GENETIC ANALYSIS IN TROPICAL MAIZE FOR PHOSPHORUS UTILIZATION IN PHOSPHORUS-LIMITED SOIL

BY

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DECLARATION

I, Chibesa Mutale Edward, hereby declare that this dissertation represents my own work and that is has not been previously submitted for a degree, diploma or other qualification at this or any other University.

Signature: .....

Date: .....

APPROVAL

This dissertation of Chibesa Mutale Edward has been approved by the University of Zambia as partial fulfilment of the requirements for the award of the degree of Master of Science in Plant Science (Agronomy)

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Board of Examiners: …….. Signature: …… Date: ……

Supervisor: ……. Signature: ……..... Date: ……...

DEDICATION

This work is dedicated to my wife Noliwe Tembo Mutale, my mother Faustina Mulenga Mutale and to all my children-Kasonde, Gideon, Chibesa Jr, Nkole and Luyando.

ABSTRACT

Maize is one of the most important economic crops on the African continent. However, its production is constrained by both abiotic and biotic factors. Phosphorus (P) deficiency is one of the major abiotic constraints in maize production. It was for this reason the study was undertaken whose objectives were to: 1) evaluate genotypes which are efficient at utilizing phosphorus in P-limited soil, 2) investigate the type of the of gene action associated with traits linked to utilization of phosphorus in P-limited soils and 3) map quantitative trait loci (QTL) associated with phosphorus utilization in P-limited soil. Thirteen inbred lines (8 females and 5 males) previously screened for phosphorus utilization were obtained from CIMMYT, Zimbabwe through the maize team at Golden Valley Research Trust (GART) in Chisamba District, Zambia. To evaluate genotypes efficiency to phosphorus utilization and determining the type of gene action, eight (8) females and five (5) with varying reactions to P utilization were mated in an 8 x 5 North Carolina Design (NCD II). Forty (40) progenies were evaluated in the screen house using Completely Randomized Design (CRD) with three replications and two treatments (0 kg P and 60 kg P). The shoot biomass, root biomass, plant biomass and plant height were determined after the plants were harvested and dried at 80 ^{o}C for 72 hrs. Five crosses were observed to be highly efficient at utilizing phosphorus in P limited soils. Specific combining ability (SCA) effects were found to be highly significant different from zero (P = 0.001) for all measured parameters. Analysis of general combining ability (GCA) effects revealed that only the root biomass was significantly different from zero (P = 0.05). The Baker's ratio for Plant height, Shoot biomass, Root biomass and Plant biomass was found to be 0.12, 0.15, 0.49 and 0.28 respectively. This implied that non additive gene action conditioned plant height, shoot biomass and plant biomass responses in P-limiting soils. On the other hand, Baker's ratio for shoot biomass was 0.49 implying that additive and non-additive gene action conditioned this trait response in P-limited soils.

Inclusive composite interval mapping (ICIM) analysis identified seven QTLs related to phosphorus utilization on chromosome 5. All the mapped QTLs were more than 5 cM from the nearest molecular marker utilized in the study. Therefore, there is need to utilize the maize genomic map to identify and test several markers near the mapped QTL, in order to locate more reliable molecular markers for marker assisted selection (MAS).

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LIST OF ABBREVIATIONS

cM	Centimorgan
CTAB	Cetyltrimethylammonium bromide
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organisation
GCA	General combining ability
ICIM	Inclusive Composite Interval Mapping
LOD	Logarithm of odds
MAB	Molecular assisted breeding
MAS	Molecular assisted selection
MB	Molecular breeding
MM	Molecular markers
P	Phosphorus
PCR	Polymerase chain reaction
Pi	Inorganic phosphorus
Po	Organic phosphorus
PR	Phosphorus rock
PVE	Phenotypic variance explained
QTL	Quantitative trait loci
SCA	Specific combining ability
SSA	Sub-Sahara Africa
SSR	Simple sequence repeats
USDA	United States Department for Agriculture

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CHAPTER ONE

INTRODUCTION

1.1 Maize Production and Uses

Maize is grown widely throughout the world in a range of agro-ecological environments. Well over 160 million hectares of maize are planted worldwide. It is estimated that in 2016/17 growing season, the total world production of maize was 1.03 billion metric tons, with the United States, China, and Brazil harvesting 37%, 23%, and 6% of the total production of maize, respectively (USDA, 2017). The African continent produced 6.5% of the world output during the same period. The top two largest African producers during 2017 were South Africa with nearly 13 million metric tons, followed by Nigeria at nearly 7.2 million metric tons. In the same year, Zambia produced nearly 2.4 million metric tons (USDA, 2017).

Maize is the most important cereal crop and a staple food (for an estimated 50% of the population) for most countries in the Sub-Saharan Africa (SSA) (Tembo et al., 2016; Edmonds et al., 2009). The calories contribution from consumed maize is about 50% in Southern Africa when compared to other sources (Banzinger and Diallo, 2002). Per capital consumption of maize grain in Zambia was estimated at 140 kg per year (Smale and Jayne, 2003). Apart from home cooking, maize is a major ingredient in stockfeed and in many industrialized food products. Maize starch can be hydrolyzed and enzymatically treated to produce syrups, particularly high fructose maize syrup - a sweetener. It is also fermented and distilled to produce grain alcohol used as a biofuel to drive motor vehicles. Maize is sometimes used as the starch source for beer. Starch from maize can also be made into plastics, fabrics, adhesives, and many other chemical products. The corn steep liquor, a by-product of maize wet milling process, is widely used in the biochemical industry and research as a culture medium to grow microorganisms.

In Zambia, maize is the most important (staple) agricultural crop and about 78% of the total area under cereal production is allocated to the crop (Smale and Jayne, 2003). It is grown in most areas, with the exception of wet, dry or infertile places where sorghum and millet are primarily grown (Reynolds et al., 2015). It is regarded as a priority crop of economic importance in Zambia and the government subsidizes input availability to ensure improved yields and ultimately food security. The production of maize is, however, hampered by both biotic and abiotic factors.

1.2 Constraints to maize production in Sub-Saharan Africa

Due to its many uses the demand for maize in Zambia and other developing countries is expected to surpass the demand for both wheat and rice by the year 2020 (Pingali and Pandey, 2001). However, average productivity of maize in several developing countries of the tropics in SSA is still considerably low. About 45 million hectares of maize is grown in the tropics, where a range of climatic, biotic and abiotic constraints severely affect productivity.

Among the abiotic stresses, yield losses due to phosphorus (P) deficiency are a major factor. Fertilizer application is one major method of replenishing P in depleted soils. However, P fertilizers are costly, nonrenewable, and potentially ineffective because of immobilization by the soil. It is estimated that the major portion (80-90%) of mineral P fertilizers applied to the soil cannot be absorbed by plants due to adsorption to iron oxides/hydroxides, Aluminium hydroxides as well as to Calcium and Magnesium carbonate surfaces and due to chemical precipitation. Moreover, the applied mineral P fertilizer may also possibly be transformed to organic form, a process known as microbial immobilization (Holford, 1997). Thus, the mineral P fertilizer recovery of crops during the year of application is usually very low (less than 20%).

Efficient phosphorus uptake and utilization are important to enhance the applied mineral P fertilizers recovery and improve its availability to maize plants. Efficient P uptake may also be useful for reducing environmental impact from fertilizer runoff and leaching (Guingo and Hebert 1997). Phosphorus utilization efficiency is a term that generally describes the ability of crop species/genotypes of a given plant species to give higher yield under P-limiting condition (Graham, 1984). Plant species as well as genotypes within the same species may differ in P utilization efficiency (Gunes et al., 2006). The ability of a crop/genotype to give higher yield under P-limiting condition may be related to: the ability to take up more P from the soil under P-limiting condition (uptake efficiency) or the ability to produce higher dry matter per unit of P in the plant tissue (utilization efficiency) or a combination of both (Gahoonia and Nielsen, 1996).Thus, developing P-efficient maize cultivars that produce reasonably high biomass in low P soils through either ways stated above can reduce mineral P fertilizer input requirement in agricultural production.

1.3 Justification of the study

More than 40% of the world soils are deficient in phosphorus and the acid-weathered soils of tropical and subtropical regions of the world are particularly prone to P deficiency (Vance et al, 2003). Furthermore, continuous cropping without commensurate nutrient replenishment is reported to contribute to low P content in many soils. This scenario is typical of the maize growing soils of Zambia where the smallholder farmers are resource poor. Fertilizer use as a strategy for replenishing soils with limiting nutrients is a critical component in improving the production of maize in the SADC region (FAO, 2004). However, P fertilizers are costly, nonrenewable, and potentially ineffective because of immobilization by the soil. Sustainable management of P in agriculture requires that professionals in the area of crop sciences discover mechanisms that either enhance plant P acquisition ability and/or efficient P

utilization ability or further exploit these adaptations to make plants more efficient to thrive under P limiting conditions.

Breeding for phosphorus utilization efficient cultivars can help resolve the problems associated with low P. Breeding for low P tolerance require an understanding of nature of inheritance of traits. Understanding the nature of inheritance will enable breeders to design appropriate breeding strategies.

Previous studies in maize have shown that tolerance to low available P is largely conditioned by additive gene effects although dominance was also important (Da Silva et al., 1992). Parentoni et al., (2008) observed that genetic analyses based on generation means in maize revealed that phosphorus utilization is a complex trait with a prevalence of dominance over additive effects. There is need to evaluate the nature of gene action conditioning phosphorus utilization efficiency in tropical maize.

Conventional breeding methods for nutrient efficiency uptake are a challenge to breeders because of their dependency on environmental effect. Moreover, results of such crop improvement programs are affected by researcher preferences and management practices. There is need to develop selection methods which are more reliable and less environmental dependent (Mbwando et al., 2016). Identification of molecular markers linked to phosphorus utilization is an initial step in coming up with a molecular marker assisted selection technique which is ideally neutral to environmental effects or management practices. Molecular markers have become widely accepted as valuable tools for crop improvement in maize (Tuberosa et al., 2003).

The use of markers for dissecting polygenic traits into their QTLs or Mendelian components increases understanding of the inheritance and gene action for traits used for selection procedures (Anderson et al, 1993). QTLs for grain yield and its components evaluated in

high- and low-P soils have been mapped mostly in temperate maize genotypes (Li et al., 2010). Thus, in our study, we applied a strategy to map QTLs in tropical maize genotypes using ICIM QTL IciMapping Version 4.1.0.0 for phosphorus utilization and its components cultivated under low-P soil. The successful mapping of phosphorus utilization loci will enable researchers to dissect these quantitative traits into their single genetic components and support the pyramiding of beneficial QTL alleles. These issues are the focus of this thesis whose objectives are presented below.

1.4 General objective

To determine the genetic control of phosphorus utilization in P-limited soils for increased maize production and productivity in Zambia.

1.*4.1 The specific objectives were to*:

1. evaluate maize genotypes for efficiency in phosphorus utilization
2. investigate the type of the of gene action associated with traits linked to utilization of phosphorus in P-limited soils
3. map QTLs associated with phosphorus utilization in P-limited soil

1.5 Research hypothesis

1. Genotypes efficient at utilizing phosphorous exist in tropical maize

2. The nature of gene action associated with traits linked to phosphorus utilization is additive

3. QTL associated with phosphorous use efficiency exist on some maize chromosomes

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and geographical distribution of maize

Maize (*Zea mays L*) is the domesticated variant of teosinte (*Euchleana Mexican schrand*). It was domesticated in Southern Mexico about 9, 000 years ago (Matsuoka et al., 2002). It belongs to *Plantae* kingdom, *Poaceae* family and genus being *Zea*. Selection soon followed, favourable alleles at loci controlling plant morphology and kernel nutritional quality were fixed at least 4,400 years ago, and further selection by Native Americans facilitated maize adaptations to varied environments (Matsuoka et. al., 2002).

The Mesoamerican region developed a trade network based on surplus and varieties of maize crops. After European contact with the Americas in the late 15th and early 16th centuries, explorers and traders carried back to Europe and introduced it to other countries. Maize spread to the rest of the world because of its ability to grow in diverse climates. By the last decade of the twentieth century a tidal wave of maize had engulfed Africa, save its driest and wettest crannies, supplanting historical African food grains like sorghum, millet, and rice. In case of Zambia, maize has been domesticated since the 1930s when it was introduced by the Europeans into the then North Rhodesia mines, for mine workers (McCann, 2007).

Initially, the large phenotypic differences between maize and teosinte obscured the identity of the wild progenitor of maize for centuries. Recent genetic analyses coupled with precision phenotyping (Doebley, 2004) confirmed earlier genetic studies showing that the defining differences between maize and teosinte reside at relatively few loci.

Geographically, maize can be grown in a number of environments from 58˚ North (e.g. Canada and the Russian Federation) to 40˚ South (e.g. Chile). Generally, tropical maize is grown between 30˚ North and 30˚ south, subtropical maize between 30˚ and 34˚ both north and south, and temperate maize beyond 34˚ latitudes. Maize can be produced in a range of

altitudes from sea level up to 3,800 metres (Farnham et al., 2003). Indeed the maize crop is produced in geographic and climatic diversity regions as can be observed from table 1 showing the top fifteen largest producers in 2016-2017 growing season.

Table 1: Top fifteen maize producers in the world, 2016/17 season

Rank	Producer	Production in Thousand Metric Tons
1	United States	384,778
2	China	219,554
3	Brazil	86,500
4	European Union	60,309
5	Argentina	36,500
6	Ukraine	28,000
7	Mexico	26,000
8	India	24,500
9	Russia	15,500
10	Canada	13,200
11	South Africa	13,000
12	Indonesia	10,200
13	Philippines	7,900
14	Serbia	7,500
15	Nigeria	7,200

(Source: USDA, 2017)

2.2 Maize structure and physiology

Maize uses C_4 photosynthesis to fix atmospheric carbon dioxide. It is a diploid, cross pollinated annual plant with 20 chromosomes (2n = 20). The maize plant is a tall, monoecious annual grass with overlapping sheaths and broad conspicuously distichous blades (Hitchcock and Chase, 1971). The maize plant stem has the appearance of a bamboo cane and is commonly composed of a number of internodes of varying lengths. A leaf grows from each node, which is generally 9 cm in width and 120 cm in length.

Ears develop above a few of the leaves in the midsection of the plant, between the stem and leaf sheath, elongating by ~3 mm/ day, to a length of 18cm (60 cm being the most the maximum observed in the subspecies) They are female inflorescence , tightly enveloped by several layers of ear leaves commonly called husks.

The apex of the stem ends in the tassel, an inflorescence of the male flowers. When the tassel is mature and conditions are suitably warm and dry, anthers on the tassel dehisce and release pollen. Maize pollen is anemophilous (dispersed by wind), and because of its large settling velocity, most pollen falls within a few meters of the tassel.

Elongated stigmas, called silks, emerge from the whorl of husk leaves at the end of the ear. At the end of each silk is a carpel, which may develop into a “kernel” if fertilized by a pollen grain. The pericarp of the fruit is fused with the seed coat referred to as ‘caryopsis”, typical of the grasses, and the entire kernel is often referred to as the seed or grain. The weight of the grain depends on the genetics of the plant as well as the environmental and cultural practices. The grain makes up about 42% of the dry weight of the plant.

The development stages of the maize plant may be divided into two physiological stages: the vegetative and the reproductive stages. The vegetative stage includes the germination and

seedling development while the reproductive stage involves the fertilization of the female structures, which will develop into ears and grains (Figure 1).

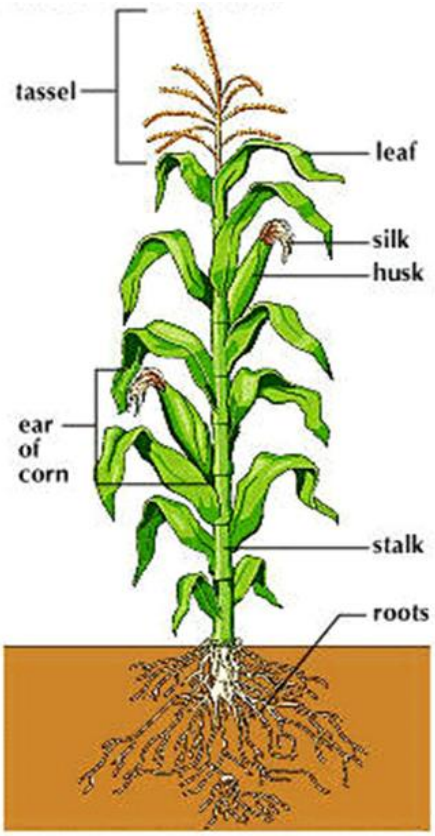


Figure 1: Diagrammatic Structure of the maize plant (Source: Kids 2018)

2.3 Factors Influencing Maize Growth

Maize is generally a crop of warm climates with adequate moisture. Maize requires a warm day time temperatures of 25°C - 30°C and cool nights. Temperatures below 8°C or above 40°C usually cause cessation of development (Birch et al, 2003).

Maize is adapted to a wide range of soils, the most suitable being heavy textured soils which are high in fertility. These soils have a higher water holding capacity than lighter soils. Soils should be well drained. Maize can also grow in lighter soils with recommended fertiliser

application. Shallow soils and soils with low pH (<4.5) or high pH (>7.0) are not suitable for maize.

Maize grown for whatever reason at commercial or small scale level has high demand for nutrients, especially nitrogen (N), phosphorus (P) and potassium (K). Depending on the soil type, the micronutrients Zinc (Zn) and Molybdenum (Mo) are also important. However, seedlings do not tolerate high levels of fertilizer and therefore basal fertilizers should be drilled at least 5 cm to the side of the plant during sowing (Hughes, 2006).

P is the world's second largest consumed nutrient in agriculture only surpassed by N (Batten, 1992) it is one of the most yield limiting factors in many tropical and sub-tropical soils (Ozanne, 1980). For this reason P is frequently the constraining factor in maize growth.

2.4 Phosphorus Availability and Utilization

Phosphorus is considered an essential nutrient for plant growth and productivity. It is a component of nucleic acid, nucleic protein and energy–rich compounds such as adenosine Triphosphate (ATP) through which plants store energy to fuel other chemical processes. P is also a constituent of numerous carbohydrates and nitrogenous compounds and furthermore it is a part of certain coenzymes (Mengel and Kirkby, 1987). P has important effects on plant metabolic processes such as photosynthesis and nitrogen fixation. It induces root development, flowering and fruiting, improvement of crop quality, and helps to prevent lodging in cereals (Pierre and Pohlman, 1933).

Soil P exists in various chemical forms including inorganic P (Pi) and organic P (Po). These P forms differ in their behavior and fate in soils. Harrison (1987) calculated that Pi usually accounts for 35% to 70% of total P in soil. The above stated P forms exist in complex equilibria with each other. P availability can be depressed by P fixation (Al, Fe, Ca, various

clays and organic matter) leading to the formation of precipitates (Al-Fe-Ca Phosphates) of low solubility.

Primary P minerals including apatites, strengite and variscite are very stable, and the release of available P from these minerals by weathering is generally too slow to meet the crop demand though direct application of phosphate rocks (i.e. apatites) has proved relatively efficient for crop growth in acidic soils. In contrast, secondary P minerals including calcium (Ca), iron (Fe), and aluminium (Al) phosphates vary in their dissolution rates, depending on size of mineral particles and soil pH (Oelkers and Valsami-Jones, 2008).

2.4.1 Soil pH

Soil pH is often referred to as the master variable of soil. The pH controls a wide range of physical, chemical, biological processes and other properties that affect soil fertility and plant growth. With increasing soil pH, solubility of Fe and Al phosphates increases but solubility of Calcium phosphate decreases, except for pH values above 8 (Hansinger, 2001). The P adsorbed on various clays and Al/Fe oxides can be released by desorption reactions. With reduction in pH below 5.3, P is complexed or fixed by Al and Fe while at high pH (>8.0) and free lime calcium carbonate, this adsorbs P in the soil solution (Available P) and increases the precipitation of calcium phosphate compounds, resulting in reduced P availability. In calcareous soils rich in $CaCO_3$ and exchangeable Ca, P may be immobilized by any or all of the following mechanisms: adsorption on active sites of $CaCO_3$, precipitation by Ca in the system and reaction with the exchangeable Ca. The amounts of calcium carbonate affect distinctly the soil properties related to plant growth, whether they are physical, such as the soil water relations and crusting, or chemical such as the availability of plant nutrients.

The map of Zambia (Figure 2) presents portions of land that classifies the soils based on soil reaction (pH). The areas coloured purple have low available phosphorus in soils.

2.4.2 Sources of phosphorus

In order to replenish the depleted phosphorus, farmers are forced to use inorganic fertilizers or manure and other P-bearing materials. One group of P –bearing materials is Phosphate rocks. Phosphate rock has been defined as the naturally occurring materials containing one or more

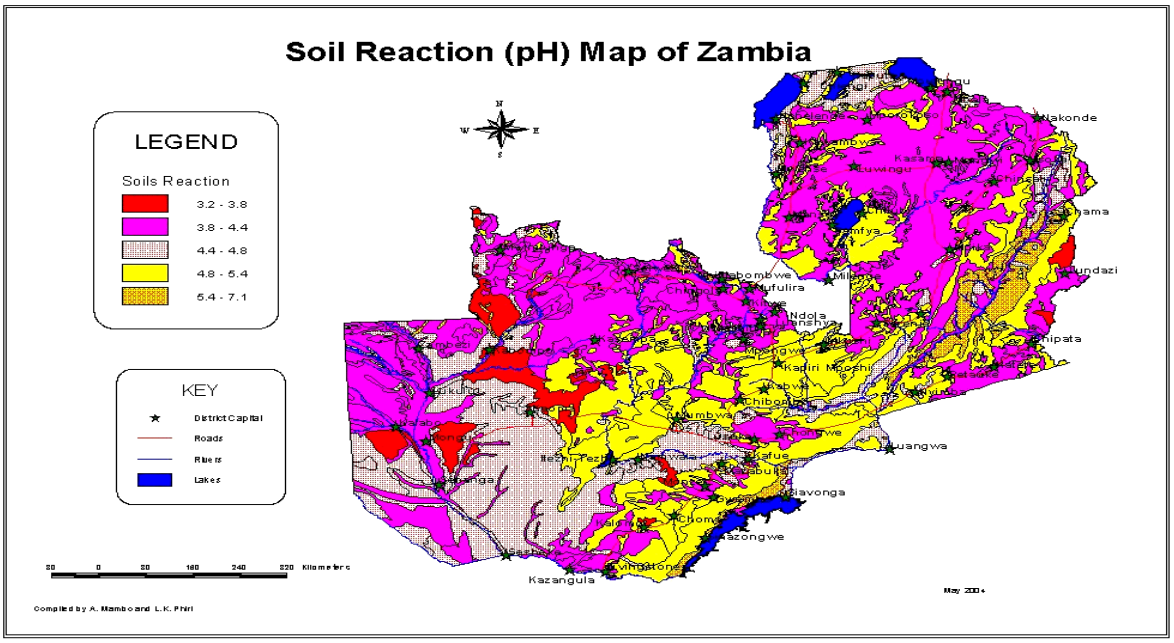


Figure 2: Soil reaction (pH) map of Zambia

(Source: ZARI, 2014)

phosphate minerals as well as possessing chemical characteristics that make it acceptable for commercial use as a source of phosphorus (Notholt, 1980). Phosphate rock is insoluble and cannot be taken up by maize plant. Acidulation using sulphuric acid or phosphoric acid and even hydrochloric acid transforms the material into soluble form for plant uptake. While the resultant products are in general effective for crop production, the production of single/triple superphosphates (SSP or TSP) as well as mono/di ammonium phosphates (MAP or DAP) require high capital investments (Van Straaten, 2007). In much of sub-Saharan Africa industries, acidulation or even partial acidulation to break down the phosphate minerals and making phosphorus more available is constrained by lack of local Sulphur or inadequate

infrastructure to allow for economical transportation of Sulphur or sulphuric acid and lack of capital.

According to Van Straaten (2002), various innovative techniques to enhance PR solubility have been investigated, including modification techniques like partial acidulation, heap leaching, thermal treatment, mechanical activation, as well as modification through biological processes. Although there are several options open to transform PR into a form that is more plant available, the options for small-scale farmers are limited. Practical alternative methods and technologies of PR transformation have to be developed for the farm level. Alternative processing techniques of PR need to be screened as to their suitability and acceptance in the local environment.

The use of organic fertilizer like compost plays an important role in the dissolution of phosphate rocks. Microorganisms help in the solubilization of phosphorus from PR by secreting organic acids, and in the process decreasing their particle size, reducing it to nearly amorphous forms. Making compost is generally laborious to farmers and the decomposition of crop residues takes longer time, usually not less than two months.

Phosphorus is absorbed as the orthophosphate ion (either as H_2PO_4- or HPO_4^{2-}) depending on soil pH. As the soil pH increases (>7.2), the relative proportion of $H_2PO_4^-$ decreases and that of HPO_4^{2-} increases. When working with phosphorus, it is necessary to distinguish between elemental phosphorus (P) and phosphate (P_2O_5) since soil test results may be reported as elemental phosphorus whereas commercial fertilizers are formulated on the basis of phosphate. Fertilizer recommendations and animal manure analysis are typically given as the amount of phosphate (Mullins, 2009).

The nonrenewable phosphate reserves in the world, which could be exploited at values of 40 dollars per Mg, should be exhausted in the second half of this century (Murrel and Fixen,

2006), indicating that research aimed at developing P efficient plants will exert a pivotal role for agriculture in the coming years.

2.4.3 Phosphorus utilization efficiency

Phosphorus efficiency may be defined as the ability of a plant to produce a certain percentage of its maximum yield (80% of maximum yield) at low level of soil P (Fohse et al., 1988). In cases of tolerance and efficiency, plants use physiological mechanisms, and sometimes, anatomic mechanisms to avoid the effect of stress and rapidly recover (Zheng et al., 2000). As a result, three main strategies have been recognized that plants use to cope with stress: one of strategy in dealing with stress is referred to as 1) specialization, meaning that, the genotype is adapted to the specific environment; the other strategy of dealing with stress is 2) generalization, a condition where the genotype has moderate suitability in most environments. 3) Phenotypic plasticity, which refers to signals from the environment interacting with the genotype and stimulating the production of alternative phenotypes, is another strategy that plants use to deal with stress (Cruz et al., 2004). Thus, according to Cruz et al, tolerance to stress is the ability of plants to produce relatively more biomass or grain yield with sub-optimal soil conditions. High yield stability is not always desirable characteristic because tolerant genotypes generally have moderate productivity, even under ideal growing conditions. Given this scenario, most of the crop breeding programs for improving the performance of the genotypes aim at increasing the resource use efficiency (RUE) or in obtaining genotypes with high phenotypic plasticity.

The farmer wants cultivars that produce a satisfactory yield when subjected to stress conditions but that have a high productivity under ideal growing conditions. The genetic control of stress tolerance and resource-use efficiency is quantitative and involves many loci distributed in different regions of the genome in cultivated species, maize inclusive (Wu et al., 2011).Thus, when the plants are subjected to limiting conditions, they would use fewer

resources to produce satisfactory results but show high yields when the conditions are ideal. Differences in phosphorus utilization efficiency may occur among plant species or genotypes of the same species due to differences in amounts of shoot dry matter produced per unit of phosphorus acquired (Rao et al., 1997).

Given this scenario, plant genotypes of a given species develop adaptive responses to phosphorus deficiency. Maize also differs greatly in adaptive mechanisms to phosphorus deficiency. To improve growth under phosphorus deficient conditions, P efficient plants have evolved two major adaptation mechanisms:

1) Enhancing phosphorus utilization efficiency.

2) Increasing phosphorus acquisition (phosphorus uptake mechanisms).

Phosphorus use efficiency in this case is the amount of phosphorus needed in the plant to produce one unit of dry matter. This is often known as internal phosphorus requirement (Vance et al., 2003). Phosphorus internal utilization efficiency can be divided into two components: the P harvest index (capacity of the plant to redistributed P from shoot to grain) and the quotient of P utilization (grain produced per unit of P in the grain). The majority of the phenotypic variation for phosphorus use in tropical maize (80.8%) has been explained by the latter (Parentoni and Souza, 2008).

On the other hand, phosphorus uptake efficiency of plants is the ability of the root system to acquire phosphorus from the soil and accumulate it in the shoots (Bhadoria et al., 2002). Plants have evolved a number of adaptation mechanisms to enhance phosphorus acquisition from soils. One set of adaptive responses is the alteration of root architecture to increase phosphorus acquisition from the soil at minimum metabolic cost (Lynch and Brown, 2001). Among the possibly beneficial root traits for phosphorus acquisition, root hairs are particularly important.

Efficiency concepts in plant mineral nutrition have been defined based on the process by which plants acquire, transport, store and use the nutrient in order to produce dry matter or grain, at low or high nutrient supply (Ciarelli et al., 1998). The concepts of nutrient acquisition efficiency, used in the sense of plant nutrient acquired from the soil, and nutrient internal utilization efficiency, defined as plant internal ability to produce yield units per unit of nutrient in the plant, have been considered as the two major components of plant nutrient use efficiency (Good et al., 2004). Evaluation of maize genotypes has shown that higher phosphorus utilization in plants has been achieved by improving both components: P acquisition and P internal utilization efficiency.

2.5 Evaluation of Genotypes for Phosphorous Utilization Efficiency

Phenotypic evaluation for phosphorus utilization in maize lines is a moderately effective approach to determine the tolerance levels of these lines to low soil phosphorus conditions. The efficiency of selection for yield in low-P environment may be improved by selecting P correlated traits. The traits related to efficient P uptake and metabolism have been suggested as selection parameters (Hinsinger 2001). In problem soils, screening for phosphorus utilization is often constrained by occurrence of companion stresses (e.g. aluminum toxicity) that restricts root growth and impairs phenotyping for P-use efficiency. Also favourable soils without stresses are often saturated with P due to continuous P fertilizer application, and this therefore entails that the endogenous P concentrations need to be reduced before genotypic differences can be determined. In order to overcome these constraints, a combination of phenotypic screening with molecular genetics such as use of molecular marker is ideal. Molecular marker information complemented by good quality phenotyping greatly facilitates the appropriate choice of parents for crosses for both inheritance and applied breeding. The availability of molecular markers can at least partially replace and/or complement phenotypic

evaluations in the field. Therefore, molecular markers are of particular value for the development of P efficient maize varieties. Molecular markers can be used to study the inheritance of phosphorus utilization traits and identify specific loci associated with the expression of these traits. Markers shown to be linked to specific genes may be used to facilitate selection of desired genotypes through marker-assisted selection (MAS) (Guingo and Hebert 1997).

2.6 Inheritance of low P tolerance in Maize

The best solution to overcoming the low P levels experienced in most Zambian soils is by breeding low P tolerant maize genotypes. The inheritance of low P tolerance is however variable among crop species due to different physical and biochemical adaptation strategies used by these plants. In temperate maize, tolerance to low available P is largely conditioned by additive gene effects although dominance was also found to be important (Da Silva *et al*., 1992). Such information is useful in guiding the breeding strategy to be used for the breeding of crops that are tolerant to low P. Little is known about the gene effects in tropical maize. Implying that different genetic information could be obtained since tropical maize has a wider genetic base (Sibov et al., 2003)

In a field evaluation on low P soils, maize genotypes efficient in P acquisition and responsive to applied P have been identified among temperate maize (Jinming et al., 2004). There is therefore, a possibility to identify material among tropical accessions that are efficient at absorbing phosphorus. Given that the ability of a plant to thrive under low P is explained by two phenomena i.e., phosphorus use efficiency which comprises of ability to acquire phosphorus from the environment and phosphorus utilization efficiency which comprises of ability to convert phosphorus once acquired into biomass or yield (Chen et al., 2009). The scope to breed and identify tropical maize genotypes for both these remains a gap that

requires further investigation of the genetics behind these complex traits. Knowledge of heritability (h^2) is required by the plant breeder to aid in selection of the desirable trait (Bhateria et al., 2006).

2.6.1 Heritability of traits associated with phosphorus utilization

Knowledge of heritability (h^2) influences the choice of selection procedures used by the plant breeder to decide which selection methods would be most useful to improve the trait. The most important function of heritability in genetic studies of quantitative traits is its predictive role to indicate the reliability of phenotypic value as a guide to breeding value. Traits with high heritability can easily be fixed with simple selection in quick progress (Bello et al., 2012). Although primary traits such as grain yield are an important criterion in selecting genotypes for stress tolerance, there is wide agreement that selection under stress is less efficient than under optimal conditions, mainly because heritability of grain yield declines under the stress (Banzinger and Cooper, 2001)

Similarly, selecting under optimal conditions (high inputs) increases genetic variance relative to environmental variance and thus increases heritability. This increases the chances of selecting superior genotypes and enhances the breeding progress. Therefore, when selecting for grain yield under stress conditions (low P or low N), additional information on secondary traits of maize such as shoot biomass and plant height should also be used to supplement the primary traits. The heritability of secondary traits may be optimized by low competition, enhancing gene fixation and conducting multiple-environment screening (Fasoulas and Fasoulas, 1997). Heritability estimates in crops were classified as high (>0.50), medium (0.30-0.50), and low (<0.30) according to Bhateria et al. (2006).

2.6.2 Gene action conditioning phosphorus utilization under P-limited soils

To develop an appropriate breeding strategy in selecting genotypes that produce high grain yield under low P soil conditions, information on gene action is important. Tolerance to low

P is a quantitatively inherited trait controlled largely by additive gene effects, although dominance and epistatic effects have also been shown to be important (Chaubey et al., 1994). Betran et al., (2003), also reported that dominance (non-additive) gene action in tropical maize was important. The information on General Combining Ability (GCA) and Specific Combining Ability (SCA) effects may be used to estimate gene action of traits.

General combining ability (GCA) and specific combining ability (SCA) are a measure of the additive and non-additive genetic variation of parents, respectively (Sprague and Tatum, 1942). It is also useful to estimate the nature of gene action involved. Examining the GCA of each parent helps in developing superior parental genotypes, while the SCA effect estimates the performance of hybrids (Cruz and Regazzi, 1994). Therefore, an analysis based on a large number of progenies from diverse parents is essential for formulating an efficient strategy for varietal improvement. Such an analysis enables broad inferences to be drawn about the nature of gene effects and the combining abilities of different varieties.

Furthermore, as GCA depends predominantly on additive effects of the genes, it informs about the potential of the segregating populations for selection of high grain yield genotypes (Ramalho et al., 1993). It has been the parameter employed to choose promising segregating populations, which should have the highest averages. As for SCA, which depends predominantly on non-additive effects of genes, it allows to identify populations which are potentially more useful to liberate variability in the segregating generations (Veiga et al., 1998).

In statistical terms, GCA effects are main effects and indicate primarily additive gene action (Falconer, 1981). Effects of GCA can also be used to select superior genotypes under low P conditions. High GCA effects under low P reflect the presence of the desired fixable low P alleles being sought. The SCA effects indicate primarily dominance (non-additive) gene

action of traits and they are non-fixable. Thus, the GCA effects are useful for selection programs in crop improvement whereas, SCA effects are important for hybrid crop development. Genotypic variation for tolerance to P-deficiency exists in maize and this has allowed selection and breeding of efficient genotypes in phosphorus deficiency soils (Da Silva and Gabelman, 1993).

2.7 Plant Breeding Techniques in Maize

Successful maize breeding and production are dependent upon the development of adapted germplasm and a continual commitment to research. Modern day breeding methods commonly used in maize may be grouped into two broad categories: population improvement and hybrid variety development. These are usually referred to as conventional or traditional methods. While significant progress has been made in relation to maize improvement using tradition breeding strategies (Dhillon and Prasanna, 2001), considerable scope exists to further enhance maize productivity. A new technique, molecular breeding (MB) has gained popularity in recent years. The use of modern molecular tools and techniques can complement conventional approaches to allow breeders to effectively address priority research areas. MB has been developed as a tool in maize breeding programs. MB may be defined in a broad-sense as the use of genetic manipulation performed at DNA molecular levels to improve characters of interest in plants. MB implies molecular marker-assisted breeding (MAB) and is defined as the application of molecular biotechnologies, specifically molecular markers, in combination with linkage maps and genomics, to alter and improve plant traits on the basis of genotypic assays. MAB as a term is used to describe several modern breeding strategies, including marker-assisted selection (MAS), marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS), and genome-wide selection (GWS) or genomic selection (GS) (Ribaut et al., 2010).

It has been reported that MB increases genetic gain per crop cycle, stacks favourable alleles at target loci and reduces the number of selection cycles. Moreover, MB cannot be affected by environment (Staub, et al., 1997) thus allowing the selection to be performed under any environmental conditions. This is very helpful for improvement of some traits (e.g. disease/pest resistance and stress tolerance) that are expressed only when favourable environmental conditions are present. For low-heritability traits that are easily affected by environments, MAB based on reliable markers tightly linked to the quantitative trait loci (QTL) for traits of interest can be more effective and produce greater progress than phenotypic selection. A QTL is a region within a genome that contains genes associated with a particular quantitative trait (Collard et al., 2005).

With MAB, selection for all kinds of traits can be carried out at seedling stage and thus reduce the time required before the phenotype of an individual plant is known. No selfing or test crossing is needed to detect the traits controlled by recessive alleles, thus saving time and accelerating breeding progress. For the traits controlled by multiple genes/QTLs, individual genes/QTLs can be identified and selected in MAB at the same time and in the same individuals, and thus MAB is particularly suitable for gene pyramiding. In traditional phenotypic selection, however, to distinguish individual genes/loci is problematic as one gene may mask the effect of additional genes.

A lot of data has been reported on the use of MB techniques like molecular markers in temperate maize but so little has been reported on tropical maize genotypes.

2.7.1 Molecular markers in maize breeding

Molecular markers (MM) are used to 'flag' the position of a particular gene or the inheritance of a particular character. MM are phenotypically neutral and are widely accepted as potentially valuable tools for crop improvement in maize (Tuberosa et al., 2003).

Molecular markers are useful in phosphorus utilization efficiency selection as they can substitute or supplement phenotypic screening in a breeding program. These markers can also be used to identify phosphorus utilization efficient lines at juvenile stages which can save time and cost of screening. Molecular markers are advantageous for traits where convectional phenotypic selection is difficult, expensive, or lacks accuracy. Molecular markers have been used to identify and characterize QTL associated with diverse traits in maize including grain yield, characters concerned with domestication, environmental adaptation, disease and insect pest resistance, and drought and heat stress tolerance (Stuber et al., 1999).

The most extensively used molecular marker in plant breeding, are the restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), microsatellites or simple sequence repeat (SSR), and single nucleotide polymorphism (SNP). However, SSR markers are frequently used for genetic and breeding studies in cultivated maize. SSR markers provide fairly comprehensive genomic coverage. They are amenable to automation, they have locus identity and they are multi-allelic. Many agronomic and quality traits show quantitative inheritance and the genes determining these traits have been quantified using QTL tools. Since the 1990s SSR markers have been extensively used in constructing genetic linkage maps, QTL mapping, marker-assisted selection and germplasm analysis in plants. At present, SSRs are the most widely used markers by maize researchers due to their availability in large numbers in the public domain (MaizeGDB; http://www.maizegdb.org), simplicity and effectiveness. These PCR-based, genetically co-dominant markers are robust, reproducible, hyper-variable, abundant, and uniformly dispersed in plant genomes (Powell et al., 1996). SSRs have been widely and successfully used in QTL mapping of phosphorus use efficiency and relative biologic characteristics associated with phosphorus utilization in maize, notably by Chen et al., (2008) and Mendes et al.,(2014) to mention a few.

Using molecular markers to genotype materials and thereafter, selecting by associating to QTL maps has the potential to hasten the intensity of selection for phosphorus utilization traits and therefore, advance the populations within a reduced time frame (Varshney et al., 2010). Construction of a genetic map in molecular breeding strategies facilitates identification of potential genomic regions for improving biotic and abiotic stress resistance.

Maize seed companies have successfully exploited marker-QTL associations in population improvement and cultivar development (Eathington et al., 2007). Some of the important factors that contributed to effective use of MAS schemes in maize breeding have been the use of year-round nurseries or continuous nurseries, high throughput genotyping and phenotyping datasets using bioinformatics tools for decision making (Eathington et al., 2007). The recent rates of conventional plus molecular plus transgenic breeding progress, and the solid prospects for important achievements in breeding for enhanced yield potential, stress tolerance (including drought tolerance) and nutrient use efficiency, have led Monsanto to boldly claim that as the world faces continued and growing demands for agricultural goods, Monsanto has committed to double crop yields in maize, soya beans and cotton by 2030 (Edgerton, 2009).

2.7.2 Quantitative trait loci (QTL) mapping

A QTL is a region within a genome that contains genes associated with a particular quantitative trait (Collard et al., 2005). QTL mapping is based on the basic principle that if there is linkage disequilibrium between the causal factor and a marker locus, mean values of the trait under study will differ among genotype groups with different genotypes at the marker locus (Zou and Zeng, 2008). In other words, QTL analysis is based on the principle of detecting an association between phenotype and the genotype of markers (Collard et al., 2005). The key requirements for mapping QTLs are trait phenotype, polymorphic markers

and genetic structure of populations (Acquaah, 2007). QTL mapping therefore involves the following steps:

1) Constructing a mapping population from two parents;

2) Identifying candidate markers and screening them for polymorphisms;

3) Constructing a linkage map;

4) Analyzing for QTL-trait association using QTL detection methods.

2.7.3 Mapping population and linkage analysis

The construction of a linkage map requires a segregating plant population (i.e. a population derived from sexual reproduction). The parents selected for the mapping population will differ for one or more traits of interest. Population sizes used in preliminary genetic mapping studies generally range from 50 to 250 individuals (Mohan et al., 1997), however larger populations are required for high-resolution mapping. If the map will be used for QTL studies, then an important point to note is that the mapping population must be phenotypically evaluated (i.e. trait data must be collected) before subsequent QTL mapping.

It is essential to develop a suitable experimental mapping population using parental lines that are highly contrasting phenotypically for the target trait, (for example, highly resistant and susceptible lines). Another important requirement is that these parental lines should be genetically divergent; this is important to enhance the possibility of identifying a large set of polymorphic markers that are well-distributed across the genome. To fulfill the second criterion, one may have to carry out molecular polymorphism survey across a set of potentially useful lines so as to identify the most suitable ones for generation of mapping population.

The choice of the mapping population is critical in QTL mapping. The breeder generates a segregating population by crossing lines with extreme phenotypic performance for the quantitative trait of interest. The most frequently used populations are derived from crossing

two inbred lines that are assumed to be homozygous with different alleles at both QTLs and genetic markers. These materials include $F_{2,}$ backcrosses, recombinant inbred lines, and doubled haploids (Acquaah, 2007).

A linkage map indicates the position and relative genetic distances between markers along chromosomes, which is analogous to signs or landmarks along a highway (Paterson, 1996). After identifying polymorphic markers, a linkage map is constructed by recording genotype data for each DNA marker on each individual of a mapping population and then using computer programmes to analyze for linkage between markers and phenotypic traits. The likelihood that particular markers are linked is usually expressed using the odds ratio, i.e., the ratio of the probability of linkage versus the probability of no linkage expressed as the logarithm of the ratio and called a Logarithm Of odds (LOD) (Collard et al., 2005). Linked markers are grouped together into linkage groups which represent chromosomal segments or entire chromosomes.

In our study, 40 F_1 genotypes were developed and evaluated for phosphorus utilization. One cross was advanced to F_2 generation. DNA was extracted from the F_2 generation. The mapping population was made up of 67 $F_{2:3}$ individuals. Simple Sequence Repeats (SSR) markers were used to map linkage groups of QTLs associated with phosphorus utilization traits in tropical maize.

Previous studies indicated contradictory results about the type of gene action conditioning P-utilization efficiency in maize (Mendes et al, 2015, Parentoni et al. 2010, Chen et al. 2008, Da Silva et al. 1992). Differences observed could arise from the germplasm understudy and environment to which the materials were subjected to. There is therefore need to evaluate the materials under our local Zambian conditions. Molecular markers can be used to study the inheritance of traits related to P utilization efficiency and identify specific loci associated

with the expression of the traits. Previous studies have indicated that most QTLs associated with phosphorus utilization efficiency were identified on chromosome 5 (Mendes et al, 2014, Chen et al. 2008). It therefore, appears that linkage group 5 holds many loci that have co-evolved to adapt to traits in maize (Shoot biomass, Root biomass and Plant height) for an association with phosphorus utilization efficiency.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Germplasm Used and Location of Experiment

The germplasm (Table 2) that was used in the study was obtained from the maize team at Golden Valley Research Trust (GART) in Chisamba District at latitude 14 ^{o} 40' south; longitude 25^{o} 01' East and at an altitude of 1140m (above sea level). Thirteen (13) inbred lines which were earlier screened for phosphorus utilization efficiency were used in the study. The screening of the

The study was conducted at the University of Zambia located at latitude 15°23´42´´South and longitude 28°20´13´´East and 1263 m (above sea level), during the period December 2014 – May 2016 .

3.2 Field and Greenhouse Experiments

3.2.1 Development of hybrids (F1 crosses)

The crossing block was set up at the field station of the School of Agricultural sciences. The crosses were made following the North Carolina Design II (NCD II). Five genotypes were used as males and eight genotypes as females. The thirteen (13) genotypes used were previously evaluated for phosphorous use efficiency. The evaluated maize genotypes were planted in 3 meters long single-row plots, spaced at 0.75 m between rows and 0.30 m between plants within the row. On the day of planting, D-compound fertilizer was applied at the recommended rate (200kg/ha). Top dressing fertilizer, urea (46%N) was applied at a rate of 200kg/ha 28 days after sowing. Other agronomic practices like weed management and pest control were done optimally in accordance with recommendations for seed production.

Forty (40) crosses were harvested at physiological maturity. The ears from crosses were hand-harvested, shelled and the F_1 (single-cross hybrid) seed was stored for evaluation.

Table 2: Maize Genotypes Used in the Study

Designation	Genotype	Pedigree	Origin	C P
Male	L60	LacarxL12-280-3-3-2-4-5-3-1-1	NTW2011/12	ME
Female	L61	LacarxL 12-280-3-3-2-4-5-3-1-2	NTW2011/12	LE
Female	L354	L12 M1 (220Gy)-150-3-2-1-1-4S6-S8-3-4-2-4-2	NTW2011/12	ME
Female	L374	L12 M1 (220Gy)-150-3-2-1-1-4S6-S8-5-1-B-2-3	NTW2011/12	ME
Female	L508	SW89300-IP5S2-5-##1-1-3-B X L917-2-8-1-2-B-B-3	NTW2011/12	ME
Female	L542	x(discard)1 X L917-1-5-2-3-6-2	NTW2011/12	ME
Female	L571	[Ent52:92SEW1-2/[DMRESR-W]EarlySel -#L-2-1-B/CML386]-B-22-1-B-4-#-B X L 1214-1-2-2-1-B-1-1	NTW2011/12	LE
Male	L584	[Ent52:92SEW1-2/[DMRESR-W]EarlySel -#L-2-1-B/CML386]-B-22-1-B-4-#-B X L 1214-2-4-1-2-4-1-6-2	NTW2011/12	ME
Male	L585	[Ent52:92SEW1-2/[DMRESR-W]EarlySel -#L-2-1-B/CML386]-B-22-1-B-4-#-B X L 1214-2-4-1-2-4-1-6-3	NTW2011/12	LE
Female	L640	[EarlyMid1/KatumaniSR]-#-169-2-4-B X L 1214-4-4-4-2-2-B-B-2	NTW2011/12	ME
Male	L655	[EarlyMid1/KatumaniSR]-#-169-2-4-B X L 1214-4-5-2-2-3-B-B-1	NTW2011/12	LE

Male	L806	[MSR123XII37TN-9-2-4-X-3/LZ946441] -B-1-5-5-BB-3 X ZEWA-8-2-2-3-5-B-1	NTW2011/12	LE
Female	L807	CML388-9 X ZEWA-14-2-7-1-B-1	NTW2011/12	LE

CP-Comment on phosphorus utilization efficiency, ME-Most efficient, LE-Less efficient

3.2.2 Evaluation of F_1 genotypes (hybrids)

Evaluation of the F_1 genotypes was carried out in the greenhouse at UNZA. Potted experiments were carried out on the determination of P utilization and work on inheritance studies. The experiment was done on P limited soil which was obtained from Ngwerere (15^o 18' 0" South, 28^o 19' 0" East) in Lusaka. The soils were randomly sampled and analysed for phosphorus content (Table 3).

Table 3: Soil characteristics of Ngwerere soils

Name	Threshold	Measured Quantity
Available Phosphorus	10mg/kg soil	5.60 mg/kg soil
pH		4.37

Forty crosses were planted following a Completely Randomized Design (CRD) with three replications and two treatments in 5kg plastic planting pots. 60kg P/ha was added to the control while 0kg P/ha was added to the experiment. The plants were harvest at 28 days and air-dried for 72 hrs. The following parameters were measured:

1) **Plant height**- Measured using a tape the average height of plants from each plot in centimetres.

2) **Shoot biomass**-The weight of the above ground total dry matter which included stalk and leaves in grams.

3) **Root biomass**- The weight of the below ground total dry matter in grams

4) **Plant biomass**- The sum of weight of the shoot biomass and the root biomass

3.3 Association of QTL to Phosphorous utilization efficiency

3.3.1 Mapping population

The 67 F_2 plants derived from cross (L354 x L585) were self-pollinated and hand-harvested at physiological maturity. The resultant $F_{2:3}$ seed was stored in labelled harvesting bag. The resultant seeds 67 $F_{2:3}$ genotypes were used in phenotypic evaluation of traits in tropical maize associated with phosphorus utilization. The $67_{2:3}$ genotypes formed the mapping population that was used in the construction of the linkage map.

67 $F_{2:3}$ were planted in P limited soil ear-to-row on 3 m long single-row plots in a Randomized Complete block Design (RCBD) with two replications at the field station, School of Agricultural Sciences, UNZA. Plants were spaced 0.75 m between rows and 0.30 m between plants. The site was chosen purposely being a soil poor in phosphorous as evident by the lab results (Table 4).

Table 4: Soil characteristics of UNZA Field Station research site

Name	Threshold	Quantity
Phosphorus	10mg/kg	2.93 mg/kg
pH		5.10

The plants were rain-fed. Two seeds were initially planted per station but were subsequently thinned to one plant per station 3 weeks after germination. Phosphorus fertiliser was not applied to the trial at any time. However, the recommended quantities of potassium (30 kg K/ha) and nitrogen (20 kg N/ha) were applied in form of potassium sulphate and urea (46% N) fertilisers at the rates of 64 kg/ha and 25 kg/ha respectively as basal dressing. Urea fertiliser was repeated at 28 days after planting at the rate of 200 kg/ha (92 kg N/ha) as top dressing. Other agronomic practices such as weed management, pest and disease control were carried out promptly. The following parameters were measured:

1) **Grain yield (GY) -** Was determined as the average total weight of shelled grain harvested from 3 m long rows in grams.

2) **100 grain weight (100GW)** – Was measured as the weight of 100 grains from the ear (cob) from the plot in grams.

3) **Plant height (PH)** – Measured using a tape the average height of matured plants in each plot from ground level to flag leaf in centimetres.

4) **Root biomass (RB)** – Was determined by weighing roots taken from the plot and finding the average in grams.

5) **Shoot biomass (SB)** – Was determined by weighing the above ground total dry matter including stalk and ears harvested from the plot and later finding the average in grams

6) **Plant biomass (PB)** – Was determined by adding the root biomass and shoot biomass.

3.3.2 Genotyping and construction of a linkage map

DNA for association of QTL to phosphorous utilization efficiency was obtained from 67 F_2 genotypes which were advanced from a cross of L585 and L354. The 67 F_2 genotypes were tagged and young leaf samples; one to two weeks old were cut from each plant for DNA extraction. DNA was extracted from the ground leaf material using the cetyltrimethylammonium bromide (CTAB) method (Hoisington et al., 1994). The extracted DNA was stored in a fridge at -20^{o}C.

Twenty four SSR primer pairs (Appendix I) were purchased from University of Cape Town, Department of Molecular and Cellular Biology (Cape Town, South Africa). SSR marker names and primer sequence information were obtained from SSR maize databases available at http://www.maizegdb.org/ssr.php. These were selected from targeted regions of the maize

genome linked to QTL for Phosphorus utilization efficiency (Wisser et al., 2006). The primers were used as part of the PCR reaction mixture. The final concentrations of reaction components were as follows: 0.2 μM each of SSR forward and reverse primers, 1× PCR buffer, 2.0 mmol MgCl2/L; 0.2 mmol/L each of dATP, dCTP, dGTP, and dTTP; 0.16 U Taq polymerase (BioLabs); and 30 ng genomic DNA and distilled sterile water to a total volume of 20 μL. Initial testing involved screening for polymorphism between the parental DNA (L585 and L354), and only five polymorphic SSR markers were subsequently used for genotyping the 67 tagged F_2 plants.

3.4 Method of Detecting Quantitative Trait Loci

Linkage map construction and QTL analysis was done using ICIM QTL IciMapping version 4.1software (Wang *et al*., 2012) which made use of the phenotypic as well as genotypic data. The DNA extracted from the F_2 plants and the phenotypic data collected from the $67_{2:3}$ genotypes was used in the genotyping and construction of the linkage map

3.5 Statistical analysis

To compare the mean performance of all measured parameters; root biomass, shoot biomass, plant length and plant biomass in P-limited soils (0 Kg P) and in P optimum soil (60 Kg P), the two-tailed T-test was used. A correlation analysis (VSN International, 2014) was done using summary statistics in Genstat Version 14.1 to estimate the degree of association of root biomass to other measured parameters. The significance of correlation (r) was done using two sided test of correlations. The total plant biomass was used to determine the most efficient genotype. According to Chen et al. (2009) phosphorus utilization efficiency is the ability to convert phosphorus once acquired into plant biomass.

Analysis of variance was performed using a fixed model and means of root biomass, shoot biomass, plant height and plant biomass were separated using the fisher protected Least Significant Difference (LSD) method, at a significant level of $\alpha = 0.05$. The variations of the

expected mean squares were estimated using the methodology explained by Singh and Choudhary (1985). Main effects due to females and males were independent estimates of general combining ability (GCA) variances while male x female interaction effects represent specific combining ability (SCA) variance. The generic ANOVA for the NCD II mating design is as shown in table 5 below.

Table 5: ANOVA for the NCD II Mating Design

Source	df	Mean square	Expected mean square
Replication	r-1		
GCA_{male}	m-1	MS_m	$\sigma_e^2 + r\sigma_{fm}^2 + rf\sigma_m^2$
GCA_{female}	f-1	MS_f	$\sigma_e^2 + r\sigma_{fm}^2 + rm\sigma_f^2$
$SCA_{Male\ x\ Female}$	(f-1) (m-1)	MS_{fm}	$\sigma_e^2 + r\sigma_{fm}^2$
Error	fm(r-1)	MS_e	σ_e^2

Where: r=replication, m=male, f=female, MS_m=Mean gca effects for males, MS_f=Mean gca effects for females, MS_{fm}=Mean squares for female-male interaction, MS_e=Mean square for error

The GCA and SCA were estimated as done by Singh and Chaudhary (1985) and is as presented:

GCA= mean of parent – test mean or overall mean

SCA = observed mean of the cross - (GCA_m+GCA_f) +test mean

The variance components for GCA and SCA were calculated as described by Singh and Chaudhary (1985).

$$\boldsymbol{\sigma^2_{sca=}\ MS_{mf} - MS_{mf}/r}$$

$$\boldsymbol{\sigma^2_{gca}m = MS_m - MS_{mf} - rf\sigma^2_m/rf}$$

$$\boldsymbol{\sigma^2_{gca}f = MS_f - MS_{mf} - rm\sigma^2_f/rm}$$

The relative contributions of GCA and SCA were estimated using the Baker's ratio (σ^2gca_f + σ^2gca_m)/(σ^2gca_f + σ^2gca_m + σ^2sca), where σ^2gca_f and σ^2gca_m are the variance components of

female GCA and male GCA respectively while σ^2sca is the variance component of SCA (Baker, 1978).

Narrow sense heritability was estimates for each set were calculated. Narrow sense heritability was calculated using the formula:

$$h^2 = (\sigma^2 gca_f + \sigma^2 gca_m)/ (\sigma^2 gca_f + \sigma^2 gca_m + \sigma^2 sca + \sigma_e^2)$$

Analysis of phenotypic variance was performed to assess if there were significant differences to P-deficient among the $F_{2:3}$ family genotypes. All the data analysis was carried out using GenStat statistical package (VSN International, 2014).

Linkage map construction and QTL analysis was done using ICIM QTL IciMapping version 4.1 software (Wang et al., 2012) which made use of phenotypic as well as genotypic data. The procedure of Inclusive Composite Interval Mapping (ICIM) was used to identify QTLs and to estimate their effects. Parameters for forward regression analysis were set at a window size of 10cM, a walk speed of 2cM and probability threshold of 0.05 each for the partial F test for both marker inclusion and exclusion. Significance threshold for QTL detection was calculated by 1,000 random permutations of the phenotypic data at 5% level, LOD thresholds were set at 2.5 for all traits.QTL positions were assigned at the point of maximum LOD score in the region under consideration. Major QTLs were identified as those with phenotypic variation explained (PVE) of >10% as described by Tembo et al. (2014).

CHAPTER FOUR

RESULTS

4.1 Evaluation of Hybrids

4.1.1 Mean performance of crosses at 0 Kg P and 60 Kg P

The t-test showed that the mean performance for all measured parameters across genotypes was higher at a rate 60 kg P/ ha that at 0 Kg/ ha (Table 6)

Table 6: Comparisons of mean performance of measured parameters at 0 kg P and 60 kg P using a paired T- test

Parameter	Mean-0^{X}	Mean-60^{Y}	Difference	P-value
Total boimass	2.00	16.90	14.90	< 0.001
Shoot biomass	1.07	13.21	12.14	< 0.001
Root biomass	0.92	3.72	2.80	< 0.001
Plant height	37.80	79.89	42.09	< 0.001

X-mean value of measured parameter at fertilizer application rate of 0 Kg P across genotypes,
Y-mean value of measured parameter at fertilizer application rate of 60 Kg P across genotypes

4.1.2 Association among traits

The results showed highly significant positive correlation (P < 0.001) amongst the four traits under consideration (Table 7).

Table 7: Simple correlation of traits

Trait	PH	SB	RB	PB
PH	-	0.79***	0.68***	0.81***
SB	-	-	0.64***	0.93***
RB	-	-	-	0.87***

PH-Plant height, SB-Shoot biomass, RB-Root biomass and PB-Plant biomass. ***significant at P < 0.001

4.1.3 Genotypic mean performance across measured parameters in P-limited soil

Significant differences ($P \leq 0.001$) were obtained among the crosses for all the measured parameters (Table 8). The SCA effects also showed highly significant differences from zero ($P \leq 0.001$) across all the measured parameters. The GCA effects showed significant differences from zero ($P \leq 0.05$) only for the root biomass.

Table 8: Mean Squares for measured parameter in Phosphorous limited soil

Source	df	PH	SB	RB	PB
Replication	2	0.50	0.01	0.04	0.07
Crosses	39	150.00***	0.98***	0.39***	2.39***
GCA_{female}	4	191.31ns	1.51ns	0.80*	4.26ns
GCA_{male}	7	169.82ns	0.89ns	0.67*	2.89ns
SCA	28	136.84***	0.87***	0.25***	1.85***
Error	78	24.07	0.11	0.08	0.25

KEY: Plant height- PH, shoot biomass -SB, root biomass -RB, plant biomass -PB.

*** Very highly significant ($P \leq 0.001$), *Significant ($P \leq 0.05$, ns=non-significant ($P > 0.05$)

4.1.4 Mean performance of the genotypes for various parameters

Table 9 presents a summary of means for the traits under consideration cultivated in P-limited soils. Further analysis revealed highly significant differences among the crosses with regards to all measured parameters. Crosses (L585 x L354), (L60 x L807), (L655 x L508) and (L806 x L374) had a higher mean performance value for plant height, shoot biomass, root biomass and plant biomass respectively.

Table 9: Means of F_1 crosses for traits used in evaluating for Phosphorus Use Efficiency

GENOTYPES	PB (g)	SB(g)	RB(g)	PH(cm)
L585 x L354	4.49	2.73	1.76	50.8
L60 x L807	3.97	2.54	1.43	49.9
L60 x L354	3.24	1.65	1.59	43.5
L806 x L374	3.2	2.06	1.13	48.1
L806 x L640	3.15	1.88	1.28	43.5
L655 x L508	3.02	1.79	1.23	48.7
L584 x L354	2.92	1.62	1.3	43.9
L60 x L374	2.88	1.41	1.47	41
L584 x L640	2.79	1.55	1.24	44.7
L655 x L354	2.78	1.29	1.49	43.6
L60 x L571	2.64	1.53	1.1	42.7
L60 x L61	2.51	1.32	1.19	42.8
L655 x L61	2.48	1.21	1.27	39.5
L806 x L354	2.41	1.5	0.91	42.9
L60 x L542	2.29	1.1	1.19	42.1
L584 x L374	2.24	1.16	1.08	44.5
L60 x L640	2.09	0.99	1.1	35.5
L585 x L571	2.08	1.15	0.92	38.3
L584 x L542	1.74	0.84	0.9	37.6
L655 x L640	1.72	0.85	0.87	39
L585 x L542	1.63	0.82	0.82	40.8
L585 x L640	1.56	0.72	0.84	40
L584 x L61	1.54	0.86	0.68	38.4
L806 x L807	1.44	0.86	0.58	38
L655 x L807	1.42	0.77	0.65	35.3
L806 x L571	1.36	0.49	0.88	28.8
L806 x L542	1.34	0.55	0.79	20.2
L60 x L508	1.33	0.73	0.6	36.7
L655 x L542	1.29	0.41	0.87	29.5
L585 x L807	1.29	0.61	0.69	35.2
L585 x L374	1.25	0.61	0.63	28.7
L584 x L571	1.22	0.58	0.64	31.4

L655 x L374	1.22	0.61	0.61	31.9
L655 x L571	1.22	0.73	0.49	34.8
L584 x L508	1.19	0.6	0.59	37.9
L585 x L508	1.17	0.59	0.58	33
L806 x L508	1.15	0.66	0.49	30
L584 x L807	1.05	0.43	0.62	29.5
L585 x L61	0.95	0.67	0.28	30.2
L806 x L61	0.77	0.45	0.32	23.6
$LSD_{0.05}$	0.82	0.53	0.45	7.98

PB (Plant biomass), SB (Shoot biomass), RB (Root biomass), PH (Plant height)

4.1.5 Combining ability effects and gene action

SCA effects were also found to be highly significant different from zero ($P \leq 0.001$) across all parameters. Table 10 presents the specific combining ability effects of the F_1 crosses for parameters that were considered in the study. From the results crosses (L60 x L807), (L585 x L354), (806 x L374) and (L655 x L508) exhibited positive significant SCA effects on all the measured parameters. Two crosses, L655 x L374 and L806 x L61 exhibited negative significant SCA effects across all the four measured parameters.

The GCA effects are shown in table 12. Analysis of GCA effects revealed that only the root biomass was significantly different from zero ($P \leq 0.05$). Further analysis of the GCA effects for root biomass showed that female parental lines L354 and L640 as well as male parental line 60 had significant effects of 0.48*, 0.14* and 0.28* respectively. Parental lines L61, L508 and L585 exhibited negative significant effect of -0.18*, -0.23* and -0.12* respectively.

Table 10: Specific combining ability effects of crosses for various traits

GENOTYPES	PH	SB	RB	PB
L60 xL61	4.0	0.09	0.16	0.24
L60 x L354	-5.37	-0.45*	-0.10	-0.54

L60 x L374	-1.67	-0.10	0.20	0.10
L60 xL508	-4.50	-0.48*	-0.38	-0.86*
L60 x L542	4.26	0.03	-0.01	0.01
L60 x L571	3.63	0.30	0.01	0.32
L60 xL640	-8.90*	-0.55*	-0.25	-0.79*
L60 x L807	8.43*	1.16***	0.36*	1.52***
L584 x L61	2.93	0.08	-0.02	0.05
L584 x L354	-1.70	-0.01	-0.07	-0.08
L584 x L374	5.10	0.12	0.14	0.24
L584 x L508	0.06	-0.15	-0.06	-0.23
L584 x L542	3.06	0.22	0.03	0.24
L584 x L571	-4.34	-0.20	-0.12	-0.33
L584 x L640	3.63	0.48*	0.22	0.69*
L584 x L807	-8.64*	-0.49*	-0.12	-0.62*
L585 x L61	-3.90	-0.15	-0.36*	-0.50
L585 x L354	6.66*	1.05***	0.46*	1.53***
L585 x L374	-9.23**	-0.47*	-0.24	-0.72*
L585 x L508	-3.44	-0.19	0.00	-0.20
L585 x L542	7.58	0.16	0.02	0.17
L585 x L571	3.96	0.34	0.23	0.58*
L585 x L640	0.36	-0.40*	-0.11	-0.51
L585 x L807	-1.54	-0.35	0.02	-0.34
L655 x L61	4.76	0.42*	0.52*	0.94*
L655 x L354	-1.24	-0.35	0.07	-0.27
L655 x L374	-6.77*	-0.45*	-0.38*	-0.83*
L655 x L508	11.53***	1.04***	0.52**	1.56***
L655 x L542	-4.37	-0.21	-0.05	-0.26
L655 x L571	-0.27	-0.06	-0.33*	-0.37
L655 x L640	-1.34	-0.24	-0.20	-0.43
L655 x L807	-2.17	-0.16	-0.15	-0.31

L806 x L61	-7.82*	-0.43*	-0.33*	-0.73*
L806 x L354	1.46	-0.25	-0.40*	-0.60*
L806 x L374	12.30***	0.91***	0.34*	1.18***
L806 x L508	-3.80	-0.20	-0.11	-0.27
L806 x L542	-10.27**	-0.18	-0.03	-0.17
L806 x L571	-2.87	-0.40*	0.16	-0.19
L806 x L640	6.50*	0.69**	0.30	1.04**
L806 x L807	3.96	-0.17	-0.12	-0.24
S.E	**2.83**	**0.19**	**0.16**	**0.29**

KEY: Standard error (S.E), plant biomass (PB), shoot biomass (SB), root biomass (RB), Plant height (PH)
*** Very highly significant (P≤ 0.001),**Highly significant (P≤0.01), *Significant (P≤0.05), ns- Non-significant (P>0.05).

The Baker's ratio for Plant height, Shoot biomass, Root biomass and Plant biomass was found to be 0.12, 0.15, 0.49 and 0.28 respectively. Parameters that were measured showed varying narrow sense heritability (h^2). Narrow sense heritability ranged from 8%-30% (Table 11)

Table 11: Narrow sense heritability

Parameter	h^2
Root biomass	0.30
Plant biomass	0.21
Shoot biomass	0.11
Plant height	0.08

Table 12: General combining ability effects for measured significant parameter

Genotype	RB
Females	
L61	-0.18*
L354	0.48*
L374	0.06
L508	-0.23*
L542	-0.01
L571	-0.12
L640	0.14*
L807	-0.14
$S.E_f$	**0.07**
Males	
L60	0.28*
L584	-0.05
L585	-0.12*
L655	0.00
L806	-0.10
$S.E_m$	**0.06**

S.Ef and S.Em**,** standard error of the effects for GCA female and GCA male respectively , *Significant (P≤0.05)

RB, root biomass

4.2 Genetic Linkage Map

From Twenty four (24) pairs of markers which were screened only five were polymorphic between test parents L585 and L354. A map with one linkage group was constructed and had 3 SSR markers (Figure 3). The three polymorphic markers were from chromosome 5. Polymorphic markers umc1194 and umc1112 located on chromosomes 4 and 7 respectively were not accounted for on the linkage map as they did not associate with any of the other markers utilized.

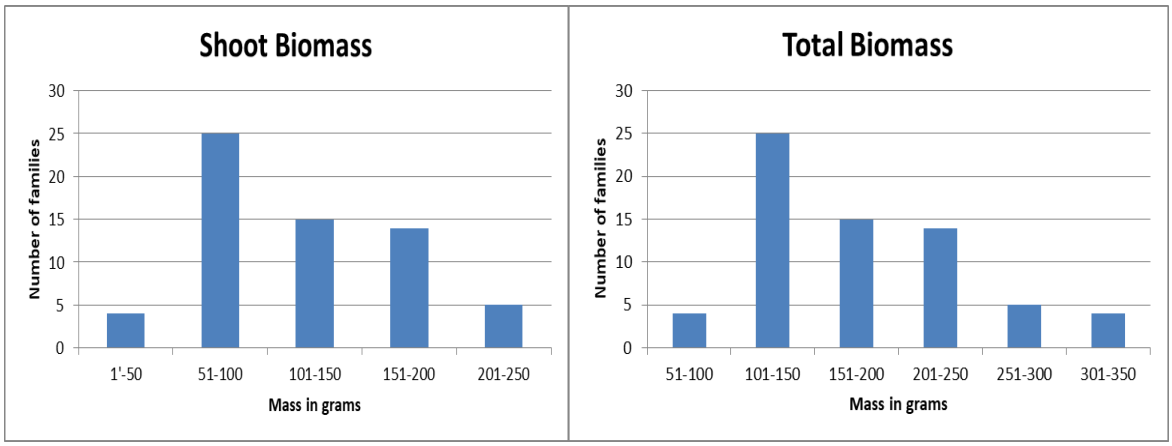
All the measured parameters in the $F_{2:3}$ population exhibited highly significant performances (P<0.001) to phosphorus utilization efficiency (Table 13).

Table 13: Analysis of variance for genotype performance for traits associated with Phosphorus Utilization Efficiency in a $F_{2:3}$ populations

		Traits					
Source	Df	GY	100SD	PH	SB	RB	PB
Rep	1	0.4	6.30	404.40	384.8	5.86	485.60
Genotype	66	1805.6***	27.76***	271.10***	4929.60***	482.63***	8157.10***
Error	66	171.3	7.53	155.60	384.0	56.79	635.80

***significant at P< 0.001

Further analysis of the $F_{2:3}$ populations in specific traits revealed distinct histogram classes. Trait 100SD had a more distinct class distribution (Figure 3).



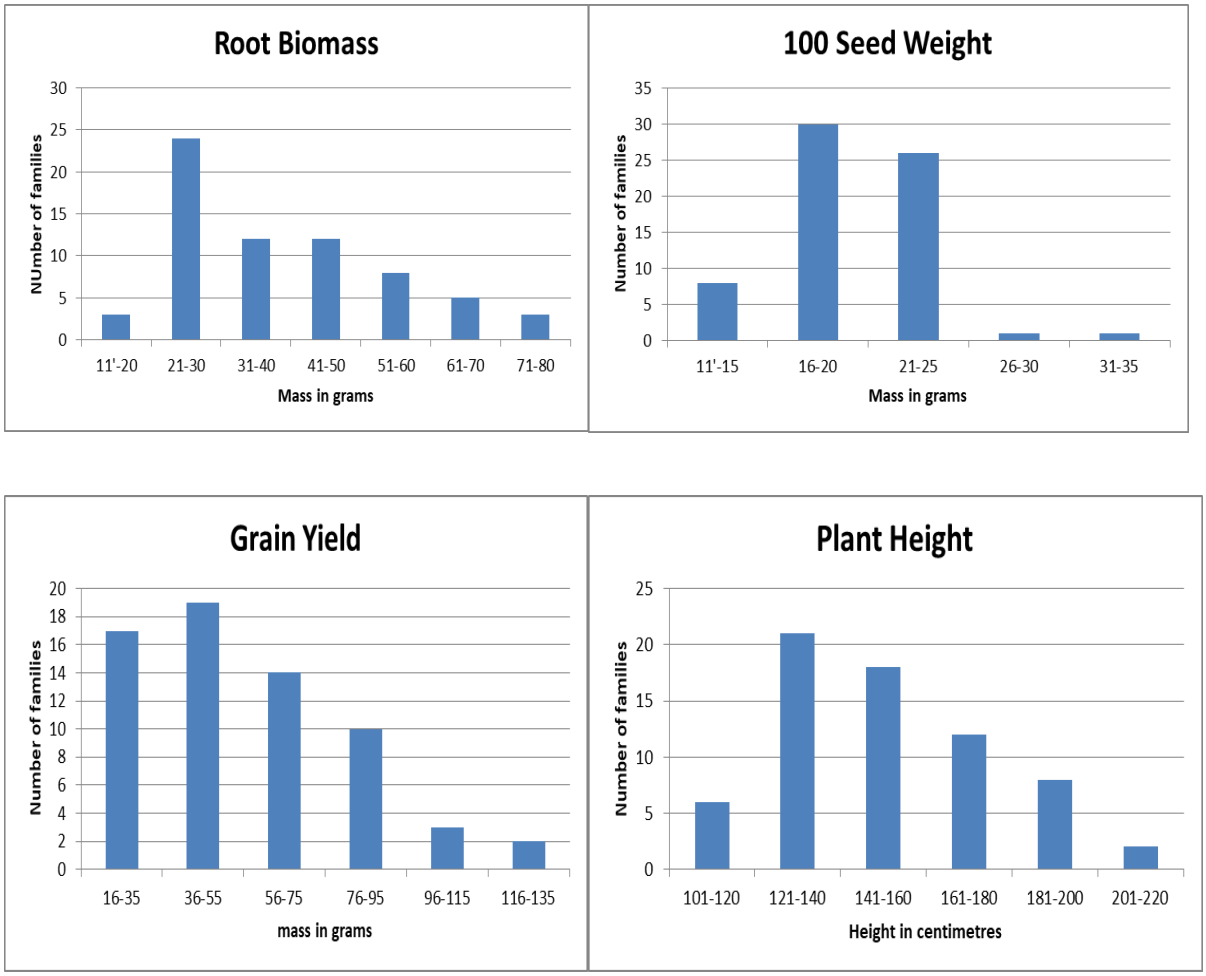


Figure 3: Frequency distribution for traits of the $F_{2:3}$ families in P-deficiency soils exhibiting number of plant responses to shoot biomass, total biomass, root biomass, 100 seed weight, grain yield and plant height

4.3 Single Marker and Quantitative Trait Loci Analysis

Single marker analysis associated with phosphorus efficient traits revealed marker umc 1194 as the most linked marker, with r^2 values of 0.189 ($P < 0.01$) followed by 0.151 ($P< 0.05$) associated with root biomass and plant biomass respectively (Table 14). However, the most significant r^2 values linked to molecular markers were found on chromosome 5 (mmc 0282 and umc 1092, Table 5). QTL detection using Inclusive Composite Interval Mapping revealed seven QTLs located on a linkage group 5 (Table 15, Figures 4 and 5). The QTLs for root biomass and grain yield, the two traits directly linked to phosphorus utilization, were detected on chromosome 5. For root biomass two QTLs were detected while one QTL was detected for grain yield. The first QTL mapped for root biomass on the linkage group with

phenotypic variation explained (PVE) by the marker of 11.49% and a likelihood of odds (LOD) score of 3.76 with an additive effect of -0.51. The other QTL for root biomass on the linkage map has a PVE value of 11.12% and a LOD score of 5.15 with an additive effect of 0.84. The QTL for grain yield had a PVE value of 16.34% and a LOD score of 4.56 with an additive effect of 1.38.

Two QTLs associated with plant height (PH) and root biomass (RB) were flanked by marker umc 1092 on the left and marker mmc 0282 on the right. Five QTLs associated with root biomass (RB), plant height (PH), plant biomass (PB), grain yield (GY) and shoot biomass (SB) were flanked by marker mmc 0282 on the left and marker umc 2136 on the right. Figures 4 and 5 shows the linkage group with detected QTLs while Table 15 shows the summarized information on QTL analysis.

Table 14: Phenotypic variation explained (r^2) by each marker for association with the analyzed traits

Marker	Chromosome	PB	SB	RB	PH	100SDWT	GY
umc 1194	4	0.151*	0.133*	0.189**	0.126*	0.103	0.126
mmc 0282	5	0.078*	0.094*	0.014	0.069	0.077*	0.079*
umc 1092	5	0.122**	0.131**	0.068*	0.068*	0.086*	0.146**
umc 1112	7	0.122**	0.114*	0.145*	0.152**	0.101*	0.106*

*, ** r^2 Significant at P= 0.05 and P=0.01 respectively. PB- Plant Biomass, SB- Shoot Biomass RB- Root Biomass, PH- Plant Height, 100SDWT-100 Seed Weight, GY-Grain Yield

Table 15: QTL Analysis for traits associated with phosphorus utilization in P-limited soil

QTL[a]	Linkage Group[b]	Position[c] (cM)	Left marker	Right marker	Bin	LOD[d] score	PVE[e] (%)	Additive[f] effect
PePH-5, 1	5	27	umc1092	mmc0282	5.04-5.05	3.22	14.69	-2.35
PeRB-5, 2	5	28	umc1092	mmc0282	5.04-5.05	3.76	11.49	-0.51
PeRB-5, 3	5	93	mmc0282	umc2136	5.05-5.08	5.15	11.12	0.84
PePH-5, 4	5	94	mmc0282	umc2136	5.05-5.08	4.22	14.61	1.27
PePB-5, 5	5	99	mmc0282	umc2136	5.05-5.08	4.32	18.28	2.97
PeGY-5, 6	5	101	mmc0282	umc2136	5.05-5.08	4.56	16.34	1.38
PeSB-5, 7	5	104	mmc0282	umc2136	5.05-5.08	4.06	18.99	4.68

Note: QTL identified between two markers, that is, between left marker and right marker

a- have been named from the trait abbreviation followed by the measured parameter abbreviation and then by the chromosome number where detected. The second number is added to show the order and the closest to zero gets position 1. Example PeRB-5, 2 means the QTL associated to phosphorus use efficiency was mapped for root biomass on chromosome 5 and it is in the second position (in terms of distance from 0 on linkage map) to another detected QTL on the same chromosome.

b -Chromosome number

c- The position of the QTL measured from the distance of the first marker listed on the linkage map

d- Logarithm of odds likelihood equivalent to $-Log_{10}$ likelihood

e-The amount of phenotypic variance explained by the detected QTL

f- Additive gene effect of detected QTL

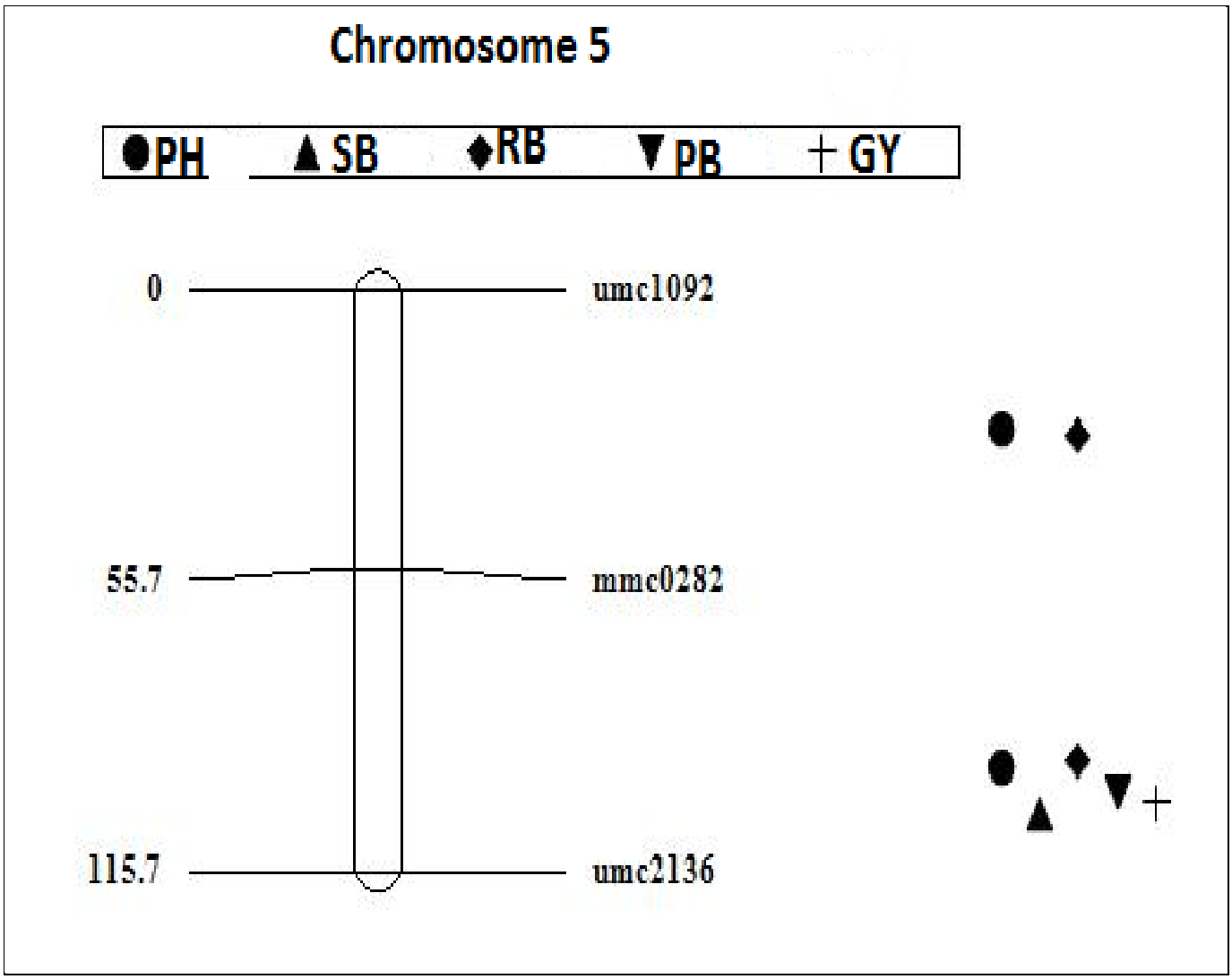


Figure 4: Linkage map on chromosome 5 showing the detected QTLs

KEY:
PH- Plant height

SB-Shoot biomass

RB-Root biomass

PB-Plant biomass

GY-Grain yield

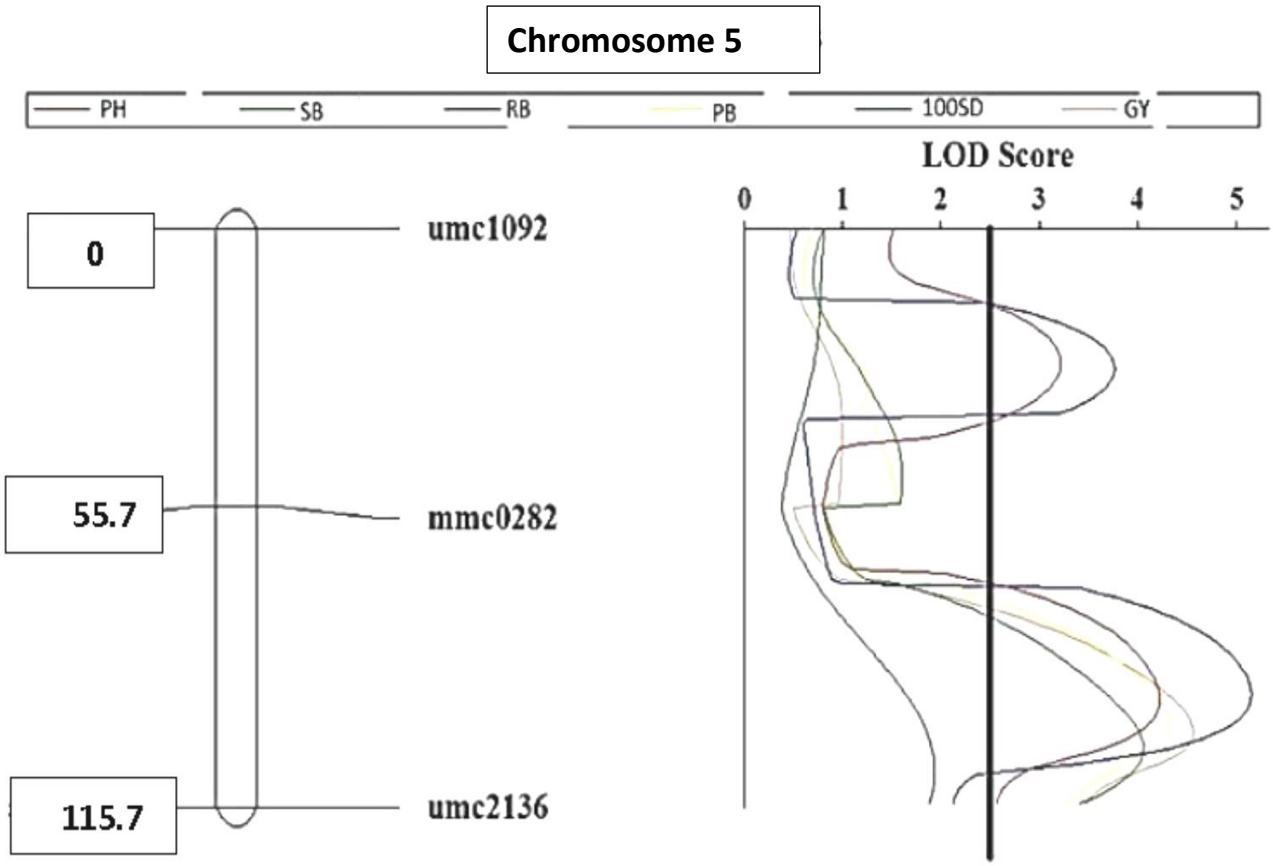


Figure 5: Linkage groups and the detected QTLs

Key:

Left side of chromosome (Ch) - Distance in centimorgans (cM)

Right side of chromosome (Ch) - Marker name

PH-Plant height

SB-Shoot biomass

RB-Root biomass

PB- Plant biomass

100SD-100 seed weight

GY-Grain weight

CHAPTER FIVE

DISCUSSION

5.1 General Statement

The results obtained shows that non-additive and additive gene action are important in the inheritance of traits associated with P utilization. In this research, parental genotypes and crosses efficient at utilizing P were identified. The use of such genotypes (efficient at utilizing P) is not only practical but feasible for small-scale farmers. In this regard the research was undertaken with an endeavor to investigate genotypes that can perform better in P-limited soils; to investigate the type of gene action conditioning association to this phosphorus utilization and map QTLs associated with phosphorus utilization in P-limited soil.

5.2 Response of Genotypes to Phosphorus Utilization

It has been established that P influences root development leading to increased biomass (Rosolem et al., 1994). In the study, root biomass showed a positive association with the plant performance as evidenced by the significant correlation (r= 0.64; P= 0.001) levels to shoot biomass and (r=0.68, P=0.001) to plant height respectively. Association of root biomass to total plant biomass was also significantly high (r=0.87 ,P=0.001) The high positive correlations of the root biomass, shoot biomass and plant height are in agreement with the findings that genotypes with a high root biomass produce high shoot biomass (Jinming et al., 2004). These genotypes would have the ability to grow and give economic yields in soil with low P. Osmort et al., (2007) observed that large root systems have a greater capacity for absorbing water and minerals as they are able to explore a larger rhizosphere.

The significant differences were observed in response to low phosphorus induced differential responses in maize genotypes grown in P-limited soil. The crosses grown in P-limited soil showed highly significant differences in plant biomass, shoot biomass, root biomass and plant

height implying that genetic variation in phosphorus use efficiency among the genotypes exists. Such variations among maize genotypes have been reported by Da Silva and Gableman (1993).

In this study, genotypes (L585 x L354), (L806 x L374), (L60 x L807) and (L655 x L508) showed exceptionally good performance across the measured parameters. Since phosphorus is a primary nutrient required for good root development of maize, a dense, spread and well-developed root system could have enabled these genotypes optimize nutrient absorption for growth and development through topsoil foraging. This agrees with earlier studies that illustrated that genotypes with superior growth in low-P environments have root traits which allows greater exploration and foraging of the topsoil (Lynch and Brown, 2001; Richardson and Simpson, 2011; Perez-Torres et. al, 2014). The aspect of foraging was not taken into consideration in this study but is however an important aspect to consider in follow-up studies

5.3 Combining Abilities and Type of Gene Action

In the study, significant positive SCA effects were exhibited by crosses (L60 x L807), (L585 x L354), (L655 x L508) and (L806 x L374) across all the measured parameters. The root biomass showed positive significant GCA effects for parental lines L60 and L354 (Table 10). In this regard positive combining ability effects are desirable because they indicate contribution of favourable alleles associated with phosphorus utilization, while it is the reverse with negative significant combining ability effects.

Baker's ratio for plant height, shoot biomass, plant biomass and root biomass was found to be 0.12, 0.15 and 0.28 and 0.49 respectively. All the traits measured except root biomass was found to be influenced by non- additive gene action. Root biomass was influenced by both additive and non-additive gene action. The findings of non-additive gene action implies that hybridization is the best option in breeding for the genotypes especially with regards to plant

height, shoot biomass and plant biomass. The presence of non-additive gene action implies that the effects of dominance and/ or epistatic gene action is at play (Adeniji and Kehinde, 2003). Therefore, significant gains in breeding for phosphorus utilization efficient genotypes can be achieved through hybridization to capitalize on the dominance/ epistatic gene effect. For the root biomass which showed both additive and non-additive, the implication is that hybridization and recurrent breeding methods can be employed.

The low narrow sense heritability for all parameters (0.08 plant height, 0.11 shoot biomass, 0.30 shoot biomass and 0.21 total biomass), obtained indicate that little transmissibility of traits between generations, and thus further prompts the use hybridization as the best breeding option in breeding, agreeing with what previous authors suggested(Adefris and Becker, 2005).

5.4 Identification of QTLs

QTLs were successfully mapped for traits associated with utilization of p in P-limited soil. A total of seven QTL were mapped on chromosome 5 associated to P utilization (Table 14). Chen et al., (2008) identified thirty one clusters of QTLs for phosphorus utilization traits on chromosome 5. It therefore appears that linkage group 5 holds many loci that have coevolved to adapt maize plants for association to phosphorus deficiency.

The amount of phenotypic variations explained (PVE) by the mapped QTLs ranged from 11% to 19%. PVE is the proportion of phenotypic variance (also designated as R^2) which is explained by a predictor of a quantitative trait and is formed using estimated effects of all markers. This depends on the number of independently measured genomic variants associated with the trait, the proportion of the total variance they explain and the sample size in the discovery sample (Wray et al., 2013). The frequency distribution graphs (Figure 3) for traits associated with phosphorus utilization efficiency indicate that non-additive genetic effects are

important in the inheritance of the traits. This is an indication that breeding efforts should be made to develop hybrids exploiting the heterosis for traits related to P utilization efficiency.

The markers associated with the first two QTLs associated with plant height and root biomass were umc1092 and mmc0282. The other five QTLs (associated with root biomass, plant height, plant biomass, grain yield and shoot biomass) were flanked by marker mmc0282 of the left and marker umc2136 on the right. Molecular-assisted selection works best when mapped QTLs are tightly linked to the markers and the tighter the linkage, the higher the probability for a marker to be inherited together with the detected QTL. For the marker to be efficient, a distance of less than 5 cM between the marker and the QTL is recommended (Collard et al., 2008, Tembo et al., 2014). In the study, the mapped QTL PeSB-5, 7 associated with shoot biomass was at 9 cM, from the nearest marker umc 2136.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The study was undertaken to investigate the possibilities of contributing towards increased maize production and productivity in Sub-Saharan Africa by the mapping of QTLs linked to phosphorus use efficiency in tropical maize (*Zea mays L.)* genotypes. The objectives of the study were: (1) to evaluate genotypes which are efficient at utilizing phosphorus in P-limited soil, (2) investigate the type of the of gene action associated with traits linked to utilization of phosphorus in P-limited soils, and (3) map QTLs associated with phosphorus utilization in P-limited soil.

Forty crosses were evaluated for efficient phosphorous utilization in P-limited soils. In this study genotypes (L585 x L354), (L806 x L374), (L60 x L807), (60 x 354) and (L655 x L508) were identified to be efficient at utilizing p in p limited soils.

The Baker's ratio for plant height, shoot biomass and plant biomass the secondary traits associated with utilizing p in P-limited soils was found to be 0.12, 0.15 and 0.28 respectively. This implied that non additive gene action conditioned plant height, shoot biomass and plant biomass responses in P-limiting soils. On the other hand, Baker's ratio for shoot biomass was 0.49 implying that additive and non-additive gene action conditioned this trait response in P-limited soils. In this regard both hybridization and recurrent breeding methods can be used in a breeding scheme for this trait.

Seven QTLs were identified on chromosome 5 with phenotypic variations ranging from 11% to 29%. All the markers used were more than 5 cM from the mapped QTLs. The closest marker, umc 2136 was 9 cM away from the mapped QTL. Therefore, there is need to utilize the maize genomic map to identify and test several markers near the mapped QTL, in order to locate more reliable molecular markers for utilization in marker-assisted selection (MAS).

6.2 Recommendation

Further research should be done in topsoil foraging to establishing whether it has a major role in utilization of phosphorus in P-limited soils among the traits under consideration, that is, shoot biomass, root biomass, plant biomass and plant height.

With regards to molecular markers associated with P utilization, a much larger mapping population should be employed and in addition many more markers should be identified between umc2136 and the mapped QTL to identify many more reliable markers for utilization in MAS.

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APPENDICES/APPENDIX

APPENDIX I

SIMPLE SEQUENCE REPEATS MOLECULAR MARKERS OBTAINED FOR STUDY

				Base Number
1	bnlg1346	Left End	CAT CAT GAA GCA ATG AAG CC	20
		Right End	CCG CGC CAT TAT CTA GTT GT	20
2	bnlg1695	Left End	ACC AAA TCC TCA TCT CGG AA	20
		Right End	CAA TCT CCC CAA AAT CTC GA	20 80
3	umc2136	Left End	CCA GAT GCG GAA GTA GAC GG	20
		Right End	GAT TCG GAG GTG ATC TGA CCT GT	23
4	mmc0282*	Left End	CTC TTT CTT TAT TTG TTC CGT T	22
		Right End	GGA CTA CAC ATC ACC AGC A	19 84
5	phi333597	Left End	AGC TCG AGT ACC TGC CGA G	19
		Right End	TGC ATC TCT GAG ACC ATC ACC	21
6	bnlg1346	Left End	CAT CAT GAA GCA ATG AAG CC	20
		Right End	CCG CGC CAT TAT CTA GTT GT	20 80
7	bnlg1695	Left End	ACC AAA TCC TCA TCT CGG AA	20
		Right End	CAA TCT CCC CAA AAT CTC GA	20
8	umc2136*	Left End	GAT TCG GAG GTG ATC TGA CCT GT	23
		Right End	CCA GAT GCG GAA GTA GAC GG	20 83
9	umc1184	Left End	CTT CCT TAC GTG TCA CCG CTC T	22
		Right End	GTG GAG TGA TGT GAT CGA TGA TG	23
10	umc1290b	Left End	CTG CTC ACG CTC ATC CTC CT	20
		Right End	AGA GAT TCA TCA GAG TGG CGA TG	23 88
11	umc1007	Left End	AAG CAA TAT CAC TAC TTT CCA GCC	24
		Right End	TAC GTA ATT CGT AGC CTT GGT CC	23
12	nc131	Left End	TTT CTT CGA TCC CAT GTC AC	20
		Right End	TAG TGT GCT AGA ACG TGC GC	20 87
13	umc1162	Left End	CAT CAG CAG GAG GAG CAG TCT C	22
		Right End	CCT GTT GAC GAG AAG AAA GAG GAA	24
14	bnlg1246	Left End	CGC AGG CCG GGG AA	14
		Right End	CCT GGC GCC CAA CC	14 74
15	umc1680	Left End	TTA ATA AAG GAG AGG GTG GGA ACC	24
		Right End	GGG GCT TAT ATG TCC CTT GAA CTC	24
16	dupssr10	Left End	AGA AAA TGG TGA GGC AGG	18
		Right End	TAT GAA ATC TGC ATC TAG AAA TTG	24 90
17	bnlg1019	Left End	ACC ATA GTT GGA CGG ACC AC	20
		Right End	ACC ACA ACA CAG ACG AGC AC	20
18	bnlg1022a	Left End	GCA AAG ATC TGT GAG GGG AC	20
		Right End	GTG TTG TCG ATC CAC TCC CT	20 80
19	bnlg1268a	Left End	TCC ACG GTG ACT GTA GAA CG	20
		Right End	CAC TTC CCC CAG ATC ATT TG	20
20	umc1148	Left End	AAA ATT ACA GAG CAT TTT GAA AGA AGA A	28
		Right End	TAG CCG TGT CAG TTT GTA GAT CCT	24 92
21	umc1194*	Left End	ACC ACC AGA CAT GGG AAA CTT CT	23
		Right End	AAG GCG GAC ACT ACT CTA CCC TCT	24
22	umc1060	Left End	ACA GGA TTT GAG CTT CTG GAC ATT	24
		Right End	GGC CTC TCC TTC ATC CTA TTC AA	23 94
23	umc1092*	Left End	TAA GGC GCA GAT GAA CTA GCC TAC	24
		Right End	CTC CAG TGA GTT CCA GCG CTA T	22
24	umc1112*	Left End	TTG GGT TCA GTT TTC ACA ACC TTT	24
		Right End	AAG ATG ATT ACT AAC TCG CGG CAG	24 94

*SSR markers which were polymorphic between the most efficient and the least efficient parents used in genotyping the $F_{2:3}$ populations.