GENETIC ANALYSIS AND SELECTION FOR PHOSPHORUS EFFICIENCY IN KENYAN MAIZE (*Zea mays* L.).

BY

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN PLANT BREEDING AND BIOTECHNOLOGY OF THE UNIVERSITY OF ELDORET.

DECLARATION BY THE CANDIDATE

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DEDICATION

To God Almighty and Jesus Christ

To my wife Linnet Evans

To my sons Joseph Geoffrey and Samuel Isaac

To my mother Rose Ouma

ABSTRACT

Low available phosphorus (P) remains a major limitation to maize (*Zea mays* L.) productivity in low P soils. Selecting maize hybrids that acquire and use P efficiently could reduce the rising costs of inorganic P. The objectives of this study were to: (i) develop P-efficient experimental maize hybrids for low P soil conditions (ii) determine the genetic effects of maize P efficiency traits under low P acid and non-acid soils (iii) determine environmental influence and stability of maize P efficiency traits in low P soils (iv) identify single nucleotide polymorphic markers (SNPs) linked to the major quantitative trait loci (QTLs) associated with P efficiency loci in a maize linkage map. A total of 30 experimental hybrids were developed using North Carolina mating design II and evaluated together with 2 checks for tolerance to low P at high P (36kgP/ha) and low P (6kgP/ha) conditions across four locations using RCBD replicated three times. For each trait, both additive and non-additive genetic effects were estimated. Environmental variation was determined using the Genotype and Genotype x Environment Interaction (GGE) and Additive Main Effect and Multiplicative Interaction (AMMI) models across 8 environments. Yield stability and superiority were determined using Finlay and Wilkinson model (FW) and Wrickes ecovalence (wi). Two hundred and twenty eight F2 individuals and 239 SNP markers were used in QTL analysis. Mean grain yield (GYLD) was significantly lower (2.49 t/ha) in the low P treatments compared to the high P sites (4.78 t/ha). Relative yield reduction (RYR) was comparable across the four locations and ranged from 42.5 - 47.7% except at Sega where it was higher (59.4%). Mean Agronomic Efficiency (AE) was 44.8 kg grain kg^{-1} P applied across the locations. Eighteen out of the 32 experimental hybrids exhibited AE above the locational mean > 44.8 $kgkg^{-1}$. Mean phosphorus efficiency ratio (PER) of 546.7 $kgkg^{-1}$ of P was obtained across the four locations with Migori exhibiting the highest mean (556.5 $kgkg^{-1}$ of P. For most of the traits, greater variation was accounted for by dominance followed by epistatic and additive genetic effects in both acid and non-acid soils. The magnitude of both additive and non-additive gene effects were always greater in non-acid compared to acid soils pointing to the detrimental effects of soil acidity on gene action. The AMMI Anova showed significant effects for genotype (G), environment (E) and GEI. For GYLD, the differences among the (E) accounted for more than half (67.6%) of the total variation while the G and GEI accounted for 11.6% and 10.3% respectively of the variation indicating the existence of mega environments. 26% of the new hybrids were more stable than the commercial hybrid (H515). Based on FW model, genotypes 1, 27, 21 and 23 were considered as superior and ideal hence can be used as reference genotype in further testing. A full multi-QTL model analysis suggested six QTLs (2 QTLs each for GYLD plant height (PHT) and ear height located on chromosomes 1, 3, 4 and 8. The two QTLs for GYLD increased yield under low P soils by 173 kg/ha while the 2 for PHT increased PHT by 18.14 cm. This study has developed potential maize hybrids that can significantly improve yields in low P soils in western Kenya. The new QTLs identified will be useful for improving maize productivity in low P soils of western Kenya.

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LIST OF ABBREVIATIONS

AE	Agronomic P use efficiency
AFLP	Amplified fragment length polymorphisms
AM	Arbuscular mycorrhizal
AMMI	Additive main effects and multiplicative interaction
ANOVA	Analysis of variance
DNA	Deoxyribonucleic Acid
FW	Finlay and Wilkinson
GEI	Genotype by Environment interactions
GGE	Genotype main effects and genotype × environment interaction effects
GPC	Grain P concentration
MAS	Marker assisted selection
NCBI	Nationl Centre for Biotechnology Information
ISSR	Inter-simple sequence repeats
MABC	Marker assisted backcrossing
METs	Multi-environment yield trials
PE	P efficiency
PAE	P acquisition efficiency
PAGE	Polyacrylamide gel electrophoresis
PUE	P utilization efficiency
PER	P efficiency ratio
PPUE	Physiological P use efficiency
PCA	Principal Component analysis
PC	Polymerase Chain Reaction

QTL	Quantitative trait Loci
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphisms
SNP	Single nucleotide polymorphisms
SSRs	Simple sequence repeats
SCAR	Sequence characterized amplified region
STS	Sequence tagged sites
STVPC	Stover P concentration

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CHAPTER ONE

INTRODUCTION

1.1. Background information

Maize (Zea mays L) is the most produced cereal in the world (Awika, 2011). Maize is widely used in animal feed, human food and for industrial purposes (Smale et al., 2011). As a human food, it is a major staple for manyof people in developing countries mainly Africa and Latin America (Sibiya et al., 2012; Awika, 2011). It is ranked second after rice in importance worldwide (Simpson & Orgazaly, 1995). In Kenya, it is a major staple food crop on which 96% of the population depend (Njenga, 2013). Maize is known to grow in a wide range of agro-ecological zones with regard to water balance, solar radiation and temperature (Downswel et al., 19999). It can be grown at varied latitudes from the equator to slightly above 50^{o} north and 50^{o} south. It is also grown in a wide range of altitudes from sea level to over 3,000 meters above the sea level, under varying conditions ranging from heavy rainfall to semi-arid, from cool to very hot climates (Donswell et al., 1999). This differential adaptation contributes to Genotype by Environment Interaction (GEI) which hinders identification of high yielding and stable maize genotypes (Akcura et al., 2011). Multi-environment yield trials (METs) are therefore essential in estimation of GEI and identification of superior genotypes for the targeted regions and to determine if the target region can be subdivided into mega-environments (Mitrovic et al., 2012). Investigation of mega-environment is a prerequisite for meaningful cultivar evaluation and recommendation (Yan et al., 2007).

Most agro-ecological zones where maize is grown in Kenya are characterized by low P acid soils (Muhammad and Underwood, 2004; Kanyanjua et al., 2002). Soil acidity covers extensive areas in tropical, subtropical and temperate zones and occupy between 30-40% of the world's arable soils (Von Uexkull and Mutert, 1995). They are found mainly in South America (26.7%), North America (19.4%), Africa (19.1%) and Asia (15.1%). The rest occur in Australia and New Zealand, Europe and Central America (Eswaran et al., 1997). Maize is cultivated in more than 140 million hectares worldwide (Pandey et al., 1994; Awika, 2011). Out of these; about 30 million hectares are located in low P acid soil environment (Von Uexkull and Mutert, 1995). On most acid soils, toxic level of aluminium (Al), manganese (Mn) and iron (Fe) as well as deficiencies of phosphorus (P), nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg) and some micro-nutrients limit maize growth (Kochian et al., 2015). Other constraints to maize productivity include moisture stress, pest and diseases and inadequate farm inputs (Ayaga, 2003; Gustavo et al., 2013). However, in acid soils most authors agree that toxic levels of aluminium and phosphorus deficiencies are the two most growth limiting factors to its productivity (Von Uexkull and Mutert, 1995; Kochian et al. 2015).

Phosphorus (P) is essential to plants and animal nutrition and is the second-most limiting nutrient after nitrogen (N) for plant growth and crop production in many agricultural areas in the tropics (Parentoni et al., 2010; Lynch, 2011). It is involved in several key plant functions including energy transfers, photosynthesis, transformation of sugar and starches, nutrient movement within the plants and transfer of genetic characteristics from one generation to the next (White and Hammond, 2008). Plant roots acquire P from the rhizosphere solution as phosphate (Pi), primarily in the form of orthophosphate ions

($H_2PO_4^-$). However, the concentration of Pi in the soil solution is often low and ranging from 2 - 10 µM. Consequently, the supply of Pi to the root surface by diffusion is slow causing P deficiency in plants (Marschner, 1995; White and Hammond, 2008). The low availability of P in these soils is mainly due to formation of poorly soluble P complexes with calcium in alkaline, and aluminium and iron in acidic soils (Oztuk et al., 2005). P deficiency is also due to inherent low soil P and insufficient fertilizer use to replace soil P removed through crop harvests (Obura et al., 2001). Plants can achieve tolerance to P deficiency by maximizing the ability of the roots to acquire and absorb P from the soil (Parentoni and Souza Junior, 2008). Plants can mobilize P through the exudation of organic acids, acid phosphatases, and ribonucleases, resulting in enhanced P availability and uptake (Hammond and White, 2008; Ma et al., 2009; Pang et al., 2009). Another strategy to cope with low-P availability is by forming mycorrhizal symbioses which increase the soil volume accessed by root systems (Li et al., 2012; Rai et al., 2013). Due to low-P mobility in tropical soils, changes in root architecture and morphology also enhance P uptake by facilitating soil exploration (Lynch, 2011; Ingram et al., 2012; Niu et al., 2013). Root structural changes leading to high P uptake include increased root hair growth (Yan et al., 20014; Haling et al., 2013; Lan et al., 2013), increased root length density and enhancement of lateral root over primary root growth (Manske etal., 2002; Wang et al., 2013).

Amelioration of P deficiency can be achieved through the use of plants that are naturally adapted to low levels of available soil phosphorus (Parentoni et al., 2010; Ouma et al., 2013, Ligeyo et al., 2014), application of inorganic sources of phosphorus like Mijingu Rock Phosphate (Obaga et al., 2005; van Kauwenberg, 2006) and the use of organic

materials as P source. Other ways include the use of soluble mineral P fertilizers which are obviously the best means to combat P deficiency even though their use is limited by their high cost that prohibits farmers access (Obura, et al., 2001).

Use of tolerant genotypes has been proposed as the most suitable, sustainable and cost effective way. This is because of the enormous genetic variations for tolerance to low P acid soils that has been reported in several studies using different germplasm, traits and methods of genetic analysis. Most stress tolerant maize varieties in the market across the world have been developed by conventional breeding. This has slowed the progress of developing maize varieties tolerant to complex quantitatively inherited traits such as tolerance to low P which are confounded by environmental influence. Besides, key parameters used to indirectly select for this trait such as grain yield and root growth characteristics have very low heritability under stressful environments and hence making their selection not feasible using phenotypic characters alone difficult (Manske et al., 2002; Parentoni et al., 2010: Yu et al., 2011). Therefore there is need to use genetic markers and molecular tools alongside the conventional strategies in order to speed up the breeding processes and to overcome the confounding effects of environment on selection.

Several genetic markers including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) have been used to identify useful genomic regions associated with yield improvement in sorghum (Leiser et al., 2014; Hufnagel et al., 2014) and maize (Maron et al., 2014; Guimarhaes et al., 2014). Majority of these

genetic markers (RFLP, AFLP, and RAPDS) are becoming obsolete because of poor reproducibility, dominant assay and complex banding patterns (Fulton et al., 2012). SSRs are still widely used in molecular biology research although they have several limitations including lack of full automation in their typing especially the PCR reaction preparations, difficulty in typing more than ten loci in a single reaction, low abundance in the genome, and time-consuming assays especially when large numbers of loci are required (Jarne and Lagoda, 1996).

Most researchers now prefer to use SNP markers (Hamblin et al., 2007; Jones et al., 2007) because of their abundance in the genomes, amenable to automation and are cheaper than other markers, (Rafalski, 2002; Morin et al. 2004). Therefore SNPs have been widely used genetic diversity studies, linkage and quantitative trait loci (QTL) mapping as well as and marker-assisted breeding (Zhu et al., 2003). Therefore in an attempt, to analyse genetic loci associated with tolerance to low P in Kenyan maize germplasm, SNP markers were used for QTL analysis.

1.2 Problem statement

Maize is the most important cereal crop in Kenya and 96% of the population depend on it as a staple food (Njenga, 2013). The country's average annual production is about 3.2 million metric tonnes while the consumption is 3.6 million metric tonnes giving a deficit of 0.4 million metric tonnes that is met through importation mainly from Zambia, Tanzania and Uganda (Snip & Kamau, 2013; USDA, 2013; MOA, 2014). Majority of the potential maize growing areas in Kenya have acidic soils and therefore suffer from low available P. Soil acidity covers over 1.6 million ha of arable land in Kenya (Kanyanjua et al., 2002). In the low P acid and non acid soils, maize yields are low and range between

0.5 - 2.0 t/ha compared to the research potential which of 8 - 12 t/ha. The low maize yields are caused mainly by high levels of aluminium saturation (> 20%) and extremely low available P (< 5 mg P/kg soil). The optimum P level considered ideal for crop growth ranges between 10.0 - 15.0 mg P/kg soils (Okalebo et al., 2002). In addition, most acid soils in Kenya contain clay minerals with high P fixation capacity (Van Straten, 2007; Obura, 2008). Thus only a small fraction of applied P becomes available to plants. Studies by Kisinyo et al. (2013) have shown that these soils have high P sorption (107-258 mg P kg). High P sorption in acid soils make crops to utilize only about 10-25% of the P fertilizer applied (Bahl & Singh, 1986). The low available P is also due to frequent nutrient removal through crop harvest and low replenishment by farmers in these regions (Okalebo et al., 2006).

Studies by Ligeyo et al. (2006) & Ouma et al. (2013) found that aluminium toxicity and low phosphorus levels associated with acid soils reduce maize yield by 16.8% and 28%, respectively.This is quite substantial considering that the two constraints often coexist and therefore their combined effect is normally higher than these individual estimates. In addition, there are no adequate P efficient maize varieties in Kenya. Although crop production in acid soils can be sustained by the application of lime, inorganic P sources and other agronomic practices (De la Fuente et al., 1997, Guimaraes et al., 2014), such technologies are not readily available for a large number of small holder farmers who are the majority. Besides, most farmers in Kenya cannot afford sufficient quantities of lime amendments and or P-based fertilizers owing to associated cost. Hence the need for low P and Al tolerant varieties in these regions.

1.3. Justification

Low available P is one of the major constraints to maize production in Kenya. Use of inorganic Pi fertilizers to maintain yields, quality and crop production is unsustainable and has raised both environmental and financial concerns. For instance, transport of soil particles loaded with P into lakes and surface waters has resulted into eutrophication (Malakouti et al., 2008). Besides, most crops such as maize do not recover all of the Pi fertilizer applied (Kisinyo et al., 2013) owing to P fixation by aluminium (Al) and iron (Fe) oxides.The impact of high fertilizer prices are greatly felt by the small holder farmers in sub Saharan Africa (SSA) because of poorly developed infrastructure and inefficient supply chains which further escalates the high prices (Van der Velde et al, 2013; Leiser et al., 2014b). Additionally, the world is currently glaring the possibility of depletion of rock P reserves estimated to last the next 40-400 years (Van Kauwenbergh, 2010, Cooper et al., 2011; Cordell and white, 2013; Obersteiner et al., 2013). Geopolitical conflicts are also likely since P reserves are heavily concentrated in a few parts of the world with Morocco holding about 75% of the global share, followed by China 6%, Algeria 3% and the rest in the USA, Near East and other African Countries (Jasinski, 2013).

Such concerns should necessitate the search for more sustainable and ecologically sound long term P management strategies. Breeding and selection approach can significantly improve the use of Pi fertilizers in agricultural systems because it leads to development of P efficient cropscapable of producing higher grain yields/biomass with comparably lower inputs of inorganic Pi fertilizers than the inefficient ones, having reduced physiological P

requirements, low tissue P concentration and/or capacity to solubilize fixed P (Ahmad, 2003; Ouma et al., 2012). Besides, the development of P-efficient maize varieties is a feasible and much more sustainable option and has been attained in other countries (Parentoni et al., 2010). However breeding and selection for complex trait such as P efficiency, grain yield or root growth characteristics based on morphological traits alone is not precise and can take ang time owing to the confounding effects of genotype by environment interactions (GXE). These traits are quantitatively inherited and have very low heritabiliy under stressful environments and are difficult to select for using phenotypic characters alone. (Manske et al., 2002; Parentoni et al., 2010: Yu et al., 2011; Ouma, et al., 2012).

Consequently, breeding for tolerance to P deficiency require the use of genetic markers and molecular tools alongside the conventional strategies. Differential capacity of plant genotypes to acquire and utilize P exists and has been exploited to develop P efficient crop varieties that produce better yields in acid soils than sensitive ones (Hammond et al., 2009, Oztuk et al., 2005, Leiser et al., 2014). Therefore such efforts can be extended in Kenya in order to improved maize productivity on low P soils of western Kenya.

1.4. Objectives

The broad objective of this study is to improve maize yield in P-deficient soils by developing adapted cultivars suitable for growing under low P acid soils.

1.5. Specific objectives

1. To develop and identify P-efficient experimental maize hybrids for low P field conditions.

2. To compare the genetic effects of maize P efficiency traits under low P acid and non-acid soils.
3. To determine environmental influence and stability of maize P efficiency traits in low P soils.
4. To identify major QTL(s) associated with P efficiency in maize using single nucleotide polymorphic markers.

1.6. Research Hypotheses

1. H_A. Experimental maize hybrids developed from inbred lines identifed for P efficiency could produce high yields and improve maize productivity in low P soils.
2. H_A. Inheritance of P efficiency traits differ in low P acid and non-acid soils and the knowledge of this could guide selection process and speed up development of P efficient maize for low P soils.
3. H_A. Genotype by Environment interactions have influence on the expression and stability of P efficiency traits and its determination could lead to accurate yield predictions in low P soils and identification of mega environments
4. HA. In the maize genome there are QTLs associated with P efficiency traits that can be identified using the abundant SNP markers and in this way precision selection for P efficiency can be made faster than using phenotypic markers that are subject to environmental influence.

CHAPTER TWO

LITERATURE REVIEW

2.1. Ecological requirements of Maize Production.

Maize requires considerable warmth from germination to flowering (http://vasatwiki.icrisat.org). The crop performs optimally with well distributed rainfall of between 600-900 mm per annum. The rainfall should be well distributed throughout the growing period. However, rainfall is most critical at flowering and silk stage. Towards harvesting dry conditions are required to facilitate the drying (www.nafis.go.ke). Maize does well in deep rich soils of the sub-tropics with abundant nitrogen and phosphorus. Its performance is best in well aerated loam or silty loams or alluvial soils with pH of 5.6-7.0.

2.2. Utilization of Maize

Maize is widely used in livestock feed as fodder, or silage, chicken feed, human food and industrial purposes (Smale et al., 2011). In human diet, maize is an important source of carbohydrates, proteins and fats. A typical maize grain has (4-4.5%) fats, (9.5-11%) protein, (70-72%) carbohydrates and 11% moisture (Ranum et al., 2014). Maize is consumed in various forms such as '*totillas'* in Mexico and Central America. In the USA, maize is processed and made available as maize flour, sweeteners and breakfast cereal (Zhou et al., 2009; Awika, 2011). In Eastern and southern Africa, the kernels are ground and mainly cooked with water and eaten in a paste or cake form (Ugali) with other accompaniments or prepared as porridge. In all parts of Africa, green (fresh) maize is boiled or roasted on its cob and served as a snack. (Donswell et al., 1996).

Industrial uses of maize include the production of ethanol, some parts of the produce is used in the production of corn sweeteners such as corn syrups, adhesives, paste, Jams and Jellies (www.vitaminsdietary.com). Micro droplets of corn starch are used as a tyre ingredient to reduce tyre weight and rolling resistance. Additionally maize-based polylactic acid (PLA) is used to make compostable plastics, packaging films and fast food serving utensils (www.agroproduct.unl.edu/plastic.html). Corn-based PLA can be blended with cotton wool and silk to make exercise clothing, suits, even a 100% corn wedding dress (http://corngilldown.com).

2.3. Global Maize Production and yield statistics.

The top maize producing countries include the USA, China, Brazil, Mexico, Argentina, India and France (Ranum et al, 2014). Over the last ten years, it's estimated that the USA, China, Brazil produce approximately 563 of the 717 million metric tons/year (Ranum et al., 2014). In 2014 worldwide maize production was about 872 million metric tones in an area of 177 million ha with an average yield of 4.9 mt/ha (www.gain.fas.usda.gov). The USA had the highest maize production in the world in 2014 and attained the highest yield per ha (Table 1). In Kenya the current national mean yield is 1.5 t/ha in about 1.8 million ha (www.gain.fas.usda.gov).

Table 1: Top maize producing countries in 2014

Country	Maize area ha	Maize production mt/ha	Average yield mt/ha
world	177379507	872066770	4.9
USA	35,359,790	273,832,130	7.8
Brazil	14,198,496	71,072,810	5.1
China	34,966,000	208,234,649	6.1
India	8,400,000	21,060,000	3.04
Mexico	6,923,900	22,069,294	3.18
Argentina	3,696,300	21,196,637	5.83
Kenya	1,800,000	2,700,000	1.5

Source: Food and Agricultural Organization of United Nations: www.faostat.fao.org and www.gain.fas.usda.gov

The low yields in Kenya is partly attributed to soil acidity coupled with frequent droughts and other production constraints notably the outbreak of Maize Lethal Necrosis (MLN) disease in many regions of maize production.

2.4. Crop production in acid soil

The production of staple food crops, and in particular grain crops, is negatively impacted by acid soils (Kochian et al., 2015). Most of the mineral reactions that occur in soil solution are influenced by many factors including pH. Variation in pH can cause the change of chemical form of a soil mineral, for example, at low pH, toxic forms of aluminium, manganese and iron are increasingly available while at high pH those of sodium and boron become prevalent and hence hinder root growth (Rural Solutions SA, 2011). While Al toxicity exhibits the most adverse effects on plant growth, hydrogen and manganese toxicities also affect plant growth negatively in acid soils. H^+ ions damage root cell membranes and other cytoskeleton structures, manganese (Mn^{2+}) ions also become toxic and affect plants negatively by causing leaf cupping or crinkling (Sparks, 2003).

2.5. Role of phosphorus in crop production

Phosphorus is involved in several key plant functions including energy transfers, photosynthesis and food formation, transformation of sugar and starches, nutrient movement within the plants and transfer of genetic characteristics from one generation to the next (White and Hammond, 2008, Lynch, 2011).

2.5.1. Effects of low P on crop production

Phosphorus starvation leads to stunted growth, thin and spindly stems with purpling of leaves (White and Hammond, 2008). Severe effects include yellowing of leaves with early leaf-senescence, delayed maturity, sparse flowering and low seed production in maize and may lead to plant death (White and Hammond, 2008; Parentoni et al., 2010; Ouma et al., 2012). Corrales et al. (2007) showed that P starvation leads to enhanced root length and root dry weight compared to when P is available. The authors showed that there was increased root elongation, dry weight, and lower shoot/root ratio in P-inefficient variety compared to P-efficient variety. This finding however contrasts with other findings where a higher root length was observed in P-efficient than in P-inefficient maize and wheat cultivars grown in P-deficient nutrient solutions (Alves et al., 2001).

2.5.2. Low P tolerance mechanisms in plants

To enhance P acquisition, plants and their root-associated microbes have evolved a series of strategies that involves modifying root growth and functioning. Common strategies include: increased root/shoot ratio (Hermans et al., 2006), modified root architecture (Lynch, 2007), decreased root diameter (Ragothama, 1999), enhanced specific root length (root length per unit root mass) (Richardson et al., 2009), higher root hair length and/or density (Ma et al., 2001), and production of aerenchyma (Lynch, 2007). According to

Brown et al., 2013, these morphological adaptations can greatly enhance the volume of soil exploited by roots, and/or benefit exploitation of P-rich patches. Bolan, 1991 also showed that associations with arbuscular mycorrhizal (AM) fungi greatly extend the soil exploration space beyond the roots for many higher plant species. Besides increasing soil volume exploited, roots and associated microbes can increase P availability from touched inorganic and organic sources by enhancing synthesis and exudation of organic acids and phosphatases (Ragothama, 1999) .Studies by Ramaekers et al., 2010 have shown that expression of high-affinity phosphate (Pi) transporters is another typical response of root functioning that facilitate increased P-uptake capacity. Plants also play an active role in acquiring hardly soluble P by excreting organic compounds capable of releasing soil-bound P. Kirk et al. (1999) estimated that P solubilisation due to organic anion excretion was responsible for the bulk of P uptake by rice from a P-deficient soil.

2.6. Inheritance of low phosphorus tolerance in plants

Significant genetic variation has been reported in maize germplasm for P-use efficiency (PUE) (Parentoni et al., 2010). Majority of authors have reported that tolerance to low P soils is under the control of both additive and non-additive gene actions with the predominance of non-additive over additive effects (Richard et al., 2015; Parentoni et al., 2010; Duncan et al., 1994; Chaubey et al., 1994 and Furlani et al., 1998). Duncan et al. (1994) reported additive, dominance, and epistatic effects in maize for tolerance to low P with additive effects being more important. Chaubey et al. (1994) and Furlani et al. (1998) reported the importance of both additive and dominance effects in controlling maize P efficiency traits while Parentoni et al. (2006) and Chen et al. (2009) reported non-additive effects being more important than additive effects for tolerance to low P

soils. Galvão and Silva (1978) reported that dominance variance was more important than additive variance for shoot and root dry weight under low P conditions. According to Da Silva and Gabelman (1992), tolerance to low P stress conditions in maize is controlled largely by additive gene effects and to a lesser extent dominance effects. Reiter (1991) demonstrated using RFLP analysis that additive gene action was predominant for all quantitative-trait loci identified. Coltman et al. (1985) showed that tolerance to low P stress in tomato and beans is quantitatively inherited. Biometric analysis of a single-cross between tolerant and susceptible tomato strains indicated that P-acquisition efficiency had higher broad sense heritability (Coltman et al., 1987). Further, generation mean analysis of six families derived from crosses between P-efficient and P-inefficient beans lines showed that P utilization efficiency was controlled by few genes with significant dominance and epistatic effects (Fawole et al., 1982). The successful introgression of alleles conferring P-efficiency into a beans variety has been demonstrated by Schettini et al. (1987). This implies the possibility of improving existing varieties using selected identified P-efficient lines. These studies give hope that selection for P efficiency is possible.

Genetic analysis of tolerance to P deficiency in rice has identified a major quantitative trait locus (QTL) for P-uptake, *pup1*. This QTL was mapped to the long arm of chromosome 12 (Ni et al., 1998; Wissuwa et al., 1998, 2002; Heuer et al., 2009). *Pup 1* breeding lines have proven effective in field trials (Wissuwa et al., 2002; Chin et al., 2011) and increased grain yield 3-folds under low P soils. According to these authors, 3 QTLs controlled dry weight and 4 QTLs controlled P uptake and explained 45.4% (dry weight) and 54.5 % (P-uptake) of the observed variation. For both traits QTL linked to

markers C443 on chromosome 12 had a major effect. Two of the 3 QTLs detected for internal P-use efficiency, including the one on chromosome 12 coincided with QTLs for P uptake. In addition to the major QTL on chromosome 12, two QTLs on chromosome 4 and 12 were identified for tiller number. These variations imply that other QTLs controlling other important traits such as grain yield would be identified and this will aid the breeding of P-efficient crops.

2.7. Field screening for tolerance to Low P and determination of P efficiency

Field screening is the most direct screening for tolerance to low P in cereals. Grain yields and total biomass are measured under field conditions (Wang et al., 2006). Such experiments are conducted in -P (very low or no added phosphorus) and +P (adequate external P supplied) and the differences in agronomic traits and P efficiency traits are compared. This allows direct determination of tolerance to low P (Ouma et al., 2012; Kisinyo et al., 2014; Richard et al., 2015). According to Leiser et al. (2014) the large difference in grain yield, the delay in flowering in the –P compared to the +P conditions indicate important growth differences that permits genetic studies for adaptation to P limited conditions. However, disadvantages of field testing include the longer growing time, problems of soil variability, vulnerability of the materials to environmental hazards such as drought, floods and lodging which may make tolerance to soil factors difficult to identify with certainty and precision (Tamas et al., 2006).

2.8. Phosphorus efficiency Concepts

Efficiency concepts in plant mineral nutrition have been defined based on the processes by which plants acquire, transport, store and use the nutrient to better produce dry matter or grain at low or high nutrient supply (Horst et al., 1993). Several measures of P

efficiency (PE) have been proposed (Moll, 1982; Blair, 1993; Baligar and Fageria 1997; Fageria, 1999; White and Hammond, 2008; White et al., 2005). However the common measures include: grain yield under low P conditions, agronomic P use efficiency (AE) which is the increase in yield per unit of added P fertilizer ($Kgkg^{-1}$), P acquisition efficiency (PAE) which is the product of the increase in plant P content per unit of added P fertilizer ($KgPkg^{-1}g$ Pf), and P utilization efficiency (PUE) which is the increase in yield per unit increase in plant P content ($Kgkg^{-1}$). PAE measures the ability of the plant to absorb available P from the soil. PUE measures the amount of grain produced per unit of absorbed P by the plant. Any species able to maintain metabolic activities at low tissue P concentration and produce more dry matter per unit of P absorbed is considered efficient in P utilization (Serpher et al., 2009). Other measures include (i) P efficiency ratio (PER), which is yield divided by the amount of P in the plant ($Kgkg^{-1}$) which is equivalent to the reciprocal of tissue P concentration if the entire plant is harvested; (ii) physiological P use efficiency (PPUE) which is yield divided by tissue P concentration at a given P concentration in the rooting medium ($Kgkg^{-1}$) and P efficiency (PE) (relative grain yield) (Hammond et al., 2009; Oztuk et al., 2005).

2.9. Determination of Genetic effects in maize

Several studies have used generation means analysis to estimate genetic effects from crosses between maize inbreds (Magnavaca et al., 1987b; Ceballos et al., 1998; Pandey et al., 2007; Vasquelez et al., 2008; Parentoni et al., 2010). Other studies using diallel crossing or North Carolina matting designs have estimated genetic effects in single crosses using General combining ability (additive genetic effects) and specific combining ability (non-additive effects) (Magnavaca et al.,1987b; Pandey et al.,1994; Salazar et

al.,1997; Richard et al., 2015). Most of these studied reported on the importance of both additive and non-additive effects in controlling maize traits. Overall, the inheritance of several important traits in maize evaluated under non-acid soils has been well documented (Furlani et al., 1998). However, information on the inheritance patterns of maize P efficiency traits in acid soils especially those in Africa are still inadequate yet this information is extremely useful in developing clear selection criteria for P efficiency in these soils.

2.10. Genotype by Environment interactions

A genotype x environment interaction (GEI) may be defined as a differential performance of genotypes across environments (Romogosa and Fox 1993). Interactions may therefore involve changes in rank/order for genotypes between environments and changes in the absolute and relative magnitude of the genetic, environmental and phenotypic variances between environments (Bowman, 1972). In practice, breeders want a broadly adapted genotype that performs better across a great area (small GEI). However, the existence of GEI complicates attainment of wide adaptation (Gauch and Zobel., 1996).

Different statistical methods are available for estimating the nature of GEI, which include parametric and non-parametric tests. For instance, type B correlation (Yamada, 1962) and joint regression (Finlay and Wilkinson, 1963; Eberhart and Russel, 1966) which are additive models. Analysis of variance as an additive model only explains the main effects and informs whether or not the GEI is a significant source of variation. However it does not provide the insight into the individual genotypes and localities which are the components of the interaction (Samonte et al., 2005). Two frequently used statistical

analyses are the additive main effects and multiplicative interaction (AMMI) and the genotype main effects and genotype × environment interaction effects (GGE) models (Gauch, 2006), because they have broader relevance for agricultural research. AMMI analysis combines ANOVA and principal component analysis (PCA) where the sources of variability in the GEI are partitioned by PCA. The interpretation of results obtained from AMMI analysis is performed with a biplot which enables visual (graphical) presentation of interaction estimate. The GGE biplot provides breeders with a more complete and visual evaluation of all aspects of the data by creating a biplot that simultaneously represents mean performance and stability, as well as identifying mega-environments and ideal genotype (Ding et al., 2007; Kang, 1993; Yan, 2001; Yan et al., 2007). The GGE biplot can be useful to display the which-won-where pattern of the data that may lead to identifying high-yielding stable cultivars and discriminating representative test environments (Yan et al., 2001). This study adopted both methods to adequately exploit GEI.

2.11. Stability and Superiority Analysis

Stability relates to the concept of consistency of performance of genotypes in a range of conditions. Performance is considered in terms of "Expected" performance and deviation from expectations. The smaller the deviation is from expectation (prediction), the more stable a genotype is. However according to Malosetti and Van Eewijk (2014) any definition of stability implies an underlying model that describes what is "Expected" hence different models, lead to different definitions of stability. There are 3 types of stability commonly used in plant breeding: type 1, 2 and 3. In type 1 (static/biological stability), the stability parameter used is variance around the mean (Becker and Leon,

1988). A stable genotype is thus able to give comparable performance across environments and hence has small variance across the environments. In type 2 stability (dynamic/agronomic stability), genotypic performance changes in a predictable way to environmental changes with respect to a model. Two models that are used for dynamic stability include Wrickes ecovalence (wi) and Finlay and Wilkinson model (sensitivity parameter - bi) (Finlay and Wilkinson, 1963 and Wrickes, 1964). In type 3 (Eberhart - Russell) stability is defined based on the deviation of response from predicted performance given the environmental conditions (deviations from FW model). In this study static stability was not considered because of the presence of high GXE interaction for most of the traits studied, instead dynamic stability was considered. Stability parameter alone does not imply superior performance since it may be related to lower yield across all environments. Hence genotypic superiority combines performance and stability (Malosetti and Van Eewijk, 2014).

2.12. Use of molecular markers as tools for selection in crops

A marker is an identifier or a "tag" of a particular aspect of phenotype and/or genotype; whose inheritance can be traced from one generation to another (De Vicente & Fulton, 2004). Markers can be morphological which are measurable on the basis of observable phenotypic traits, biochemical which include allelic variants of enzymes called isozymes or molecular which arise from variations in DNA sequences of organisms (Collard & Mackill, 2008).

Selection using molecular markers is based the genetic diversity which is the variation, or differences between, organisms at the DNA sequence level. The variations may be caused

by natural or artificial selection, mutations, recombination and other mechanisms (Collard *et al*., 2005). The existence of variations within a population means that some individuals within that population can adapt to certain environmental conditions because of possessing favourable genes which can be selected for by the molecular markers and exploited in crop improvement (De Vicente & Fulton, 2004). Selecting for particular traits using DNA markers also involves the construction of a linkage maps which are used to identify chromosomal regions that contain genes controlling simple traits (controlled by single gene) and quantitative traits using QTL analysis (Magalhaes et al., 2007; Maron et al., 2014, Guimaraes et al., 2014; Matonyei, 2015). Such genomic regions can then be introgressed into useful crops using Marker assisted backcrossing (MABC) or used as molecular tools for Marker assisted selection (MAS) (Ribaut and Hoisington, 2002).

Use of molecular markers has several advantages over other marker types including: their abundance in the genome, they are not affected by the environmental factors and/ developmental stage of the plant (Collard et al., 2005). Several molecular markers are available to scientists for use in selective breeding. Some of the commonly used molecular markers include restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), microsatellites and single nucleotide polymorphisms (SNP). DNA markers can broadly be classifies as non-PCR based and PCR based DNA markers.

2.12.1. Restriction Fragment Length Polymorphism

Among the non-PCR based markers, RFLP are the most widely used non-PCR based molecular markers (Semagn *et al*., 2006). RFLP originates in the DNA rearrangements that arise from evolutionary processes, point mutations, insertions or deletions within the fragments and unequal crossing over (Schlotterer, 2004). They are moderately polymorphic, exhibit high reproducibility, co-dominant, abundant and evenly distributed in the genome. RFLPs are very reliable markers in linkage analysis and breeding and can easily be used to determine if a linked trait is present in a homozygous or heterozygous state; information that is highly desirable for recessive traits (Winter & Kahl, 1995). These markers are however; rarely used because of the large quantities (1–10 μg) of pure and high molecular weight DNA required. Moreover, analysis of organisms with large genomes requires more quantities of their DNA samples. Besides, use of radioactive isotopes in analysis makes them relatively expensive and hazardous (Winter & Kahl, 1995).

2.12.2. Random amplified polymorphic DNAs (RAPDs)

These are molecular markers that use single short primer sequence of about 10 bp to amplify anonymous sequence(s) distributed throughout the genome (Williams et al., 1990). They are in high abundance in most genomes (Williams et al., 1990). They are inexpensive, easy to perform and analyse. However, they have poor reproducibility and are dominant making them unsuitable for marker assisted breeding. Despite their unreliability, RAPDs have been used as species specific markers in diversity and evolutionary relationship studies (Karp et al., 1996).

2.12.3. Microsatellites or simple sequence repeats (SSRs)

SSRs are sections of DNA made up of tandemly repeating units of short nucleotide units (1 – 10 bp) arranged throughout genomes of eukaryotic organisms (Powell et al., 1996). The number of repeats may vary considerably between individual members of species. Microsatellites are highly reproducible, co-dominant; easily analyzed are polymorphic (Powell et al., 1996). These characteristics make SSRs widely used in MAB and in the assessment of genetic variation in germplasm collections (Mohammadi & Prasanna, 2003). The major disadvantage of SSRs is the high cost of their development if adequate primer sequences are unavailable for a species being studied (Mohammadi & Prasanna, 2003).

2.12.4. Amplified fragment length polymorphism and other markers (AFLP)

AFLP technique is based on selective amplification of a set of restriction fragments from a mixture of DNA fragments following endonuclease digestion. The AFLPs are DNA fragments of lengths between 80 – 500 bps obtained by digestion of DNA by restriction enzymes followed by ligation by oligonucleotide adapters to the digestion products (Vos *et al*., 1995). These fragments are amplified by PCR reaction and scored for polymorphisms. The combination of restriction digestion and selective PCR generate a large number of markers (Vos *et al*., 1995). Polymorphisms are identified based on differences on lengths of fragments and are visualized on PAGE or gel electrophoresis (GE) (Vos *et al*., 1995). These markers however, require high resolution visualization platform and are difficult to analyse since they exhibit complex banding patterns (Young *et al*., 2001, Saal & Wricke, 2002). Other PCR based markers include Sequence tagged

sites, Expressed sequence tags (ESTs), Sequence characterized amplified region (SCAR, Inter-simple sequence repeat (Wricke, 2002).

2.12.5. Single nucleotide polymorphisms (SNPS)

A SNP is a difference in DNA sequence of just one nucleotide. Sometimes differences involving a small number of nucleotides is considered a SNP. They were discovered based on DNA sequence information (Gupta et al., 2008). When SNPs occur in genic regions, they are often neutral but in some cases they may be associated with amino acid change of a gene product (Troggio et al., 2007). Most SNPs are however, distributed in the non-coding regions of genomes (Gupta et al., 2008).

SNPs are useful in genetic applications such as linkage analysis and QTL mapping and MAB (Zhu et al., 2003). The markers have found many uses due to their high abundance and distribution in genomes of various species (Nasu et al., 2002), for example maize has 1 SNP every 60 – 120 bps along DNA sequence (Ching et al., 2002). The amenability of SNP markers to automation has made them become the most preferred markers for genomic studies. Many genotyping platforms are now available for SNP analysis.

Currently, chip-based technology is the most high-throughput SNP genotyping platform. The Illumina chip-based SNP detection technology is useful for a broad range of applications to genotype samples with different possible levels of multiplexing, from 48 to 384 (Bead Xpress) and 1536 (Golden Gate) to 55,000 SNPs (Infinium). Such chip-based genotyping platforms are suitable for large-scale studies that require genotyping of individual samples with thousands of SNPs (Low et al., 2006). They may be unsuitable for studies where only a small to moderate number of SNPs are needed over a large

number of samples, as is the case in mapping, marker assisted recurrent selection, marker assisted back- crossing, and quality control applications. In such cases, uniplex SNP genotyping platforms such as the competitive allele specific PCR (KASP) systems are more suitable (Low et al., 2006).

In multiplex genotyping platforms, certain proportion of SNPs often become uninformative (Dvornyk *et al*., 2004), in such cases, SNPs that provide a good level of discrimination are selected for each population under study (Semagn *et al*., 2012).

2.13. Application of DNA markers in QTL detection and genetic linkage mapping

The innumerable polymorphisms revealed by molecular markers can be valuable in QTL detection only if their genomic locations are known (Kumar, 1999). This information is obtained by construction of linkage maps. A linkage map is a representation of the positions of genes and/or markers along a chromosome as determined by linkage analysis (Kumar, 1999). Linkage is determined by the behaviour of homologous chromosomes during meiosis in crossing over events where recombinant gametes are formed. The frequency with which loci located on the same chromosome recombine is a function of the physical distance between them (Collard *et al*., 2005). Loci that are far apart on the chromosome often and not always have high recombination frequencies while very close loci exhibit low frequencies. Recombination frequencies of 0.5 indicate a completely independent assortment of genes and mean that the genes are not linked (Kumar, 1999). Any departure from this frequency infers gene linkage. The concept of recombination frequencies is used in genetic linkage mapping using markers. Many pairwise linkage

tests are made before they are compiled into a linkage group or chromosome (Mohan *et al*., 1997).

The identification of QTL(s) controlling a trait of interest is only possible when there is mapping population with many polymorphic markers between the populations contrasting for traits of interest (Collard *et al*., 2005). The population sizes usually required for preliminary mapping range from 50 – 500 individuals. High resolution mapping, however, require larger populations usually over 500 individuals (Collard *et al*., 2005).

Many different statistical analyses can be performed to detect QTLs of interest. Examples of commonly used analyses are the t-test to determine the effects of treatments on discerning the trait(s) in mapping populations, analysis of variance to test the significance of genetic variation among the mapping populations and linear regression to test the association between phenotype and genotype, hence allowing detection of QTLs (Nguyen *et al*., 2003). Based on these different methods, QTL identification may be achieved using single marker analysis, simple interval mapping or composite interval mapping (Tansley, 1993, Collard *et al*., 2005).

Several QTLSs for various traits have been identified using molecular markers. For example Riede & Anderson (1996) identified Al tolerance QTLs in wheat using RFLP molecular markers while Nguyen *et al*., (2003) used a combination of RFLP, AFLP and SSR to identify Al tolerance QTLs in rice. Guimaraes et al. (2014) identified 5 genomic regions associated with Al tolerance using SNPS; Maron et al. (2014) also detected 3 copies of MATE1 gene to be associated with Al tolerance in maize.

At present phosphorus uptake 1 (*Pup 1*) gene is the only P- related QTL that has been identified in rice variety kasalath (Ni et al., 1998; Wissuwa et al., 1998, 2002; Heuer et al., 2009). Further sequencing and characterization of the *Pup 1* locus showed the presence of *Pup1* specific protein kinase gene named phosphorus starvation tolerance 1(*PSTOL1*) (Gamuyao et al., 2012). These authors further showed that overexpression of the *PSTOL1* significantly increased grain yield by 30% using a physiological mechanism based on the enhancement of early root growth and development under phosphorus deficient soils thereby enabling plants to acquire more phosphorus and other nutrients. Hufnagel et al. (2014) investigated the role of homologs of *PSTOL1* in sorghum (*SbPSTOL1*) under low P soils in Mali and Brazil and reported that *SbPSTOL1* genes colocalized with QTLs for traits underlying root morphology and dry weight accumulation under low P. These authors therefore suggested that *PSTOL 1* gene enhanced P acquisition and performance of sorghum under low P soils. So far no QTLs/genes have been reported to be responsible for tolerance to low P in maize.

CHAPTER THREE

3.0. DEVELOPMENT OF MAIZE HYBRIDS FOR PHOSPHORUS EFFICIENCY IN P DEFICIENT ACID SOILS OF WESTERN KENYA

3.1 Abstract

Low available phosphorus (P) remains a major limitation to maize (*Zea mays* L.) productivity in low P soils. The environmental and financial costs of using inorganic phosphate fertilizers to maintain or improve crop yields and quality are high. Breeding maize hybrids that acquire and use P more efficiently could reduce these costs. The objective of this study was to develop and identify P-efficient experimental maize hybrids under field conditions. A total of 30 experimental hybrids were developed from inbred lines selected for P efficiency using the North Carolina mating design II. The hybrids were evaluated together with 2 checks for tolerance to low P at high P (36kgP/ha) and low P (6kgP/ha) conditions across four locations using RCBD replicated three times. Mean grain yield was significantly lower (2.49 t/ha) across the low P treatment compared to the high P treatments (4.78 t/ha). Relative yield reduction (RYR) was comparable across the four locations and ranged from 42.5 - 47.7% except at Sega where it was a little higher (59.4%). A 48.9% mean yield reduction was observed at the low P treatment compared with the high P treatment across the locations. Agronomic Efficiency (AE) ranged from 22.7 - 72.9 kg grain kg-^{1} P with a mean of 44.8 kg grain kg^{-1} P applied. Eighteen out of the 32 experimental hybrids exhibited AE above the locational mean > 44.8 kg grain per kg P applied. Mean phosphorus efficiency ratio (PER) of 546.7 kgkg^{-1} P was obtained across the four locations with Migori exhibiting the highest mean (556.5 kgkg P^{-1}. Majority of the experimental hybrids (57%) had higher phosphorus acquisition efficiency (PAE) than the average of all the genotypes. Phosphorus use efficiency (PUE) ranged from 208.8 - 977.5 kgkg^{-1} with majority of the hybrids (63%) giving lower values than the average (553.4 kgkg^{-1}) while a phosphorus efficiency (PE) mean of 71.6 % was recorded across the locations. In most cases, hybrids showing higher PE also exhibited higher PER and PAE and genotypes showing higher P efficiency traits (PE, PAE, PUE, AE, PER) had higher grain yield production under low P supply . Consequently, their correlation with the grain yield at low P supply was highly significant. (PE & GYLD r = 0.68***, AE & GYLD r= 0.52**, PAE and GYLD, r=0.36* and PUE & GYLD r= 0.34*). Grain yield was positively correlated with Relative Root Density (RRLD) at both P levels although the correlations were higher at high P (r_g= 0.37*) compared to low P (r_g=0.34*). The correlation between grain and shoot P concentration and grain P content with majority of the P efficiency indices (PAE,PE,PUE) at both high and low P supply was always low or tended to be negative and non-significant showing that seed P reserve, and stover P concentration, had either none or minimal contribution to differential P efficiency. The relationship between PE and shoot P content (SPCNT) was highly significant (r= 0.66**), suggesting that SPCNT is a reliable parameter in selecting for P efficiency in maize during vegetative growth. There was moderate to high broad sense heritability among the studied traits suggesting that the exhibited variations were heritable and hence selectable and could have considerable potential for breeding new P efficient maize varieties. Grain yield at low P had strong positive genetic and phenotypic correlation with most of the traits studied indicating that both correlations are suitable models for selection and yield improvement in maize in low P soils. This study has developed potential maize hybrids that can significantly improve yields in low P soils in western Kenya.

Key words: genetic correlation, grain yield, heritability Maize, phosphorus efficiency

3.2. Introduction

Phosphorus (P) is essential to plants and animal nutrition and is the second most limiting nutrient after nitrogen (N) for plant growth and crop production in many agricultural areas in the tropics (Parentoni et al., 2010; Lynch, 2011). It is involved in several key plant functions including energy transfers, photosynthesis, transformation of sugar and starches, nutrient movement within the plants and transfer of genetic characteristics from one generation to the next (White and Hammond, 2008). Phosphorus exists in various mineral forms in the soil including phosphate rock (PR), which is partially made of apatite (an impure tri-calcium phosphate mineral); it is an important commercial source because of the high concentration of P minerals it contains (van Kauwenberg, 2006). Deposits of PR have been discovered in many parts of the world but the ones that account for most of the world PR production are in Morocco, Algeria, USA, the Near East, China and other African countries. Approximately 90% of the entire PR that is mined is used for food production, fertilizers, feed and food additives and it can either be used as raw materials in the industrial manufacture of water-soluble phosphate (WSP) fertilizers or as P sources for direct application in agriculture (Cordell, 2008b).

Plant roots acquire P from the rhizosphere solution as phosphate (Pi), primarily in the form of orthophosphate ions ($H_2PO_4^-$) (White and Hammond, 2008). The concentration of Pi in the soil solution is often low (2–10 µM) and, consequently, the supply of Pi to the root surface by diffusion is slow. P deficiency is therefore a common mineral nutritional problem in both calcareous and acidic soils (Marschner, 1995). The low available P in these soils is mainly due to the formation of poorly soluble P complexes with calcium in

alkaline and aluminium and iron in acidic soils (Oztuk et al., 2005). P deficiency is also due to inherent low soil P and insufficient fertilizer use to replace soil P removed through crop harvests (Obura et al., 2001). Sanchez et al., (1997) suggest that P depletion due to crop harvest alone occurs at the rate of 2.5 kg P/ha/year from the soil warranting constant replenishments. In general, it is estimated that P availability to plant roots is limited in nearly 67% of the cultivated soils, causing an important constraint to crop production (Batjes, 1997).

The available P in western Kenyan acid soils ranges between 2 to 5 mg P/kg soil against the optimal range (10 to 15 mg P/kg soil) required for high crop productivity (Kisinyo et al., 2013a). Moreover, these soils have high P sorption (107-258 mg P kg) because of the predominant high clay fractions mainly kaolinite, Al and Fe oxides which have large surface area exposed for P sorption (Tisdale et al., 1990, Obura, 2008; Kisinyo et al., 2013a). Crops are frequently supplied with inorganic Pi fertilizers to maintain yields, quality and crop production. However most crops such as maize do not use all of the Pi fertilizer applied (Kisinyo et al., 2013b) owing to P fixation, leaching and other factors. The environmental and financial costs of using inorganic Pi fertilizers are high. For instance, transport of soil particles loaded with P into lakes and surface waters results into eutrophication (Malakouti et al., 2008) of lakes. Besides, the inorganic P fertilizer prices have been on the rise since 2007 due to constant increase in demands for feed, food and fuel production (Cordell et al., 2009, Leiser et al., 2014b). Such fluctuations in fertilizer prices economically affect farmers worldwide, however small holder farmers in sub Saharan Africa (SSA) are worst affected by such changes because of poorly developed infrastructure and inefficient supply chains which further escalates the already high prizes

further (Van der Velde et al, 2013). In some cases fertilizer may even be out of reach of the farmers in remote places or may arrive too late to be useful. Hence fertilizer use has really been wanting in SSA and western Kenya in particular. Additionally, the world may face depletion of world's rock P reserves estimated to last for the next 40-400 years (Van Kauwenbergh, 2010, Cooper et al., 2011; Cordell and white, 2013; Obersteiner et al., 2013). Geopolitical conflicts are also likely since P reserves are heavily concentrated in various parts of the world with Morocco holding about 75% of the global share, followed by China 6%, Algeria 3% and the rest in the USA, Near East and other African Countries (Jasinski, 2013).

These factors should necessitate diversification to more sustainable and ecologically sound crop production strategies aiming at increasing P acquisition and utilization efficiency in agriculture and environmental conservation. Research strategies aimed at developing P efficient cultivars therefore remain very relevant in achieving sustainable and competitive agricultural production systems. Breeding of crops that acquire and/or use P more efficiently is one strategy that can reduce the use of Pi fertilizers in agricultural systems. Differential capacity of plant genotypes to acquire and utilize P has been shown to exist and has encouraged researchers to study P efficiency as influenced by both P absorption by roots and utilization in plants, and to develop P efficient crop genotypes (Ligeyo et al., 2014, Parentoni et al., 2010, Hammond et al., 2009, Oztuk et al., 2005, Leiser et al., 2014). These authors showed that P efficient crops produced comparable yields/biomass with lower inputs of inorganic Pi fertilizers and had reduced physiological P requirements and tissue P concentrations, thus significantly reduced the amount of P removed by the crop and, thereby, the amount of P needed to maintain the

availability of Pi in the soil. Batten, 1992 considered selection of efficient varieties as supplementary to, or even as a replacement for, fertilizer application in agricultural systems.

Besides the enormous contribution of the genetic potential, environmental factors also influence nutrient efficiency in plants (Gahoonia et al., 1994). According to Ceccarelli (1994), response to selection under low input conditions is often considered less efficient due to low heritability as a result of higher experimental error and lower genetic correlations expected. However contrary results have been reported for this assumption. Further studies by Ceccarelli (1996) and Burger et al. (2008) reported higher genetic variation under highly stressed environments especially with the inclusion of locally adapted lines in the trial. These authors concluded that heritability under low input conditions can be comparable to high input conditions or even higher if appropriate genetic materials are included in the study and if experimental error is of similar magnitude.

Efficiency concepts in plant mineral nutrition have been defined based on the processes in which plants acquire, transport, store and use the nutrient to better produce dry matter or grain at low or high nutrient supply (Horst et al., 1993). Gahoonia and Nielsen (1996) demonstrated that, a genotype efficient in P absorption is the one which can both dissolve soil P and absorb it efficiently. Several measures of P efficiency (PE) have therefore been proposed (Moll, 1982; Blair, 1993; Baligar and Fageria, 1997; Fageria, 1999, White et al., 2005; White and Hammond, 2008). However the common measures include: grain yield under low P conditions, agronomic P use efficiency (AE) which is the increase in

yield per unit of added P fertilizer ($Kgkg^{-1}$), P acquisition efficiency (PAE) which is the product of the increase in plant P content per unit of added P fertilizer ($KgPkg^{-1}g$ Pf), and P utilization efficiency (PUE) which is the increase in yield per unit increase in plant P content ($Kgkg^{-1}$). PAE measures the ability of the plant to absorb available P from the soil. PUE measures the amount of grain produced per unit of absorbed P by the plant. Any species able to maintain metabolic activities at low tissue P concentration and produce more dry matter per unit of P absorbed is considered efficient in P utilization (Serpher et al., 2009). Other measures include (i) P efficiency ratio (PER), which is yield divided by the amount of P in the plant ($Kgkg^{-1}$) which is equivalent to the reciprocal of tissue P concentration if the entire plant is harvested; (ii) physiological P use efficiency (PPUE) which is yield divided by tissue P concentration at a given P concentration in the rooting medium ($Kgkg^{-1}$) and P efficiency (PE) (relative grain yield) (Oztuk et al., 2005; Hammond et al., 2009). In this study selection of plant genotypes efficient in acquiring and utilizing P as well as producing reasonable yield under low P conditions were considered as important selection strategies under the low P soils of western Kenya. The objective of this study was to develop and identify P-efficient experimental maize hybrids for low P soils of western Kenya.

3.3 Materials and Methods

3.3.1 Experimental locations

The selected locations for this study were Kenya Agricultural Research Institute (KARI)/CIMMYTT research centre at Kibos North, Sega, Koyonzo, Migori and Chepkoilel. Kibos location was selected as suitable for developing the crosses because it has two seasons in a year and the availability of irrigation facilities (sprinkler) while

Sega, Koyonzo, Migori and Chepkoilel locations were suitable for studying genetic effects of phosphorus deficiency in the soil because their soils are characterized by low available phosphorus. Besides maize is a major food crop in these regions.

Kibos North ($0^0$00'53S, and 34^0 52'55''E) is 14 Km North East of Kisumu city in Kisumu County and located at an altitude of 1679 m above sea level (m.a.s.l). It receives a mean annual rainfall of between 400 – 700 mm with a mean minimum temperature range of between 16 – 19^0C while mean maximum range is between 27 – 30^0C (KESREF, 2001; Jaetzold and Schmidt, 1983). The soils are classified as Vertisols with dark greyish brown to dark brown sandy clay loam, texture underlain by brownish to greyish brown clay loam to light clay (FAO, 1988).

Sega is located at 0^o 15'N and 34^o 20'E in Siaya County in western Kenya. It has an elevation of between 1140 – 1400 m above sea level (m.a.s.l) with a bimodal annual average rainfall pattern of between 800 – 1200 mm. The mean minimum temperature range is 15 – 17^0C while the mean maximum range is between 27 – 30 ^{0}C. The soils are Orthic Acrisols characterized by low pH (4.5), high Al saturation (44%) and low available P of 2.2 mgP/kg of soil (Jaetzold and Schmidt, 1983; Kisinyo, 2011).

Koyonzo site is located at 0^o 25'N & 34^o 25'E in Kakamega county in western Kenya. It has an elevation of between 1310 m.a.s.l. and a bimodal rainfall distribution pattern with an average annual rainfall of between 1200 – 1600 mm. The mean annual temperature range is 20 – 22^oC. The soils are luvisols with a pH of 5.4, Al saturation (15 %) and low available P of 3 mgP/kg soil (Jaetzold Schmidt, 1983 Kisinyo et al., 2009).

Chepkoilel location is located at 0^o 37'N; E 035^o 15' in Uasin Gishu County. It lies at an altitude of 2143 ma.s.l with a mean annual rainfall of 900 – 1100 mm received in a unimodal pattern. Most of the rains fall between March and September while dry spells are experienced between October through to February. The mean maximum and minimum temperatures are 26 and 10^oC, respectively. The soils are chromic ferralsols characterized by low pH (4.8), high Al saturation (45.6%) and low available P levels of 4.4mgP/kg of soil (Kisinyo, 2011).

Migori site is located at 1^o 03'S & 34^o 24'E in Migori county in western Kenya. It has an elevation of between 1381 m.a.s.l with an average annual rainfall of between 1000 – 1400 mm. The mean annual temperature range is 22 – 24^oC. The soils are humic ferralsols with a pH of 5.75, Al saturation (12%) and low available P of 2.56 mgP/kg (Jaetzold Schmidt, 1983, Ouma, 2013).

3.3.2 Soil Sampling, Preparation and Characterization

Soil sampling was done by taking six sub-samples with a soil auger at the 0 - 20 cm soil depth in a zig-zag pattern at the four locations (Migori, Sega, Chepkoilel and Koyonzo). The sub-samples were thoroughly mixed and about 1.2 kg composite samples packed in a black polythene bag and transported to the lab where they were air-dried, ground and passed through a 2 mm sieve. They were then analysed for texture, pH (1:2.5 (soil: water), available P, exchangeable bases (Ca^{2+}, Mg^{2+}, K^{2+}) and Al^{3+} using the procedures Okalebo et al. (2002) and Smith (1981).

3.3.3 Developing maize hybrids for P efficiency

A total of eleven maize inbred lines were used as parents in this study (Appendix 1). These lines were developed at KARI-Kitale by the Moi University – KARI-Kitale joint maize breeding program and have undergone between 8-16 generations of self-pollination. These parental inbred lines were selected among 175 maize inbred lines based on higher grain yield capacity compared with the inefficient ones under low available soil phosphorus (Ouma et al., 2012). Selection was also based on their general and specific combining abilities (GCA/SCA) (information provided by KARI-Kitale).

3.3.4 Generation of the crosses

North Carolina mating Design II (NCD II) procedures as described by Comstock and Robinson (1948, 1952) were used to generate maize hybrids using the selected inbred lines. The linear model of NCDII is $x_{ijk}=\mu+r_k+f_i+m_j+(m\times f)_{ij}+e_{ijk,}$ where μ is the mean, r_k is the effect of k^{th} replication ($k=1,2, \ldots, r$), fi is the effect of the i^{th} female parent ($i=1,2,\ldots,j$), m_j is the effect of the j^{th} male parent ($j=1,2,\ldots,m$) and $(m\times f)_{ij}$ is the interaction effect between parent i and j, and e_{ijk} is the experimental error of the observation x_{ijk} (Lee *et al.* 2005). Three types of hybrids were developed single cross hybrids (SCHs), Three-way cross hybrids (TWCHs), double cross hybrids (DCHs) .The inbred lines were grouped into two. Group A (Male parents) and B (female parents). The classification into two groups was based on prior information on heterotic patterns obtained from KARI-Moi University maize breeding program (Ligeyo, Personal comm.).

3.3.5 Single crosses, 3-way crosses and Double crosses

Ten maize inbred lines contrasting for P efficiency (five from group A and 5 from B) were planted at Kibos centre in August 2011 and crossed in NCRD II to generate 25 single cross hybrids. Each female parent row was planted side-by-side with each male parent row in a two row plot of 3-m in length. Five of the SCHs (KML 036 X S 396-16-1, HS L3 X 5046-2 X MUL 229, HS 228 x 5046-16 X MUL 229, KML 036 X S 396-16-1 and HS L3 X 5046-2 X S 396-16-1) were crossed to other 5 selected inbred lines (MUAP II SR and POOL 9 A BASF, K4, K15 and K17) to generate twenty five TWCHs (Table 3). Six SCHs (KML 036 X MUL 229, HS 228 X 5046-16 X MUL 229, HS L3 X 5046-2 X MUL 229, KML 036 X S 396-16-1, HS L3 X 5046-2 X S 396-16-1 and KML X MUAP II SR were further selected and crossed in NCRD II to generate 9 DCHs.

3.3.6 Pollination and field procedure

In all the crosses, pollen was transferred manually from the tassel of the male parents and deposited on the silks of the female parents. Before silks appeared out of the husks, a transparent silking paper bag was placed on the ear to prevent any contamination from foreign pollen sources. A day before pollination a brown paper bag was placed on the tassel to collect the pollen. The next morning pollen was collected by carefully shaking the tassel to ensure pollen deposited into the bag then it was removed. After placement of the pollen on the silk, the pollination bag was left on the ear until it maturited to prevent any further contamination (Clave, 2008). Off types, plants with severe disease symptoms and plants with malformations were eliminated. At maturity, only well-formed ears without ear rot or any infections were harvested.

3.4. Evaluation of maize hybrids for P efficiency under field conditions

3.4.1 Experimental Design

A total of 32 experimental maize hybrids comprising 9 TWCHs, 5 DCHs, 9 backcrosses (BC1), 5 SCH, 2 parental lines and two checks (H 515 and a P-efficient synthetic) were evaluated for tolerance to low P in a replicated trial at Sega, Chepkoilel, Migori and Koyonzo locations during the long rain of 2013. The experiment was laid out in a split plot arrangement in RCBD replicated three times. Main plot contained 2 levels of P (6 KgP/ha and 36 KgP/ha supplied as TSP) while the genotypes were randomized in the sub plot. Each genotype was planted in a two row plot measuring three meters long with inter and intra-row spacing of 0.75 m x 0.30 m respectively. Two seeds were sown per hill and later thinned to one per hill. Digger program in Genstat was used to generate randomization design and field layout. All the plots were side-dressed using calcium Ammonium Nitrate (CAN) at the rate of 75 Kg N/ha. All standard agronomic practices were followed. Soil analysis for soil available P at all the locations was done using the procedures of Olsen et al. (1954).

3.4.2 Data collection

Data was collected during anthesis and at maturity. During anthesis, destructive sampling was done on 6 randomly selected plants according to Bell and Fischer, (1994). Root sampling was done using the root box technique as described by Vepraskas and Hoyt, (1988) and Manske, (2002) in order to determine root length per unit of soil volume (root length density). The line-intercept method developed by Newman (1966) and modified by Tennant (1975) was used for measuring root length density. This method counts the

total number of intersections between roots and the vertical and horizontal lines of a grid drawn on a petri dish. Root length was estimated by the equation:

R = N x fresh weight of total sample/ fresh weight of counted subsample. Where R is the total root length density, and N is the number of intersections between the roots and random horizontal grid lines in the petri dish.

At maturity, data was collected on grain yield, (GYLD-t/ha), plant height (PHT-cm), Stover yield (STV= leaves, stalks, ear husks and cobs- t/ha), ear height (EHT-cm), internode length (INL-cm), grain P concentration (GPC %), grain P content (GPcnt Kg/ha), days to 50% silking (DASLK) and days to 50% anthesis (DANTH). At maturity, all the cobs in a row for each entry were harvested and adjusted to 13% moisture content while assuming an 80% shelling percentage. The moisture content was then determined from a sample of 7 randomly selected cobs. PHT was recorded in 10 competitive plants per plot, from the soil surface to the tip of the highest tassel branch, and the plot means used for analysis. Stover samples were collected from 6 plants and a sample of 200g of grain obtained from each plot. These samples were oven dried at 80^{o}C to a constant weight and grain and stover dry matter determined. Grain and stover samples were ground and analyzed for P concentration using the vanadomolybdate method (Westerman, 1990). Based on grain and stover dry matter yields, and on P concentration in these plant components, the phosphorus content in the grain and in the stover were determined. The P efficiency parameters were then obtained on a plot basis following the procedures of Moll et al. (1982, Hammond et al. (2009) and Parentoni et al. (2010) as follows:

a. Agronomic P use efficiency (AE) =Y_{high}–Y_{low})/D_{Papp} (kg/Kg Pf)

b. P uptake efficiency (PAE) = [(P_{high} xY_{high})–(P_{low}xY_{low})]/D_{Papp} (KgP/kgPf)

c. P utilization efficiency (PUE) = (Y_{high}–Y_{low})/[(P_{high}xY_{high})–(P_{low} xY_{low})] (kg/ kg)

d. P efficiency ratio (PER) = Y_{high}/(P_{high}xY_{high}) or Y_{low}/(P_{low}xY_{low}) kg/kg

e. Phosphorus Efficiency (PE) = $Y_{low/}$ Y_{high} x 100%

Where: Y_{high} - is the yield on a high P or fertilized soil; Y_{low} - is the yield on a low P/unfertilized soil; P_{high} – is the tissue P concentration on a high P or fertilized soil; P_{low} - tissue P concentration on a low P or unfertilized soil; D_{Papp} - difference in amount of P applied as fertilizer between high and low P treatments; Pf- P fertilizer.

3.4.3. Statistical Analysis

All means computation and variance analysis (ANOVA) were done using Genstat Version 18 (Payne et al., 2014). The protected least significant difference (LSD) was used to separate the mean. An individual Anova was done for all the traits in each of the 2 P levels per location. A combined ANOVA for the two P levels across the locations was done after verifying data homogeneity. ANOVA was done by fitting the split plot model for the data:

$$Y_{ijklm} = \mu + Si + B_{k(i)} + P_j + SP_{ij} + \grave{\varepsilon}_{ijk} + G_{km} + SG_{ikm} + SPG_{ijm} + \underline{\grave{\varepsilon}}_{ijkm}$$

Where Y_{ijkm} is the observation on the $ijkm^{th}$ plot,

$\mu -$ the general mean,

S_i the effect due to the i^{th} location,

$B_{k(i)}$ the effect due to the k^{th} replication in i^{th} location,

P_j effect due to the j^{th} phosphorus level

SP_{ij} effects due the interaction of the j^{th} phosphorus level with the i^{th} location

$è_{ijkl}$ is the residual effect due to *ijkl* th whole plot

G_m is the effect due to the m^{th} genotype in the k^{th} replicate

SG_{im} is the effect due to the m^{th} genotype in the k^{th} replicate in the i^{th} location

SPG_{ijm} is the effect due to the m^{th} genotype in j^{th} level of phosphorus in the k^{th} replicate in the i^{th} location $è_{ijkm}$ is the residual effect due to *subplot*

3.4.4. Estimation of heritability

Broad sense heritability (H^2) was estimated by variance components using linear mixed models (REML) of Genstat version 18. It was calculated as follows:

$$H^2 = \sigma^2_g / \{(\sigma^2_g + \sigma_{ge}{}^2_+ (\sigma_{error}{}^2/r)\}$$

Where H^2 is broad sense heritability,

σ^2_g is the generic variance; $\sigma_{ge}{}^2$ is the variance due to Genotype x environment interactions,

$\sigma_{error}{}^2$ is the error variance, r is the number of replicates per genotype (Knapp et al. 1985; Ribaut et al., 1996; Oakley et al., 2006).

3.4.5 Genetic correlations

Data from 10 different pairs of traits (GYLD, PHT, EHT, Cob L, INTL, STV, STVP (%), GPC(%), GPNT and STVPCNT) measured at both high and low P levels at each location was used for genetic correlation studies. The genetic coefficient of correlation (r_g) of traits X and Y was calculated according to Kearsey and Pooni (1996) as follows:

- $r_g = \sigma xy / \text{sqrt}(\sigma^2 x * \sigma^2 y)$
- σxy = covariance between x and y while $\sigma^2 x$ and $\sigma^2 y$ the variances of traits x and y, respectively

The variance and the covariance components were estimated using REML output of Genstat (Payne et al., 2014). Analysis of variance and covariance was done considering genotypic affects as fixed and phosphorus levels and location as random effects. Relative Yield Reduction (RYR) was calculated according to Leiser et al. (2012) where RYR =1-($MeanYield_{-P}/MeanYield_{+P}$ *100%.

3.5 Results and Discussion

3.5.1 Initial Soil Characteristics of the study Locations

The soils were found to be generally of low fertility. Sega and Chepkoilel soils were strongly acidic (30-45% Al saturation), while Migori and Koyonzo soils were non-acidic (< 20% Al saturation). However, soil available P was low at all the locations. Total N, organic carbon, Ca and cation exchange capacity (CEC) were also low at all the locations. Koyonzo and Chepkoilel experienced the highest amount of rainfall per year of about 1400 mm while Sega received the lowest (1000 mm) during the experimental period (Table 2). The annual temperature range was 21 - 25 ^{o}C with Sega experiencing

the highest temperature regimes. All the soils were P deficient (2.3 - 4.4 mg P kg^{-1}) and unsuitable for maize production (Table 3). According to Okalebo et al. (2002), bicarbonate extractable P levels below 10 mg P kg^{-1} of soil are considered inadequate for good and healthy plant growth. Besides, all the locations had low C (5.28-7.11 cmol kg^{-1}) and exchangeable Ca (2-4 cmol kg^{-1}) except Koyonzo (Ca=4 cmol kg^{-1}). According to Landon (1983 and 1984), a CEC < 15 cmol kg^{-1} and exchangeable Ca^{2+} < 4.0 cmol kg^{-1} are considered low for crop production. Chepkoilel location had the highest percent clay (66%) while Koyonzo had the least (29%). The later had the highest sand (54%) whereas Sega had the least (28%). These results compares well with those of Kisinyo et al. (2013a) who also reported low available P (2.13-6.08 mg P kg^{-1}), low CEC (6.01-7.08 cmol kg^{-1}) and high % Al saturation in both the highland East of Rift valley (RV) and West of RV soils. In this study chepkoilel is located in the highland East of the RV while, Sega, Migori and Koyonzo are on the western side of the RV. Contrarily this study found higher clay content in the highland of RV soils (66%) compared to those of the western side of the RV (29-56%). This is contrary to the findings of Kisinyo et al. (2013a) who found higher clay content in sites that were on the western side of the RV. This is probably because different sites were used in the two studies. Moreover the findings for similar sites such as Sega between the two studies are very close for most soil physical and chemical properties investigated. From the soil chemical and physical properties observed (with low base cations, available P, C, and N), it was evident that the western Kenya soils are depleted and unsuitable for healthy maize growth. These findings agree well with those of Landon (1984); Ligeyo, (2007) and Kisinyo et al. (2013a) who also reported highly depleted soils in many parts of western Kenya. These soils could have

lost most base cations due to leaching by rainfall. The low available P is attributed to P fixation to clay minerals (Al and Fe oxide) (Van straten, 2007; Obura et al., 2008). The low P and N could also have been due to continuous cultivation, hence nutrient removal through crop harvest without proper soil replenishment. Moreover, studies by Okalebo et al. (2006) showed that most farmers in western Kenya do not replenish their soils.

Table 2: Location agro ecology, soil chemical and physical characteristics

Experimental site	mean Rainfall (mm)	Mean. Temp (°C)	pH	P (mg/kg)	%N	%C	cmo/kg					%Al	%Sand	%Clay	%Silt	Textural Class
							K	Ca	Mg	Al	ECEC					
Koyonzo	1400	22	5.4	3.4	0.12	2.69	0.06	3.52	2.46	1.07	7.11	15	54	29	17	sandy clay loam
Chepkoilel	1400	21	4.8	4.4	0.13	3.51	0.07	1.93	1.76	2.2	5.28	45	18	66	16	clay
Sega	1000	25	4.65	2.3	0.13	2.69	0.04	2.81	1.72	2.1	6.54	30	28	56	16	Clay
Migori	1200	24	5.8	2.66	0.1	1.6	0.8	3.47	1.73	1.02	6.4	12	32	33	15	sandy clay loam

ECEC = effective cation exchange capacity.

3.5.2 Development of experimental maize hybrids

Table 3 shows the list of the crosses that were successful. A total of 12 SCHs, 9 FWCs and 18 TWCHs were developed at Kibos in 2011.

Table 3: List of single, three-way and four way crosses developed

Single Crosses	Pedigree	Four way Crosses	Pedigree
SCH1	KML 036 x MUL 229	FWCH1	(KML 036 X MUL 229) X (HS L3 X 5046-2 X MUL 229)
SCH2	KML 036 X S 396-16-1	FWCH2	(KML 036 X MUL 229) X (HS L3 X 5046-2 X S 396-16-1)
SCH3	KML 036 XK15	FWHC3	(KML 036 X MUL 229) X (KML 036 X MUAP II SR)
SCH4	KML 036 X MUAP II SR	FWCH4	(HS 228 X 5046-16 X MUL 229) X (KML 036 X S 396-16-1)
SCH5	KML036 XAO89	FWCH5	(HS 228 X 5046-16 X MUL 229) X (HS L3 X 5046-2 X S 396-16-1)
SCH6	HS L3 x 5046-2 X MUL 229	FWCH6	(HS 228 X 5046-16 X MUL 229) X (KML 036 X MUAP II SR)
SCH7	HS L3 x 5046-2 X S 396-16-1	FWHC7	(KML 036 X S 396-16-1) X (HS L3 X 5046-2 X MUL 229)
SCH8	HS L3 x 5046-2 X K15	FWHC8	(KML 036 X S 396-16-1) X (HS L3 X 5046-2 X S 396-16-1)
SCH9	HS L3 x 5046-2X MUAP II SR	FWHC9	(KML 036 X S 396-16-1) X (KML 036 X MUAP II SR)
SCH10	HS L3 x 5046-2 X AO89		
SCH11	HS 228 x 5046-16 X MUL 229		
SCH12	HS 228 x 5046-16X S 396-16-1		
Three way Crosses	Pedigree	Three way Crosses	Pedigree
TWCH1	KML 036 x MUL 229 x MUAP I ISR	TWCH10	HS L3 x 5046-2 XS 396-16-1 X POOL 9A BASF
TWCH2	HS L3 x 5046-2 X MUL 229 X MUAPII SR	TWHC11	HS L3 x 5046-2 X MUL 229 X K4
TWHC3	HS 228 x 5046-16 X MUL 229 X MUAP II	TWCH12	HS 228 x 5046-16 X MUL 229 X K4
TWCH4	KML 036 x S 396-16-1 x MUAP I ISR	TWCH13	HS L3 x 5046-2 XS 396-16-1 X K4
TWCH5	HS L3 x 5046-2 XS 396-16-1 X MUAPII SI	TWCH14	KML 036 x MUL 229 x K15
TWCH6	KML 036 x MUL 229 x POOL 9A BASF	TWHC15	HS L3 x 5046-2 X MUL 229 X K15
TWHC7	HS L3 x 5046-2 X MUL 229 X POOL 9A B	TWCH16	HS 228 x 5046-16 X MUL 229 X K15
TWCH8	HS 228 x 5046-16 X MUL 229 X POOL 9A	TWCH17	HS L3 x 5046-2 XS 396-16-1 X K15
TWCH9	KML 036 x S 396-16-1 x POOL 9A BASF	TWCH18	KML 036 x MUL 229 x K17

3.5.3. Response of maize hybrids to P fertilizer application across four locations.

Analysis of variance for agronomic traits and means

ANOVA Table 4 shows highly significant variations ($p \leq 0.01$) for locations (Loc) for all the traits measured. Phosphorus levels (PL) had siginificant effects for all the traits. This implied that Locations were different and that P fertilizer application had an effect on the performance of maize hybrids. The interaction Loc*PL was significant ($p < 0.05$) for majority of the traits implying differential location response to P application. This was expected because of the variation in P levels measured across the locations. Genotype

differences were highly significant ($p \leq 0.01$) for all the traits measured. This can be attributed to genetic variation in P efficiency amongst the genotypes. Loc x Geno was significant for for all the traits measured while PLxGeno level was only significant for GYLD, PHT and EHT. Such substantial genetic variation in response to P deficiency and P supply has been shown previously in maize hybrids (Parentoni et al., 2010, Ligeyo et al., 2014), sorghum (Leiser et al., 2013),wheat cultivars grown in Australia (Batten, 1986; Osborne and Rengel, 2002a, Manske et al., 2000) and in rice, Wissuwa et al. (2002).

Table 4: Mean square for grain yield and other agronomic traits for maize hybrids tested across 4 locations

Source of variation	d.f	GYLD	PLHT	EHT	EANO	DSLK	DANTH	INTL	STV
Replication	2	0.07	142.25	286.02	42.36	43.031	28.552	12.313	0.8237
Location (Loc)	3	524.645***	418061.26***	137360.46***	6218.05***	55182.28***	57884.58***	3833.26***	3000.93***
Phosphorus level (PL)	1	347.01***	118265.81***	50195.3***	1002.02***	6737.16***	6325.345***	734.79***	1209.30***
Loc.PL	3	19.63***	603.48***	63.87	816.15***	429.54***	546.04***	13.46*	110.4318***
Pooled Error (A)	14	0.45	361.02	178.41	72	84.506	18.622	26.794	5.3892
Genotype (Geno)	31	10.44***	4304.92***	1959.65***	55.75***	89.464***	79.012***	12.49***	68.0895
Loc.Geno	93	2.34***	771.01***	412.59***	19.64***	26.642***	25.667***	3.72***	22.0055
PL.Geno	31	0.55***	346.82***	195.52***	14.92	8.113	6.545	1.833	3.5465
Loc.PL.Geno	93	0.67***	339.34***	128.64***	12.86	8.147	7.504***	2.30**	4.9123
Pooled Error (B)	493	0.13	63.80	34.64	10.94	6.685	4.733	1.561	0.8976
Grand mean		4.11	193.33	79.46	14.35	79.3	77.4	12.32	8.5
CV		8.7	4.10	7.40	23	3.3	2.8	10.1	11.1

Note. GYLD-grain yield, PLHT-plant height, EHT-Ear height, INT-Internode length, STV-stover yield, DANTH-days to 50% anthesis, DSLK-Days to 50% silking.

3.5.4. Agronomic performance of hybrids under high and low P levels

The low P treatment generally exhibited reduced GYLD, PLHT, EHT, STV and delayed flowering (DANTH and DASILK), relative to the corresponding high P treatment (Table 5). Mean grain yield was significantly lower (2.49 t/ha) across the low P treatment compared to the high P treatment (4.78 t/ha) although there was a rather big range (35-95%) for relative yield reduction (RYR) among the hybrids. Most of the maize hybrids showed substantial yield differences between the P levels. The large yield and height reductions, delayed flowering between the two P levels demonstrated that the two conditions did differ, were contrasting for P stress and therefore suitable for selection. The application of P fertilizer increased grain yield because of the increased soil available P, which is necessary for healthy plant growth, proper root development, vegetative growth, seed formation and faster grain maturity (Tisdale et al., 1990). Such yield increments have been reported by Kisinyo et al. (2013b) and Ligeyo et al. (2014). A 48.9% mean yield reduction across soil P levels was observed, which compares well with those of Fox (1978) and Parentoni et al. (2010) who reported mean grain yield reductions of 35% and 47%, respectively in maize hybrids due to low P levels. Yield reductions, delay in flowering and reduced height in low P compared to high P have also been reported for several crops (Rossiter, 1978; Fageria et al., 1988; Atlin and Frey, 1989; Wissuwa and Ae, 2001; Manske et al., 2001; Turk et al., 2003; Chen et al., 2008; Cichy et al., 2009, Ouma et al., 2013; Ligeyo et al., 2014). This implies that our selection criteria based on yield differences, height reduction and delay in flowering was appropriate.

Across the 4 locations the highest grain yield was realised in hybrid 40 (6.49 t/ha) followed by 28 (5.59 t/ha) while the least yielder was parental inbred line 31 (0.12 t/ha). Measurement of grain yield under low P conditions has been proposed as a suitable criterion for discriminating P-efficient from P-inefficient genotypes (Parentoni et al., 2010). Further to this, Gourley et al., (1993) defined the basic conditions to consider when selecting genotypes for nutrient efficiency. They proposed a concept to distinguish "superior genotypes" from "efficient ones based on the idea that a superior metabolic activity is likely to result in higher yields independent of P availability. Accordingly, a "superior", rather than an "efficient" genotype would be identified. A key condition to categorize a pair of genotypes as "P efficient" and "P inefficient" would therefore be that they should achieve comparable yields with optimum P availability and should show significant differences under low P supply. Therefore considering the grain yields presented in Table 5, hybrids 1, 39, 25, 16 among others are P-efficient while 31, 7 and 11 are P-inefficient. Hybrids 28 and 40 can be categorised as P efficient as well as good responders to P fertilization while genotypes 23, 2, 30, and 20 are only good responders probably due to superior cell metabolism. These results compare well with those of Parentoni et al. (2006) and (2010) who distinguished P efficient maize from inefficient based on their yields at low P.

PLHT, EHT and STV were reduced by 18.5%, 12.1% and 26 % respectively across the locations. Under high P conditions, the tallest genotype was hybrid 25 (232.25 cm) followed by 40 (224.37 cm) while the shortest was parental line 31 (170.48 cm) followed by hybrid 11 (183.62) while under low P the tallest was hybrid 39 (212 cm) followed by 40 (207 cm) and the shortest one 31 (129.4 cm) followed by 27 (161.21 cm). Comparable

results were obtained for biomass production (STV) where in high P, hybrid 40 was the leading (16.36 t/ha) followed by 39 (12.83 t/ha) while the least was hybrid 14 (7.61 t/ha) followed by 11 (7.62 t/ha). Under low P, hybrid 40 still had the highest biomass production (12.68 t/ha) while parental line 31 gave the least (4.43 t/ha) (Table 5).

Table 5: Mean yields of maize hybrids tested for P-efficiency across 4 locations in western Kenya.

	GYD (t/ha)		RYR	PLHT (cm)		EHT (cm)		STV (t/ha)		DANTH (days)		DSLK (days)	
Hybrid	36 kgP/ha	6 kgP/ha	%	36 kgP/ha	6 kgP/ha	36 kgP/ha	6 kgP/ha	36 kgP/ha	6 kgP/ha	36 kgP/ha	6 kgP/ha	36 kgP/ha	6 kgP/ha
1	5.43	3.05	43.81	212.06	189.40	88.17	66.67	10.87	8.93	73.36	78.58	75.08	80.83
2	5.56	2.89	47.99	223.54	196.77	91.52	70.50	11.11	7.91	75.00	80.08	76.75	82.00
3	5.14	2.61	49.14	213.04	181.47	90.10	73.83	10.21	7.65	75.17	80.78	76.42	82.99
5	5.00	2.47	50.68	195.31	179.40	83.54	66.61	8.04	5.78	74.00	79.08	76.08	80.50
6	5.17	2.69	47.92	205.33	185.50	84.48	72.19	8.81	6.25	72.17	78.42	73.92	80.00
7	4.32	2.00	53.74	198.00	177.10	82.33	70.62	8.36	7.23	76.83	83.33	79.75	86.42
8	4.11	2.06	49.91	200.71	176.90	81.88	65.88	9.60	6.64	76.42	83.50	79.33	85.67
9	4.15	2.17	47.65	196.52	179.81	80.48	67.77	9.04	6.94	72.42	78.92	74.58	80.42
10	4.62	2.20	52.37	203.85	174.23	77.10	61.40	7.80	5.70	73.58	78.42	75.08	80.50
11	3.89	1.58	59.45	183.62	169.92	76.12	65.46	7.62	4.72	74.02	78.92	76.14	81.08
13	4.53	2.29	49.41	186.94	161.21	72.50	57.96	8.66	6.11	77.92	80.75	79.08	82.42
14	4.06	2.10	48.26	194.82	162.46	85.98	69.73	7.61	5.98	73.90	80.17	76.31	82.58
15	4.47	2.14	52.12	203.96	189.41	86.00	70.40	9.39	6.51	73.92	80.33	76.25	82.58
16	5.01	3.24	35.32	212.83	190.42	94.37	71.67	10.16	8.34	74.92	80.08	77.77	82.42
17	4.90	2.47	49.63	207.81	185.67	85.54	73.94	9.16	6.53	71.33	77.58	72.95	79.75
18	4.56	2.93	35.67	201.84	173.67	91.26	70.81	8.72	7.43	73.08	79.50	75.42	80.83
19	4.56	2.27	50.20	202.73	178.52	86.67	67.38	8.98	6.90	74.17	79.25	75.42	81.50
20	5.30	2.75	48.17	207.17	168.10	87.71	61.40	10.80	7.98	72.92	79.75	74.00	80.92
21	5.36	3.05	43.04	202.54	181.50	85.67	73.48	9.43	7.52	72.83	79.25	74.25	81.92
22	4.59	2.31	49.73	211.27	196.36	86.52	72.69	11.11	8.36	74.48	80.58	77.31	82.58
23	5.23	2.75	47.37	208.77	187.21	81.46	73.58	10.68	7.58	75.00	77.98	76.25	80.72
24	4.89	2.77	43.44	217.25	186.81	94.09	78.85	9.12	6.82	75.61	81.42	77.79	83.17
25	5.42	3.27	39.64	232.25	198.08	107.54	86.26	11.44	9.31	79.80	84.79	81.47	86.48
26	4.34	2.54	41.48	207.44	183.77	88.12	72.36	12.06	7.54	73.83	78.00	75.25	80.25
27	4.51	2.46	45.54	197.48	163.62	83.79	62.77	8.02	5.50	73.33	79.92	74.50	80.33
28	5.59	2.90	48.08	210.85	189.35	91.67	73.56	9.93	7.73	73.73	79.42	75.39	81.00
29	5.03	2.64	47.54	209.02	172.40	91.58	67.75	8.94	6.44	72.83	79.92	75.33	81.83
30	4.98	2.44	50.91	218.94	186.17	93.40	75.25	11.56	8.38	73.42	79.08	74.17	80.83
31	2.20	0.12	94.78	170.48	129.40	74.40	46.73	8.88	4.43	77.83	84.42	78.92	86.83
33	4.16	2.00	51.84	198.15	175.85	85.11	72.83	8.09	6.51	73.23	79.92	74.69	84.25
39	5.60	3.21	42.66	224.37	212.02	103.77	104.29	12.83	10.74	76.25	82.42	78.25	83.67
40	6.41	3.27	48.96	224.83	207.05	108.67	99.54	16.36	12.68	76.92	83.33	78.92	85.08
G.MEAN	4.78	2.49	48.95	205.74	180.92	87.55	71.38	9.79	7.28	74.51	80.25	76.34	82.26
SE	**0.34**	**0.33**		**4.58**	**4.39**	**3.26**	**3.07**	**0.54**	**0.44**	**0.94**	**1.03**	**1.56**	**0.83**
SED	**0.12**	**0.11**		**1.83**	**1.93**	**1.20**	**1.48**	**0.26**	**0.25**	**0.29**	**0.29**	**0.35**	**0.58**
LSD (0.05)	0.32	0.25		13.02	9.00	4.20	3.40	0.90	0.53	1.90	1.10	1.50	0.70

Note. GYD-grain yield, RYR-relative yield reduction, PLHT-plant height, EHT-Ear height, Cob L-Cob length, STV-stover yield, DANTH-days to 50% anthesis, DSLK-Days to 50% silking

Mean grain yield was highest at Chepkoilel location for both P conditions (6.7 and 3.6 t/ha) and lowest at Sega location (2.1 and 0.9 t/ha) (Table 6). The high grain yield at Chepkoilel is attributable to the longer growth period experienced resulting in higher accumulation of the photosynthates hence higher yield and biomass production. RYR was fairly comparable across the four locations (42.5-47.7%) except at Sega where it was slightly higher (59.4%) (Table 6). Migori location exhibited higher biomass (STV) than Chepkoilel which was contrary to the expectation. The reason was that the Chepkoilel trial was hit by hailstones both at anthesis and at grain filling stages resulting in significant reduction in leave size and number. Sega location exhibited the lowest biomass accumulation probably because of the confounding effects of pre-flowering drought that had set in unexpectedly during the experimental period even though the plants later recovered. The hybrids were tallest at Migori under the two P conditions compared to the rest of the sites (240 and 219 cm) although this was similar to Koyonzo location (230.5 and 219.8 cm) (Table 6). The observations for EHT were consistent with those of PHT with Migori and sega locations exhibiting the highest and the lowest EHT respectively. Concerning days to 50 % anthesis (DANTH), Koyonzo site had the shortest growth time to flowering under both P conditions (63 and 67 days) while Chepkoilel the longest (97 and 108 days). Similar trend was observed for days to 50% silking with Koyonzo and Chepkoilel taking the shortest and the longest time to silk respectively (Table 6).

Table 6: Locational mean grain yield, and other agronomic traits for maize hybrids

Location	P level	PHT	EHT	STV	DANTH	DASLK	GYL	RYR
		cm	cm	t/ha	days	days	t/ha	%
Chepkoilel	36 kgP/ha	214.30	92.59	9.47	97.27	98.64	6.74	47.66
	6 kgP/ha	189.40	78.19	7.43	108.04	109.14	3.60	
Migori	36 kgP/ha	240.35	104.83	13.98	65.03	66.17	5.20	43.89
	6 kgP/ha	219.85	87.50	11.23	68.93	70.34	2.97	
Koyonzo	36 kgP/ha	230.46	104.26	9.12	63.28	65.35	5.14	42.49
	6 kgP/ha	201.28	87.63	6.88	67.07	70.06	2.94	
Sega	36 kgP/ha	137.86	47.77	4.99	72.28	75.20	2.12	59.35
	6 kgP/ha	113.17	32.30	3.46	76.96	79.59	0.89	
LSD (0.05)	36 kgP/ha	20.50	10.10	1.16	8.50	9.60	0.42	
	6 kgP/ha	18.30	7.22	0.64	3.92	4.30	0.19	

Note. GYL-grain yield, RYR-relative yield reduction, PHT-plant height, EHT-Ear height, INT-Internode length, STV-stover yield, DANTH-days to 50% anthesis, DSLK-Days to 50% silking.

3.5.5. Variation in Phosphorus efficiency traits among maize hybrids

There were significant variations ($p<0.05$) for Agronomic P efficiency (AE), P-efficiency ratio (PER), P-acquisition efficiency (PAE), P-efficiency (PE) and P-utilization efficiency for the 32 maize genotypes across 4 locations (Table 7). The P-efficiency traits measured were generally higher in low P tolerant hybrids compared to the low P sensitive checks/parents. AE was in the range of 22.7-72.9-kgkg^{-1} with a mean of 44.8 kgkg^{-1}. Highest AE was realized in hybrid 40 followed by 28 and least in hybrid 18. Eighteen out of the 32 genotypes exhibited AE above the mean > 44.8 kgkg^{-1} while 13 of the hybrids had higher AE than the commercial hybrid (H515) across the four locations (Table 7). The highest PER was measured in hybrid 25 (1248.7 kgkg^{-1}) followed by H515 (764.5 kgkg^{-1}) while parental line S396-16-1 gave the least (232.1 kgkg^{-1}). The genotypes attained a mean PER of 546.7 kgkg^{-1} across the four locations. PAE ranged from 0.06 - 0.2 kgPkgf^{-}. Hybrid 19, 24 and 25 exhibited the highest PAE while hybrid 8 and 14 the lowest. Majority of the genotypes (57%) had higher PAE than the average of all the

genotypes. Eight of the hybrids (19, 24, 25,29,1,7,28 and 40) also showed higher PAE than the commercial check (H515) across the locations. Mean % PE was 71.6 % PE (relative grain yield increase) across locations with the highest in hybrid 18 (85.2%) and the lowest in line 31(48.4%). In most cases, genotypes showing higher PE also exhibited higher PER and PAE. Nine of the hybrids had higher PE than the commercial check while 13 genotypes exhibited higher PE than the average (Table 7). PUE ranged from 208.8 $kgkg^{-1}$ (hybrid 18) to 977.5 $kgkg^{-1}$ (hybrid 17). Majority of the genotypes (63%) gave lower values for PUE than the average (553.4 $kgkg^{-1}$). In most cases genotypes with higher values of PUE also expressed higher values of PAE. A total of 12 hybrids were selected based on PUE above the average across the four locations. Majority of these were also the best performers across the locations.

Table 8 shows AE, PER, PAE, PE and PUE across 4 locations. AE was highest at Chepkoilel (104.5 $kgkg^{-1}$) and lowest at Sega (41 $kgkg^{-1}$). Koyonzo and Migori gave comparable AE. PER was highest at Migori (556.5 $kgkg^{-1}$) although this did not differ significantly with Sega (536.2) location while the lowest PER was realized at Koyonzo PAE was highest at both Chepkoilel and Migori and lowest at Sega location while the highest PUE was realized at Sega followed by Koyonzo and was least at Chepkoilel (Table 8).

Table 7: Variation in phosphorus efficiency indicesof maize hybrids tested across 4 low P soils

Maize Hybrid	AE Kg/Kg	PER Kg/Kg	PAE KgP/kgf	PE %	PUE Kg/Kg
KML 036 XS396-16-1	47.6	481.56	0.18	73.69	464.87
HS 228-5046-16XS396-16-1	57.33	610.83	0.15	69.09	519.91
HS L3-5046-2XS396-16-1	52.47	696.96	0.13	69.35	554.06
HS L3-5046-2XMUL 229	52.8	597.01	0.14	68.32	670.27
KML 036 XMUL 229	50.97	645.44	0.09	70.44	711.05
HS 228-5046-16XS396-16-1XHS 228-5046-16	45.63	529.64	0.18	68.27	595.03
HS 228-5046-16XS396-16-1XS396-16-1	36.73	442.94	0.06	73.19	747.88
KML 036 XS396-16-1XKMLO36	34.27	452.35	0.13	75.23	349.59
KML 036 XMUL 229XKMLO36	48.97	604.8	0.07	68.2	897.25
KML 036 XMUL 229XMUL 229	45.37	445.5	0.15	64.99	487.66
HS L3-5046-2XMUL 229XHS L3-5046-2	42.93	443.12	0.1	71.56	507.55
HS 228-5046-16XMUL 229XMUL229	33.63	413.85	0.06	75.14	458.27
HS 228-5046-16XMUL 229XHS 228-5046-16	46.07	506.17	0.13	69.11	586.88
HS L3-5046-2XS396-16-1XHS L3-5046-2	27.27	555.23	0.07	83.66	398.61
KML 036 XAO89XMUAPII SR	49.37	510.15	0.08	69.76	977.49
MUL 229XHS 228-5046-16XHS L3-5046-2	22.57	400.42	0.09	85.16	208.8
MUL 229XHS 228-5046-2XMUAP II SR	44.57	578.62	0.2	70.65	713.51
HS L3-5046-2XMUL 229XMUAP II SR	53.43	637.33	0.11	69.75	699.84
KML O36 XMUL 229XMUAP II SR	45.17	506.24	0.12	74.7	477.36
KML 036 XS396-16-1XPOOL 9A BASF	44.33	504.64	0.15	70.99	489.16
KML O36 XMUL 229XMUAP II SR	50.83	514.79	0.16	70.81	449.39
HS L3-5046-2XMUL 229XPOOL 9A BASF	39.13	535.85	0.2	75.99	428.93
S596-41-2-2-MULXBRS1OO1XKRSTOPVX82	39.9	1248.73	0.2	77.9	538.64
KML 036 XMUL 229XKML 036 XS396-16-1	28.4	583.88	0.07	80.39	498.24
KML 036 XMUL 229XHS L3-5046-2XMUL 229	36.77	431.53	0.16	75.53	383.96
S396-16-1XHS L3-5046-2XHS L3-5046-2XMUL 229	57.97	474.8	0.17	68.91	458.14
MUL 229XHS 228-5046-16XHS L3-5046	48.03	413.57	0.19	71.35	411.11
MUL 229XHS 228-5046-16XKML 036 XS396	52.77	495.79	0.16	68.18	498.55
S396-16-1	37.87	232.06	0.14	48.39	435.15
AO89	40.17	484.34	0.17	71.01	438.07
H515	47.93	765.52	0.16	74.31	793.29
MEDIUM ALTITUDE SYNTHETIC	72.93	749.45	0.17	65.86	860.18
G.MEAN	**44.82**	**546.66**	**0.14**	**71.56**	**553.4**
LSD (0.05)	**4.29**	**50.03**	**0.01**	**4.89**	**42.85**

Agronomic P efficiency (AE), P efficiency ratio (PER), P-acquisition efficiency (PAE), P efficiency (PE) and P use efficiency (PUE)

Table 8: Locational mean P efficiency indices of maize hybrids tested in 2013

Location	AE	PER	PAE	PE	PUE
	Kg/Kg	Kg/Kg	KgP/kgf	%	Kg/Kg
Chepkoilel	104.5a	494.4a	0.21a	52.3b	723.5a
Migori	74.4b	556.5b	0.20a	56.1a	778.5ab
Koyonzo	73.2b	486.2a	0.15b	57.5a	969.6c
Sega	41.0c	536.2b	0.09c	40.6c	971.6c

Means in the same column followed by the same letter are not significantly different**:** AE-agronomic efficiency, PER- phosphorus efficiency ratio, PAE-P acquisition efficiency, PE-phosphorus efficiency, PUE- phosphorus utilization efficiency

Estimates of overall efficiency of applied P fertilizer have been reported to be about or less than 10%. Plants that are efficient in absorption and utilization of nutrients greatly enhance the efficiency of applied fertilizers hence reducing cost of inputs, and preventing losses of nutrients to ecosystems (Fageria and Baligar, 1999). The present study shows the existence of substantial variation for phosphorus efficiencies which are known to be under genetic and physiological control and is modified by plant interactions with environmental variables (Baliger et al., 2001, Baligar and Fageria, 1997). Such substantial genetic variation in response to P deficiency and P supply was also shown for a large number of wheat cultivars grown in Australia (Osborne and Rengel, 2002a, Oztuk et al., 2005). Measurements of lower P-efficiency traits in low P sensitive maize lines and vice versa is consistent with those of Jiang et al. (2010) who reported lower grain P utilization in low P tolerant maize compared to their tolerant counterparts regardless of whether they were planted in low or high P conditions. The results for AE compare well with previous studies such as those of Baligar et al. (2001) and Baligar and Fageria (1997) although they reported higher AE (79 $kgkg^{-1}$) than observed in this study (72.9

$kgkg^{-1}$). This is probably because of some of the major soil chemical constraints reported in the western Kenya soils where this study was conducted such as high levels of Al toxicities, elemental deficiencies (very low N and P levels), and low organic matter content (Table 1). These constraints can greatly reduce AE (Baligar and Bennet, 1986a, Baligar and Fageia, 1997). According to these authors, these factors affect mineralization and immobilization, fixation by adsorption, precipitation mechanisms, leaching, run- off, and gaseous losses via denitrification and ammonia volatilization. The soil organic matter for instance helps to maintain good aggregation and increase water holding capacity and exchangeable K, Ca, and Mg. It also reduces P fixation, leaching of nutrients and decreases toxicities of Al and Mn and hence increases AE (Fageria, 1992). These findings also compare well with those of Kisinyo et al. (2013b) who reported on average slightly higher range of values for AE in the long season experiments (55-70 $kgkg^{-1}$), compared to this study (22.9-72) probably because of the inclusion of lime amendments. Liming is an effective way to correct soil chemical constraints by improving the availability of Ca, Mg, Mo, P, soil structure, and CEC (Adams, 1984).The average PER for the 32 genotypes was 546.7 kg/kg which is well within the range reported by other studies, (525-625kg/kg) for P-efficient maize (Fageria and Baligar, 1999). From the results presented in Table 7, majority of the genotypes expressing higher PE also showed higher PAE, PUE and PER implying a good correlation between these traits. Overall, efficient entries (higher PE values) were far superior in utilization of absorbed nutrients than the inefficient entries. The finding that both PUE and PAE exhibited larger range than PER and PE implies that PUE and PAE contributed more to the observed genetic variations in P-efficiency traits than the latter parameters. The mean PE and PUE was

553.4 kg/kg and 71.6, respectively which compare well with the values of Sepehr et al. (2009) who reported a mean PE and PUE of 550 kg/kg and 71%, respectively in genotypes of wheat, rye and triticale. They also compare well with those of Parentoni et al. (2010) who reported PUE of 400 kg/kg in tropical maize and those of Fageria et al. (2006) who reported PUE of 388 $kgkg^{-1}$in maize cultivated in red oxisols. The disparity with the findings of Fageria et al. (2006) could be attributed to differences in soil available P used in the two studies. In the present study soil available P was extremely low (2.3-4.7 mgP/kg of soil) across the locations while in Fageria et al. (2006) study soil available P was in the range of 4.4 -7.37 mgP/kg of soil. The natural genetic variation observed among genotypes of maize demonstrates the potential for breeding cultivars with improved nutrient use efficiencies (NUE), which will ultimately acquire and utilize applied inorganic Pi fertilizers more efficiently

3.5.6. Effects of P application on grain and stover P concentration and P content

Grain and Stover P concentrations were generally higher with high P regimes than low ones for all the genotypes. With the application of high P the average grain P concentration increased significantly from 0.15% to 0.19% while that of stover P from 0.03 to 0.06% (Table 9). Under high P supply, grain P was highest in hybrids 25, 18 40, 23 and 1 (0.21%) while lowest with hybrid 27 (0.18%), while stover P concentration was highest and lowest (0.09, 0.05 %) in, genotypes 31 and 39, respectively. Under low P supply, grain P concentration (GPC) ranged from 0.14 to 0.17 % while stover P concentration (STVPC) from 0.01 -0.05 % (Table 9). These findings compare well with those of Hammond et al. (2009) who reported 4.9 fold increases in STVPC in *Brassica*

Oleraceae. They are also in agreement with the results reported by Liao et al. (2005); Sepehr et al. (2009) and Ozturk et al. (2005) who reported significant increase in stover P as a result of high P regimes. The genotypes with lower P concentration in their tissues were able to utilize P better while those with higher P concentrations are more efficient in P absorption. Therefore, a good breeding strategy for increasing phosphorus utilization in maize would be to select for reduced GPC. According to Parentoni et al. (2010), reduction in GPC would have a positive impact on animal nutrition, since grain P is stored as the anti-nutritional factor (phytate). A lower GPC will also reduce environmental pollution from high P manure produced by large animal feeding facilities. However, the strategy of reducing GPC should have a limit, since grain P is needed in the grain filling process and is also important in seed germination.

For grain and stover P contents, application of high P level resulted in 2 folds increment in these parameters (Table 9). Moreover, genetic differences were evident among the genotypes. In similar studies, Batten (1986) using 23 wheat genotypes and Osborne and Rangel (2002a) using 106 cereal genotypes also reported large genotypic differences in P content. The differences were attributed to root size, root morphology and changes in the rhizosphere. Grain P content was generally higher than stover P content at both P levels for the 32 maize genotypes across the four locations. Low P supply resulted into significant reduction (up to 52%) in grain P content and up to 85% in stover P content across the four locations (Table 9). For grain P content the reduction due to low P supply ranged from 1.6 kg/ha at Sega to 4.7 kg/ha at Chepkoilel. Similarly, stover P content reduced from 0.5 kg/ha in Sega to 3 kg/ha in Chepkoilel location.

Table 9: Effects of P variation on grain and Stover P concentration of maize hybrids across 4 locations

Maize	Grain P conc. (%)		Stover P conc. (%)		GPCNT (Kg/ha)		SPCNT (Kg/ha)	
Hybrid	36 kgP/ha	6kgP/ha	36kgP/ha	6kgP/ha	36kgP/ha	6kgP/ha	36kgP/ha	6kgP/ha
1	0.21	0.14	0.07	0.03	11.3	6.5	6.4	2.6
2	0.20	0.15	0.06	0.03	9.1	4.1	4.1	1.6
3	0.19	0.15	0.06	0.04	7.4	2.8	4.0	1.4
5	0.18	0.15	0.07	0.04	8.4	4.3	5.5	2.3
6	0.20	0.15	0.06	0.03	8.0	4.2	3.7	1.7
7	0.19	0.15	0.06	0.01	8.1	4.1	5.6	1.0
8	0.19	0.15	0.06	0.04	9.3	6.1	5.1	3.2
9	0.19	0.14	0.06	0.04	9.2	4.5	4.3	1.8
10	0.19	0.16	0.06	0.03	7.6	4.3	4.3	2.3
11	0.19	0.15	0.06	0.02	8.7	4.2	4.5	1.5
13	0.19	0.17	0.06	0.04	10.2	6.0	4.9	3.0
14	0.18	0.15	0.04	0.03	9.8	5.9	3.5	2.4
15	0.19	0.15	0.06	0.03	8.8	4.8	5.6	2.7
16	0.17	0.15	0.05	0.04	9.0	5.3	4.3	2.9
17	0.19	0.17	0.05	0.03	9.6	6.4	3.8	1.5
18	0.21	0.15	0.05	0.05	11.4	6.4	5.0	4.1
19	0.19	0.15	0.08	0.02	7.9	4.3	7.3	1.7
20	0.19	0.15	0.06	0.03	8.3	4.3	4.1	1.5
21	0.20	0.15	0.05	0.04	10.6	6.0	4.6	2.6
22	0.18	0.14	0.05	0.02	9.1	4.7	4.5	1.4
23	0.21	0.16	0.07	0.05	10.1	5.1	5.9	3.1
24	0.19	0.15	0.06	0.02	9.1	4.7	6.9	2.3
25	0.21	0.15	0.08	0.06	4.3	0.4	7.7	2.4
26	0.18	0.16	0.05	0.04	7.4	4.0	4.1	2.3
27	0.18	0.15	0.07	0.03	10.4	5.9	7.7	4.3
28	0.19	0.15	0.05	0.04	11.8	6.3	5.0	2.3
29	0.20	0.16	0.08	0.04	12.2	7.0	10.2	6.7
30	0.20	0.17	0.05	0.02	10.0	5.2	4.2	1.2
31	0.19	0.15	0.05	0.03	9.5	5.2	4.4	1.4
33	0.20	0.16	0.06	0.02	8.6	4.1	5.3	1.6
39	0.19	0.17	0.09	0.03	7.3	3.8	6.4	2.0
40	0.21	0.16	0.08	0.05	8.6	4.3	6.5	2.5
G.MEAN	0.19	0.15	0.06	0.03	9.10	4.85	5.29	2.36
SE	0.02	0.02	0.01	0.01	1.08	0.93	0.74	0.63
SED	0.01	0.01	0.01	0.01	0.27	0.20	0.18	0.14
lsd (0.05)	0.011	0.009	0.004	0.001	0.71	0.33	0.30	0.23

Note: GPCNT-grain P content, STVPCNT-Stover P content

Chepkoilel location exhibited the highest grain P content while Sega the lowest. This is probably attributable to the total available P which was highest at Chepkoilel and lowest

at Sega. However, for stover P content, Migori was the leading followed by Koyonzo while Sega still produced the least implying that the genotypes had better P acquisition efficiency at Migori and Koyonzo probably because of low levels of Al concentration in these soils (Fig 1).

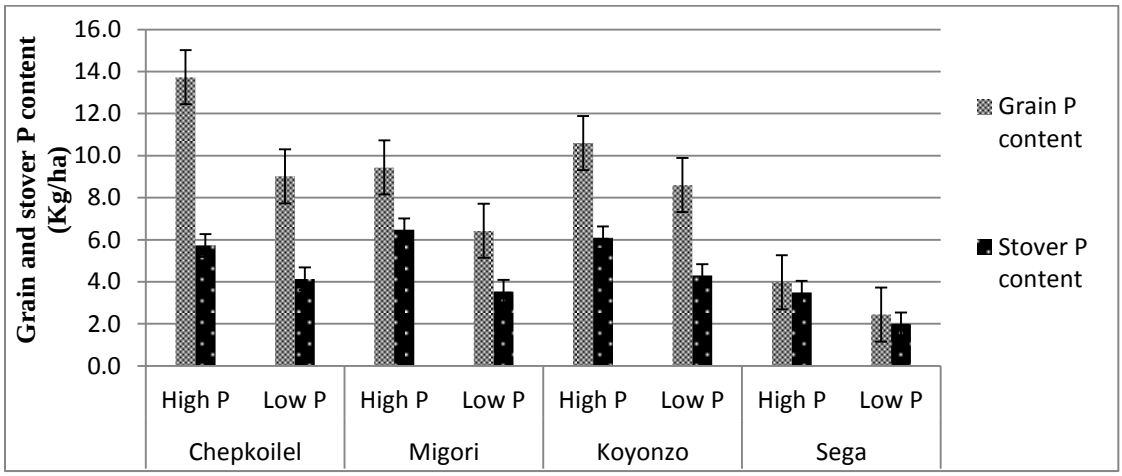

Figure 1: Grain P and Stover P content of maize hybrids across 4 locations

3.5.7. Effects of low P on Root Length Density of maize experimental hybrids

RLD was generally higher at higher P compared to lower P supply for all the genotypes across the locations (Table 10). The mean RLD ranged from 6 to 16.9 cm/cm^3 under high P supply while it ranged from 1.38 to 9.47 cm/cm^3 under low P supply. High P supply resulted in an increase of RLD from 5.9 to 11.6cm/cm^3 across the locations (Table 10). The relative root length density reduction % (RRLDR) due to low P supply ranged from 41-77% across the locations with a mean of 49.6%. Migori exhibited the highest RLD under high P supply while it was highest at Koyonzo under low P supply. However RLD was lowest under both P conditions at Sega (Fig 2). Consequently % RRLR was highest at Sega (57%) followed by Koyonzo (53%) and least at Chepkoilel (39%) (Fig 3).

Table 10: Root length density of maize hybrids under varying P conditions across four locations

Maize	Chepkoilel		Sega		Migori		Koyonzo		Mean across sites		RRLVR
Hybrid	36 kgP/ha	6 kgP/ha	36 kgP/ha	6 kgP/ha	36 kgP/ha	6 kgP/ha	36 kgP/ha	6 kgP/ha	36 kgP/ha	6 kgP/ha	(%)
KML 036 XS396-16-1	8.2	5.3	5.7	2.3	16.8	8.6	16.7	5.1	11.85	5.31	55.16
HS 228-5046-16XS396-16-1	20.5	7.0	3.1	1.2	28.4	15.8	10.2	5.7	15.52	7.41	52.25
HS L3-5046-2XS396-16-1	11.2	5.1	3.5	1.6	20.1	5.1	8.9	4.6	10.94	4.09	62.57
HS L3-5046-2XMUL 229	12.6	3.7	2.4	0.9	21.9	17.3	10.0	6.7	11.72	7.16	38.94
KML 036 XMUL 229	14.7	10.5	2.1	0.6	34.2	17.5	13.4	9.3	16.10	9.47	41.16
HS 228-5046-16XS396-16-1XHS 228-5046-16	16.0	9.4	3.5	2.0	22.5	15.7	14.3	3.0	14.07	7.52	46.57
HS 228-5046-16XS396-16-1XS396-16-1	8.9	4.5	2.7	1.5	24.0	13.3	9.4	6.5	11.22	6.45	42.50
KML 036 XS396-16-1XKMLO36	18.3	7.6	3.4	1.9	33.9	7.9	10.6	6.1	16.55	5.88	64.44
KML 036 XMUL 229XKMLO36	8.7	5.4	3.3	1.3	21.2	13.3	6.8	4.3	10.00	6.07	39.34
KML 036 XMUL 229XMUL 229	5.0	1.8	3.9	1.6	7.5	2.5	9.7	2.3	6.52	2.05	68.50
HS L3-5046-2XMUL 229XHS L3-5046-2	11.3	5.4	4.4	1.6	20.3	12.3	11.2	4.1	11.81	5.86	50.37
HS 228-5046-16XMUL 229XMUL229	20.0	10.8	3.9	1.8	13.5	10.4	9.4	4.7	11.69	6.94	40.67
HS 228-5046-16XMUL 229XHS 228-5046-16	12.8	2.6	2.6	1.4	18.0	15.3	20.3	9.3	13.45	7.16	46.77
HS L3-5046-2XS396-16-1XHS L3-5046-2	13.0	9.2	3.8	0.9	19.8	13.5	10.5	3.8	11.78	6.84	41.96
KML 036 XAO89XMUAPII SR	6.5	3.8	5.4	2.8	16.4	12.2	14.0	6.9	10.58	6.43	39.17
MUL 229XHS 228-5046-16XHS L3-5046-2	10.8	4.1	2.1	1.1	28.9	5.7	6.7	3.5	12.11	3.62	70.10
MUL 229XHS 228-5046-2XMUAP II SR	7.3	2.2	4.3	3.2	22.5	18.3	13.3	8.8	11.83	8.14	31.19
HS L3-5046-2XMUL 229XMUAP II SR	8.6	3.8	5.2	2.2	16.4	10.7	9.7	6.8	9.95	5.86	41.11
KML O36 XMUL 229XMUAP II SR	9.7	5.6	3.5	0.7	23.9	9.1	13.0	4.3	12.50	4.90	60.77
KML 036 XS396-16-1XPOOL 9A BASF	12.3	6.9	6.2	3.9	26.2	11.5	10.3	5.1	13.73	6.86	50.03
KML O36 XMUL 229XMUAP II SR	13.7	8.0	5.6	2.3	14.5	6.5	11.5	1.6	11.33	4.57	59.64
HS L3-5046-2XMUL 229XPOOL 9A BASF	8.1	6.3	6.1	1.1	25.8	10.3	11.7	2.6	12.92	5.08	60.69
S596-41-2-2-MUL XBRS1OO1XKRISTLOPVX82-93	16.0	10.6	4.5	1.9	27.1	17.0	11.6	2.5	14.80	7.99	45.98
KML 036 XMUL 229XKML 036 XS396-16-1	6.7	2.5	1.6	0.6	7.7	4.2	8.7	2.4	6.17	2.46	60.14
KML 036 XMUL 229XHS L3-5046-2XMUL 229	7.4	3.6	1.9	1.4	9.9	6.8	7.1	4.7	6.56	4.14	36.97
S396-16-1XHS L3-5046-2XHS L3-5046-2XMUL 229	11.0	6.5	3.4	1.9	15.2	8.1	10.5	2.3	10.03	4.70	53.07
MUL 229XHS 228-5046-16XHS L3-5046-2XMUL 229	12.1	9.2	3.5	2.0	31.7	21.7	20.4	3.8	16.93	9.18	45.77
MUL 229XHS 228-5046-16XKML 036 XS396-16-1	9.5	5.7	6.2	0.7	10.3	8.5	9.7	7.2	8.90	5.51	38.02
S396-16-1	4.0	1.0	4.4	2.0	6.5	1.5	9.9	1.0	6.00	1.38	76.94
AO89	13.1	5.1	3.0	1.3	15.8	12.7	11.7	8.7	10.89	6.95	36.18
H515	9.6	5.7	1.7	0.5	18.2	10.3	8.9	7.7	9.59	6.06	36.88
MEDIUM ALTITUDE SYNTHETIC	16.0	4.5	3.4	1.9	18.6	9.0	11.2	7.5	12.32	5.73	53.48
MEAN	11.3	5.7	3.8	1.6	19.9	11.0	11.3	5.1	11.6	5.9	49.6
H2	0.9	0.9	0.9	0.8	1.0	0.9	0.9	0.7	0.93	0.84	
SED	0.4	0.3	0.1	0.1	0.7	0.5	0.4	0.3	0.41	0.29	
Lsd (0.05)	0.9	0.4	0.2	0.1	1.6	1.2	0.8	0.4	0.99	0.42	

RRLVR - % relative root length density reduction.

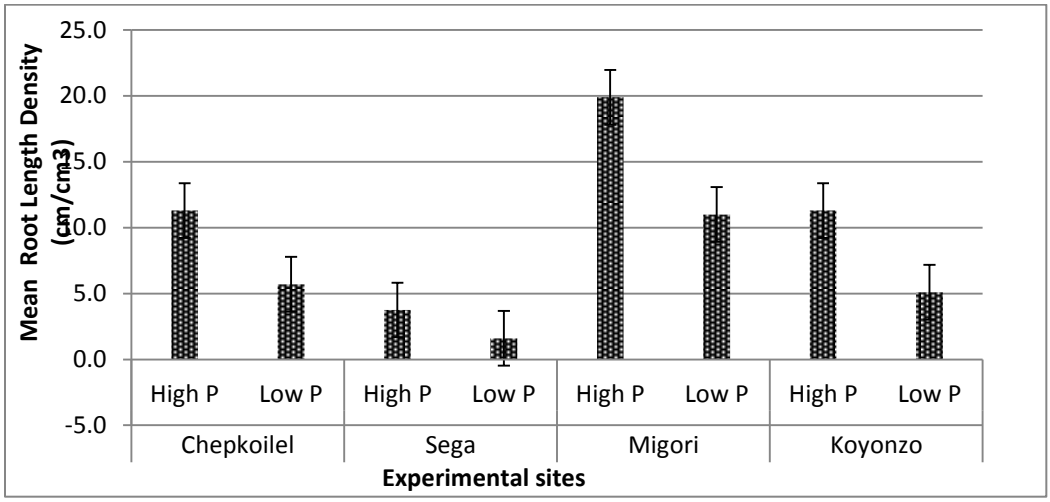

Figure 2: Effects of P on RLD of maize hybrids across 4 locations.

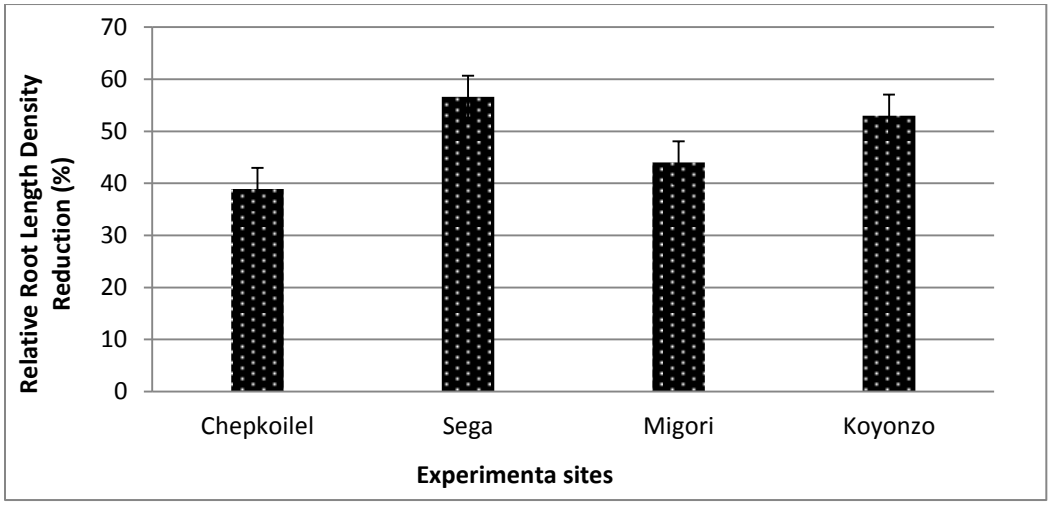

Figure 3: RLD reduction under low P conditions across four locations

Good root growth is a prerequisite for improved shoot growth and higher yields, especially in marginal environments (Manske and Vlek, 2002). Plants may respond to nutrient and water stress by altering rate of uptake per unit root length or weight partitioning between roots and shoots, root exudates and by a lower demand for nutrients and water. Each of these parameters can be altered by selection and breeding. Root length is a major determinant of the absorbing surface area and is one of the most important

parameters for P acquisition from the soil (Manske, 2000). RLD is defined as root length per unit of soil volume. Genotypes with higher root length density are able to take up more phosphorus (Manske et al., 2000a). Therefore plants can be rated and selected on the basis of their RLD. The best advantage is to have more roots per unit volume of soil. In this study, RLD was generally higher with high P application because of positive root response to a higher amount of available P in the soil. These results are in agreement with those of Manske et al. (2000) who reported higher mean RLD under high P compared to Low P application in wheat germplasm. The higher RLD at Migori is probably because of high pH of these soils condusive for plant root growth. However, the lower RLD at chepkoilel and Sega are attributed to the effect of aluminium toxicity that inhibits root growth and development in acid soils (pH < 5.5). It could also have been due to very low available P especially at Sega hence lower root response compared to other location.

3.5.8. Phenotypic correlation between grain yield and P-efficiency traits

There was no significant correlation between P acquisition efficiency (PAE) and P utilization efficiency (PUE) or between PAE and grain P concentration (GPC) in both low and high P conditions (Table 11a & b). Correlation between grain yield and GPC was not significant at both high (r = 0.18) and low P (r= 0.085). The lack of correlation between grain yield and grain P concentration has also been observed in wheat (Schulthess et al., 1997) and in maize (Parentoni et al., 2010). The correlation between stover P concentration (SPC) and the P efficiency indices was also low or tended to be negative except for PER where it was significant under both Low (r= 0.37*) and high P

conditions (r=0.50**). These findings suggest that GPC and SPC may not be suitable criteria for determining P efficiency in maize.

Jones et al. (1992) and Fageria and Baligar (1999) also reported a lack of correlation between plant P concentration and P efficiency in wheat cultivars. Other studies such as (Zhu and Smith, 2001) have suggested that Seed P concentration can greatly affect plant performance under low P supply especially at early growth stages. According to Liao and Yan (1999), higher seed size and higher P concentration of seed can contribute to higher P efficiency in larger crops like bean, and therefore, should be considered in evaluation of genotypes for P efficiency. In contrast, this study did not find significant correlation between grain P concentration and P efficiency parameters studied indicating that genotypic variation for P efficiency found in the present study is inherent and not related to seed P concentration. However grain yield at both low (r= 0.45*) and high P supply (r= 0.43*) significantly correlated with stover P content although the correlation with grain P content was negative and non-significant at both P levels. Seemingly, grain P content, like grain P concentration, had a minimal contribution to differential P efficiency in all genotypes. These results imply no or very low contribution of seed P reserves to the presented variation in P efficiency observed in maize. The results of this study further compares well with those of Oztuk et al. (2005); Seperhr et al. (2009) Parentoni et al. (2010) who also reported minimal contribution of seed P reserve to P tolerance variation in Wheat, maize, Barley and Oat genotypes.

Table 11a: Correlation between Grain yield and other agronomic traits of maize hybrids across four locations under high P.

	PHT (cm)	STV (t/ha)	GYLD (t/ha)	GPC (%)	STPC (%)	GPCNT kg/ha	STPCNT kg/ha	AE Kg/Kg	PER Kg/Kg	PAE KgP/kgf	PE (%)	PUE Kg/Kg
PHT	-											
STV	0.7	-										
GYLD	0.82***	0.60***	-									
GPC	0.33	0.33	0.18	-								
STPC	0.37*	0.34*	0.22	0.35*	-							
GPCNT	-0.27	-0.21	-0.031	0.087	-0.32	-						
STPCNT	0.26	0.1	0.43*	0.29	0.76***	0.053	-					
AE	0.35*	0.44*	0.59***	0.34	0.36*	0.00031	0.076	-				
PER	0.71***	0.49**	0.58***	0.26	0.50**	_0.77***	0.21	0.27	-			
PAE	0.3	0.21	0.44*	0.039	0.6	-0.05	0.69	0.37	0.25	-		
PE	0.41*	0.045	0.35*	-0.051	-0.018	-0.023	0.11	_0.52**	0.25	-0.22	-	
PUE	0.29	0.37*	0.54*	0.11	0.27	-0.33	-0.045	0.80***	0.39*	0.045	_0.43*	-

NB.PHT-plant height, STV-stover yield, GYLD-grain yield, GPC- grain P concentration, STPC-stover P concentration, GPCNT-grain P content, STVPCNT-stover P content, AE-agronomic efficiency, PER- phosphorus efficiency ratio, PAE-P acquisition efficiency, PE-phosphorus efficiency, PUE- phosphorus utilization efficiency

Table 11b: Correlation between Grain yield and other P-efficiency traits of maize hybrids across four locations under low P

	PHT (cm)	STV (t/ha)	GYLD (t/ha)	GPC (%)	STPC (%)	GPCNT kg/ha	STPCNT kg/ha	AE Kg/Kg	PER Kg/Kg	PAE KgP/kgf	PE (%)	PUE Kg/Kg
PHT	-											
STV	0.78***	-										
GYLD	0.77***	0.71***	-									
GPC	0.09	0.13	0.085	-								
STPC	0.17	0.32	0.25	0.043	-							
GPCNT	-0.32	-0.26	-0.095	0.073	-0.084	-						
STPCNT	-0.1	-0.064	0.45*	0.076	0.46**	0.48**	-					
AE	0.37*	0.39*	0.52**	0.22	-0.036	-0.13	-0.22	-				
PER	0.61**	0.57**	0.57**	0.077	0.37*	-0.80***	-0.176	0.23	-			
PAE	0.24	0.27	0.36*	-0.13	-0.17	-0.3	-0.0038	0.38*	0.27	-		
PE	0.41*	0.27	0.68***	-0.073	0.30	0.041	0.36*	_0.52**	0.25	-0.22	-	
PUE	0.35*	0.32	0.34*	0.35*	-0.1	-0.24	-0.34	0.80***	0.39*	0.05	_0.43*	-

NB.PHT-plant height, STV-stover yield, GYLD-grain yield, GPC- grain P concentration, STPC-stover P concentration, GPCNT-grain P content, STVPCNT- stover P content, AE-agronomic efficiency, PER- phosphorus efficiency ratio, PAE- P acquisition efficiency, PE-phosphorus efficiency, PUE- phosphorus utilization efficiency

The better relationship between stover P content and P efficiency traits of genotypes may indicate a contribution of enhanced P uptake in expression of high P efficiency in studies where the total amount of P per shoot or per plant (shoot or stover P content) is considered as 'P uptake'. Such studies include: Gill et al. (1994); Jones et al. (1992) and Fageria and Baligar (1999).

In most cases, genotypes showing higher P efficiency traits (PE, PAE, PUE, AE, PER) had higher grain yield production under low supply of P. Consequently, their correlation with the grain yield of genotypes at low P supply was highly significant. (PE & GYLD r = 0.68***, AE & GYLD r= 0.52**, PAE & GYLD, r=0.36* and PUE & GYLD r= 0.34*). These correlations were equally significant at high P level. From the correlation analysis presented in Table 11 a and b, grain yield under P deficiency appears to be the most reliable parameter for screening genotypes for P efficiency and this finding is in agreement with that of many researchers (Jiang et al., 2010; Ligeyo et al., 2014; Parentoni et al., 2010).

3.5.9. Heritability and genetic correlation between grain yield and other agronomic traits.

Low, medium and high estimates of broad sense heritability (H^2) were measured for different plant traits (Table 12). This may be an indicator of the modifying effects of the various locations and the presence of genotype by environment interactions (GXE) in determining H^2. For grain yield under high P, the highest heritability was attained at Koyonzo (0.94) while the lowest was at Chepkoilel (0.89).Under low P, the highest H^2 was realized at Chepkoilel (0.91) and was lowest at Migori (0.89). These results compare

well with results from other researchers (Aminu and Izge, 2012 and Hasib, 2012). Similar studies by Hasib (2005) reported highest estimated H^2 in grain yield (0.993) and plant height (0.90) of rice among the traits under study. Overall, moderate values for H^2 were measured for internode Length, days to 50% anthesis and days to 50% silking. Studies by Olakojo and Olaoye (2011) and Wannows (2010) also reported moderate heritability for these traits in maize hybrids. Moderate to high estimate of broad sense heritability of the various traits reported in this study showed that a large proportion of the observed variations were transmissible to the subsequent generations and indicated the potential for developing high yielding varieties through selection. Broad sense heritability was generally higher under low P compared to high P conditions across the four locations although this was not consistent for all the traits. This is an indication that selection for tolerance to low phosphorus is more feasible under low P compared to high P conditions. Under low P, Ear height exhibited the highest heritability (0.87) followed by grain yield (0.85) while the lowest heritability was recorded in grain and stover P concentration. This shows that grain and stover P concentration was greatly affected by the confounding environmental variations. This observation was expected due to the variations in soil available P among the locations. The implication is that the duo traits may not be suitable criteria for selections under P deficient soils.

Table 12: Heritability of maize hybrids in 4 locations

Location	Phosphorus level	PHT Cm	EHT cm	INT cm	STV t/ha	DANTH days	DASLK days	GYLD t/ha	GPCNT Kg/ha
Chepkoilel	36 kgP/ha	0.88	0.84	0.63	0.95	0.90	0.78	0.89	0.59
	6 kgP/ha	0.95	0.96	0.69	0.95	0.95	0.80	0.91	0.70
Migori	36 kgP/ha	0.87	0.91	0.53	0.92	0.75	0.72	0.92	0.781
	6 kgP/ha	0.88	0.88	0.71	0.9	0.77	0.76	0.890	0.696
Koyonzo	36 kgP/ha	0.88	0.83	0.24	0.71	0.82	0.76	0.94	0.89
	6 kgP/ha	0.77	0.74	0.18	0.87	0.20	0.38	0.90	0.79
Sega	36 kgP/ha	0.90	0.87	0.82	0.88	0.62	0.49	0.92	0.83
	6 kgP/ha	0.91	0.85	0.78	0.88	0.20	0.38	0.90	0.78

GYLD-grain yield, PHT-plant height, STV-stover yield, DANTH-days to 50% anthesis, DSLK-Days to 50% silking, STVPCNT- stover P content, GPCNT-grain P content

Genetic correlations between trait pairs were significantly different among the tested maize experimental hybrids under the 2 P conditions. Under Low P, grain yield (GYD) was highly correlated with plant height (r_g = 0.72**) ear height (r_g = 0.54*), internode Length (r_g = 0.73**), cob Length (r_g =0.81***) and stover yield (r_g = 0.61**) (Table 13). However grain yield was negatively correlated with days to anthesis and silking. GYD also exhibited high positive correlation with grain P content (r_g = 0.90***). Under high P conditions greater magnitudes of the genetic correlation coefficient (r_g) were observed for PHT (0.74**), EHT (0.56*) and Cob L (0.56*) while the r_g values were lesser in magnitude for STV (0.54*), days to anthesis (-0.16) and days to silking (-0.15) (Table 14). GYD was also positively correlated with Root Length Density (RLD) at both P levels although the correlations were higher at high P (r_g= 0.37*) compared to low P (r_g=0.34) (Fig 4a & b). These results agree with those of Manske (2000) who reported positive correlation between GYD and RLD in wheat.These authors also reported higher correlation under high P compared to low P conditions.

Table13: Genetic Correlations between Grain yield and agronomic traits of maize hybrids in four locations under low P

	PHT (cm)	EHT (cm)	INTL (cm)	CobL (cm)	STV (t/ha)	DANTH (days)	DSLK (days)	GYD (t/ha)	STVP (%)	GRP (%)	GPCNT (Kg/ha)
PLHT (cm)	-										
EHT (cm)	0.85***	-									
INTL (cm)	0.88***	0.78**	-								
CobL (cm)	0.74**	0.63**	0.71**	-							
SYLD (t/ha)	0.77**	0.80***	0.72	0.65	-						
DANTH (days)	–0.23	–0.01	–0.33	–0.31	0.006	-					
DSLK (days)	–0.27	–0.05	–0.40*	–0.40*	–0.03	0.95***	-				
GYD (t/ha)	0.72**	0.54*	0.73**	0.81***	0.61**	–0.36	–0.44*	-			
STVP (%)	-	-	-	-	-	-	-	-	-		
GRP (%)	-	-	-	-	-	-	-	-	-	-	
GPCNT (Kg/ha)	0.72**	0.57*	0.73**	0.75**	0.67**	–0.39	–0.45*	0.90***	-	-	-
STVP CNT(Kg/ha)	0.48*	0.62**	0.41*	0.45*	0.87***	0.26	0.24	0.37	-	-	0.41*

Note. PHT-plant height, EHT-Ear height, INTL-Internode length, Cob L-Cob length, STV-stover yield, DANTH-days to 50% anthesis, DSLK-Days to 50% silking, GYD-grain yield , STVP-stover P concentration, GRP- grain P concentration, GPCNT-grain P content, STVPCNT- stover P content.

Table14: Genetic correlations between Grain yield and agronomic traits of maize hybrids across four locations under high P

	PHT (cm)	EHT (cm)	INTL (cm)	Cob L (cm)	STV (t/ha)	DANTH (days)	DSLK (days)	GYLD (t/ha)	STVP (%)	GPCNT (Kg/ha)
PLHT (cm)	-									
EHT (cm)	0.80***	-								
INTL (cm)	0.78**	0.54*	-							
CobL (cm)	0.77**	0.79**	0.65*	-						
SYLD (t/ha)	0.71**	0.75**	0.50*	0.62**	-					
DANTH (days)	–0.11	0.069	–0.22	–0.034	0.21	-				
DSLK (days)	–0.086	0.15	–0.20	0.046*	0.21	0.93***	-			
GYD (t/ha)	0.74**	0.56*	0.65**	0.81***	0.54*	–0.16	–0.15	-		
STVP (%)	–0.28	–0.13	–0.35	–0.24	0.12	0.40*	0.43*	–0.35	-	
GPCNT (Kg/ha)	0.69**	0.52*	0.65**	0.79*	0.47*	–0.10	–0.12	0.95***	–0.29	-
STVPCNT (Kg/ha)	0.25	0.39	0.055	0.20	0.72**	0.45*	0.45*	0.042	0.73	0.037

Note.PHT-plant height, EHT-Ear height, INTL-Internode length, Cob L-Cob length, STV-stover yield, DANTH-days to 50% anthesis, DSLK-Days to 50% silking, GYLD-grain yield , STVP-stover P concentration, GRP- grain P concentration, GPCNT-grain P content, STVPCNT- stover P content

These findings also agree well with those of Aminu and Izge, (2012); Rafiq et al. (2010) and Mohan et al. (2002) who reported significant genetic correlation between GYD in maize and other agronomic attributes such as plant height, ear height and days to 50% flowering. However the results of the present study contrast those of Sumathi et al. (2005) who reported low genetic correlation between plant height and GYD. Under both low and high P conditions, there was no genetic correlation between GYD and both grain P and stover P concentration implying that both grain and stover P concentration are not suitable indices for selecting maize for tolerance to low P. According to Yasien (1993), genetic correlation is the heritable association between two variables. It facilitates reliance on other parameters while selecting for others. The extent of reliability in such a selection therefore depends on the degree of the genetic correlation between the traits in question. From this study therefore selection for any of the above traits which are significantly correlated with GYD will lead to indirect selection for GYD under high and low P conditions.

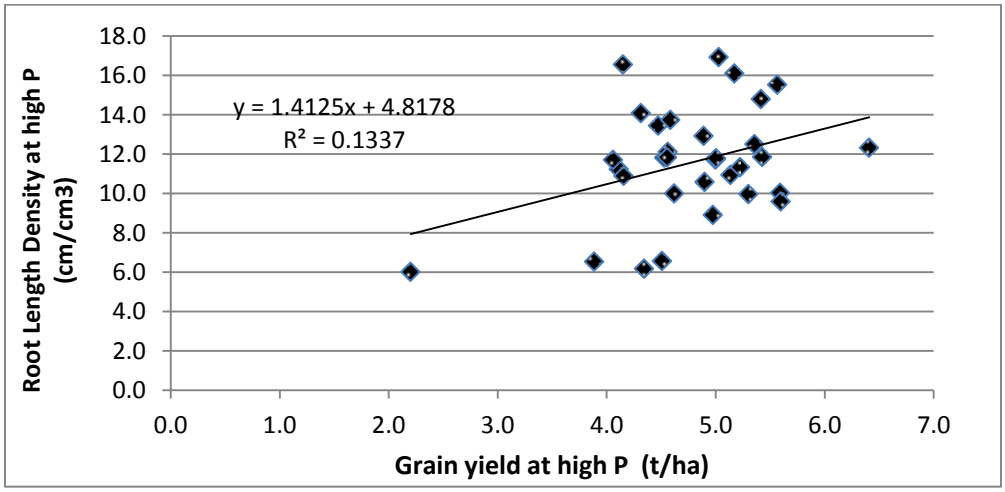

Figure 4a: Genetic Correlation between GYLD and RLD of maize hybrids in high P

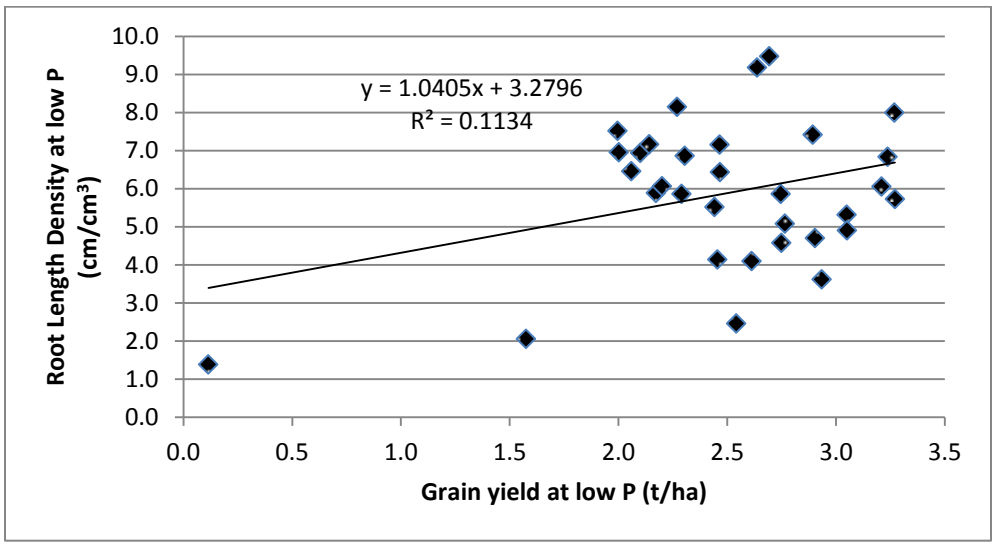

Fig 4b: Genetic Correlation between GYLD and RLD of maize hybrids tested in low P

3.10. Conclusions and Recomendations

This study has developed and evaluated 30 experimental maize hybrids out of which 20 were selected as very suitable for growing in low P soils of western Kenya based on the genetic variation in P efficiency that existed amongst the hybrids both at low P supply and in response to P application. The correlation between grain and stover P concentration, grain P content with majority of the P efficiency indices (PAE, PE, PUE) at both high and low P supply was always low or tended to be negative and non-significant implying that seed P reserve, and stover P concentration, had minimal or no contribution to differential P efficiency observed in all genotypes and may not be suitable criteria for determining P efficiency in maize. The natural genetic variation observed between the maize genotypes demonstrates the potential for breeding cultivars with

improved phosphorus efficiency. The study recommends further testing of these hybrids for consideration for release in Kenya.

CHAPTER FOUR

4.0. INHERITANCE OF MAIZE P EFFICIENCY IN ACID AND NON ACID SOILS OF WESTERN KENYA.

4.1 Abstract

Soil acidity is a major constraint to maize (*Zea mays* L.) productivity in tropical soils due to toxic levels of aluminium (Al) and phosphorus (P) deficiency that hinder maize growth and development. Breeding programs for acid-soil tolerance are desirable as a relatively inexpensive and sustainable way for increasing maize yields on these soils. The objectives of this study were to: (i) investigate the inheritance of traits associated with phosphorus efficiency in maize (ii) compare the genetic control of maize P efficiency traits in acid and non-acid soils. Six F1 single crosses derived from acid soil tolerant and susceptible lines were used in this study. The parental inbred lines, the F1's, F2's, BC1P1, BC1P2, from each of the six crosses were evaluated in two low P acid and two low P non-acid soils of western Kenya. P efficiency traits evaluated included: shoot dry matter (SDM), grain yield (GYLD), plant height (PHT), root length density (RLD), P acquisition efficiency (PAE) and P utilization efficiency (PUE) and phosphorus efficiency (PE) For each trait, mean genetic effect (m), additive genetic effects (a), dominant genetic effects (d) and epistatic digenic effects (aa, ad, dd) were estimated. Means for all the traits studied were significantly higher at high P conditions in non-acid soils compared to acid soils for all the generations. However, under low P conditions, certain traits (eg. SDM) exhibited higher means in acid compared to non-acid soils. Highest phosphorus efficiency (PE) was exhibited by the F1s in both acid and non-acid soils although it was higher in non-acid (59%) compared to acid soils (54 %). There was higher reduction in PE in acid soils (25-50%) compared to non-acid soils (15 to 30%). In both acid and non-acid soils with low P, single cross hybrids showed high PE and also exhibited high SDM. Mean heritability was generally higher under non-acid soils compared to acid soils although this depended on the generation, the trait and the level of P applied. Additive and non-additive effects were detected in both acid and non-acids soils for majority of the traits examined although this also was dependent on the trait studied and the level of P supplied. For most of the traits, the major part of the variation was accounted for by dominance followed by epistatic and then additive genetic effects in both acid and non-acid soils. The magnitude of both additive and non-additive gene effects were always greater in non-acid soils compared to acid soils pointing to the detrimental effects of soil acidity on the detection of gene actions in maize. These results suggest that the inheritance of GYLD and other P efficiency traits did not differ in acid and non-acid soils even though soil acidity affected the magnitude of the gene effects.

Key words: acid soils, additive, dominant, epistatic effects, generation analysis, maize.

4.2 Introduction

Maize (*Zea mays* L) is the most cultivated cereal in the world and is widely used for animal feed, human food and industrial purposes. Maize is a major staple food crop for most people in developing countries, mainly in Africa and Latin America where soil acidity and low available P are common (Lopes and Lakirns, 1991; Awika, 2011). Its yields are low in acid soils mainly because of Al toxicity and P deficiency (Kochian et al., 2015). Soil acidity covers extensive areas in tropical, subtropical and temperate zones and occurs in 30-40% of the world's arable soils (Von Uexkull and Mutert, 1995). They are found mainly in South America (26.7%), North America (19.4%), Africa (19.1%) and Asia (15.1%). The rest occur in Australia and New Zealand, Europe and Central America (Eswaran et al., 1997). Some acid soils contain levels of aluminium that are toxic to plants. Al toxicity limits plant growth mainly through its adverse effects on root growth and development besides reducing the agronomic and recovery efficiencies of nutrients such as P, N and Ca by plants (Giller et al., 2002). Phosphorus starvation leads to stunted growth, thin and spindly stems with purpling of leaves. Severe effects include yellowing of leaves with early leaf senescence, delayed maturity, sparse flowering and reduced grain yields of between 20-50% (OlSen., 1972; Parentoni et al., 2010; Ouma et al., 2012; Ligeyo et al., 2014).

Different strategies have been suggested to improve the productivity of these soils including lime application, P replenishment through inorganic P sources and the development of tolerant cultivars (Pandey et al., 2007; Kisinyo et al., 2013a; Ouma et al., 2013; Ligeyo et al., 2014). All these authors have considered the latter approach as the most suitable, sustainable and cost effective because of the enormous genetic variation

for tolerance to soil acidity that has been reported in several studies using different germplasm, traits and genetic analysis methods. Duncan et al. (1994) reported additive, dominance, and epistatic effects for P efficiency in maize with additive effects being more important whileChaubey et al. (1994) and Furlani et al. (1998) reported the importance of both additive and dominance effects in controlling maize P efficiency traits. Other reports by Parentoni et al. (2006) and Chen et al. (2009) showed non-additive effects to be more important than additive effects for tolerance to low P soils. Additionally, Narro et al. (2000) reported that dominance variance was either similar to or of greater importance than additive variance for yield in acid soils. Further results from diallel cross studies carried out in acid soils by Magnavaca et al. (1987a); Pandey et al. (1994); Salazar et al. (1997) have shown that for grain yield, additive effects accounted for the major part of the total genetic variance, although non-additive effects were also significant.

Generation means analysis has also been used by several authors to estimate genetic effects from crosses between maize inbreds with different levels of tolerance to acid soil (Magnavaca et al., 1987b; Ceballos et al., 1998; Pandey et al., 2007; Vasquelez et al., 2008; Parentoni et al., 2010). Vasqualez et al. (2008) reported that dominance followed by additive effects were more important for grain yield, plant height and days to anthesis in both acid and non-acid soils. According to studies by Pandey et al. (2007), both additive and dominance effects were more important than epistatic effects in the inheritance of grain yield in acid soils. Ceballos et al. (1998) further reported that for grain yield the additive-dominance model accounted for 91.1% of the variation in non-acid soils and 70.0% of the variation in acid soils, and that epistatic effects were more

important in acid than in non- acid soils. These studies give hope that selection for P efficiency under acid and non-acid soils is possible.

Overall, the inheritance of several important traits in maize evaluated under non-acid soils has been well documented (Hallauer et al., 1988; Hallauer and Miranda Filho, 1988; Duncan et al., 1994; Chaubey et al., 1994; Furlani et al., 1998). However information on the inheritance patterns of maize agronomic traits especially those related to P efficiency in acid soils is still inadequate given that the area under maize production in acid soils is substantial especially in South America, North America, Africa and Asia. Besides, with increased use of inorganic P-based fertilizers routinely in agricultural systems to overcome P- deficiency and to restore soil fertility, it is expected that the world acid soil area will increase in the future because some of the available inorganic P sources contribute to further soil acidification (Clotta et al., 2002).

A clear selection criteria for P efficiency in acid and non-acid soils and a better understanding of their genetic control is still limiting yet this information is crusual in selecting for target traits for yield improvement, QTLs mapping studies and also for the choice of adequate mapping populations. For example, recombinant inbred lines would be more appropriate for traits which are predominantly inherited by additivity, or by additive x additive epistasis, while predominantly non-additive inherited traits should preferentially be mapped in top-crosses or F2-F3 populations. Therefore further efforts to elucidate the genetic basis and the inheritance of tolerance to low P in maize under both acid and non-acid soils is expected to accelerate the development of P efficient maize cultivars. The objectives of this study were to (i) investigate the inheritance of traits

associated with to phosphorus efficiency in maize (ii) compare the genetic control of maize P efficiency traits in acid and non-acid soils.

4.3 Materials and Methods

4.3.1 Experimental Design and Field Evaluation

A total of six single crosses (KML 036 X MUL 229, HSL3 X 5046-2 X S396-16-1, KML 036 X S396-16-1, HSL3 X 5046-2XMUL 229, HS 228 X S 396-16-1 and HS 228 X MUL 229) were used to estimate the genetic effects under low P acid soils at (Sega and Chepkoilel sites) and low P non-acid soils at Migori and Koyonzo based on procedures described by Gambles (1962). The parents were selected based on tolerance to low P conditions and combining ability (Ouma et al., 2013). For each cross, the F1 was advanced by selfing to obtain F2 generation. Backcross 1 to each parent (BC1P1 and BC1P2), were also obtained by crossing the F1 for each cross with each of its 2 parents, with the F1 as the female parent. At least 10 ears were saved and balance bulked to represent each generation. The pollination procedure and the experimental sites have been described in section 3.31 and 3.37. A total of 23 maize genotypes comprising 6 backcrosses (BC1), 6 F1 single crosses (SCH), 5 parental lines and 6 F2s were evaluated for tolerance to low P in a replicated trial at Sega, Chepkoilel, Migori and Koyonzo sites during the long rains of 2013. The experiment was laid out in an RCBD replicated three times. Treatment consisted of the 24 genotypes and 2 levels of P described as low P (6 KgP/ha) and high P (36 KgP/ha) supplied as TSP). A two row plot measuring three metres long, with inter and intra-row spacing 0.75 m x 0.30 m for each generation except the F2 where four row plots were used. Two seeds were sown per hill and later thinned to

one. Genstat software (Payne et al., 2014) was used to generate the randomization and field layout. All the plots were side-dressed using calcium Ammonium Nitrate (CAN) at the rate of 75 Kg N/ha.Standard agronomic practices were followed to maintain the experimental plots.

4.3.2 Data collection

Root length density (RLD), shoot dry matter (SDM), phosphorus utilization efficiency (PUE), Shoot P concentration (SPC) and P efficiency (PE) were measured at anthesis. Destructive sampling was done on 7 randomly selected plants according to Bell and Fischer, 1994 for all generations except F2 where 15 samples were analysed. Root sampling was done using the root box technique as described by Vepraskas and Hoyt (1988) and Manske (2002) in order to determine Root length per unit of soil volume (root length density). The line-intercept method developed by Newman (1966) and modified by Tennant (1975) was used to determine root length density. This method counts the total number of intersections between roots and the vertical and horizontal lines of a grid drawn on a Petri dish. Root length was estimated by the equation: R = N x fresh weight of total sample/ fresh weight of counted subsample. Where R is the total root length density, and N is the number of intersections between the roots and random horizontal grid lines in the petridish. Shoot samples were oven dried at 80^{o}C, ground and ashed at 550 ◦C for determination of P concentration in the whole shoot. The ground samples were then dissolved in 3.3% HCl and analyzed for P by using the method of Barton (1948). Based on shoot dry matter yield, and on P concentration in these plant components, the phosphorus content in the shoot and PUE were determined using the method of

Hammond et al. (2009). The P efficiency ratio (relative shoot growth) was calculated as the ratio of shoot dry matter production under low P to that under adequate P supply. At maturity, data was collected on grain yield, (GYLD-t/ha), plant height (PHT-cm); PHT was recorded in 10 competitive plants per plot, from the soil surface to the tip of the highest tassel branch, and the plot means used for analysis. Stover samples were collected, oven dried at 80^{o}C to constant weight ground and analyzed for P concentration analysis using the vanadomolybdate method (Westerman, 1990). Based on grain yields, and P concentration in these plant tissues, the P efficiency parameters were determined on a plot basis according to Hammond et al. (2009), Parentoni et al. (2010); and Moll et al. (1982) as follows:

a. Agronomic P use efficiency (APE) =Y_{high}–Y_{low})/D_{Papp} (kg/Kg Pf)

b. P utilization efficiency (PUE) = (Y_{high}–Y_{low})/[(P_{high}xY_{high})–(P_{low} xY_{low})] (kg/ kg)

c. P efficiency ratio (PER) = Y_{high}/(P_{high}xY_{high}) or Y_{low}/(P_{low}xY_{low}) kg/kg

d. Phosphorus Efficiency (PE) = $Y_{low/}$ Y_{high} x 100%

Where: Y_{high}-yield on a high P or fertilized soil; Y_{low} -yield on a low P or unfertilized soil; P_{high}-tissue P concentration on a high Por fertilized soil; P_{low}-tissue P concentration on a low P or unfertilized soil; D_{Papp}-difference in amount of P applied as fertilizer between high and low P treatments; Pf- P fertilizer.

4.3.3. Data Analysis

Generation means for each cross and P treatment were used to estimate the gene effects according to the Malther and Jinks (1982) model following Gamble's (1962) notation to define the genetic parameters in the model. This model is as follows:

$Y_k = m + \alpha a + \beta d + \alpha^2 aa + \alpha\beta ad + \beta^2 dd$, where α and β are the coefficients for additive and dominance effects,

Y_k = the observed mean across locations of the k^{th} generation

m = mean of all possible homozygous locus, considering all locus controlling the trait;

a = pooled additive effects

d = pooled dominance effects

aa = additive x additive gene interaction effects

ad = additive x dominance gene interaction effects

dd = dominance x dominance gene interaction effects

The expectation of the generation mean parameters are therefore as follows:

m = mean of all possible homozygous loci, considering all locus controlling the trait;

a = BCP1- BCP2

d = F1-4F2-0.5P1 -0.5P2 +2BCP1 + 2BCP2

aa = 2BCP1 +2BCP2 – 4F2

ad = BCP1 -0.5P1- BCP2 + 0.5P2

dd = P1 + P2 + 2 F1 + 4F2 – 4BCP1 – 4BCP2

Estimates of additive, dominance and epistatic effects were computed for each cross by weighted least square regression analysis (Malther and Jinks, 1982) using the equation $b = (X' D^{-1X})^{-1}(X' D^{-1}y)$, where b is the vector of genetic effects (m, a, d, aa, ad, and dd), X is the incidence matrix of the genetic effects coefficients (α, β, α^2, $\alpha\beta$, and β^2), y is the column vector of the generation means and D^{-1} is a weighted diagonal matrix, where the diagonal elements were the reciprocals of the variances of each generation mean computed for each generation (P's, F1's, F2's, and BC's). A mixed general linear model

of SAS (PROC MIXED) was used to estimate the genetic effects from the generation means of each cross at each P level and combined over locations. F test of the sum of squares for the genetic effects was used to reduce the model appropriately. In the selected model, genetic parameters having significant effects were included and all the non-significant parameters excluded from the model. For each cross in each trait, the ratios a/m, d/m and epistasis/m were calculated using absolute values. Only data where the parameter estimates (a, d and epistasis) were significant were used in these calculations. For each trait and at the two P levels across the locations, a general mean of the ratios a/m and d/m was calculated using data from all crosses with significant effects.

4.5. Results

4.5.1. Means and heritabilities of P efficiency traits at Chepkoilel and Sega sites

In low phosphorus (P) acid soils, shoot dry matter yields (SDM) were significantly higher at high P compared to low P ones for all the generations tested (Parents, F1s, BC1s and F2s). Higher P supply increased mean SDM from 0.17 to 0. 25 kg per plant in the parentals, 0.26 to 0.46 kg/plant, in the F1s, 0.25 to 0.45 kg/plant for the backcrosses and 0.13 to 0.26 kg/plant for the F2s (Table 15). The F1s attained the highest SDM under both high (0.46 kg/plant) and low P (0.26 kg/plant) supplies while the parental lines yielded the least (0.25 kg/plant) under high P while the F2s gave the least under low P (0.13). Mean broad sense heritability (H^2) for SDM was generally higher under high P compared to low P conditions for all the generations except for the F2s where the reverse was true. The F1s exhibited the highest heritability at high (0.603) and low P conditions (0.57) (Table 15). The highest mean root length density (RLD) was obtained in the F1 at high P (10.23 cm/cm^3) and lowest in parentals (6.66 cm/cm^3) while the backcrosses

exhibited the highest RLD (6.37 cm/cm^3) under low P, although this was not significantly different from the score for the F1s. The highest heritability for RLD was obtained in the backcrosses while lowest in the F2s. Low P supply resulted in a significant reduction (23 to 50%) in shoot P concentration of genotypes in all the generation. Backcrosses exhibited the highest mean PUE (559.28 gSDM/gP) while parentals the least (520.9 gSDM/gP) although the difference was not large. Genotypes showing higher P efficiency (PE) had higher SDM production under low P supply. The highest mean PE (54%) was measured in the F1s that also exhibited very high mean SDM under low P conditions.

Table 15: Mean SDM, RLD, and PC of maize genotypes at Sega and Chepkoilel sites.

ENTRY	SDM (kg/plant)		RLD (cm/cm3)		PC (%)		PUTE	PE
	P36	P6	P36	P6	P36	P6	gSDM/gP	%
S1 (Parents)	0.248	0.185	5.657	3.244	0.163	0.126	570	48
K1	0.278	0.187	7.69	4.407	0.161	0.107	488	51
H2	0.253	0.172	7.455	3.349	0.17	0.13	382	56
H1	0.273	0.185	8.391	4.572	0.142	0.117	582	50
H3	0.176	0.13	5.422	3.611	0.157	0.121	566	48
M1	0.245	0.143	5.359	3.569	0.153	0.12	537	42
MEAN	0.25	0.17	6.66	3.79	0.16	0.12	520.98	49.22
H2	0.581	0.395	0.227	0.607	0.645	0.404		
SE	0.02	0.02	1.162	0.854	0.01	0.006		
LSD (0.05)	0.016	0.011	0.77	0.47	0.01	0.008	35.2	
K1XS1 (F1s)	0.485	0.253	8.457	6.455	0.149	0.125	583	48
H1XS1	0.452	0.287	9.372	6.147	0.144	0.116	463	59
H2XS1	0.46	0.233	8.591	5.011	0.155	0.117	588	45
H1XM1	0.385	0.237	10.974	5.435	0.156	0.11	491	65
H2XM1	0.477	0.252	9.331	5.844	0.133	0.127	567	48
K1XM1	0.518	0.297	14.681	8.898	0.139	0.103	474	60
MEAN	0.46	0.26	10.23	6.3	0.15	0.116	527.66	54.03
H2	0.603	0.576	0.638	0.583	0.1	0.441		
SE	0.025	0.019	1.009	0.821	0.006	0.005		
LSD (0.05)	0.038	0.021	0.79	0.48	0.01	0.008	37.4	
H1XS1*H1(B	0.458	0.242	13.255	6.759	0.158	0.127	589	52
H1XS1*S1	0.493	0.222	8.844	6.016	0.167	0.112	773	40
K1XS1*K1	0.46	0.278	8.989	4.944	0.156	0.13	641	58
K1XM1*K1	0.447	0.27	9.899	8.202	0.157	0.131	538	56
K1XM1*M1	0.388	0.186	11.226	8.339	0.162	0.12	602	45
H2XM1*H2	0.418	0.232	7.27	5.627	0.179	0.114	406	51
H1XM1*M1	0.487	0.247	9.516	5.828	0.156	0.133	553	52
H1XM1*H1	0.412	0.262	7.135	5.734	0.266	0.117	384	57
H2XS1*H2	0.467	0.277	11.198	5.856	0.167	0.116	548	56
MEAN	0.45a	0.25a	9.70b	6.37a	0.17	0.12	559.28	51.84
H2	0.749	0.471	0.67	0.721	0.1	0.384		
SE	0.02	0.016	0.546	0.156	0.019	0.006		
LSD (0.05)	0.029	0.017	0.7	0.63	0.009	0.0075	36.8	
K1XM1(F2 s)	0.26	0.135	4.626	3.862	0.141	0.117	689	42
H1XM1	0.31	0.175	7.394	4.905	0.16	0.1	492	50
H1XS1	0.243	0.133	8.25	4.44	0.145	0.128	391	50
K1XS1	0.27	0.113	7.341	5.01	0.145	0.098	689	40
H2XM1	0.23	0.147	10.714	5.184	0.143	0.115	294	62
H2XS1	0.231	0.087	6.197	4.271	0.149	0.112	682	49
MEAN	0.26	0.13	7.42	4.61	0.15	0.11	539.38	49.02
H2	0.407	0.549	0.417	0.504	0.256	0.15		
SE	0.019	0.015	1.212	0.598	0.006	0.006		
LSD (0.05)	0.017	0.008	0.9	0.51	0.008	0.006	40.4	

Shoot dry matter (SDM), root length Density, (RLD) and P concentration (PC), H1-HSL3 X 5046-2, H2-HS 228, M1-MUL 229, S1-S396-16-1

4.5.2. Means and heritabilities of P efficiency traits at Migori and Koyonzo sites.

All the studied traits showed significant variations among the genotypes and between the generations (Table 16). Under low P non-acid soils, SDM yields were significantly higher under high P compared to low ones for all the generations. Mean SDM was increased at least two folds by the application of higher P in all the generations. The F1s attained the highest SDM under both high (0.52 kg/plant) and low P (0.229 kgP/plant) supplies while the F2s yielded the lowest under high P (0.24 kg/plant) and low P (0.12 kg/plant). Mean broad sense heritability for SDM did not show a clear pattern. It was higher under low P for parentals and F1s while under high P, it was higher for backcrosses and the F2 generation. The BC1 exhibited the highest heritability i.e o.757 and 0.716 under high and low P respectively (Table 16). Under high P supply, the highest RLD mean was obtained in the BC1 (20 cm/cm^3) and the lowest in the F2 generation (11.87 cm/cm^3) while at low P, the backcrosses exhibited the highest RLD (14.1 cm/cm^3) and the F2 generation the lowest (8.16 cm/cm^3). The highest mean H^2 was obtained in the F1s and BC1s under high and low P respectively while the lowest in the F2s. Low P supply resulted in a significant reduction (15.5 to 31%) in shoot P concentration (SPC) of genotypes in all the generations. In the case of P utilization efficiency (PUE), backcrosses exhibited the highest mean PUE (645.1 gSDM/gP) while parentals the least (485.88 gSDM/gP). Genotypes showing higher P efficiency (PE) had higher shoot dry matter production under low supply of P. The highest mean PE (59%) was measured in the F1s that also exhibited high mean SDM under low P conditions.

Table 16: Mean SDM, RLD, PC and PCNT of maize genotypes at migori and Koonzo sites

ENTRY	SDM (kg/plant)		RTLD (cm/cm3)		PC (%)		PUTE	PE
	P36	P6	P36	P6	P36	P6	gSDM/gP	%
S1 (Parents)	0.353	0.107	11.430	8.074	0.177	0.133	409	43
K1	0.258	0.120	12.906	10.650	0.174	0.163	693	53
H2	0.362	0.157	13.376	6.730	0.159	0.145	608	66
H1	0.338	0.217	13.091	10.958	0.165	0.133	307	65
H3	0.277	0.140	11.126	10.644	0.155	0.160	557	58
M1	0.262	0.140	12.704	7.489	0.134	0.110	341	54
MEAN	0.31	0.15	12.44	9.09	0.161	0.141	485.88	56.53
H2	0.391	0.703	0.718	0.485	0.591	0.30		
SE	0.035	0.024	0.868	0.703	0.009	0.026		
Lsd (0.05)	0.022	0.0107	0.85	0.64	0.0115	0.010	35.14	
K1XS1 (F1s)	0.492	0.203	20.000	12.575	0.144	0.142	630	59
H1XS1	0.561	0.263	18.998	12.976	0.127	0.130	608	62
H2XS1	0.574	0.227	21.998	11.214	0.141	0.142	760	52
H1XM1	0.518	0.277	10.606	10.407	0.164	0.132	361	73
H2XM1	0.497	0.177	13.215	12.622	0.189	0.123	457	47
K1XM1	0.483	0.230	25.291	17.333	0.158	0.136	413	64
MEAN	0.521	0.229	18.351	12.855	0.154	0.134	538.298	59.432
H2	0.504	0.618	0.781	0.621	0.598	0.159		
SE	0.025	0.017	1.281	0.865	0.011	0.024		
Lsd (0.05)	0.025	0.015	1.42	0.85	0.0107	0.010	37.23	
H1XS1*H1(Bcs)	0.365	0.320	22.540	15.465	0.156	0.132	440	75
H1XS1*S1	0.378	0.240	17.778	9.570	0.141	0.113	659	70
K1XS1*K1	0.416	0.250	19.201	16.769	0.146	0.134	530	63
K1XM1*K1	0.462	0.237	27.044	18.140	0.150	0.116	694	58
K1XM1*M1	0.341	0.177	21.663	14.094	0.158	0.157	752	49
H2XM1*H2	0.418	0.187	19.928	14.680	0.166	0.140	716	49
H1XM1*M1	0.371	0.203	17.008	15.469	0.142	0.100	492	59
H1XM1*H1	0.362	0.167	16.801	13.777	0.162	0.102	874	47
H2XS1*H2	0.435	0.200	18.182	9.005	0.156	0.114	650	47
MEAN	0.394	0.220	20.016	14.108	0.153	0.123	645.107	57.406
H2	0.757	0.716	0.747	0.78	0.123	0.190		
SE	0.021	0.019	0.941	0.851	0.006	0.028		
Lsd (0.05)	0.034	0.025	1.33	0.75	0.011	0.007	46.07	
K1XM1(F2 s)	0.236	0.143	11.796	7.893	0.170	0.126	393	64
H1XM1	0.300	0.113	12.568	9.808	0.167	0.101	653	42
H1XS1	0.231	0.123	11.897	9.063	0.172	0.105	329	53
K1XS1	0.205	0.150	13.229	8.317	0.153	0.124	482	67
H2XM1	0.236	0.103	10.350	8.608	0.167	0.128	591	46
H2XS1	0.244	0.097	11.401	5.291	0.187	0.114	648	41
MEAN	0.242	0.122	11.873	8.163	0.169	0.117	515.892	52.290
H2	0.524	0.45	0.454	0.509	0.316	0.17		
SE	0.023	0.016	2.156	1.808	0.010	0.014		
Lsd (0.05)	0.0184	0.0093	0.91	0.63	0.013	0.0092	37.80	

H1-HSL3 X 5046-2, H2-HS 228, M1-MUL 229, S1-S396-16-1, SDM- shoot dry matter, RLD- root length density, PC- P concentration and PCNT- P content

4.5.3. Discussions

Maize genotypes differed significantly in shoot and root growth at both low P and higherP supply. Such substantial genetic variation in response to P deficiency and P supply was also observed in past studies for maize hybrids (Parentoni et al., 2010; Yan et al. 2014, Ligeyo et al., 2014), sorghum (Hufnagel et al., 2014; Leiser et al., 2014), *Brassica oleracea* (Hammond et al., 2009) and wheat (Oztuk et al., 2005). The application of high P fertilizer increased SDM, RLD, PE and PUE in both acid and non-acid soils because of the increased soil available P, which is necessary for healthy plant growth and high grain production. Soil P availability is critical for the early growth and development of maize as it affects root morphological and physiological characteristics that are important for eventual P uptake since P is immobile and often unavailable in most soils (Hajabbasi and Schumacher, 1994, Obura et al., 2008). Similar results have been reported in maize for increased root length density, grain yield, PE and PUE due to increased P application. (Hajabbasi and Schumacher 1994; Jiang et al., 2010) and in wheat (Monasterio et al., 2002; Oztuk et al., 2005). The increments in measured P efficiency traits measured were also due to the fact that P is involved in several key plant functions such as energy transfers, photosynthesis, transformation of sugar and starches, nutrient movement within the plants and transfer of genetic characteristics from one generation to the next (White and Hammond, 2008) and hence the increase in SDM, root growth and development. The results for RLD is however contrary to those of Cichy et al. (2009) who recorded a decrease in root growth as a result of increased P supply in bean recombinant inbred lines. The contrasting results could be explained by the difference in plant growth habits and adaptation between maize and bean.

There was a general reduction in SDM and other P efficiency traits in acid soils compared to non-acid soils. This was in addition to the higher reduction in PE in acid soils (25-50%) compared to non-acid soils (15 to 30%). These results compare well with those of Velasquez et al. (2008) who observed significant decrease in maize grain yields and plant height for parentals, F1, F2 and backcross generations. Manse et al. (2002) also reported decrease in RLD as a result of soil acidity in bread wheat genotypes. These observations can be attributed to the detrimental effects of soil acidity on maize performance, grain yield and other agronomic traits (Borrero et al. 1995, Ceballos et al., 1998, Parentoni et al., 2010; Kisinyo et al., 2013a; Ouma et al., 2013 and Ligeyo et al., 2014). Apart from the low available P, the crops in the acid soils most likely suffered an additional constraint mainly Al toxicity since there is high Al saturation at Chepkoilel and Sega (section 3.5.1). Aluminium toxicity causes a decrease in cell division in maize roots leading to restricted development and a subsequent reduction in nutrient and water uptake (Doncheva et al., 2005) hence reduced SDM, RLD and PE in acid soils. In both acid and non-acid soils, genotypes showing higher PE also exhibited higher SDM under low P suggesting that SDM is a suitable index for selecting maize genotypes for P efficiency at vegetative stage.

Lower broad sense heritabilities in acid soils were probably due to high experimental error and low generic variations depicted under such conditions. These findings compare well with those of Ceccarelli (1994) who obtained lower heritabilities estimates under stress environments. However, in some cases in the present study, higher heritabilities were reported in acid soils compared to non-acid soils. This is probably because of differences in genetic constitution of various genotypes and genetic control in different

traits. This could be true considering that some of the parental (K1 and H1) inbred lines included in this trial were P efficient and hence considered to be well adapted to low P conditions. These findings compare well with those of Ceccarelli (1996), Burger et al. (2008) ad Mandal et al. (2010) who reported greater genetic variation under stress environments and suggested that heritability in such environments can sometimes be comparable to non-stress environments or even higher if the experimental error is of the same magnitude.

4.5.4. Estimates of Gene effects in low P acid and non acid soils

Shoot dry matter (SDM)

In high P acid soils, majority of the crosses (83%) exhibited significant dominance gene action compared to additivity (33%) although additivity was more pronounced under low P supply (67%) for SDM (Table 17). Consequently the magnitude of mean dominance was higher under both P conditions compared to mean additive genetic effects (0.88 and 0.55 vs. 0.075 and 0.085) for dominance and additive under high and low P respectively. Epistatic effects (additive x additive) and (dominant x dominant) were only detected for a few crosses under both P conditions. The mean of ratio “a/m”, “d/m”, and “epistasis/m” at high P was 0.27, 3.58 and 1.69 respectively while they were 0.74, 6.1 and 5.6 at low P, respectively. This indicates that dominance and epistatic effects were more important in the expression of SDM than additive effects under both P conditions in acid soils. However, both dominance and additive effects had higher magnitude under low P conditions (d/m, a/m ratios 6.1 and 0.74) compared to high P conditions (d/m, a/m ratios of 3.5 and 0.0.27) (Table 19). In non-acid soils, majority of the crosses exhibited

significant dominance gene action (83% and 67%) compared to additive (33 and 17%) gene action under high and low P conditions respectively for SDM. Therefore mean dominance was higher compared to mean additive genetic effects (0.81 vs. 0.11 and 0.55 vs. 0.14) in high and low P conditions. Significant epistatic effects (dominance x dominance) were detected at both P levels. The magnitude of epistatic effects were greater than dominance under both P conditions, although they were more pronounced under low P (6.13) compared to high P(4.29) (Table 18).

Table 17: Estimates of genetic effects for shoot dry matter in two acid soils

(a) Shoot dry matter at High P (acid soils of Chepkoilel and Sega)									
Cross	m	a	D	aa	ad	dd	a/m	d/m	Epist/m
K1XS1	0.27**	0.05	0.80**	-	-	-	-	2.96	
H1XS1	0.24**	-0.04	1.04**	0.93*	-	-1.25*	-	4.28	1.33
H2XS1	0.23**	0.05	0.98**	0.85*	-	-1.04*	-	4.23	0.82
H1XM1	0.31**	-0.08*	0.60	-	-	-0.91*	0.24	-	2.93
H2XM1	0.23**	0.07*	0.76*	-	-	-	0.30	3.32	-
K1XM1	0.26**	0.06	0.81*	-	-	-	-	3.11	-
Mean	0.26	0.075	0.88	-	-	-	0.27	3.58	1.69
(b) Shoot dry matter) at Low P (acid soils of Chepkoilel and Sega)									
Cross	m	a	D	aa	ad	dd	a/m	d/m	Epis/tm
K1XS1	0.11*	0.10*	0.54*	0.47*	-	-	0.85	4.80	4.3
H1XS1	0.13*	0.02	0.50	-	-	-	-	-	-
H2XS1	0.09*	0.09*	0.64*	-	-	-0.69*	1.00	7.35	6.9
H1XM1	0.18*	0.02	0.37	-	-	-	-	-	-
H2XM1	0.15*	0.08*	0.27	-	-	-	0.52	-	-
K1XM1	0.14*	0.08*	0.48	-	-	-	0.62	-	-
Mean	0.13	0.085	0.59	-	-	-	0.74	6.1	5.6

m: mean; a: additive; d: dominance; aa, ad and dd are additive x additive, additive x dominance and dominance x dominance epistasis, respectively; Significant at 5% (*) and 1% (**) probability levels.

Root Length Density (RLD)

For root length density, the number of crosses under high P acid soils with significant additive effects was larger (100%) than those with significant additive effects in low P (50%) (Table 19). No epistasis was detected for RLD in acid soils. The overall mean of

ratio “a/m”, “d/m”, for RLD at high P was 0.39 and 2.88, respectively while they were 0.17 and 2.13 respectively at low P. This shows that dominance was more important than additive effects in the inheritance of RLD in both P conditions.

Table 18: Estimates of genetic effects for shoot dry matter in two non-acid soils

(a) Shoot dry matter (Kg/plant) at High P (non-acid soils of Migori and Koyonzo)									
Cross	m	a	d	aa	ad	dd	a/m	d/m	Epist/m
K1XS1	0.20**	-0.04	0.98**	--	--	-1.37*	-	4.78	6.85
H1XS1	0.23**	-0.01	0.64*	--	--	--	-	2.75	-
H2XS1	0.24**	-0.09*	1.01**	--	--	-1.26*	0.35	4.16	5.25
H1XM1	0.30**	-0.01	0.34	--	---	--	-	-	-
H2XM1	0.24**	0.04	0.70*	--	--	-0.91*	-	2.95	0.67
K1XM1	0.24**	0.12*	0.74**	--	--	-1.06*	0.51	3.14	4.41
Mean	0.242	0.11	0.81	-	-	-1.23	0.43	3.56	4.29
(b) Shoot dry matter (Kg/plant) at Low P (non-acid soils of Migori and Koyonzo)									
Cross	m	a	d	aa	ad	dd	a/m	d/m	Epist/m
K1XS1	0.14**	0.05	0.15	-	-	-	-	-	-
H1XS1	0.12**	0.01	0.59*	-	-	-0.72*	-	4.92	6.0
H2XS1	0.11**	-0.02	0.71*	-	-	-0.86*	-	6.46	7.8
H1XM1	0.13**	-0.06	0.46*	-	-	-	0.46	3.44	-
H2XM1	0.13**	0.05	0.21	-	-	-	-	-	-
K1XM1	0.15**	0.14*	0.46*	-	-	-0.69*	0.93	3.18	4.6
Mean	0.13	0.14	0.55	-	-	-0.75	0.67	4.5	6.13

m: mean; a: additive; d: dominance; aa, ad and dd are additive x additive, additive x dominance and dominance x dominance epistasis, respectively; Significant at 5% (*) and 1% (**) probability levels.

Table 19: Estimates of genetic effects for Root Length Density in two acid soils

(a) Root Length density (cm/cm^3) at High P (acid soils of Chepkoilel and Sega)									
Cross	m	a	d	aa	ad	dd	a/m	d/m	Epist/m
K1XS1	7.34**	2.99*	2.40	-	-	-	0.41	-	-
H1XS1	8.25**	4.41*	13.55*	-	-	-	0.53	1.64	-
H2XS1	6.20**	4.20*	13.64*	-	-	-	0.68	2.20	-
H1XM1	7.39**	2.38*	6.82	-	-	-	0.32	-	-
H2XM1	10.71**	1.27*	-13.39*	-	-	-	0.12	1.25	-
K1XM1	4.63**	-1.33*	29.90**	-	-	-	0.29	6.46	-
Mean	7.42	2.76	15.46				0.39	2.88	-
(b) Root Length density (cm/cm^3) at Low P acid soils of Chepkoilel and Sega)									
Cross	m	a	d	Aa	ad	dd	a/m	d/m	Epist/m
K1XS1	5.01**	0.62*	0.27	-	-	-	0.12	-	-
H1XS1	4.44**	0.74*	8.78*	-	-	-	0.17	1.98	-
H2XS1	4.27**	0.89*	5.84*	-	-	-	0.21	1.37	-
H1XM1	4.91**	-0.29	4.12*	-	-	-	-	0.84	-
H2XM1	5.18**	-0.25	4.46*	-	-	-	-	0.86	-
K1XM1	3.86**	-0.34	21.8*	-	-	-	-	-	-
Mean	4.61	0.75	9.00				0.17	2.13	-

m: mean; a: additive; d: dominance; aa, ad and dd are additive x additive, additive x dominance and dominance x dominance epistasis, respectively; Significant at 5% (*) and 1% (**) probability levels.

The number of crosses with significant additive effects was smaller (33.3%) in high P compared to low P (50%) non-acid soils (Table 20). At both P conditions dominance effects were more important followed by epistatic effects and additive effects, although both dominance and additive effects had higher magnitude under low P conditions (d/m, a/m ratios of 3.55 and 0.36) than under high P conditions (d/m, a/m ratios of 2.0 and 0.33). Epistatic effects (aa and dd) for RLD were detected at both P conditions in non-acid soil in four out of the six crosses although they were more pronounced at high P (epist/m ratio of 1.77) compared to low P (epist/m ratio of 1.08).

Table 20: Estimates of genetic effects for Root Length density in two low P non-acid soils

(a) Root Length density (cm/cm^3) at High P (non-acid soils of Migori and Koyonzo)

Cross	m	a	d	Aa	ad	dd	a/m	d/m	Epist/m
K1XS1	13.2**	4.77*	32.1**	24.27*	-	-	0.36	2.43	1.83
H1XS1	11.9**	5.89*	20.6**	-	-	-	0.50	1.76	-
H2XS1	11.4**	3.00	18.44*	-	-	-30*	-	1.62	2.6
H1XM1	12.6**	2.31	8.97	-	-	-	-	-	-
H2XM1	10.3**	3.68	17.10*	16.93*	-	-	-	1.65	1.64
K1XM1	11.8**	4.05*	45.4**	32.89*	-	-21.17*	0.24	3.85	0.99
Mean	11.87	4.9	26.7	-	-	-	0.37	2.26	1.77

(b) Root Length density (cm/cm^3) at Low P (non-acid soils of Migori and Koyonzo)

Cross	m	a	d	Aa	ad	dd	a/m	d/m	Epist/m
K1XS1	8.32**	1.77	36.48**	30.27*	-	-43.9*	-	4.39	1.63
H1XS1	9.06**	5.89*	20.28**	-	-	-	0.65	2.24	-
H2XS1	5.29**	1.00	19.66**	-	-	-	-	3.72	-
H1XM1	9.81**	-1.69	23.44**	19.26	-	-32.49*	-	2.39	1.32
H2XM1	8.61**	3.68	25.44**	16.93	-	-22.82*	-	2.96	0.68
K1XM1	7.89**	4.05*	44.16**	32.89*	-	-38.56*	0.51	5.59	0.718
Mean	8.16	4.97	28.24	-	-	-	0.58	3.55	1.08

m: mean; a: additive; d: dominance; aa, ad and dd are additive x additive, additive x dominance and dominance x dominance epistasis, respectively; **, * Significant at 5% (*) and 1% (**) probability levels.

Grain Yield

In high P acid soils, significant epistatic effects were detected for grain yield, additive x additive (aa) in two crosses, additive x dominance epistasis (ad) in four crosses and dominance x dominance (dd) in two crosses. For grain yield the mean value of the ratios a/m, d/m, and epist/m were 0.24, 1.98 and 0.8, respectively indicating that dominance effects, followed by epistatic effects were more important than additive effects. In low P, similar results were reported with ratios a/m, d/m, and epist/m being increased at least 3.6 folds (0.81, 7.73 and 3.5, respectively (Table 21).

Table 21: Estimates genetic effects for Grain yield evaluated in two low P acid soils

(a) Grain yield (t/ha) at High P (acid soils of Chepkoilel and Sega)

Cross	m	a	d	aa	ad	dd	a/m	d/m	Epist/m
K1XS1	2.6**	0.63	5.67*	-	-	-	-	2.18	
H1XS1	3.6**	0.47	3.22	-	-1.10*	-	-	-	0.31
H2XS1	3.0**	0.73*	4.20*	-	-	-1	0.24	1.40	
H1XM1	2.9**	0.70*	7.60**	4.29*	-1.12*	-	0.24	2.59	1.0
H2XM1	4.0**	0.29	4.72*	-	-1.72*	-	-	1.17	0.43
K1XM1	2.9**	-0.31	7.38**	5.21*	-1.58*	-7.96*	-	2.57	1.49
Mean	3.2	0.71	5.91	-	-	-	0.24	1.98	0.80

(b) Grain yield (t/ha) at low P (acid soils of Chepkoilel and Sega)

Cross	m	a	d	aa	ad	dd	a/m	d/m	Epist/m
K1XS1	2.4**	-0.25	2.93*	-	-1.75*	-	-		0.73
H1XS1	2.6**	-0.13	1.48	-	-1.29*	-	-		0.50
H2XS1	2.6**	-0.34	2.48*	-	-1.44*	-	-		0.55
H1XM1	1.1**	-0.60	5.02**	-	-2.23**	-	-	7.49	2.0
H2XM1	1.3*	1.07*	7.00**	7.94*	-	12.12*	0.81	7.80	9.3
K1XM1	1.0*	-0.08	5.00**	6.27*	-1.17*	-8.03*	-	7.90	8
Mean	1.8	1.07	4.4	-	-	-	0.81	2.4	3.5

m: mean; a: additive; d: dominance; aa, ad and dd are additive x additive, additive x dominance and dominance x dominance epistasis, respectively; Significant at 5% (*) and 1% (**) probability levels.

In non-acid soils, the ratios a/m, d/m, and epist/m were 0.41, 1.4 and 1.98 respectively with high P while they were 0.44, 2.9, 1.97 with low P indicating that dominance effects, followed by epistatic effects were more important than additive effects for the inheritance of grain yield in non-acid soils (Table 22).

Table 22: Estimates of gene effects for Grain yield evaluated in two low P non-acid soils

(a) Grain yield (t/ha) at High P (non-acid soils of Migori and Koyonzo)									
Cross	m	a	d	aa	ad	Dd	a/m	d/m	Epist/m
K1XS1	4.10**	0.61	4.63*	-	-	-	-	1.13	-
H1XS1	5.01**	0.60	4.38*	-	-	-	-	0.87	-
H2XS1	4.48**	0.62	-0.96	-	-	-	-	-	-
H1XM1	4.36**	0.73	3.28*	-	-	-	-	0.75	-
H2XM1	3.30**	1.52*	8.49*	7.88*		-14.42*	0.46	2.57	1.98
K1XM1	3.72**	1.33*	6.36*	-	-	--	0.36	1.71	
Mean	4.2	1.43	5.4	-	-	-	0.41	1.4	1.98
(b) Grain yield (t/ha) at low P (non-acid soils of Migori and Koyonzo)									
Cross	m	a	d	aa	ad	Dd	a/m	d/m	Epist/m
K1XS1	1.83*	0.88*	3.30*	-	-	-	0.48	1.8	-
H1XS1	3.24**	0.99*	0.56	-	-	-	0.30	-	-
H2XS1	2.18**	1.09*	4.25*	-	-	-	0.50	1.95	-
H1XM1	2.14**	0.45	5.62*	-	-	-	-	2.62	-
H2XM1	1.88*	0.90*	8.38*	7.97*	-	-13.67-*	0.48	4.47	3.03
K1XM1	2.14**	0.96*	7.58*	5.27*	-	-7.01-*	0.45	3.53	0.92
Mean	2.2	0.96	5.8	-	-	-	0.44	2.9	1.97

m: mean; a: additive; d: dominance; aa, ad and dd are additive x additive, additive x dominance and dominance x dominance epistasis, respectively; Significant at 5% (*) and 1% (**) probability levels

4.5.5. Discussions on gene effects

According to Gambles, (1962), the relative importance of additive (a), dominance (d), and epistatic (epist) effects, compared with the mean effect (m) can be obtained for each cross where the parameters are significant. An overall mean of these ratios from the different crosses can then be used to verify the relative importance of these gene effects in trait expression. Using these ratios, epistasis followed by dominance was more important in SDM inheritance compared to additive effects in non-acid soils. In both acid and non-acid soils, dominance and epistasis were more important than additive portion although epistatic effects were more pronounced in non-acid soils while dominance more pronounced in acid soils. The magnitude of additive, dominance and epistasis was always

higher in low P compared to high P in both acid and non-acid soils. This implies that selecting for SDM is more successful under low P soils in both acid and non-acid soils. These findings compare well with those of Oztuk et al. (2005); Cichy et al. (2009) and Hammond et al. (2009) who found SDM as a suitable selection criteria for P efficiency under low P conditions for both beans, brassica and wheat genotypes.

For RLD both dominance and additive effects were more pronounced at high P conditions compared to low P conditions. These results agree with those of Monyo and Whittington (1970) and Kazemi et al. (1979) who suggested that root systems traits are polygenically inherited and largely controlled by weak additive gene effects. The additive genetic effects can allow breeding progress to be made by selecting for the particular root trait of interest. From these results, it seems that selection for RLD in acid soils is more suitable under high P conditions because of high additive effect although this kind of selection may lead to identifying good responders rather than efficient genotypes. However, such selection strategy would still be suitable in acid soils considering that a large proportion of soil P is held very tightly to the surface of soil particles as organic phosphorus compounds and hence unavailable even at high P supplementation. In contrast to acid soils, selection for RLD in non-acid soils is more suitable at low P levels following high additivity and dominance gene effects. Epistatic effects (aa and dd) for RLD were detected at both P conditions in non-acid soil. These results agree with those of Wolf and Hallauer (1997) who concluded that epistasis in maize seems to be more important in either poorer or better environments. These findings also suggest that variation in P levels in non-acid soils did not affect the detection of epistatic effects for RLD.

For GYLD, at high P in acid soils dominance effects, followed by epistatic effects were more important than additive effects. In low P, similar results were reported with ratios a/m, d/m, and epist/m being increased. In non-acid soils dominance effects, followed by epistatic effects were still more important than additive effects for the inheritance. These results also suggest that for grain yield, the pooled additive effects were more important in explaining genetic variations between the generations in acid soils (0.51t/ha) compared to non-acid soils (0.43 t/ha). The mean additive effects did not differ significantly (0.51 t/ha vs. 0.43 t/ha) between acid and non-acid soils suggesting that estimates of pooled additive effects were not affected by soil acidity. These results imply the suitability of selecting for grain yields in low P conditions under acid soils because of increased magnitude of additive genetic effects or using either P level in non-acid soils since the magnitude of additivity did not differ significantly. These findings compare well with those of Parentoni et al. (2010) and Richard et al. (2015) who reported the importance of dominance effects, followed by epistatic effects than additive effects for maize grain yield in acid soils. They also compare well with those of Vasqualez et al. (2008) who reported higher magnitude of pooled additive effects in acid compared to non-acid soils.

The pooled dominance effects for grain yield were significant for 83.3% and for 100% crosses in acid and non-acid soils, respectively. The magnitude of these effects was greater than the mean parameter, except for one cross (H1XS1) in both acid soil and non-acid soil (Table 24). Also, the effects were positive for most of the crosses, except one cross in non-acid soil (H2XS1). These findings compare well with those of Vasqualez et al. (2008) who reported significant pooled additive variance in 78.6% of the crosses studied and 100% significant additive variance in non-acid soils. For acid soil the

significant dominance effects averaged 4.4 tha^{-1} and ranged from 2.48-7.0 t ha^{-1}.For non-acid soil the dominance effects averaged 5.6 tha^{-1} and ranged from 4.38 to 8.49 t ha^{-1}. Thus, contrary to the additive effects, the magnitudes of the dominance effects were significantly affected by soil acidity. These findings further compares well with those of many researchers (Gamble, 1962a; Cockerham and Zeng, 1996; Vasqualez et al., 2008 and Richard et al., 2015) who reported on the importance of dominance genetic effects for the inheritance of grain yield in maize in non-acid soils. They also agree with those of Ceballos et al. (1998) who found out that the estimates of dominance effects were influenced by soil acidity and it accounted for 63.0% and 81.0% of the total sum of squares in acid and non-acid soils, respectively for grain yield. Studies by Vasqualez et al. (2008) further reported higher estimates of dominance effect in non-acid soil compared to acid soils. Epistatic effects for grain yield were detected in all the crosses in acid soils, with aa effects in three crosses, ad effects in six crosses, and dd in three crosses while in non-acid soils, only 33% of the crosses presented significant epistatic effects with aa and dd effects in three crosses. The number of crosses with epistatic effects was greater in acid compared to non-acid soils. Besides, the magnitudes of these effects were also larger in acid than in non-acid soils. These results partially agree with those of Ceballos et al. (1998) who reported that epistasis was more important for grain yield in acid compared to non-acid soils. In our results epistatic effects were detected in both soil types, suggesting that soil acidity affected the detection of epistatic effects. These results also disagree with those of Vasqualez et al. (2008) who found no effects of soil acidity in the detection of epistasis. The results further disagreed with those of Wolf and Hallauer (1997) who suggested that epistasis in maize seems to be more important in

either poorer or better environments. Studies by Jinks et al. (1973) also showed greater frequency and magnitude of epistasis in both extremes of a range of environments in tobacco (*Nicotiana tabacum* L.). In this study epistatic effects for grain yield were more important in acid compared to non-acid soils

4.6. Conclusions and Recommendations

1. Both additive and non-additive effects were detected in both acid and non-acids soils Dominance effects played a more important role than epistatic effects and the latter were more important than additive effects in the inheritance of majority of P efficiency traits studied in maize in both acid and non-acid soils. The magnitude of both additive and non-additive gene effects were always greater in non-acid soils compared to acid soils pointing to the detrimental effects of soil acidity in the detection of gene actions in maize.
2. Our results suggest that the inheritance of grain yield Root Length Density and Shoot Dry matter did not differ in acid and non-acid soils.
3. From this syudy similar breeding strategies are recommended for use in both acid and acid soils under similar P conditions

CHAPTER FIVE

5.0. GENOTYPE BY ENVIRONMENT INTERACTION AND STABILITY ANALYSIS FOR MAIZE P EFFICIENCY TRAITS IN LOW PHOSPHORUS SOILS OF WESTERN KENYA

5.1. Abstract

Most developing countries are food insecure and rely heavily on maize (*Zea mays* L.) as a staple food yet its yields are significantly reduced by low available phosphorus (P) among other constraints. In Kenya the crop grows in a wide range of environmental conditions hence stability of genotype is an important consideration. The present study was conducted to: (i) determine genotype by environment interaction (GEI) and stability of grain yield and other agronomic traits of experimental maize hybrids grown across eight low P environments in western Kenya. Thirty maize hybrids and 2 checks were planted in RCBD replicated three times in 2013. Data on grain yield, agronomic traits and environmental variables were analysed using the Genotype and Genotype x Environment Interaction (GGE) and Additive Main Effect and Multiplicative Interaction (AMMI) models. Yield stability and superiority estimates were done using Finlay and Wilkinson model (bi) and Wrickes ecovalence (wi) stability parameters. The AMMI ANOVA showed significant effects for genotype (G), environment (E) and GEI. For grain yield, the differences among the environments accounted for (67.6%) of the total variation while the G and GEI accounted for 11.6% and 10.3% respectively of the variation. The larger E variation relative to G suggested the existence of different mega-environments. The AMMI score for grain yield was highest at environment K2 (0.89) and lowest at C2 (-1.69). Both AMMI and GGE models categorized the eight environments into 3 mega environments. Using GGE biplots the genotypes 29, 30, 28 and 27 were identified as the best performers across the environments while 25 as the poorest performer. The most stable genotype was 9 while the least 29. Twenty six percent of the newly developed hybrids were more stable than the commercial hybrid (H515) based on Wrickes (wi) ecovalence. Based on GGE biplot and superiority analysis (Pi), genotypes 1, 27, 21 and 23 could be considered as ideal and most superior. They had better mean yield and stability in low P soils of western Kenya (Pi= 0.4-1.4) while genotypes 25, 3, 26, 39 and 31 were the least superior (3-10.5). The ideal genotypes identified can be used for broad selection and for reference in genotype evaluation and further testing. The scientific information obtained from these results could be useful to plant breeders in supporting breeding program decisions for wide adaptation.

Key words: AMMI, GGE, grain yield, low P, Maize, stability, superiority

5.2. Introduction

Maize (*Zea mays* L) is a major staple food crop for the majority of people living in developing countries especially in the sub-Saharan-Africa (Lopes and Lakirns, 1996, Sibiya et al., 2011). In Kenya it is the main staple food crop that 90% of the population depend on. Maize production in Kenya has been on the downward trend in many parts of the country. This has been due to , moisture stress, aluminium (Al) toxicity and phosphorus (P) deficiency, maize lethal necrosis amongst other constraints (Kisinyo et al., 2013a; Ouma et al., 2013). Maize is known to grow in a wide range of agro-ecological zones with regard to water balance, solar radiation and temperature. It can be grown between the equator to latitutes slightly above 50^{o} north and 50^{o} south. It is also grown at altitudes from zeroto over 3,000 meters above the sea level and under conditions ranging from heavy rainfall to semi-arid, cool to very hot climates (Donswell et al., 1996; Hill et al., 1997). This differential adaptation contributes to Genotype by Environment Interaction (GEI) which hinders identification of high yielding and stable maize genotypes (Akcura et al., 2011). GEI may be defined as a change in the relative performance of two or more genotypes measured in two or more environments. Interactions may therefore involve changes in rank/order for genotypes between environments and changes in the absolute and relative magnitude of the genetic, environmental and phenotypic variances between environments (Bowman, 1972).

Multi-environment yield trials (METs) are essential in estimation of GEI and identification of superior genotypes in the final selection cycles and to develop an understanding of the target region and in particular to determine if the target region can

be subdivided into mega-environments. Mega environments correspond to a set of environment that shows less GXE among one another hence sharing the best or the worst genotype (Mitrovic et al., 2012). Investigation of mega-environment is therefore a prerequisite for meaningful cultivar evaluation and recommendation (Yan and Hunt, 1998).

In practice, breeders, seed producers and distributors want a broadly adapted genotype that performs better across a great area (has small GEI). The development of high yielding cultivars with wide adaptability is therefore the ultimate aim of plant breeders. However, the existence if GEI complicates development of widely adapted cutivars (Gauch and Zobel., 1996) because it frequently changes the genotypic ranks in different environments due to cross interaction making their proper selection difficult. It is therefore essential that GEI is properly understood and taken into account rather than ignored.

GEI and yield stability analysis therefore remains important in measuring varietal suitability for cultivation across seasons and ecological zones (Ariyo et al., 2011). Different methods are available for statistical analysis, including parametric and non-parametric tests, to estimate the nature of GEI and its control. Examples include, type B correlation (Yamada, 1962) and and joint regression (Finlay and Wilkinson, 1963; Eberhart and Russel, 1966; Perkins and Jinks, 1968) which are additive models. Analysis of variance as an additive model only explains the main effects and informs whether or not the GEI is a significant source of variation. It does not provide insight into the individual genotypes and localities which are the components of the interaction (Samonte

et al., 2005). Two frequently used statistical analyses are the additive main effects and multiplicative interaction (AMMI) and the genotype main effects and genotype × environment interaction effect (GGE) models (Gauch, 2006). These two statistical analyses (AMMI and GGE) have broader relevance for agricultural researchers because they pertain to any two-way data matrices, and such are the data that emerge from many kinds of agricultural experiments. AMMI analysis combines ANOVA and principal component analysis (PCA) where the sources of variability in the genotype by environment interaction are partitioned by PCA. The interpretation of results obtained from AMMI analysis is performed with a biplot that relates genotypic means to the first or some of the principal interaction components (Yan et al., 2007). GGE biplot analysis enables visual (graphical) presentation of interaction estimate. This method also combines ANOVA and PCA by partitioning together sums of squares of genotypes and sums of squares of GEI (which are relevant in genotype evaluation) using PCA method. It shows the first two principal components (PC1 and PC2) which are obtained by singular values decomposition (SVD) of multi-location trials yield data (Yan and Rajcan, 2002). The GGE biplot provides breeders with a more complete and visual evaluation of all aspects of the data by creating a biplot that simultaneously represents mean performance and stability, as well as identifying mega-environments and ideal genotype (Ding et al., 2007; Kang, 1993; Yan, 2001; Yan et al., 2007). The GGE biplot can be useful to display the which-won-where pattern of the data that may lead to identifying high-yielding stable cultivars and discriminating representative test environments (Yan et al., 2001). The difference from AMMI is that GGE biplot analysis is based on environment-centred PCA, whereas AMMI analysis refers to double-centred PCA. The

objective of this study was to determine GEI and stability of grain yield and other agronomic traits of experimental maize hybrids grown across eight low P environments in western Kenya.

5.3. Materials and Methods

A total of 32 maize genotypes were used in this study. The maize genotypes comprised 9-Three way crosses (TWCHs), 5-double crosses (DCHs), 9 backcrosses (BC1), 5 single crosses (SCH), 2 parental lines and two checks (H 515 and P-efficient synthetic). The various crosses were derived from a total of eleven maize inbred lines (which have been described in Table 1 section 3.3.3) using North Carolina mating Design II (NCD II) procedures as described by Comstock and Robinson (1948, 1952). The inbred lines were developed at KARI-Kitale on the Moi University – KARI-Kitale joint maize breeding program and have between 8-16 generations of self-pollination. These inbreds were selected from among 175 maize inbred lines based on higher grain yield capacity for the efficient lines compared with the inefficient ones under low available soil phosphorus (Ouma et al., 2012). The statistical model and the pollination procedures used to develop the various hybrids have been described in sections 3.3.5 and 3.3.6. The genotypes were evaluated in a replicated trial across 8 environments (four stressed and 4 non-stressed). The stressed environments received inadequate P supply (6 Kg P/ha) while the non-stressed environments received adequate P (36 Kg P/ha) and were located at Sega, Chepkoilel, Migori and Koyonzo. All the environments have been described in section 3.3.1. The two experiments were laid out in RCBD replicated 3 times during the long rain of 2013. Each genotype was planted in a two row plot measuring three meters long with

inter and intra-row spacing of 0.75 m x 0.30 m respectively. Two seeds were sown per hill and thinned to one upon establishement

5.3.1. Data collections

Data was collected for grain yield, (GYLD-t/ha), plant height (PH-cm), Stover yield (STV= leafs, stalks, ear husks and cobs- t/ha), ear height (EHT-cm), internode length (INL-cm), grain P concentration (GPC %), grain P content (GPcnt Kg/ha), days to 50% silking (DASLK), days to 50% anthesis (DANTH). At maturity, all the cobs in a row for each entry were harvested and adjusted to 13% moisture content while assuming and 80% shelling percentage. The moisture content was then determined from a seed sample of 7 randomly selected cobs. PHT was recorded in 10 competitive plants per plot, from the soil surface to the tip of the highest tassel branch, and the plot means used for analysis. Stover samples were collected from 7 plants and a sample of 200g of grain obtained from each environment. These samples were oven dried at 80^{o}C and grain and stover dry matter determined. Grain and stover samples were ground and analyzed for P concentration using the vanadomolybdate method (Westerman, 1990). Based on grain and stover dry matter yields, and on P concentration in these plant components, the phosphorus content in the grain and in the stove were determined.

5.3.2. Data Analysis

The means of all parameters recorded were computed using Genstat Version 18 (Payne et al., 2014). The protected least significant difference (LSD) was used for mean separation. Genotype by environment interaction was analyzed using Finlay and Wilkinson (FW) model (1963), Additive Main effect and Multiplicative interaction (AMMI), and GGE

biplots (Gauch, 2006, Yan et al., 2007) using breeding view program in Genstat version 18. Stability and superiority measures were calculated based on FW model and Wrickes ecovalence (wi) (Wrickes, 1964) using breeding view stand-alone program.

The statistical models fitted were as follows:

I. Finlay and Wilkinson Model: $y_{ij} = \mu + Gi + Ej + \beta iEj + \epsilon ij$

- y_{ij} the measured mean of the ith genotype in the jth environment
- G_i, genotypic effect ,
- E_j environmental effect , plus an effect of the combination of genotype and environment GE_{ij} given by a genotypic-specific sensitivity parameter (βi) such that $GE_{ij} = \beta_i E_j + \epsilon_{ij}$

II. AMMI model: $y_{ij} = \mu + Gi + Ej + \sum \lambda_k \gamma_{ki} \eta_{kj} + \delta ij$

- y_{ij} is the measured mean of the ith genotype in the jth environment ,
- G_i the genotypic effect, Ej, environmental effect
- $\sum \lambda_k \gamma_{ki} \eta_{kj}$ a sum of multiplicative terms accounting for environmental effects, δij the residual variance

III. GGE Model: $yij = \mu + Ej + \sum \lambda_k \gamma_{ki} \eta_{kj} + \delta ij$

- yij is the measured mean of the ith genotype in the jth environment ,
- Ej, environmental effect
- $\sum \lambda_k \gamma_{ki} \eta_{kj}$ a sum of multiplicative terms accounting for both the genotypic performance and environmental effects, δij the residual variance

5.4. Results and Discussion

5.4.1. Performance of maize hybrids across environments

The environmental mean and variances for grain yields, plant height, ear height and other agronomic traits for maize hybrids are presented in Table 23. The environmental mean corresponds to the mean of all genotypes grown in a given environment, and gives an indication of how favourable the environments are for the general adaptation. Mean grain yield (GYLD) ranged from 1.4 -6.7 t/ha. The highest GYLD of 6.7 t/ha was recorded at C2 followed by M2 (5.2 t/ha) while the lowest was 1.4 t/ha at S1.This is an indication that C2 was the most favourable environment for grain yield while SI the least favourable. Environments which received higher phosphorus (P) supply exhibited higher grain yields than those with lower P supply. However yield at Sega with higher P supply (S2) were still lower than those of Chepkoilel, Migori and Koyonzo with low P (C1, M1 and K1, respectively) (Table 23). This was expected because of the extremely low available P (2.2 mg/kg of soil) in the Sega soils. According to Kisinyo et al. (2013) Sega soils are extremely depleted and deficient in available P and require 52kg P/ha to be applied after every one cropping season for replenishment.

The highest maize growth (plant height, Ear heights and internode length) was recorded at M2 (240 cm, 105 cm and 16.5 cm respectively) while the lowest was at S1 (113.2 cm, 32.3 cm and 5 cm respectively). Mean stover yields ranged from 1.6-3.5 t/ha with environment M2 giving the highest and S1 the lowest scores. Environments C1 and C2 recorded lower stover yields that environment MI and M2. This could have been because C1 and C2 were hit by hailstones towards physiological maturity. Mean days to Anthesis (77.4) was lower than that for silking (79.3) across the environments. The maize hybrids

took the shortest time (63 and 65 days) to reach both anthesis and silking respectively at environment K2 while they took the longest time (108 and 109 days) to attain the same at environment C1. Anther-silk interval (ASI) was highest at S2 followed by S1 and lowest at M2 and C1. Prevalence of the grey leaf spot disease was highest at K2 followed by K1 and lowest at M2 with an average score of 1.9 across the environments.

There was heterogeneity of variance among the environments for all the traits measured. For grain yield, the highest variance was at C1 while lowest at S1, for plant height, the highest variance was realised at C1 and lowest at M2, for days to 50% anthesis the highest variance was at C2 and the lowest at K2 while for biomass (STV) C2 exhibited the highest and S1 lowest variance. These results indicate that SI discriminated less between genotypes for most of the traits. This is reflected in the smaller variance. Heterogeneity of variance among environments suggests that genotypes showed a more variable adaptation in some environments than in others which is an indicator of the presence of genotype by environment interaction. This implies that different hybrids could be selected for different agro ecologies (Derera et al., 2008).

Table 23: Means and variance of GYLD and other agronomic traits for maize hybrids tested in 8 environments.

Trait	GYLD (t/ha)		Plant Height (cm)		Ear Height (cm)		INTL (cm)		STV (t/ha)	
ENV	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance
C 1	4.552	1.1567	189.4	533.6	78.18	384.7	13.22	1.548	7.434	10.938
C 2	6.738	1.5993	214.3	347.6	92.59	139.2	15.05	0.783	9.468	13.264
K 1	3.894	0.3553	201.3	201.3	87.63	109.2	13.18	0.912	6.876	2.551
K 2	5.143	0.7614	230.5	270.6	104.26	89.0	15.29	1.693	9.117	3.182
M 1	3.919	0.8603	219.8	430.1	87.50	218.2	13.91	3.279	11.233	8.045
M 2	5.201	1.3615	240.4	193.3	104.83	168.7	16.52	1.483	15.988	11.535
S 1	1.427	0.3399	113.2	296.3	32.29	45.5	4.99	0.405	3.464	0.410
S 2	2.123	0.4827	137.9	389.2	47.77	104.3	6.35	0.551	4.578	0.900
Trait	DSLK (days)		DANTH (days)		GLS (scale 1-5)		ASI (day)		Ear Number	
ENV	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance
C 1	109.14	15.166	108.04	16.258	1.875	0.2922	1.119	1.483	21.06	10.723
C 2	98.64	16.278	97.27	18.190	1.854	0.2432	1.388	1.595	21.92	7.963
K 1	70.06	7.574	67.07	2.535	2.324	0.2609	2.989	2.807	15.53	3.282
K 2	65.35	5.792	63.28	3.263	2.525	0.2570	2.083	1.999	15.54	7.216
M 1	70.34	7.615	68.93	7.447	1.615	0.3885	1.417	1.497	16.42	7.040
M 2	66.17	3.655	65.03	4.496	1.468	0.1859	1.136	0.779	8.18	1.799
S 1	79.59	3.130	76.96	3.640	1.807	0.4826	2.615	1.529	8.79	10.140
S 2	75.20	9.201	72.28	5.505	1.883	0.4861	2.897	3.319	7.08	8.364

Note: GYLD- grain yield,INTL-internode length, STV-stover, DANTH-days to anthesis, DASLK-days to silking, ASI-anther-silk interval, CI-Chepkoilel with 6KgP/ha, C2-Chepkoilel with 36 KgP/ha, MI-Migori with 6 Kg P/ha, M2- Migori with 36KgP/ha, K1-Koyonzo with 6KgP/ha K2-Koyonzo with 36KgP/ha, SI- Sega with 6KgP/ha, S2-Sega with 36 Kg P/ha.

5.4.2. Partitioning Genotype by Environment Interactions using Finlay and Wilkinson Analysis

According to Finlay and Wilkinson (FW) model (1963), genotype and environment main effects and their sensitivity parameters were highly significantly different (Table 24). For grain yield, the differences between the environments accounted for (75.4%) of the total sum of squares, genotypic differences accounted for 13.03 % of the total sum of squares

and the sensitivity parameter was also significant but only accounted for 2.5 % of the total sum of squares. The rest of the sum of squares was accounted for by the residual. For plant height, differences between the environments accounted for 84.7% of the total sum of squares. The genotypes were significantly different and accounted for 8.2 % of the total sum of squares and the sensitivity parameter was also significant but only accounted for 1.1 % of the total sum of squares. The rest of the sum of squares was accounted for by the residual. These results show that there was significant contribution of environmental variance to majority of the diferrenes observed with the studied P-efficiency parameters. This was expected as majority of them were quantitatively inherited traits. Similar results have been previously reported for grain yield in maize (Makumbi et al., 2005; Menkir and Ayode, 2005 and Nzuve et al., 2013)

5.4.3. Partitioning of Genotype by Environment Interactions using AMMI Analysis

AMMI biplots are useful for visualization of GEI patterns, genotypic adaptation and stability. The combined analysis of variance showed that genotype and environment main effects and their interaction were highly significant for all the traits measured (Table 25). For grain yield, the AMMI analysis of variance revealed that differences between the environments accounted for (67.6%) of the total sum of squares (SS). The genotypes and the genotype x environment interaction (GEI) also accounted significantly for 11.6% and 10.3% respectively of the total sum of squares. A large SS for environments indicated that the environments were varied, with large differences among environmental means causing most of the variation in the plant grain yields. The larger GXE relative to Genotype suggests the possible existence of different mega-environments. These findings agree with those of Rad et al. (2013) who reported significant effects of environmental

variance on bread wheat grain yield. They are also in agreement with those of many others (Yan et al., 2000 and Ahmed et al., 2011) who reported more variation due to environmental variance (69-80%) than genotype x environment (10-21%) and genotype a lone (9-10%) for wheat grain yield and rice grain arsenic concentration respectively.

Table 24: Partitioning of the GEI for grain yield and other agronomic traits of maize hybrids using F.W model

Source of variation	d.f.	GYLD	PHT	EHT	INTL	STV	DANTH	DSLK	ASI	GLS	EANO
Genotypes	31	3.7***	1435.3***	652.9***	4.2***	22.3***	26.4***	29.8***	2.0ns	0.8089	18.6***
Environments	7	94.1***	65431***	21910***	582***	500***	8644***	8292***	20.***	3.8724	1058***
Sensitivities	31	0.7*	189.7ns	68.9ns	1.0ns	7.2**	15.4***	15.4***	3.2**	0.1742	7.1ns
Residual	186.0	0.4	172.8	89.5	0.9	3.5	3.3	3.9	1.6	0.3	5.2
Total	255.0	3.4	2119.8	754.5	17.3	19.9	244.7	235.9	2.4	0.4	35.9

Table 25: Partitioning of the GEI for grain yield and other agronomic traits of maize hybrids using the AMMI model

Source	d.f.	GYLD	PHT	EHT	INTL	STV	DANTH	DSLK	ASI	GLS	EANO
Genotypes	31	3.7***	1435***	653***	4.2***	22.3***	26***	30***	2 ns	0.81***	18.6***
Environments	7	94.1***	65431***	21910***	582.2***	500***	8644***	8292***	20.1***	3.9***	1057.6***
Interactions	217	0.5	175	87	0.9	4.1	5	6	1.858	0.255	5.4
IPCA 1	37	1.1***	356***	215***	1.9***	11.5***	15***	16***	3***	0.6***	9.4***
IPCA 2	35	0.7***	234**	146***	1.4***	6.6***	4**	6***	2.7**	0.3**	7.4**
Residuals	145.0	0.2	115.0	40.0	0.6	1.6	2.0	3.0	1.4	0.2	3.9

Note: GYLD-grain yield, PHT-plant height, INTL-internode length, EHT-ear height. STV stover yield, DANTH-days to 50% anthesis, DSLK- days to 50% silking ,ASI- anther silking interval,EANO-ear number

The GXE interaction was partitioned between the first two Interaction Principal Components Axes (IPCA). For grain yield, the first IPCA was highly significant, capturing 42% of the total variation in the GxE interaction SS and 17 % of the interaction degrees of freedom. The second IPCA was also significant ($p < 0.05$) and explained 22.9% of the total variation. The first two IPCA axes jointly accounted for 64.9% of the GxE interaction SS. Results from the AMMI biplots showed that C2 was the major contributor for GXE and S1 the least contributor (longest and shortest vector lengths, respectively). Genotype 23, 27 and 28 showed the largest GXE across the environments (further apart from the origin). The genotypes 23, 30, 16 and 24 had positive interaction with environments S1, K1 and S2 while they had negative interaction with C1 and M1.The genotypes 10, 11, 12 and 19 positively interacted with M1, M2 and K2 and no interaction with the rest of the sites (Fig 5 a). For Plant height, differences between the environments accounted for 79 % of the total sum of squares. The genotypes and the GxE interaction also accounted significantly for 8% and 7%, respectively of the total sum of squares. The first IPCA was significant, capturing 34.6% of the total variation in the GxE interaction SS while the second interaction PCA was also significant ($p < 0.05$). The first two IPCA axes jointly accounted for 56.1% of the GxE interaction SS. K2, S2 and C1 were the major contributors for GXE while S1 the least contributor. Genotype 31 showed the largest GXE across the environments while genotypes 16 and 22 had the least, being located near the origin. Genotypes 29 and 26 showed positive interaction with K1 and K2 and negative interaction with M1, SI and S2. The genotypes 5, 9 and 10 interacted positively with C1, K1 and C2 and negatively with M1, M2 and S2 (Fig 5 b). For grain P concentration, the first two IPCA accounted for 55.84% of the variation with the first

IPCA capturing a larger part (34.94%) of this variation. MI and M2 were the major contributors for GXE while K1 contributed the least. Genotypes 17, 22 and 26 showed the largest GXE across the environments while 1 showed the least. Genotype 17 interacted positively with S2, C2, K1 and K2 but had negative interaction with M1, SI. The genotype 26 showed positive interaction with S1 and S2 while its interaction with M2 and K2 was negative (Fig 5 c). The first IPCA captured 37.09 % of the total variation for grain P content while the second IPCA explained 26.4%. M2 and C2 were the major contributors for GXE and M1 the least. Genotype 12 interacted positively with K2 and C2 but negatively with M2. Genotype 31 had positive interaction with K1, K2 and M1 and negative interaction with C1, C2 and M2 (Fig 5 d).

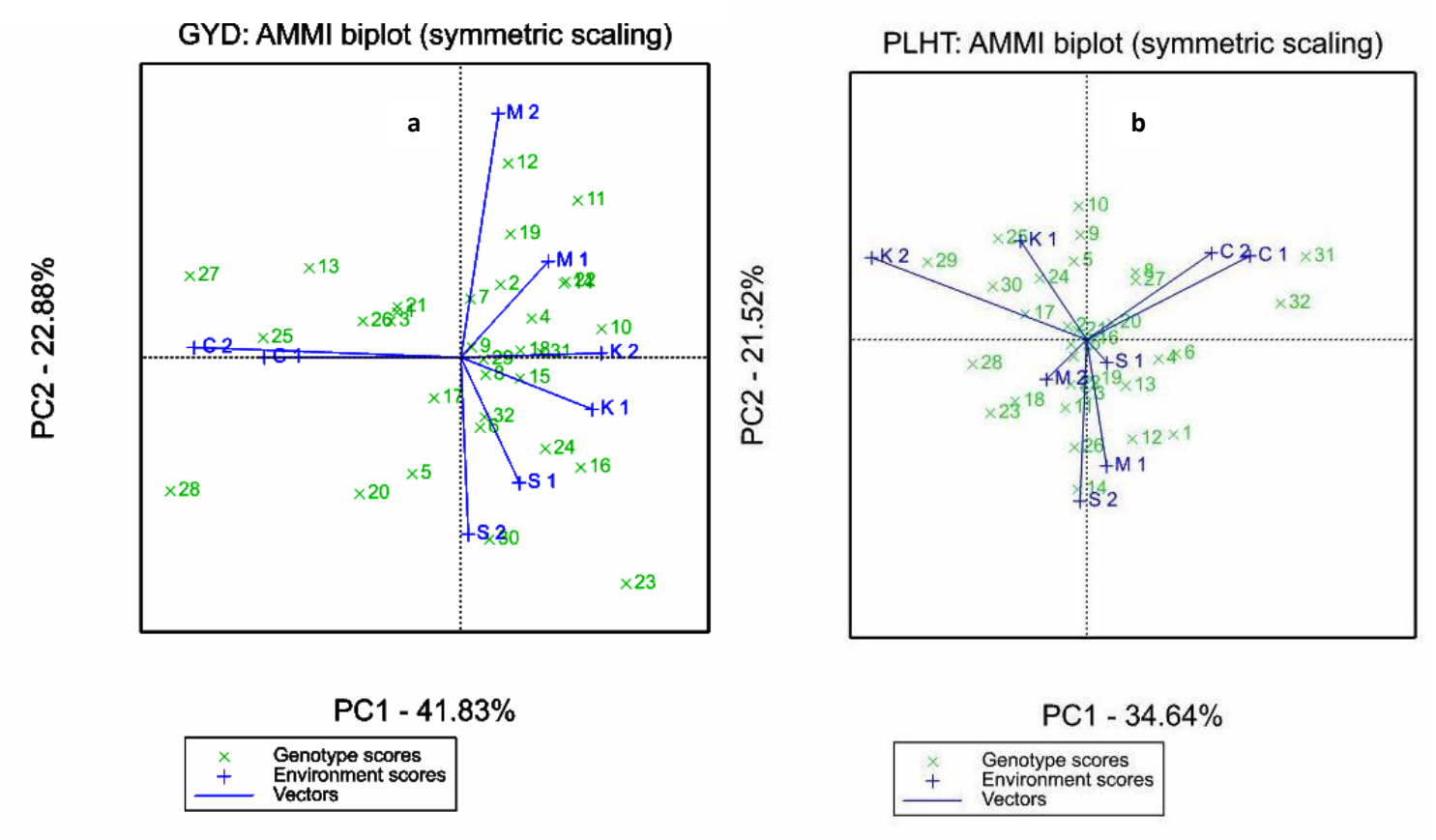

Figure 5 a-b: Biplots for AMMI Model for Grain yield (a) and plant height (b) of maize hybrid tested in western Kenya in 2013

Note* Genotypes are represented by multiplication sign (x). Environments are shown with addition sign (+) CI-Chepkoilel with 6 Kg P/ha, C2-Chepkoilel with 36 KgP/ha, MI-Migori with 6KgP/ha, M2- Migori with 36 Kg P/ha, K1-Koyonzo with 6 KgP/ha K2-Koyonzo with 36 KgP/ha, SI-Sega with 6 KgP/ha, S2-Sega with 36 Kg P/ha. GYD-grain yield, PLHT-Plant height

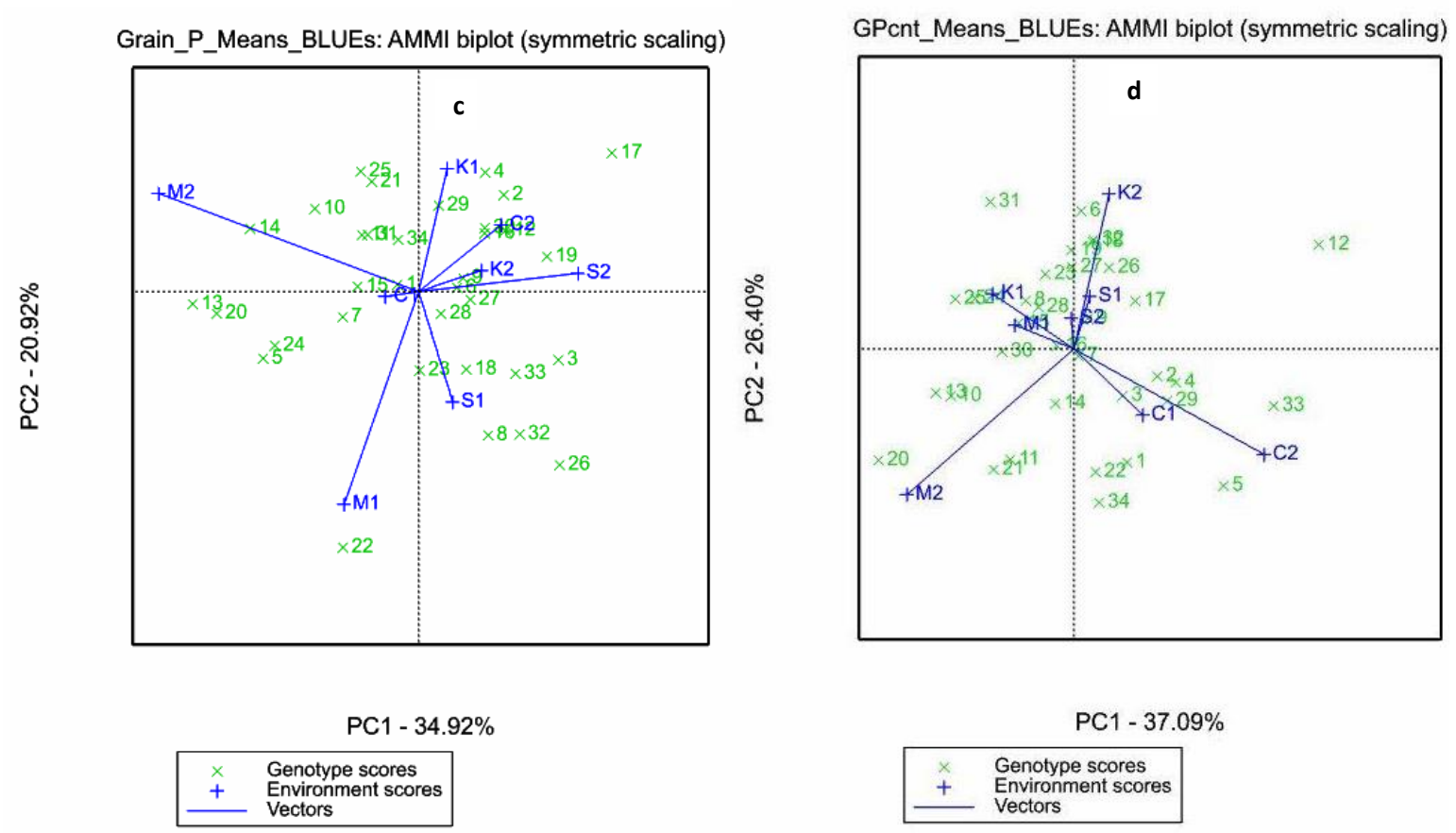

Figure 5 c-d: Biplots for AMMI Model for grain P concentration (c) and grain P content (d) of maize hybrid tested in western Kenya in 2013

Note* Genotypes are represented by multiplication sign (x). Environments are shown with addition sign (+) CI-Chepkoilel with 6 KgP/ha, C2-Chepkoilel with 36 KgP/ha, MI-Migori with 6 KgP/ha, M2- Migori with 36 KgP/ha, K1-Koyonzo with 6 KgP/ha K2-Koyonzo with 36 KgP/ha, SI- Sega with 6 KgP/ha, S2-Sega with 36 KgP/ha, Grain-P- grain P concentration and GPcnt-grain P content.

5.4.3. AMMI selections

The first four best genotypes per environment were selected based on AMMI model score for grain yield and other agronomic traits (Table 26). The AMMI score for grain yield was highest at K2 (0.89) and lowest at C2 (-1.69). Genotype 25 was selected as the best genotype at K2, K1 and S2, genotype 21 was best at both M1 and M2 while genotype 34 was best at C1 and C2. These results indicate specific adaptation of these genotypes to these environments. However, genotype 34 was also selected among the best four genotypes at S1, S2 and M2 showing that this genotype had wide adaptation to the test environments. Other genotypes showing wide adaptation for grain yield included genotypes 21 and 28. It was observed that none of the hybrid checks were among the best four genotypes across the environments. This demonstrates the potential of utilizing the newly developed hybrids to improve maize grain yields in low P soils of western Kenya. For plant height, the highest AMMI score was recorded in K2 (7.28) and the lowest in C2 (-5.7). Genotype 25 was best in 5 environments (K2, K1, M2, S2 and M1) while genotype 33 in the remaining environments (S1, C1 and C2). The other well adapted genotypes included 2 and 4. The highest AMMI score for biomass was attained at K2 (1.7) and lowest at C2 (-2.78). The best AMMI selection at K2 was genotype 23 while the best genotype across the remaining environments was 34. For days to 50% tasselling, the best environment was K1 (1.26) while the worst was C2 (-3.4). The first four selected genotypes at K1 were 34, 7, 8 and 13 while those selected at C2 were 25, 31, 33 and 25. S2 had the highest AMMI score for days to 50% silking (1.66) while C2 the lowest (-3.28). The most favourable genotypes across environments included G7, G34, G25, G31 and G8. For grain P content and concentration, the best environments included M2 and

S2 and the worst C2 and M1. Genotype 20 was best at M2 for grain p content and G33 at C2 for the same trait (Table 26). G 25 was selected among the first four best genotypes in more than half (63%) of the test environments for both P content and concentration implying that this genotype may be good at both P acquisition from the soil and P-utilizationl. Other genotypes dominating in P concentration, included G9, 8, and G24 (Table 28).

Table 26: First four AMMI selections per environment for agronomic traits across 8 environments.

	Grain yield (t/ha)							Plant height (cm)					
ENV	**Mean**	**Score**	**1**	**2**	**3**	**4**	**ENV**	**Mean**	**Score**	**1**	**2**	**3**	**4**
K2	5.161	0.8977	G25	G20	G28	G21	K2	230.6	7.28	G25	G30	G2	G26
K1	3.91	0.8394	G25	G4	G28	G23	K1	201.3	2.388	G25	G2	G33	G4
M1	3.911	0.5611	G21	G20	G28	G4	M2	240.2	1.507	G25	G2	G34	G16
S1	1.429	0.3704	G25	G4	G34	G29	S2	137.6	0.326	G25	G16	G28	G1
M2	5.221	0.2443	G21	G20	G34	G28	M1	219.6	-0.538	G25	G16	G34	G1
S2	2.118	0.0436	G25	G4	G29	G34	S1	113.5	-0.721	G33	G34	G25	G2
C1	4.54	-1.2592	G34	G33	G5	G4	C2	214.4	-4.479	G33	G34	G4	G2
C2	6.758	-1.6974	G34	G33	G5	G4	C1	189.4	-5.764	G33	G34	G4	G2
	Ear height							Biomass (stover yield t/ha)					
K2	103.8	3.864	G25	G30	G19	G18	K2	9.168	1.764	G23	G6	G2	G30
S2	47.53	3.543	G25	G34	G28	G16	S1	3.47	1.51	G34	G23	G30	G2
S1	32.24	2.104	G33	G34	G25	G30	K1	6.882	1.221	G34	G25	G20	G23
K1	87.52	1.016	G34	G33	G25	G30	S2	4.569	1.209	G34	G25	G23	G20
M2	104.9	-0.586	G34	G25	G24	G33	M1	11.18	-0.134	G34	G25	G20	G33
M1	87.38	-1.245	G34	G25	G33	G24	M2	15.9	-0.739	G34	G25	G20	G22
C2	92.53	-1.463	G33	G34	G25	G30	C1	7.369	-2.05	G34	G33	G1	G26
C1	77.63	-7.233	G33	G34	G22	G30	C2	9.411	-2.782	G34	G33	G1	G26
	Days to 50% Anthesis (days)							Days to 50% silking (days)					
K1	67.11	1.268	G34	G7	G8	G13	S2	75.18	1.662	G13	G4	G7	G24
S1	76.86	1.196	G8	G24	G22	G26	K1	70.06	1.374	G7	G8	G34	G13
S2	72.25	1.139	G8	G24	G34	G22	S1	79.53	0.872	G7	G8	G34	G31
M2	65	0.911	G34	G7	G31	G13	M2	66.14	0.769	G7	G34	G8	G31
K2	63.37	0.864	G34	G7	G31	G13	K2	65.38	0.745	G7	G8	G34	G31
M1	68.93	0.518	G7	G34	G31	G13	M1	70.3	0.449	G31	G34	G7	G8
C1	108	-2.496	G25	G31	G33	G7	C1	109.1	-2.582	G25	G31	G33	G23
C2	97.18	-3.4	G25	G31	G33	G23	C2	98.56	-3.288	G25	G31	G23	G33
	Grain P concentration (%)							Grain P content (kg/ha)					
S2	0.186	0.203	G3	G17	G9	G19	M2	8.884	2.341	G20	G34	G21	G25
C2	0.205	0.105	G9	G3	G17	G25	K1	7.924	1.141	G25	G20	G24	G28
K2	0.204	0.080	G9	G3	G8	G31	M1	7.159	0.831	G25	G20	G34	G24
S1	0.182	0.044	G8	G22	G3	G26	S2	3.75	0.037	G25	G34	G4	G28
K1	0.206	0.036	G25	G9	G31	G4	S1	2.417	-0.224	G4	G25	G34	G28
C1	0.199	-0.043	G5	G9	G20	G24	K2	10.63	-0.493	G6	G25	G18	G4
M1	0.183	-0.095	G22	G5	G8	G24	C1	9.092	-0.965	G34	G4	G33	G1
M2	0.176	-0.331	G20	G13	G14	G5	C2	13.84	-2.669	G33	G5	G34	G4

5.4.4. Comparing the performance of genotypes using GGE Biplots

For grain yield, the first two IPCA axes jointly accounted for 77.68% of the total variation with the first axis explaining the major part of the variance (60.47%). For stover yield GGE biplot accounted for 80.98% of the total variation. For grain and stover yields, environment C2 and M2 exhibited the largest genetic variance while S1 and S2 had the lowest (Fig 6 a & b). The First PCA1 is normally represented on the x-axis and is used to estimate performance of genotypes hence genotypes with higher PC1 values were considered more productive. They included genotypes 1, 27, 28 and 29 (Fig 6 a). The genotypes were also grouped into high yielding or low yielding by the average ordinate environment (AOE) (Mohammadi and Amri, 2009). The thick line shown in Fig 6a is the performance line which passes through the origin of the biplot and helps to determine the mean performance of a genotype with the arrow on the performance line showing increasing mean grain yield. For grain yield, the highest nominal yields were attributed to hybrid 29, 30, 28 and 27 which were were identified as best performers. The poorest performer was hybrids 25. This genotype is referred to as vertex genotype characterised by the longest distance from the origin of the biplot in its direction (opposite the direction of ordinance line). From this study, Hybrid 29 was the best performer in environment C1 and C2 followed by hybrid 27 while the least performer in these environments was hybrid 25. Hybrid 28 and 1 were best at S1 and S2 while 21, 16 and 13 were best at K1. These hybrids (29, 27, 28, 21, 16 and 13) were found very close to certain environments and were regarded as responsive and showed specific adaptation. These findings compares well with those of Abay et al., 2009 who reported several genotypes clustering around specific locations. According to Sharma et al., 2010, such genotypes normally show

decreased stability across environments. Majority of the hybrids showed average performance in all the environments (found at the origin of the biplot) except for the case of days to 50% silking, stover yields and grain P concentration (Fig 6a-f).

Hybrid 29 produced the highest biomass and won in 4 environments (M1, M2, C1 and C2). However, Hybrid 25 was the best performer for plant height at K2 followed by hybrid 30 while parental line 31 was the worst. (Fig 6 b-c). These findings agree well with those of Nzuve et al., 2013 who also used the AOE to categorise maize hybrids into different yield classes. There was high genetic positive correlation for grain yield between the following environments K1 and K2 (r_g=0.6**), K1 and M1 (r_g =0.72**), K2 and M1 (r_g =0.65**) and C1 and C2 (r_g 0.88***). In contrast very low correlation was observed between K2 and S2 (r_g =0.25), K2 and C2 (r_g =0.33) (Fig 6 a).

For both grain P concentration and grain P content, GGE biplot accounted for 53.13% and 70.71% of the total variation. For grain P, there was high positive correlation between S2 and K2, S1 and K2, C2 and K1 and negative correlation between M1 and C2, C2 and S2, C2 and S1. The winning genotype for grain P at M1 was hybrid 22, the best at S1 included hybrid 26, 33 and 3, and at M2 were hybrid 20 and 13 while at C2 was 30. For this trait, majority of the hybrids showed specific adaptation to particular environments (hybrids scattered towards the environments) (Fig 6 e & f). For grain P content, most of the hybrids exhibited average performance (concentrated around the origin although certain hybrids still showed preference to certain environment for instance hybrid 39 showed preference to C2 while 24 and 25 were the best at K1 and M1 (Fig 6 e & f).

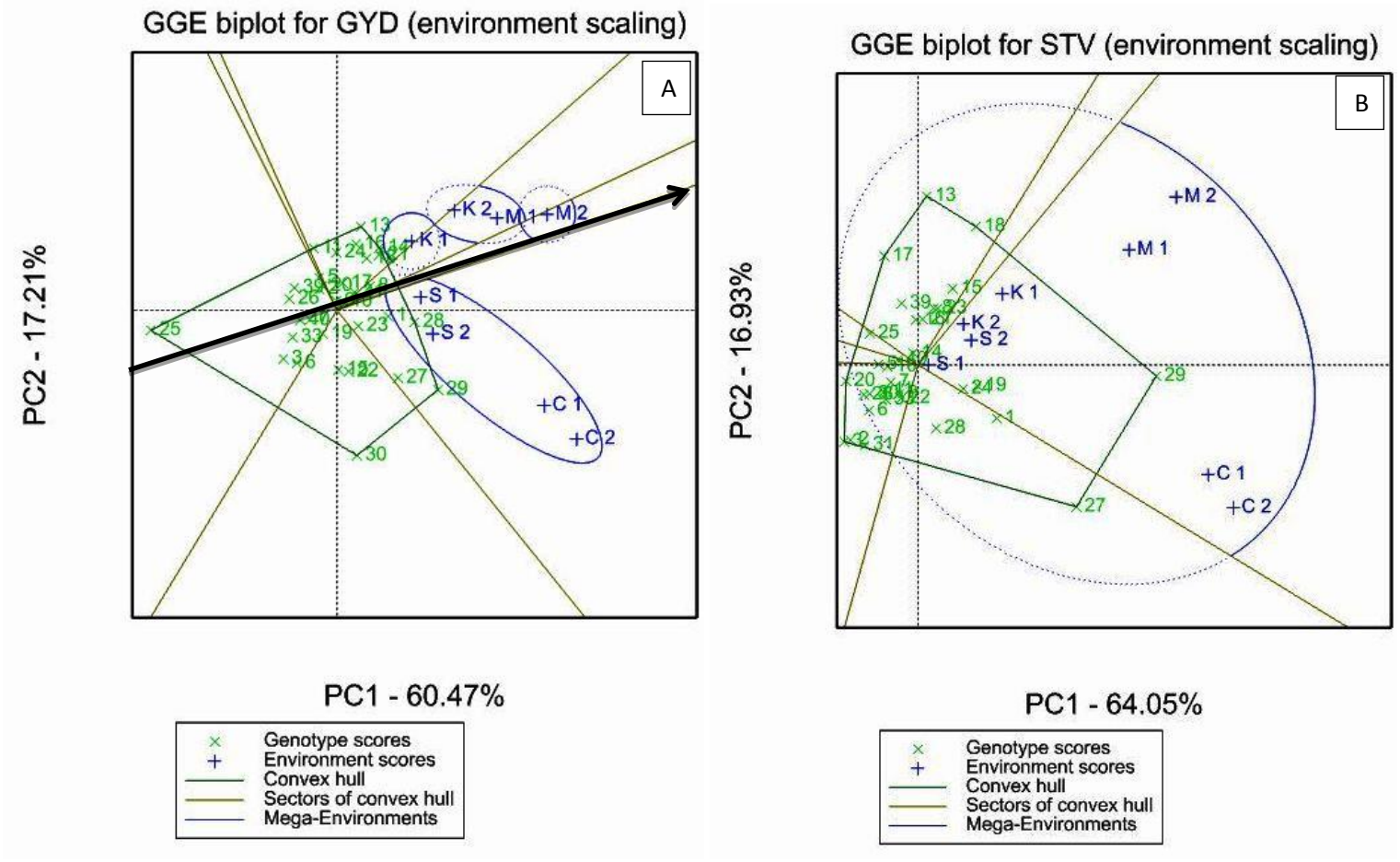

Figure 6 a-b: GGE Biplots for Grain and stover yield of maize hybrids conducted in western Kenya

Note* Genotypes are represented by multiplication sign (x). Environments are shown with addition sign (+) CI-Chepkoilel with 6 KgP/ha, C2-Chepkoilel with 36 KgP/ha, MI-Migori with 6 KgP/ha, M2- Migori with 36 K gP/ha, K1-Koyonzo with 6KgP/ha K2-Koyonzo with 36 Kg P/ha, SI- Sega with 6 KgP/ha, S2-Sega with 36 KgP/ha. GYD-grain yield, STV-biomass/stover yield

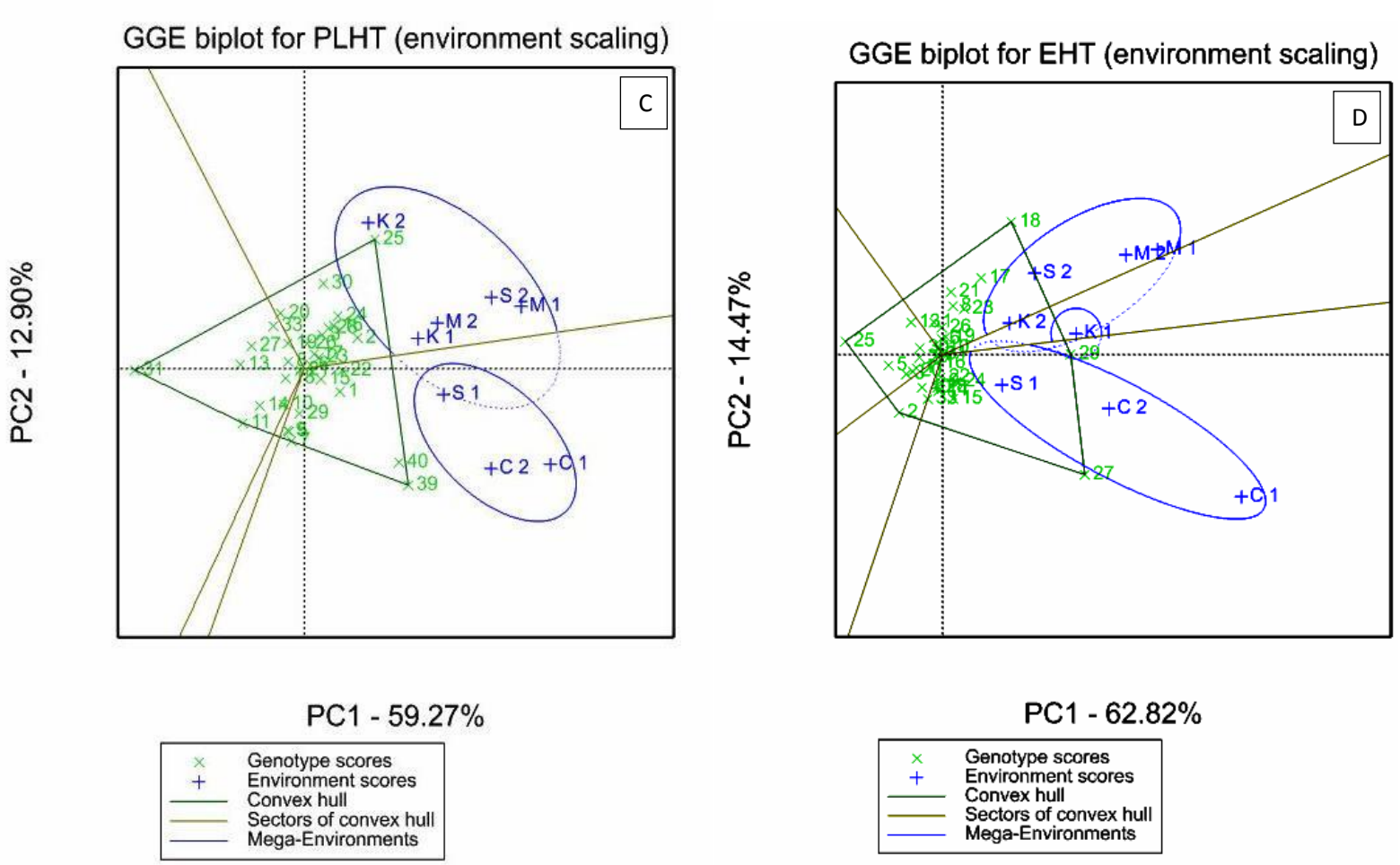

Figure 6 c-d: GGE Biplots for plant height and ear height of maize hybrids conducted in western Kenya

Note* Genotypes are represented by multiplication sign (x). Environments are shown with addition sign (+) CI-Chepkoilel with 6 KgP/ha, C2-Chepkoilel with 36 KgP/ha, MI-Migori with 6 KgP/ha, M2- Migori with 36 KgP/ha, K1-Koyonzo with 6 KgP/ha K2-Koyonzo with 36 KgP/ha, SI- Sega with 6 KgP/ha, S2-Sega with 36 KgP/ha. PLHT-plant height, EHT-Ear height

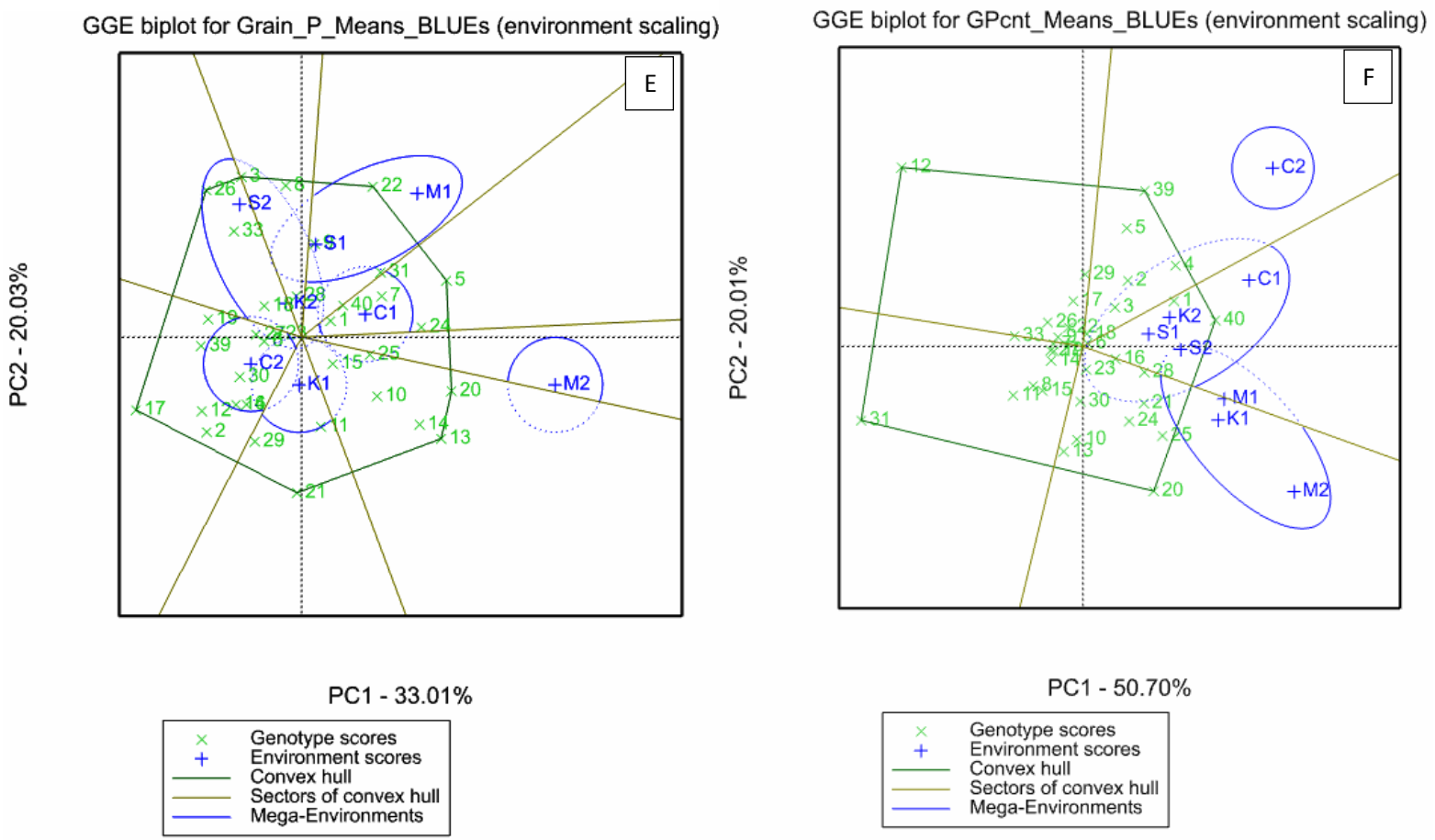

Figure 6 e-f: GGE Biplots for grain P and grain P content of maize hybrids conducted in western Kenya

Note* Genotypes are represented by multiplication sign (x). Environments are shown with addition sign (+) CI-Chepkoilel with 6KgP/ha, C2-Chepkoilel with 36 KgP/ha, MI-Migori with 6 KgP/ha, M2- Migori with 36 KgP/ha, K1-Koyonzo with 6 KgP/ha K2-Koyonzo with 36 KgP/ha, SI- Sega with 6 KgP/ha, S2-Sega with 36KgP/ha. Grain P-grain phosphorus content, GPcnt-grain phosphorus content

5.4.5. Genotypic Stability Analysis

For grain yield, majority of the maize hybrids (50%) showed below average sensitivity (bi < 1) which represents above average stability for these hybrids and is an indication that they were well adapted to unfavourable low P environments (Table 27). The results also showed that 9% of the tested hybrids expressed average sensitivity (Average slope bi =1) which shows average stability and good adaptation to both low P and high P environments. The remaining hybrids (41%) exhibited above average sensitivity (bi > 1), which indicates below average stability and shows that these genotypes were well adapted to high P (favourable) environments. The most adapted genotype to both low P and high P environments was genotype 1(Average slope bi =1.01) while the least adapted to both environments was hybrid 29 (bi =1.38). However the best adapted genotype to low P environments was hybrid 25 (bi =0.57). The commercial check, genotype 39 was only moderately adapted to low P environments (bi =0.91). These results show the great potential of the newly developed maize hybrids to the low P regions of western Kenya. For plant height the most stable hybrid was 7 (bi= 0.82) while the least stable was 3 (bi = 1.22) while for ear height, the most stable was hybrid 14 and the least was hybrid 29 (Table 27). For grain yield the ecovalence stability parameter ranged from 0.4 to 11.8 (Table 28). The most stable genotype was hybrid 9 (wi= 0.4) followed by 7 (wi=0.6) while the least stable was hybrid 30 (wi=11.8) followed by 29 (wi=9.2). Twenty six percent of the newly developed hybrids were more stable across the environments than the check (39- hybrid 515). However, for plant height, the most stable hybrid was 27 followed by 14 while the least stable was hybrid 39 followed by 31 (Table 28).

Table 27: Dynamic stability for maize hybrids tested across eight environments

Hybrid	GYLD t/ha	PHT cm	EH cm	INTL cm	STV t/ha	DTANTH Days	DTSL days	ANSLK days	EANO	GLS
1	1.07	0.98	0.83	1.05	1.24	0.94	0.91	1.56	0.93	1.20
2	1.01	1.04	0.95	1.04	0.57	0.96	1.01	0.97	1.13	0.69
3	0.97	1.22	1.05	0.95	0.71	0.93	0.90	2.05	1.20	1.40
5	0.87	0.77	0.92	0.99	0.91	0.94	0.94	2.98	0.99	1.71
6	0.85	1.13	0.96	0.90	0.83	1.02	1.02	0.75	0.89	0.37
7	0.87	0.82	0.92	1.00	0.82	0.93	0.97	0.30	0.88	0.90
8	0.88	1.10	0.95	1.03	1.05	1.00	1.03	0.57	0.99	0.49
9	0.97	0.94	0.90	0.99	0.95	0.98	0.99	0.71	1.08	0.31
10	1.06	1.06	0.97	0.87	0.76	0.98	1.03	0.05	0.94	0.94
11	0.98	1.14	0.81	1.04	0.74	1.05	1.03	0.93	0.89	0.04
13	1.03	1.06	0.95	1.02	1.35	0.93	0.88	1.41	1.20	0.77
14	1.18	0.90	0.80	0.97	0.97	1.02	1.04	0.83	1.16	1.75
15	1.28	0.85	1.05	1.20	1.22	1.00	1.00	1.51	1.32	0.78
16	1.12	0.77	0.89	0.87	0.96	1.10	1.15	0.54	0.95	0.78
17	0.96	0.91	1.22	1.15	1.11	0.99	1.02	0.77	1.18	1.43
18	0.59	0.99	1.14	1.03	1.40	1.38	1.37	0.86	0.76	0.96
19	0.99	0.94	1.10	1.08	1.25	0.94	0.94	0.14	1.13	1.17
20	1.04	1.05	1.12	1.08	0.74	0.91	0.92	-0.24	0.92	1.50
21	1.19	0.93	1.01	0.99	1.05	1.00	1.02	-0.26	0.65	1.26
22	0.90	1.06	0.96	1.06	0.93	1.01	1.01	-0.44	0.88	0.77
23	1.10	0.99	1.21	1.06	1.22	0.96	0.93	1.62	1.02	0.65
24	0.89	1.10	0.94	0.98	1.01	1.01	1.00	0.58	1.09	1.72
25	0.57	1.11	0.98	0.81	1.25	1.14	1.12	1.18	0.64	1.43
26	0.80	1.08	1.16	0.95	0.69	1.00	0.99	1.57	1.03	1.03
27	1.34	1.15	1.15	1.12	1.25	1.15	1.09	1.27	0.99	1.40
28	1.17	0.90	0.93	1.06	0.94	0.97	1.02	1.94	0.83	1.31
29	1.38	0.92	1.25	1.02	1.71	0.92	0.91	0.94	1.16	1.19
30	1.18	0.90	0.90	0.84	0.89	0.96	0.94	0.78	1.00	1.48
31	1.16	1.09	1.11	1.02	0.56	0.93	0.97	0.66	1.08	0.73
33	0.74	1.14	0.92	0.97	0.75	0.97	0.89	3.12	0.82	0.53
39	0.91	0.92	1.01	1.03	1.16	1.03	0.96	1.97	0.83	1.02
40	0.97	1.04	0.92	0.84	0.95	0.98	1.01	-0.24	1.40	0.12

Note: GYLD-grain yield, PHT-plant height, EHT-ear height, INTL-internode length, STV-stover, DANTH-days to anthesis, DASLK-days to silking, ASLKI-anther-silk interval, EANO-ear number

In static/biological stability a stable genotype is able to give comparable performance across environments and hence has small variance across the environments. The stability

parameter used is variance around the mean (Becker and Leon, 1988). In this study static stability was not considered because of the presence of high GXE interaction for most of the traits studied, instead dynamic stability was considered. In dynamic/agronomic stability, genotypic performance changes in a predictable way to environmental changes with respect to a model. Two models that were used for dynamic stability include Wrickes ecovalence (wi) and Finlay and Wilkinson model (sensitivity parameter - bi) (Finlay and Wilkinson, 1963 and Wrickes, 1964). Stability parameter alone is insufficient because it may be related to lower yield across all environments. The ideal situation is therefore to have a stability parameter along with the highest mean in any environment. The ideal genotype should have the highest mean performance and be absolutely stable (Yan and Kang, 2003, Sharma et al., 2010). Genotypic superiority combines performance and stability.

Table 28: Agronomic stability measure coefficients for maize hybrids tested across 8 environments in western Kenya

	GYLD	PHT	EHT	INTL	STV	DANTH	DASLk	ASLKI	EANO
Hybrid	t/ha	cm	cm	cm	t/ha	Days	days	days	
1	0.8	1324.8	659.5	7.3	52.3	12	18.3	5.9	59
2	1	489.7	252.8	1.1	22.5	8.4	7.6	6.9	16
3	2.7	1412	261.1	1.5	13.8	26.9	49.4	14.6	29.7
5	2.3	1004.4	489.1	3.2	4.9	35.9	56.1	24.6	63.8
6	2.2	755.5	255.3	3.5	10	17.4	27.3	10.5	17.3
7	0.6	1004.4	34.9	1.8	6.9	22.5	4.9	10.8	45
8	2.5	393.8	298.2	6.1	16.7	14.2	14.9	13.9	37.9
9	0.4	1059.8	296.7	2.1	2.3	24.5	23.5	4.8	9.2
10	1.9	1147.3	343.5	3.4	11.3	6.8	8.2	15.3	14.3
11	3.1	1711.2	963.5	5.8	9.5	23.4	11.9	7.7	29.8
13	4.6	712.8	415.5	10.8	55.5	20	37.3	6.7	67.3
14	3.9	979.4	436	2.6	1.3	5.8	12.5	9.5	32.7
15	4.2	486.4	389.1	6.9	21.9	20.5	12.6	10.5	61.5
16	3.1	1718.1	396.5	7.5	14.5	107.9	95	11.5	8
17	1.1	388.8	952.8	5.6	33.6	24	36.5	5.3	41.9
18	4.2	133.8	1230.3	8.9	42.2	318.9	294	4	73.9
19	1.1	647.7	112.3	3.9	29.3	32.6	25.1	13	52.9
20	1.9	925	333	8.3	18.8	37.7	17.5	11.1	32.4
21	2.4	301.4	377.6	7.5	6.3	5.8	12.8	13.1	36.4
22	3	572.7	498.1	3.3	8.7	20.2	35.8	15.9	32
23	1.4	327.4	770.6	1	14.8	26	37.5	7.6	31.2
24	3.4	1371.7	324.1	2.3	13.3	6.2	22.3	14.8	23.3
25	7.7	1729.3	985.5	24.7	43.4	77.2	87	12.7	59.7
26	2.4	586.5	421.1	5.9	20.6	10.9	47.6	24.6	24.2
27	6.1	1488.3	4256.7	11.3	147.5	55.3	28.9	11.2	22.7
28	3.5	765.4	133.5	2.5	21.8	20.2	49.6	40.6	26
29	9.2	1995.3	1765.2	9.2	145.2	22.3	39.4	10.7	38.6
30	11.8	1440.7	213.8	5.1	7.4	5.4	13.3	3.5	25.3
31	2.6	3190.4	361.6	6.6	53.6	20.4	12.1	5.8	20.6
33	3.3	1129.7	357.5	6.6	17	26.7	37.6	26.1	45.9
39	1.5	3817.9	31.9	2.9	13.3	22.4	12.1	20.6	32.5
40	0.9	3013.4	169.3	21.9	3.4	5.3	7.6	9.4	65.2

Note: GYLD-grain yield, PHT-plant height, EHT-ear height, INTL-internode length, STV-stover, DANTH-days to anthesis, DASLK-days to silking, ASLKI-anther-silk interval, EANO-ear number

5.4.6. Superiority Analysis

There was siginificant variation for superiority measure among the genotypes for various traits measured. Genotypes 29, 1,27,21,23, 18 were more superior (Pi = 0.4-1.40) while genotypes 25, 3, and 26, 39 and 33 (Pi =.3-10.5) were more variable and hence inferior for grain yield (Table 29). The most superior genotypes for plant height included 39, 40, 25, 2, 22 (Pi =181.5 to 543.8) while genotypes 12, 11, 13, 13, 31 (Pi = 1609-3885) were least superior. Genotype 29, 27, 1 and 19 were most superior for biomass production (Pi = 0.3-17) while 6, 25, 2, 20 and 3 least superior (Pi = 41.1-47.8). Some of the genotypes that were stable were not necessarily selected for superiority. From the above results, it is evident that selection based on genotypic stability a lone may not results into tracking the ideal genotypes. A breeder must therefore consider both stability measure parameter and the mean performance of a cultivar across all environments. These findings agree well with those of Bernardo, 2002 who also recommended the consideration of both stability parameter as well as the genotypic mean performance across environments.

Table 29: Superiority measure coefficients for maize hybrids tested across 8 environments in western Kenya for one year.

Hybrid	GYLD t/ha	PHT cm	EH cm	INTL cm	STV t/ha	DTANTH days	DTSL days	ANSLK days	EANO	GLS scale 1-5
29	0.40	934.3	141.9	1.8	0.3	30.1	27.7	7.2	18.9	1.4
1	0.80	679.8	931.6	1.8	14.9	58.4	58.2	6.9	16.8	1.3
27	0.80	1519.3	83.3	0.9	6.5	21.1	23.9	9.0	6.3	1.6
21	1.20	962.1	802.9	3.6	27.0	48.6	47.3	9.8	4.6	1.0
23	1.40	713.9	727.8	3.2	24.8	42.2	44.3	7.6	17.7	1.1
18	1.40	1078.4	498.4	1.5	19.4	6.2	7.3	8.6	10.7	1.6
8	1.40	1126.9	755.1	3.4	25.3	42.9	32.4	6.3	8.1	1.5
14	1.40	1609.0	837.8	3.6	28.4	52.1	49.0	7.8	7.3	1.1
22	1.60	543.8	812.7	5.4	32.6	51.2	46.4	7.8	9.7	0.7
31	1.60	3885.4	877.1	2.9	41.1	66.5	64.7	8.6	14.9	1.0
16	1.70	758.3	886.6	4.3	27.8	46.0	41.2	7.7	7.1	1.4
13	1.80	1868.5	1119.6	3.1	29.0	57.4	65.7	10.2	13.6	0.7
17	1.90	763.6	611.8	2.0	37.5	37.9	34.7	7.1	17.1	1.2
10	1.90	1098.8	834.5	4.6	34.3	53.5	49.2	8.8	14.0	0.4
9	1.90	1130.7	825.6	3.5	31.6	71.3	68.8	8.0	11.6	0.7
30	2.00	734.2	1042.3	3.5	40.4	51.9	52.8	7.9	15.8	0.8
24	2.20	609.7	683.0	3.8	18.0	51.8	58.2	11.7	4.4	0.7
15	2.30	746.5	722.2	2.1	21.4	43.0	36.7	4.8	23.0	1.0
19	2.40	1036.2	810.9	1.9	17.0	61.7	59.7	8.7	12.8	0.9
20	2.50	1206.4	1096.2	3.5	45.7	58.2	63.1	13.0	14.0	0.7
2	2.60	424.1	1229.6	2.5	45.0	55.4	52.7	7.8	10.8	1.2
7	2.70	1091.4	856.9	4.4	33.8	49.4	40.6	7.2	13.9	0.8
5	2.70	1136.5	1422.6	7.9	37.4	32.6	35.4	8.9	15.7	0.9
11	2.80	1789.0	1050.6	3.2	34.8	46.6	47.3	8.6	9.0	1.3
40	3.10	261.8	1006.4	5.5	32.1	59.0	55.8	8.4	14.9	1.1
6	3.30	777.2	846.9	5.7	41.1	43.2	39.3	5.4	19.4	0.3
33	3.30	1258.7	944.3	6.1	35.3	28.2	26.3	4.1	11.5	0.9
39	3.40	181.5	1033.5	3.7	32.2	25.3	23.4	5.3	24.0	0.6
26	3.50	801.9	886.2	5.0	40.6	48.1	41.7	6.1	11.3	1.1
3	4.10	816.3	1192.8	6.1	47.8	57.1	55.7	6.0	23.5	0.3
25	10.50	408.0	1902.6	13.9	43.8	12.3	14.3	8.8	65.3	0.3

Note: GYLD-grain yield, PHT-plant height, EHT-ear height, INTL-internode length, STV-stover, DANTH-days to anthesis, DASLK-days to silking, ASLKI-anther-silk interval, EANO-ear number

5.5. Conclusions and Reommendation

The results indicated that grain yield and other agronomic traits were highly influenced by GE interaction; the magnitude of environment effect was about six times that of genotype effect. Twenty six percent of the newly developed hybrids were more stable than the commercial hybrid based on Wrickes (wi) stability parameter. According to the GGE biplot and superiority analysis (Pi) genotypes 1, 27, 21 and 23 can be characterised as genotypes with the appropriate mean yield and stability (most superior) in low P soils of western Kenya (Pi= 0.4-1.4) while genotypes 25, 3, 26, 39 and 31 as least superior (3-10.5). The scientific information obtained from these results could be useful to plant breeders in supporting breeding program decisions on cultivar stability and adaptation. This study recommends further testing of these maize hybrids in more seasons for validation of the present findings and for commercial release of the experimental maize hybrids given the current low productivity of maize in Kenya.

CHAPTER SIX

6.0. GENETIC MAP AND QTLS ASSOCIATED WITH TOLERANCE TO LOW PHOSPHORUS IN MAIZE USING SNPS.

6.1. Abstract

Low available phosphorus (P) is a major constraint to maize (*Zea mays* L.) productivity in tropical soils. Selection for P efficient varieties is one useful strategy to combat this constraint. However, selection using morphological trait alone is slow and always confounded by environmental influence on the major traits of importance. The objective of this study was to identify major QTL(s) associated with P efficiency in maize using single nucleotide polymorphic markers. 228 F2 individuals derived from a cross between maize inbred lines KML 036 and S396-16-1 tohether with 239 SNPs were used in the study. The 239 SNPswere mapped onto ten linkage groups (LGs) spanning 2255 centiMorgans (cM) with an average inter-marker distance of 9.44 cM. On average, one LG contained 23.9 markers that spanned an average of 94.4 cM. The median distance between markers ranged from 0.5-41 cM with an average of 2.6 cM. Majority of the SNP markers (63 %) followed the Mendelian segregation and were fairly distributed in all the LGs. Mean performance for all the traits in the F3 population were higher than the parental values, which suggested transgressive segregation for all traits. Low to moderate broad sense heritability (0.35-0.50) was measured in the F3 population for grain yield (GYLD), plant height (PHT) and Ear height (EHT) which indicated that tolerance to low P is controlled by complex multi genetic factors. A full multi-QTL model analysis suggested six QTLs (2 QTLs each for GYLD, PHT and EHT) located on chromosomes 1, 3, 4 and 8. For GYLD, both the high value and the dominant alleles for the 2 QTLs always came from the first parent (KML 036), however for PHT and EHT, the dominant allele was sometimes coming from the second parent (S396-16-1). The two QTLs for GYLD increased maize yield under low P soils by 173 kg/ha (0.173 t/ha) with KML 036 being the contributor of the favourable alleles resulting to the yield increase while the 2 QTLs for PHT increased plant growth by 18.14 cm. The % phenotypic variance explained by these QTLs under low P environments had a wide range (0.242 -53.34%) and were much lower for GYLD compared to plant growth. Both additive and dominance gene actions contributed differentially to the observed phenotypic variance for tolerance to low P soils with dominance contribution being more important compared to the additive one for majority of the QTLs. The new QTLs identified will be useful for improving maize productivity in low P soils of western Kenya.

Key words: Additive, dominance, heritability, Low P, maize, Quantitative trait loci

6.2. Introduction

Phosphorus is one of the most important plant nutrients, contributing approximately 0.2% of a plant's dry weight, and is a component of key organic molecules such as nucleic acids, phospholipids and energy transfers (White and Hammond, 2008; Schachtman et al., 1998). However, in the tropics, it is mainly unavailable to plants even when the total amount of soil P is high (Marschner, 1995). It is estimated that P availability to plant roots is limited in nearly 67% of the cultivated soils, causing an important constraint to crop production (Lynch, 2011; Batjes, 1997). Therefore sustainable agricultural production requires improved P management (Tilman et al., 2002). P deficiency in both calcareous and acidic soils are majorly due to formation of poorly soluble P complexes with calcium in alkaline and aluminium and iron in acidic soils (Oztuk et al., 2005; Marschner, 1995). It is also due to inherent low soil P and insufficient fertilizer use to replace soil P removed through crop harvests (Obura et al., 2001). According to Sanchez et al., 1997, P depletion due to crop harvest is at the rate of 2.5 kg P/ha/year from the soils warranting constant replenishments. To date both modern high intensive agriculture and traditional subsistence farming strongly relies on phosphorus (P) fertilization to maintain yields and quality of crops. However, this approach is currently faced with several challenges. On one hand, there is increasing concern about P scarcity because the world's main source of P fertilizers, phosphate rock, is a limited and non-renewable resource which might only last for the next 40-400 years (Kauwenbergh, 2010; Cooper et al., 2011; Cordell and white, 2013; Obersteiner et al., 2013). On the other hand, P fertilization can harm the environment by contributing to the eutrophication of water bodies (Conly et al., 2009). Moreover, inappropriate P fertilization also accelerates the

soil P imbalance in croplands worldwide and may affect the inherent potential of maize roots to obtain P from the soil surface (Deng et al., 2014). Other concerns include high and fluctuations in fertilizer prices which economically affect farmers worldwide (Van der Velde et al, 2013).

Such concerns have in the resent years necessitated the diversification to more sustainable and ecologically sound crop production strategies aiming at increasing P acquisition and utilization efficiency in agriculture and environmental conservation. Apart from the need for long term P management strategies, the development of P-efficient maize varieties is a valid and necessary approach to improving yield and enhancing food security especially in the western Kenya region where maize is the most important food crop to the small holder farmers who are the majority in this region and the soils are extremely low in available P (<5mgP/Kg soil). Breeding and selection approach can significantly reduce the use of Pi fertilizers in agricultural systems as it results into crops that produce comparable yields/biomass with lower inputs of inorganic Pi fertilizers or have reduced physiological P requirements and tissue P concentrations. These traits enables the reduction in the amount of P removed by the crop and subsequently the amount of P needed to maintain the availability of Pi in the soil (Ahmad, 2003; Ouma et al., 2012).

However breeding and selection using morphological trait alone is often confounded by genotype by environment interactions (GXE) on some of the major parameters used to select for tolerance to low P under field conditions. Traits such as grain yield and root growth characteristics that are useful for selecting for tolerance to low P are complex

quantitatively inherited trait P and have very low heritability under stressful environments and hence the difficulty to select for them using phenotypic characters alone. (Manske et al., 2002; Parentoni et al., 2010: Yu et al., 2011; Ouma, et al., 2012). Additionally conventional breeding alone for tolerance to P deficiency is slow and hence the use of genetic markers and molecular tools alongside the conventional strategies are essential if this constraint is to be overcomed. In maize, the genetic control of P efficiency is dependent on the selection criteria used, growing stage of the plants and the environmental conditions where the experiment is conducted, whether under field, green house or in nutrient culture solution (Parentoni et al., 2010). Tolerance to low P is controlled largely by additive gene effects, although dominance and epistatic effects have also been shown to be important (Chaubey et al., 1994; Parentoni et al., 2010). Furthermore, P acquisition efficiency has been shown to have higher broad sense heritability hence the potential for selection (Coltman et al., 1987). Indeed, genetic based solutions to low P soil limitation in agriculture is still wanting in Kenya as is evident by lack of improved low P soil tolerant varieties in the market. Therefore the use of genetic markers alongside conventional breeding could speed up the breeding process.

Several genetic markers have been used including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) to identify useful QTLs for yield improvement in sorghum (Leiser et al., 2014; Hufnagel et al., 2014), maize (Maron et al., 2014; Guimarhaes et al., 2014). Although microsatellites have widely been used in molecular biology research, they have several limitations including: low level automation of their methods, difficulty in typing more than ten loci in a single

reaction, low abundance in the genome, and time-consuming analysis requiring arge numbers of loci (Jarne and Lagoda, 1996).

Therefore recent advances in molecular technology have preferred SNP markers (Hamblin et al., 2007; Jones et al., 2007)over (or in addition to) microsatellites and other markers in mapping studies because SNPs have high genomic abundance meaning that any genomic location can be analysed, have potential for high through put analysis and lower genotyping error rates, can easily be typed on a much larger scale, low cost per data point, locus-specificity, and codominance (Rafalski, 2002; Morin et al. 2004). According to Slate (2008), some SNP platforms have error rates lower than the mutation rate of some microsatellites and so map error or map inflation due to typing error are likely to be less problematic than is the case for microsatellites. Therefore SNPs have emerged as a powerful tool for many other genetic applications, including genetic diversity studies, linkage and quantitative trait loci (QTL) mapping, and marker-assisted breeding (Zhu et al., 2003). Consequently this study adopted the use of SNPs for QTL analysis in maize.

Currently, chip-based technology is the most high-throughput SNP genotyping platform. The Illumina chip-based SNP detection technology is useful for a broad range of applications to genotype samples with different possible levels of multiplexing, from 48 to 384 (Bead Xpress) and 1536 (Golden Gate) to 55,000 SNPs (Infinium). Such chip-based genotyping platforms are suitable for large-scale studies that require genotyping of individual samples with thousands of SNPs (Low et al., 2006). They may be unsuitable for studies where only a small to moderate number of SNPs are needed over a large

number of samples, as is the case in mapping, marker assisted recurrent selection, marker assisted back- crossing, and quality control applications. In such cases, uniplex SNP genotyping platforms such as the competitive allele specific PCR (KASP) systems are more suitable (Low et al., 2006). Hence the genotyping in this study was Kasp based.

At present phosphorus uptake 1 (*Pup 1*) gene is the only P- related QTL that has been identified in rice variety kasalath. This QTL was mapped to the long arm of chromosome 12 (Ni et al., 1998; Wissuwa et al., 1998, 2002; Heuer et al., 2009). *Pup 1* breeding lines have proven effective in field trials (Wissuwa et al., 2002; Chin et al., 2011) and increased grain yield 3-folds under low P soils. Further sequencing and characterization of the *Pup 1* locus showed the presence of *Pup1* specific protein kinase gene named phosphorus starvation tolerance 1(*PSTOL1*) (Gamuyao et al., 2012). These authors further showed that overexpression of the *PSTOL1* significantly increased grain yield by 30% using a physiological mechanism based on the enhancement of early root growth and development under phosphorus deficient soils thereby enabling plants to acquire more phosphorus and other nutrients. Hufnagel et al. (2014) investigated the role of homologs of *PSTOL1* in sorghum (*SbPSTOL1*) under low P soils in Mali and Brazil and reported that *SbPSTOL1* genes colocalized with quantitative trait loci for traits underlying root morphology and dry weight accumulation under low P. The *SbPSTOL1* alleles reduced root diameter which is associated with enhanced P uptake under low P while both Sb03g006765 and Sb03g0031680 alleles increased root surface area resulting into increased grain yield in low-P soils. These authors therefore suggested that *PSTOL 1* gene enhanced P acquisition and performance of sorghum under low P soils. So far only one study, Mendes et al. (2015) has reported QTLs related to P efficiency in tropical

maize using grain yield and other phenotypic data in tropical conditions. The information needed for breeding for tolerance to low P is therefore still scarce.This study therefore intended to identify single nucleotide polymorphic markers (SNPs) that are linked to the major QTL(s) associated with P efficiency loci using 208 F2 individuals derived from a cross between maize inbred lines KML 036 and S396-16-1 contrasting in tolerance to low P.

6.3. Materials and Methods

6.3.1. Genetic material

A total of two hundred and twenty eight F2 plants derived from KML 036 x S 396-16-1 maize inbred lines contrasting for P-efficiency were used in this study. The inbred line KML 036 is P-efficient while S 396-16-1 is P-inefficient (Ouma et al., 2012). Both lines are white seeded and have been described in Section 3.3.3. The 2 inbred lines were crossed in 2011 to generate F1 progenies which were advanced to F2 through selfing. Two hundred and twenty eight F2:3 were raised during the long season of 2012 at Migori site. In this study, the F2 segregating population was used for genetic linkage map construction since it's the best population for preliminary mapping. Besides, it requires less time and minimum efforts for development and gives the highest level of segregation when compared to other populations (Singh and Prasanna, 2008).

6.3.2. Leaf sampling, Genomic DNA extraction and quantification

Maize leaf samples were obtained from 3 weeks old maize seedlings using LGC genomics leaf sampling Kit contained 96-well storage plate from Kibos site. Sample leaves were placed on the Harris cutting mat and leaf disks were cut in rolling circular

motion using 6mm clean Harris Uni-Core cutting tool. The plunger on the cutting tool was then depressed swiftly to release the leaf disc into the appropriate wells. The cutting end of Harris Uni-core cutting tool was rinsed several times in 2% NaClO (sodium hypochlorite) washed 5 times in water and dried on paper towel before using it in the next leaf sample (http://www.finnzymes.com/directpcr/harris_unicore.html). The above procedure was repeated until all the parental and the F2 samples were completed. The plates were then sealed with a perforated (gas permeable) heat seal, packaged in a heavy duty and sealed in the presence of a desiccant (Silica gel) to dehydrate and hence preserve the leaf tissue during transit at ambient temperature and shipped to LGC genomics in the UK.

Genomic DNA was extracted from dried young leaf samples using kleargene leaf DNA extraction Miniprep Kit which utilizes a glass fibre solid support inserted to microtitre plate in 96 and 384-well formats. The method is Centyl Trimethyl Ammonium Bromide (CTAB) based (Hoisington et al., 1994; http://www.lgcgenomics.com/kleargene-spin-plate). DNA pellets were kept at room temperature for 30 minutes and then dissolved into 200 uL of 0.1xTE buffer. DNA was quantified using Quant-iTTM PicoGreen dsDNA Assay Kit (Invitrogen San Diego, CA) and the fluorescence measured using the Microtiter plate reader (Varioscan from Thermo Scientific). Samples were adjusted to 40 ng/µl using Tris-EDTA buffer. Note that the total amount of DNA quantity of 3-30 ng is required for KASP PCR reactions (Gaur et al., 2012).

6.3.3. Selection of polymorphic SNP markers

The DNA from the two parental lines (KML 036 and S 396-16-1) were genotyped for polymorphism using a total of 1250 random SNP chip developed at Cornell University, out which the 466 polymorphic SNPs were selected based on the Nucleotide SNP calling of the parental samples (Semagn et al., 2012)**.** The F2 DNA samples were then assayed using 466 polymorphic SNPs at LGC genomics laboratory in the UK.

6.3.4. SNP genotyping and PCR Amplification

SNP genotyping was carried out using Kompetitive Allele Specific PCR (KASP) using the LGC genomics KASP system (lgcgenomics.com; Yuan et al., 2014). KASP assay components used for SNP genotyping comprised: primer mix and KASP PCR master mix. The primer mix contained 0.05-0.07 µM of each of the 2 unlabelled allele specific forward primers and 0.07-0.20 µM of one common unlabelled reverse primer. The KASP PCR master mix contained 0.2-0.5 uM of klear *Taq* polymerase, 0.05-0.20 Mm of each dNTPs, 1-2 µl of 1x PCR Buffer (10mM Tris-HCl, Ph 8.3), 1.8-2.8 mM $MgCl_2$ and two distinct FRET (fluorescence resonant energy transfer) cassettes; one labelled with FAM™ dye (Emission wave length 485- 520 nm) and the other with HEX™ dye (Emission wave length 535-M556 nm) in the corresponding quencher. The passive reference dye succinimidyl ester (ROX) (Emission wave length 575- 610 nm) was used to allow normalisation of variations in signal caused by differences in well to well liquid volume. The KASP homogenous assay was added to each of the 2-2.5 µl (1-10 ng/ µl) DNA samples with total reaction optimized to 4-10 µl volume in each of the 384 well PCR plates (http://www.lgcgenomics.com/kasp-genotyping-reagents). Two no template

controls (NTCs) were included on each genotyping plate. The volumes of the reagents and reaction volumes were calculated using a standard procedure given by the manufacturer in a spread sheet found at www.kbioscience.co.uk/download/index.html. The PCR plates were then sealed with a clear seal using Fusion Laser welding system and placed into the Hydrocycler water bath-based thermocycler where the PCR reaction was initiated. The thermocycler regimes were set at initial denaturation at 94^{o}C for 15 minutes followed by 10 cycles of 20s at 94^{o}C, annealing for 60s at touch down temperatures declining from 65-57^{o}C (dropping by 0.8^{o}C per cycle) and extension for 10s at 72 ^{o}C. Then another 26 cycles for 20s at 94^{o}C, 60s at 57^{o}C and extension for 40s at 72 ^{o}C (www.lgcgenomics/KASP_manual.pdf).

6.3.4. Plate Reading and Analysis of SNP genotyping data

An in point reading of KASP PCR data was done using Real time PCR machine (RTPCR) (Applied Biosystems - http://www.appliedbiosystems.com) at between 20-40^{o}C in order to capture both the FAM™ and the HEX™ dye signals. KASP uses the fluorescence FAM and HEX for distinguishing genotypes (**www.kbioscience**.co.uk//KASP_manual.pdf**)**. The data was then imported into the KlusterCaller software **www.kbioscience.co.uk/software/klustercaller** for automatic SNP calling for each locus. Data was automatically read by the software and checked manually for errors and rescored while designating homozygous and heterozygous clusters. Using this software, the FAM and HEX data were plotted on the x and y axes, respectively which automatically created a genotype cluster diagram for all genotypes at each SNP. Passive reference dye (ROX) was used to normalise the data by dividing FAM and HEX values by the passive reference value for the particular wells, thus removing the

variable of liquid volume. Genotypic classes were then determined according to sample clusters using the FAM and the HEX fluorescence. The presence of the same fluorescence dye signal alone (FAM) (Red) indicated that the DNA sample was homozygous for one allele while the HEX (Blue) dye signal alone indicated that the DNA sample was homozygous for the other allele. A 50/50 mixture (Green) of the two dyes indicated heterozygous DNA samples. The Cluster data was viewed graphically using SNP Viewer version 3.2.

6.3.5. Construction of Genetic Linkage Map

The map was generated based on 239 polymorphic SNPs and 228 F2 families (2 F2 families were omitted for having > 80% missing data). The results of the SNP alleles were converted to marker data by formatting using Microsoft excel into 2, 0, 1 and -1 which is the format required by ICiMapping software version 3.2 (Jiankang et al., 2012). Where 2 and 0 are KML 036 and S 396-16-1 respectively while 1 and -1 represents heterozygotes and missing data respectively. Goodness of fit test was performed using *Chi-square* test (p=0.05) for the conformity to the expected Mendelian segregation ratio of 1:2:1. Markers were ordered with the regression mapping algorithm and were classified into Linkage groups (LGs) using the grouping module at LOD thresholds of 7-8.0 at an increment of 0.5.

Linkage groups were determined at LOD 8.0 with a recombination frequency smaller than 0.49 and a maximum threshold value of 5 cM for the jump. The best marker order was determined using the 'Ripple' function (value of 1). Recombination frequencies between marker loci was estimated using the maximum likelihood estimate (MLE) of the

recombination fraction and converted to map distances in centiMorgans (cM) using the Kosambi mapping function (Kosambi, 1944).

6.3.6. Phenotyping of F2:3 populations in low P soils.

Out of the 228 F2:3 progenies of the cross KML 036 X S 396-16-1 genotyped, only 208 were evaluated at Migori site in the long rain season of 2014. The rest did dot have adequate seeds for evaluation. The experiment was laid out in a 16 x 13 resolvable alpha lattice incomplete block design replicated three times. Sixteen genotypes were blocked together in each of the 13 incomplete blocks. Randomization and field layout was generated by Genstat version 17 (Payne et al., 2014). The plants were grown in single row plots of 3 m long using a spacing of 0.75 x 0.30 m. Six weeks after sowing, all the plots were top dressed using Calcium Ammonium nitrate (CAN) at 75 kg N/ha. Weeding ase done manually thrice and the crop protected from stalk borer (*Buseolafusca*L.) damage using 2-3 granules of Beta-cyhalothrin (Bulldock GR 0.05) at a rate of 6 Kg ha^{-1} applied in the whorl of each plant after thinning. Data was scored for grain yield, plant height and ear height from a sample of 8 plants per plot drawn from inner rows.

6.3.7. Statistical Analysis

Field data was analysed by Linear mixed models (REML) using Genstat version 18 to obtain means and variance components under low P among the 208 genotypes. The genotypes were considered fixed while the blocks, as random effects when fitting the mixed model in order to determine the genotypic effects. The genotypic mean of the F2:3 families (BLUEs-best linear

Unbiased estimates) were used for QTL analysis. The following model was fitted and used to analyse the data

$\underline{Y}_{ijm} = \mu + \rho_j + \underline{B}_{m(j)} + G_i + \underline{\grave{\varepsilon}}_{ijm}$ (Kersey and Pooni, 1998).

Where Y_{ijm} is the observation on the ijm^{th} plot,

μ is the general mean, ρj is the effect due to the fixed j^{th} replication, $B_{m(j)}$ is the effect due to the m^{th} random incomplete block nested within replicate

G_i is the effect due to the i^{th} genotype in the m^{th} block, of the jth replicate

$\grave{\varepsilon}_{ijm}$ is the residual effect due to ijm^{th} plot

6.3.8. Estimation of heritability

Broad sense heritability (H^2) was estimated by variance components using linear mixed models (REML) of Genstat version 18. Broad-sense heritability was calculated as follows:

$H^2 = \sigma^2_g / \{(\sigma^2_g + (\sigma_{error}{}^2/r)\}$

Where $H^{2\ is}$ broad sense heritability, σ^2_g is the generic variance; $\sigma_{error}{}^2$ is the error variance; r is the number of replicates per genotype (Ribaut et al., 1996).

6.3.9. QTL Analysis

Phenotypic mean values of 208 F2:3 families and linkage map data were used to perform QTL analysis for plant height, ear height and grain yield. A composite interval mapping method (CIM) (Jansen and Stam 1994; Zeng 1994) was implemented in Breeding view

software (Genstat based) (Payne et al., 2014). QTL detections were performed every 5 cM along the chromosomes. In the first step, simple interval mapping was performed and cofactors selected (Lander and Botstein, 1989). For co-factor selection, F-to enter and F-to drop threshold was set at 6.0 to avoid selecting multiple markers linked to one QTL as co-factors (Wissuwa et al., 2002). Using these cofactors to reduce the residual variation, QTLs were detected using composite interval mapping (CIM) Zheng, 1994) where further runs were done with all markers on chromosomes selected as co-factors in order to detect multiple QTLs on chromosomes with greater resolutions (Utz and Melchinger, 1996). QTL detections were performed every 5 cM along the chromosomes and a final multi-QTL model was fitted (Jiankang et al., 2012). Likelihood ratio statisticts based on permutations (-log10 (P)) with LOD score of >3.0 considered significant for QTL detection (Liu, 1998).

6.4. Results and Discussion

6.4.1. Screening SNP markers for polymorphism

A total of 1250 useful SNP markers from maize genome were used to genotype the two maize parental lines (KML 036, S396-16-1) for polymorphism out of which 1165 (93.2%) were reported. The remaining (85) were not included in the analysis due weak amplication, irreproducibility in allele calling or had more than 10% missing data. Out of the 1165 SNPs, 79 were mono-allelic and were also excluded from the subsequent analysis hence only 1085 markers were analysed for polymorphism in the two parental lines. In the 1085 SNPs, base changes involved A/C (182), A/G (708), A/T (68) and C/G (127) with the A/G changes accounting for thehighest (65.3%) and A/T the lowest (6.2%) of the informative SNPs (Fig 7).

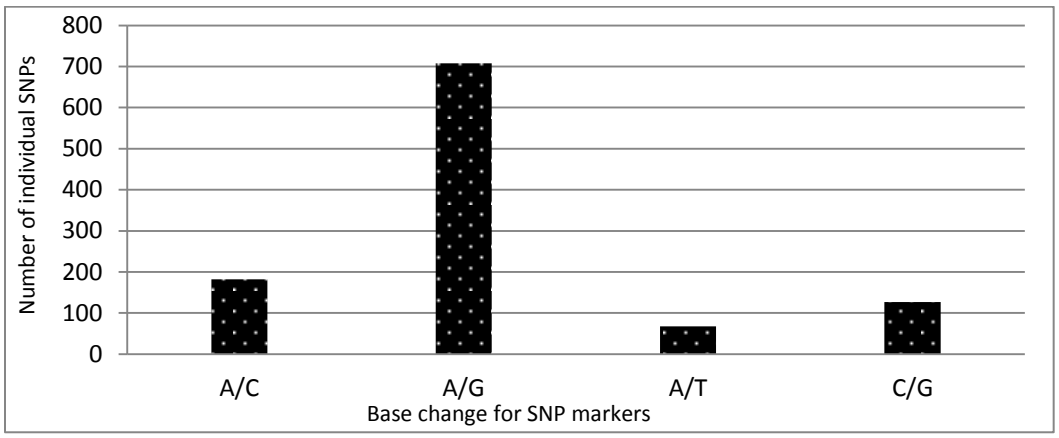

Figure 7: Frequency of allelic base change in polymorphic SNP used to genotype

6.4.2. SNP genotyping of F2 segregating population

The 228 maize F2 families were genotyped with the 466 polymorphic SNPs using KASP (Kompetitive allele specific PCR) genotyping system and data scored using Klustercaller software version 3.2. For each SNP, the genotyping data representation included three main clusters corresponding to AA/GG homozygotes, AG/AC/AT heterozygotes and CC/TT homozygotes. Fig 8 shows the appearance of an end point fluorescence scatter plots of the genotyping data scored with Klustercaller software with and without normalization with Passive reference dye (ROX).

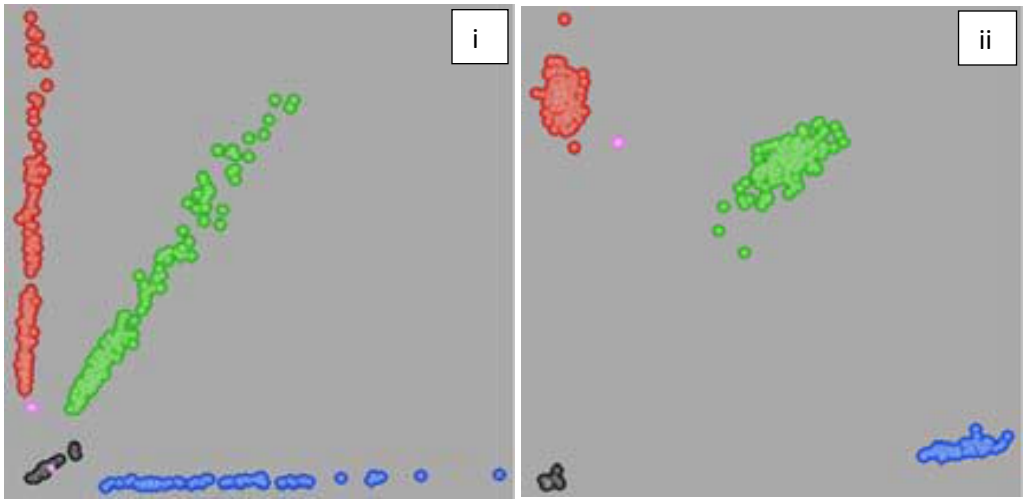

Figure 8: Cluster plot results for maize sample SNP genotyping

In this study, an F2 segregating population was used, which is expected to contribute very many heterozygotes therefore most of the SNP markers produced three main clusters representing the two homozygous and one heterozygous genotypes (Fig 9 A). However some markers produced only two clusters representing the two homozygous groups while others one cluster representing one homozygous group (Fig 9 B, C). Some data points were also sometimes ambiguously and located outside these clusters (indicated by arrows in Fig 9 D) and represented those for which no calls were generated and were therefore scored as missing data.

A total of 436 SNPs were successfully genotyped in the F2 segregating population giving a success rate of 93.2%. The remaining 30 (6.8%) SNP markers did not produce clearly well separated clustering patterns hence were considered as technically unsuccessful and were excluded from the analysis. Out of the 436 successful SNPs, 52 SNP markers (11.92%) were considered false (failed to detect an SNP in the F2 segregating population) that grouped into a single cluster (e.g. PHM1870_20 in Fig 9 C).

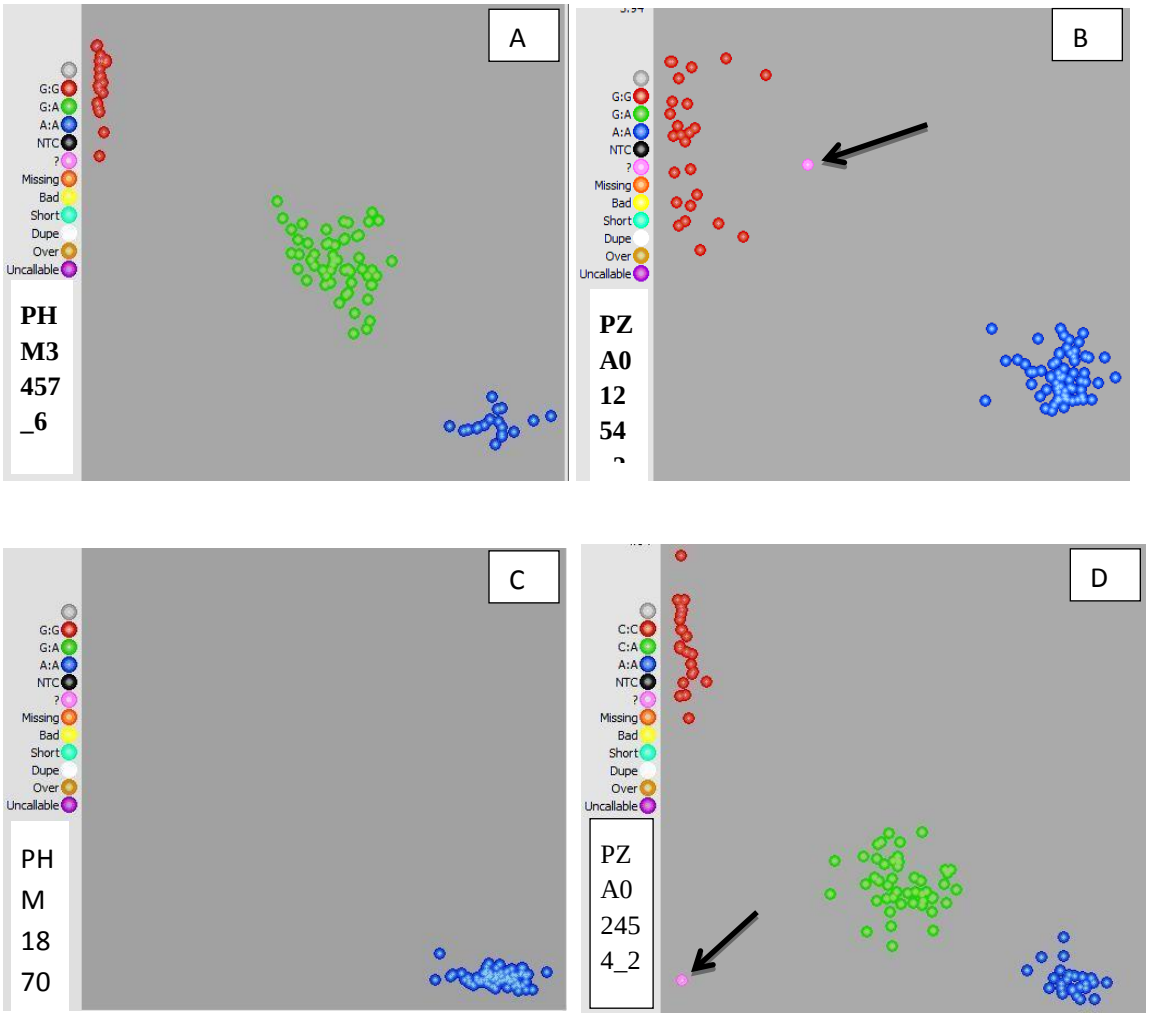

Figure 9 A-D: Representative clustering patterns generated by the KASP SNP Genotyping assay.

Additionally, of the 384 SNPs that showed clear clustering pattern, 145 were found to be heterozygous in at least one of the parents and hence were excluded from linkage analysis. In general therefore, out of the 436 SNP which were successfully genotyped, only 239 were considered informative in the maize F2 segregating population which demonstrated a success rate of 54.82% with the KASP assay in the Kenyan maize F2 population studied. These results compare well with those of Gaur et al. (2012) who genotyped 768 SNPs in Chick pea (*Cicer arietinum* L.) recombinant inbred lines (RILs)

using Illumina Golden Gate assays (GGGT) and reported similar SNP clustering pattern and obtained a success rate of 90.75% .They also agree well with those of Semagn et al. (2012) who reported missing SNP data, ambiguity, irreproducibility in allele calling in 30.7% of the SNPS they used in diverse CIMMYT maize inbred lines. A sample view of the segregation of the maize F2:3 in chromosomes 2 and 8 as viewed using flapjack software (Milne et al., 2010) are shown in Fig 10.

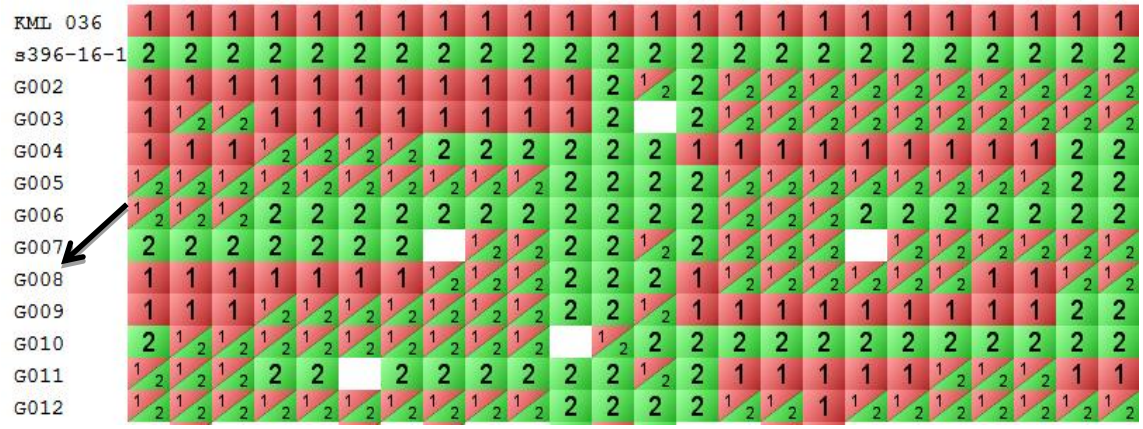

Figure 10: Segregation of maize F2 genotypic data on chromosome 2.

6.4.3. SNP mapping

The genotyping data of 239 polymorphic SNPs screened on 228 F_2 segregating populations was used for map construction and linkage analysis. Two hundred and thirty nine (239) markers were mapped onto the ten linkage groups spanning 2255 centiMorgans (cM) with an average inter-marker distance of 9.44 cM. The LGs were numbered (1 to 10) based on the common marker positions shared between corresponding LGs from previous studies (Yanli et al., 2009; Semagn et al., 2012) and according to public maize genetic maps (Fan et al., 2006). The genetic length of the LGs ranged from 117.818 cM (LG 6) to 425.52 cM (LG2) (Table 30). The markers were

unevenly and non-randomly distributed in the LGs with LG 8 being the most saturated (43 markers) with an average marker density of 7.72 cM, whereas LG7 had the least number of markers (only 9) (Table 30). On an average, one linkage group contained 23.9 markers that spanned an average of 94.4 cM. The median distance between markers ranged from 0.5-41 cM with an average of 2.6 cM.

Table 30: Distribution of SNP markers on the ten maize linkage groups

Linkage group	Length Cm	Number of markers	Average Length(cM)	Median distance between markers	95% percentile of distances (cM)
1	154.3	20	7.72	1.8	50.5
2	425.4	36	11.82	5.9	40.3
3	138.9	27	5.14	0.5	41.1
4	243.4	20	12.17	3.1	78.2
5	118.1	22	5.37	0.7	30.4
6	117.8	21	5.61	1.2	38.7
7	413.9	9	45.99	4.1	136.6
8	331.8	43	7.72	3.3	34.8
9	145.4	26	5.59	3.2	19.1
10	166.4	15	11.09	1.7	72.9
Genome	2255.5	239	9.44	2.6	45.7

The x^2 test performed showed segregation distortion (SDST) for 37 % of the marker loci. However these markers were finally integrated into the map in order to minimize loss of genetic information related to these markers. Moreover, the distorted markers were found to be widely distributed throughout all the LGs even though the ratios varied from one LG to another. For example LG 6 showed the highest distortion (8.3%) while LG 3 the least (2.1%) (Fig 11). The overall segregation distortion of 37% observed in this study was compared well with those of Choudhary et al. (2012) and Gaur et al. (2012) who reported SDST of 41.3 and 42% in bean population.

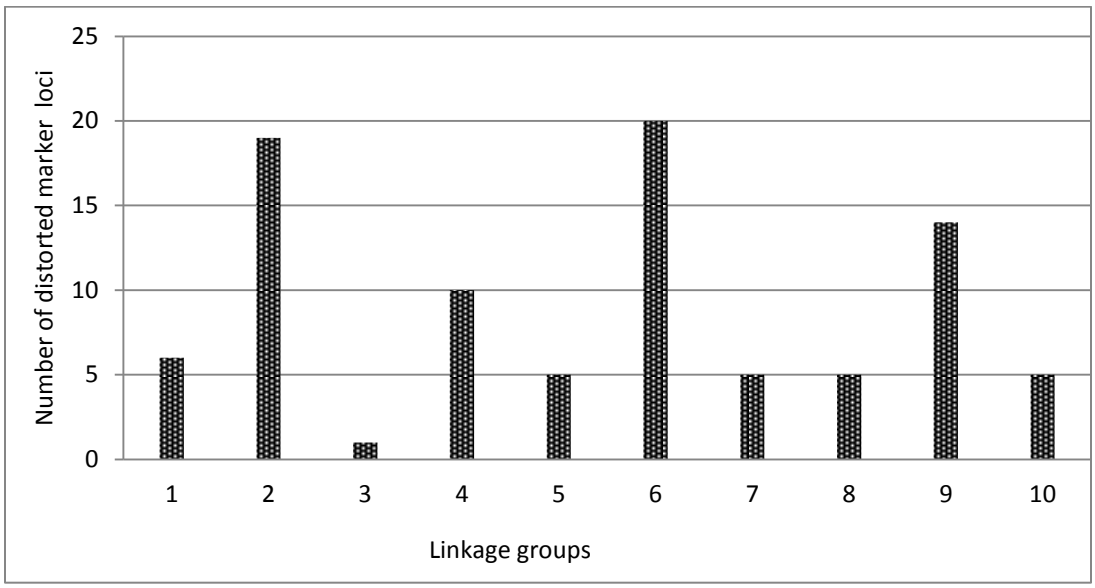

Figure 11: Marker loci showing distorted segregation resulting from x^2 test.

6.4.4. Phenotypic distribution, heritability and correlations

Means and broad-sense heritabilities for GYLD, PHT and EHT are presented in Table 30. Mean performances observed in all the traits in F3 population was higher than the parental values, which suggested transgressive segregation with respect to parental values for all traits. This finding may also suggest the absence of epistasis for the inheritance of these traits under low P soils (Table 31).These findings compare well with those of Huang et al., 2010 who also reported intermediary performance of the segregating F3 populations in comparison with the parental lines. In contrary to the findings of this study, the authors reported higher mean parental values compared to the segregating F3 population and suggested some epistatic gene actions. In this study F3 means were higher than the parental means.Broad sense heritability ranged from 0.35-0.50 among the phenotypic traits studied and was highest PHT and lowest in GYLD. The low to moderate heritability values based on family means for the various traits (GYLD, PHT

and EHT) indicate that tolerance to low P and the measured traits are complex multi genetic factors each regarded as quantitative trait loci (QTL) as have been suggested by Wissuwa et al. (2002) and Yu et al. (2011). The low to moderate heritability under stress conditions has also been reported by Weber et al. (2012) and Edmeades et al. (1999) and was generally attributed to the larger environmental component to the variance associated with stressed environments. The findings of this study further compares well with those of Semagn et al. (2013) who reported heritabilities ranging from 0.23 to 0.58 for grain yield and anther silk interval for 18 maize bi-parental populations.

Table 31: Heritabilities for GYLD, PHT and EHT of maize F3 population in low P soils

Parents				F3 segregating population					
Trait	KML 036	S396-16-1	Mean	Median	Lower quartile	Upper quartile	Mean	Standard deviation	Heritability
GYLD (t/ha)	2.5	0.4	1.5	2.4	1.6	3.4	2.7	1.5	0.32
PHT (cm)	150	125	137.5	170.0	153.3	186.7	169.2	24.3	0.55
EHT (cm)	60	40	50.0	53.3	43.3	63.3	53.9	15.9	0.38

GYLD-grain yield, PHT-plant height, EHT-ear height.

There was significant and high to moderate positive genetic correlation between GYLD, PHT and EHT (Table 1). The high positive and significant genetic correlation between PHT and EHT (r_g = 0.78**) under low P conditions may suggest that the duo traits may be controlled by similar QTLs or those located in the same chromosomal position. Studies by Semagn et al., 2013 also reported low to medium significant genetic correlations between GYLD and anther silk interval (ASI) although their correlations were negative because of the inverse relationship expected between grain yields and flowering characteristics. These findings also agree well with those of Mohan et al., 2002; Rafiq et al., 2010 and Aminu and Izge, (2012) who all reported significant genetic

correlation between grain yield in maize and other agronomic attributes such as plant height, ear height and days to 50% flowering.

Table 31: Genetic correlations between grain yield pant height and Ear heights of F2:3 segregating populations

	PHT	EHT	GYLD
PHT	-		
EHT	0.788**	-	
GYLD	0.56*	0.45*	-

6.4.5. QTL detection in F2:3 populations

QTLs for various traits were identified based on two rounds of composite interval mapping (CIM) and by finally fitting the full-multi QTL model. Permutation tests were employed to decide on the LOD score at $p< 0.05$ (Jiakang et al., 2012). For the QTLs detected, the LOD score (-log10 (P)) ranged from 3.12-3.97 with an average of 3.47. A total of 6 QTL were detected: 2, QTLs each for GYLD designated (CIP49 and PZAO2454_2), PHT (C8P114 and C8P247) and EHT (C3P122 and PHM 4586_12). The QTLs were non-uniformly distributed across the chromosomes (Table 32, Fig 12 a-c). For GYLD, they were located on chromosome 1 and 4 while for both PHT and EHT, the 3 QTLs were located on chromosome 8 while one for EHT on chromosome 3. Both additive and dominance gene actions contributed differentially to the observed phenotypic variance for tolerance to low p soils with dominance contribution being more important compared to the additive ones for majority of the QTLs. For grain yield the two QTLs increased grain yield by 173 kg/ha (0.173 t/ha) (additive effects) with KML 036 being the contributor of the favourable alleles resulting in the yield increase (Table 32).

The 2 QTLs for plant height increased plant height by 18.14 cm while the EHT QTLs gave an increase of 3.67 cm. For grain yield, both the high value and the dominant alleles for the 2 QTLs always came from the first parent (KML 036), however for PHT and EHT, the dominant allele was sometimes coming from the second parent (S396-16-1). The % phenotypic variance explained by these QTLs under low P environments had a wide range (0.242 -53.34%) and were much lower for grain yield compared to plant growth. These results compare well with those of semagn et al. (2012) who reported between 1.3 to% to 8.4% explained variance in maize under drought stress conditions. It also agrees with those of Chen et al. (2009) who reported 24-35% range for P utilization efficiency in maize and those of Mendes et al.(2015) who reported a range of 38-64 % of explained variance for phosphorus efficiency QTLS. The latter authors also identified QTLs for P aquisiion efficiency and Utilization efficiency on chromosomes 1, 3, 4, 5, 7 and 8 which coincides with the ones in the current study that were identified on chromosome 1, 3, 4 and 8. The information presented here is useful and will guide further breeding for tolerance to low P soils.

Table 32: QTLs associated with low P tolerance traits their position and effects in maize F2:3 populations.

Grain yield (t/ha)									
Locus no.	Locus name	Linkage group	QTL Position	%Expl. Var.	Add. eff.	High value allele	Dom. eff.	Dominant allele	~-log10(P)
20	C1P49	1	49	1.227	0.119	KML_036	0.351	KML_036	3.662
228	PZA02454_2	4	76.8	0.242	0.053	KML_036	0.339	KML_036	3.559
Plant height (cm)									
462	C8P114	8	113.76	53.347	14.733	KML_036	11.092	S396-16-1	3.29
494	C8P247	8	246.54	2.856	3.409	KML_036	10.162	KML_036	3.972
Ear height (cm)									
191	C3P122	3	121.81	7.075	3.016	KML_036	*	*	3.134
497	PHM4586_12	8	259.5	0.336	0.657	KML_036	3.518	S396-16-1	3.238

Fig 12: shows the genetic map of the identified QTLs fitted using breeding view in Genstat version 18, Payne al. (2012).

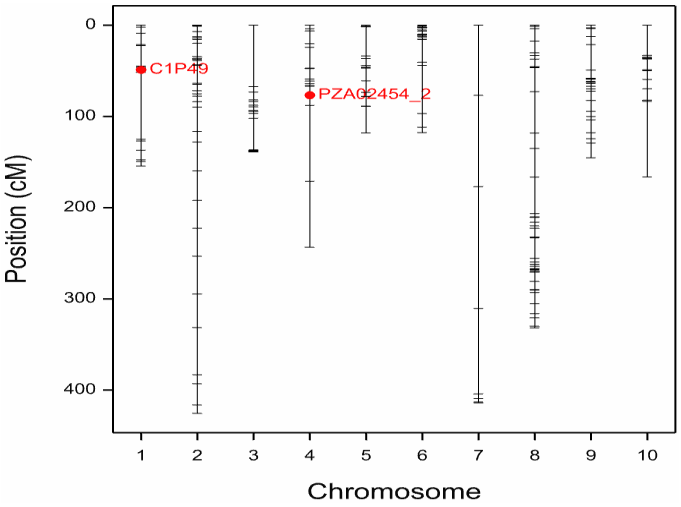

Figure 12 a: The genetic map of the identified grain yield QTLs

The red bulletin shows the locus name and the QTL position on the genetic map

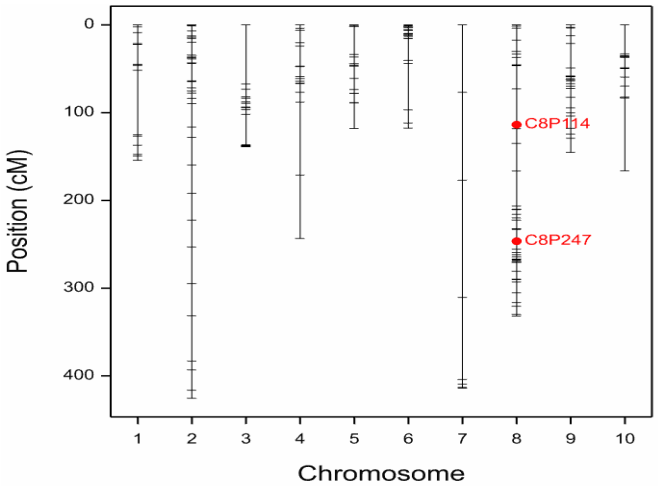

Fig 13 b: The genetic map of the identified PHT QTLs.

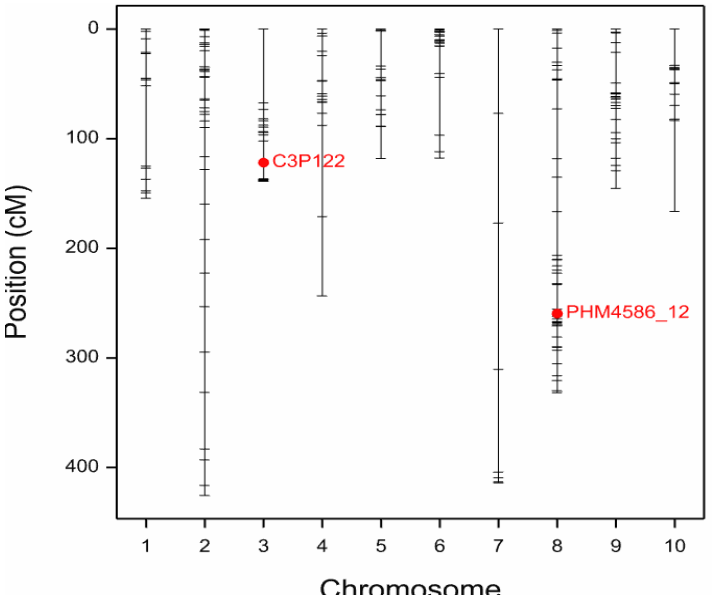

Fig 13 c: The genetic map of the identified EHT QTLs

6.5. Conclusions and Recommendations

A total of 6 QTLs located on chromosomes 1, 3, 4 and 8 were detected: 2 QTLs each for GYLD, PHT and EHT. The QTLs were non-uniformly distributed across the chromosomes and coincided with those identified in previous studies. Both additive and dominance gene actions contributed differentially to the observed phenotypic variance for tolerance to low P soils with dominance contribution being more important compared to the additive one for majority of the QTLs. The newly QTLs identified under low P conditions will be useful for improving maize productivity in low P soils of western Kenya. It's recommended that further studies be done to validate the identified QTLs in other populations and also to further characterize the QTL loci to identify the specific genes responsible for tolerance to low P.

CHAPTER SEVEN

7.0. GENERAL CONCLUSSIONS AND RECOMMENDATIONS

The present study was conducted in order to: (i) develop and identify P-efficient experimental maize hybrids for adaptation to low P field conditions (ii) compare the genetic effects of maize P efficiency traits under low P acid and non-acid soils (iii) determine environmental influence and stability of maize P efficiency traits in low P soils and (iii) identify major QTL(s) associated with P efficiency in maize using single nucleotide polymorphic markers. This study has developed and evaluated 30 experimental maize hybrids out of which 20 were identified as very suitable for growing in low P soils of western Kenya based on the genetic variation in P efficiency that existed amongst the hybrids both at low P supply and in response to P application. The correlation between grain and Stover P concentration and grain P content with majority of the P efficiency indices (PAE, PE, PUE) at both high and low P supply was always low or tended to be negative and non-significant implying that seed P reserve, and Stover P concentration, had minimal contribution to differential P efficiency observed in all genotypes and may not be a suitable criteria for determining P efficiency in maize. On the other hand, grain yield under low P correlated well with other P efficiency parameters studied (PAE, PUE, PER, AE and PE, RLD) implying that these parameters can be considered for indirect selection for tolerance to low P in maize. Grain yield at low P had strong positive genetic and phenotypic correlation with most of the traits studied indicating that both genotypic and phenotypic correlations are suitable models for selection and yield improvement in maize under low P soils.

Both additive and non-additive effects were detected in both acid and non-acids soils although this was more dependent on the trait studied and the level of available P. Dominance effects played a more important role than epistatic effects and the latter were more important than additive effects in the inheritance of majority of P efficiency traits studied in maize in both acid and non-acid soils. In most cases, epistasis was specifically more important in non-acid soils while dominance in acid soils. Additive gene effects were fairly of similar magnitude across the acid and non-acid soils. The magnitude of both additive and non-additive gene effects were always greater in non-acid soils compared to acid soils pointing to the detrimental effects of soil acidity in the detection of gene actions in maize. Our results suggest that the inheritance of grain yield and other P efficiency traits (plant height, Root Length Density, P acquisition Efficiency, Shoot Dry matter and P utilization Efficiency) did not differ in acid and non-acid soils. The overall results of this study showed that soil acidity significantly reduced P efficiency traits in maize and affected the detection of the genetic effects for these traits

Grain yield and other agronomic trait performance of maize were highly influenced by GE interaction; the magnitude of environment effect was about six times that of genotype effect. Twenty six percent of the newly developed hybrids were more stable than the commercial hybrid based on Wrickes (wi) stability parameter. According to the GGE biplot and superiority analysis (Pi), Genotypes 1, 27, 21 and 23 can be characterised as genotypes with the appropriate mean yield and stability (most superior) in low P soils of western Kenya (Pi= 0.4-1.4) while genotypes 25, 3, 26, 39 and 31 as least superior (3-10.5).

A total of 6 QTLs located on chromosomes 1, 3, 4 and 8 were detected: 2 QTLs each for GYLD, PHT and EHT. The QTLs were non-uniformly distributed across the chromosomes and coincided with those identified in previous studies. Both additive and dominance gene actions contributed differentially to the observed phenotypic variance for tolerance to low P soils with dominance contribution being more important compared to the additive one for majority of the QTLs.

In this study SNP alleles A/G accounted for the largest (65.3%) base change of the informative SNPs. Majority of the SNP markers (63 %) followed the Mendelian segregation and showed allelic frequencies according to the expectation and were widely distributed in all the chromosomes. Low to moderate broad sense heritability (0.35-0.50) measured in the F3 population for grain yield, plant height and Ear height indicated that tolerance to low P is controlled by complex multi genetic factors.

The natural genetic variation observed between the maize genotypes demonstrates the potential for breeding cultivars with improved phosphorus efficiency. The scientific information obtained from these results and the newly QTLs identified under low P conditions could be useful to plant breeders in improving maize productivity under low P and supporting breeding program decisions on cultivar stability and adaptation.

This study recommends further testing of these maize hybrids in more seasons for validation of the present findings and for commercial release of the experimental maize hybrids given the current low productivity of maize in Kenya. It also recommends further studies on QTL validation in other populations and further characterization to identify the specific genes responsible for tolerance to low P in Kenyan maize.

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8.0. Appendices

Appendix I: Description of the parental maize inbred lines was used to produce F1 hybrids

Inbred line	Source	P-efficiency class	Designated Male/Female
1.KML 036	KARI KAKAMEGA	Highly efficient	Female parent
2.HS L3 x 5046-2	An inbred line previously selected under low P from a Brazilian single cross hybrid	Highly efficient	Female parent
3.S 396-16-1	KARI KAKAMEGA	Inefficient	Male parent
4. MUL 229	KARI MUGUGA	Inefficient	Male parent
5.HS228 x 5046-16	An inbred line derived from a Brazilian single cross hybrid	Efficient	Female parent
6.MUAP II SR	KARI KITALE	Medium efficient	Male parent
7.POOL 9A BAS	KARI-KAKAMEGA	Medium efficient	Male parent
8.K15	KARI-KITALE	Medium efficient	Male parent
9.K4	KARI-KITALE	Medium efficient	Female parent
10.K17	KARI-KITALE	Medium efficient	Female parent
11.AO89	KARI-KITALE	Medium efficient	Male

Source: Ouma et al. (2012).

Appendix II: Key used for SNP data scoring

Type/Colour	Legend example	Title	Details
		Empty	Wells that have deliberately not been filled and are to be ignored
	G:G/ A:A/T:T	1:1	Calls that have been assigned to allele 1. These are homozygous for one allele. The genotype is displayed on the legend
	G:A/A:T/G:C	1:2	Calls that have been assigned to allele 1&2. These are heterozygous for the two allele. The genotype is displayed on the legend
	A:A/T:T/C:C	2:2	Calls that have been assigned to allele 2. These are homozygous for the other allele. The genotype is displayed on the legend.
	NTC	NTC	No template controls. Water control wells to be genotyped
	?	Unused	Calls that have not been scored because they were unreliable. These show up as "?" in the results file.
		Missing	Wells that should be filled but have been manually flagged missing
	Bad	Bad	DNA that the observed genotyping indicates a problem with the sample. This is usually caused by a sample constantly providing unreliable genotype results over several SNPs.