0.1643
Phosphorus*Inbred	5	12.78	2.56	1.04	0.4101
Error Replication *Phosphorus*Inbred	30	73.39	2.45		
Total	47	122.53			
Grand Mean		6.15			
CV (Replication*Phosphorus)		24.43			
CV (Replication*Phosphorus*Inbred)		25.45			

Appendix 40 Tap root diameter.

Source	DF	SS	MS	F	P
Replication	3	0.24	0.08		
Phosphorus	1	0.00	0.00	0.14	0.7296
Error Replication *Phosphorus	3	0.07	0.02		
Inbred	5	0.32	0.06	1.75	0.1544
Phosphorus*Inbred	5	0.12	0.02	0.63	0.6753
Error Replication *Inbred*Phosphorus	30	1.10	0.04		
Total	47	1.85			
Grand Mean	1.35				
CV (Replication*Phosphorus)	11.31				
CV (Replication*Phosphorus*Inbred)	14.12				

Appendix 41 Lateral root length.

Source	DF	SS	MS	F	P
Replication	3	3895.10	1298.37		
Phosphorus	1	1138.50	1138.54	0.85	0.4244
Error Replication *Phosphorus	3	4015.40	1338.48		
Inbred	5	12986.20	2597.25	4.91	0.0021
Inbred*Phosphorus	5	7145.00	1428.99	2.70	0.0394
Error Replication *Inbred*Phosphorus	30	15867.90	528.93		
Total	47	45048.20			
Grand Mean	209.47				
CV (Replication*Phosphorus)	17.47				
CV (Replication*Phosphorus*Inbred)	10.98				

Appendix 42 Lateral branching frequency.

Source	DF	SS	MS	F	P
Replication	3	425.16	141.71		
Phosphorus	1	1.74	1.745	0.02	0.8933
Error Replication *Phosphorus	3	246.02	82.00		
Inbred	5	580.14	116.02	2.22	0.0788
Phosphorus*Inbred	5	67.89	13.57	0.26	0.9317
Error Replication *Phosphorus*Inbred	30	1571.43	52.38		
Total	47	2892.38			
Grand Mean		15.68			
CV (Replication*Phosphorus)		57.73			
CV (Replication*Phosphorus*Inbred)		46.14			

Appendix 43 Distance to the first lateral root.

Source	DF	SS	MS	F	P
Replication	3	59.63	19.88		
Phosphorus	1	0.18	0.18	0.02	0.9023
Error Replication *Phosphorus	3	30.19	10.06		
Inbred	5	21.79	4.36	1.07	0.3942
Phosphorus*Inbred	5	27.25	5.45	1.34	0.2732
Error Replication *Inbred*Phosphorus	30	121.68	4.06		
Total	47	260.73			
Grand Mean	1.97				
CV (Replication*Phosphorus)	160.48				
CV (Replication*Phosphorus*Inbred)	101.88				

Appendix 44 First whorl angle.

Source	DF	SS	MS	F	P
Replication	3	105.85	35.28		
Phosphorus	1	73.34	73.34	2.65	0.2021
Error Replication *Phosphorus	3	83.07	27.69		
inbred	5	389.7	77.94	2.61	0.0448
Phosphorus*Inbred	5	244.38	48.88	1.64	0.1805
Error Replication *Inbred*Phosphorus	30	895.54	29.85		
Total	47	1791.88			
Grand Mean		62.931			
CV (Replication*Phosphorus)		8.36			
CV (Replication*Phosphorus*Inbred)		8.68			

Appendix 45 Second whorl angle.

Source	DF	SS	MS	F	P
Replication	3	342.98	114.33		
Phosphorus	1	58.38	58.38	1.43	0.3171
Error Replication *Phosphorus	3	122.13	40.71		
inbred	5	247.93	49.59	1.8	0.144
Phosphorus*Inbred	5	122.67	24.54	0.89	0.5011
Error Replication *Inbred*Phosphorus	30	828.51	27.62		
Total	47	1722.60			
Grand Mean		66.73			
CV (Replication*Phosphorus)		9.56			
CV (Replication*Phosphorus*Inbred)		7.88			

Appendix 46 Third whorl angle.

Source	DF	SS	MS	F	P
Replication	3	117.01	39.00		
Phosphorus	1	191.74	191.74	3.41	0.1619
Error Replication *Phosphorus	3	168.58	56.19		
Inbred	5	312.01	62.40	1.56	0.2025
Phosphorus*Inbred	5	96.21	19.24	0.48	0.7883
Error Replication *Phosphorus*Inbred	30	1202.72	40.09		
Total	47	2088.26			
Grand Mean		67.48			
CV (Replication*Phosphorus)		11.11			
CV (Replication*Phosphorus*Inbred)		9.38			

Appendix 47 Fourth whorl angle.

Source	DF	SS	MS	F	P
Replication	3	82.86	27.62		
Phosphorus	1	75.33	75.33	2.71	0.1981
Error Replication *Phosphorus	3	83.28	27.76		
Inbred	5	476.02	95.20	2.95	0.028
Phosphorus*Inbred	5	134.2	26.84	0.83	0.5383
Error Replication *Phosphorus*Inbred	30	969.53	32.32		
Total	47	1821.21			
Grand Mean		65.128			
CV (Replication*Phosphorus)		8.09			
CV (Replication*Phosphorus*Inbred)		8.73			

Appendix 48 Fifth whorl angle.

Source	DF	SS	MS	F	P
Replication	3	749.50	249.82		
Phosphorus	1	897.30	897.27	2.70	0.1992
Error Replication *Phosphorus	3	998.80	332.92		
Inbred	5	2747.70	549.53	2.85	0.032
Phosphorus*Inbred	5	1484.60	296.92	1.54	0.2075
Error Replication *Phosphorus*Inbred	30	5785.70	192.86		
Total	47	12663.50			
Grand Mean		64.72			
CV (Replication*Phosphorus)		28.19			
CV (Replication*Phosphorus*Inbred)		21.46			

Appendix 49 Sixth whorl angle.

Source	DF	SS	MS	F	P
Replication	3	1861.30	620.43		
Phosphorus	1	1716.80	1716.81	1.16	0.361
Error Replication *Phosphorus	3	4454.10	1484.70		
Inbred	5	10814.50	2162.91	2.07	0.097
Phosphorus*Inbred	5	4208.60	841.71	0.81	0.5547
Error Replication*Phosphorus*Inbred	30	31344.40	1044.81		
Total	47	54399.70			
Grand Mean		42.10			
CV (Replication*Phosphorus)		91.51			
CV (Replication*Phosphorus*Inbred)		76.77			

Appendix 50 Number of whorls.

Source	DF	SS	MS	F	P
Replication	3	0.73	0.24		
Phosphorus	1	0.52	0.52	1.47	0.312
Error Replication *Phosphorus	3	1.06	0.35		
Inbred	5	2.85	0.57	1.81	0.1409
Phosphorus*Inbred	5	0.85	0.17	0.54	0.743
Error Replication *Phosphorus*Inbred	30	9.46	0.32		
Total	47	15.48			
Grand Mean		5.60			
CV (Replication*Phosphorus)		10.62			
CV (Replication*Phosphorus*inbred)		10.02			

Appendix 51 Partial ANOVA for either nitrogen or phosphorus experiment.

Source of variation	Degree of freedom
Replication	(r-1)=(4-1)=3
Main plot factor(A)	(a-1)=(2-1)=1
Error(a)	(r-1)(a-1)=(4-1)(2-1)=3
Subplot factor B	(b-1)=(10-1)=9
A×B	(a-1)(b-1)=(2-1)(10-1)=9
Error(b)	(a(r-1)(b-1))=(2(4-1)(10-1))=54
Total	(r×a×b-1)=(4×2×10-1)=79