INHERITANCE OF PHOSPHORUS USE EFFICIENCY AND RESISTANCE TO ANTHRACNOSE IN SELECTED SORGHUM GENOTYPES GROWN IN THE ACID SOILS OF WESTERN KENYA

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DECLARATION

I certify that the work presented in this thesis is original and has not been submitted, either in whole or in part, for an award of a degree in any other University. Contributions of others have been clearly indicated, with due reference to the literature, and acknowledgement of collaborative research and discussions.

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DEDICATION

In loving memory of my late dad Leo Henry Nyambok; your contributions in my life and academics, love and care will forever be missed by me. To my wonderful mother Teresa Akech, my brothers and sisters, Aunt Babra and Uncle Ineah Orawo, for your motivation, encouragements, love and care.

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Table of Contents

List of Tables

List of Plates

Abstract

The acid soils of western Kenya where most of the sorghum is grown have very low available phosphorus, at 2-5mg P/kg soil compared to the recommended levels of 10-15mg P/ kg soil required for optimal crop productivity. In these soils, sorghum grain yield is low (1.0 t/ha) and has continued to decline due to low P availability in the soil, use of low yielding varieties and anthracnose susceptibility, among other factors. Inorganic phosphorus fertilizer supplementation is not economically viable for the resource constrained farmers in these areas. In addition, only between 10-30% of the applied P is available to the crop while the rest get fixed by clay minerals. The poor plant mineral nutrition in western Kenya is further compounded by the prevalence of foliar diseases notably anthracnose (*Colletotrichum sublineolum)* which causes yield loss of up to 70% in susceptible cultivars. Since both problems tend to occur together in sorghum growing areas, there is a need to develop cultivars that are not only anthracnose resistant but are also efficient in phosphorus use. At present, such cultivars are not available to farmers in western Kenya. This study sought to determine the mode of gene action governing the inheritance to phosphorus use efficiency (PUE) and resistance to anthracnose to support breeding for multiple stress tolerance in sorghum. Six stable inbred lines (G2, C1, K5e, L6, O2 and P5), contrasting for the two traits were used as parents in this experiment. A six-parent half diallel cross was used to generate F1 seed using the hot emasculation technique. F1s were advanced to F2. Genetic analysis was conducted on F2 progeny for both traits. Recombinant F2 populations plus their parents were screened at Sega, a low P site. For the anthracnose study, screening of recombinant F2 populations plus their parents was done at Kibos; the disease hotspot. Both sites lie in the major sorghum growing region in western Kenya. Standard statistical data analyses were used to determine the mode of gene action governing the inheritance of PUE and resistance to anthracnose disease in sorghum. Both additive and non-additive genetic effects were found to govern the inheritance of PUE with the predominance of non-additive genetic effects over additive effects. In study two, both were found to govern the inheritance of resistance to anthracnose with additive effects largely determining resistance to the disease. This study identified three parental genotypes (G2, C1 and O2) and two crosses (G2xL6 and G2xO2) which combine efficiency in phosphorus use and resistance to anthracnose disease. These should be used in the development of multiple stress tolerant sorghum varieties for farmers to improve sorghum yield in the low P and anthracnose endemic soils of western Kenya and Eastern Uganda.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Economic importance and challenges to increased productivity

Sorghum (*Sorghum bicolor* (L.) Moench) is an important cereal and is ranked second most important in Sub-Saharan Africa after maize (Zidenga, 2004; FAO, 2009). Globally, it is an important crop in arid to semi agro-ecologies because of its ability to withstand harsh environmental conditions including high temperatures, moisture deficit and water stagnation (Harlan and de Wet, 1972; Devries and Toeniessen, 2001; Sleper and Poehlman,2006; Balole and Legwaila, 2006). Sorghum is a major source of food globally which accounts for over 65 % of the carbohydrate requirements and 39% of the daily calorie intake for over 300 millions of people in developing economies of Africa and Asia (Godwin and Gray, 2000; Rooney and Waniska, 2004).Other uses of sorghum include the manufacture of beers, feeds, silage and direct pasture for livestock, textile dyes, ethanol for biofuel and other industrial purposes as well as molasses and syrups from sweet sorghum (Harlan and de Wet, 1972; Wortmann *et al.*, 2006). In Kenya, it is grown principally in drought-prone, marginal agricultural areas of Eastern, Nyanza, Western and Coastal provinces (EPZA, 2005).Western Kenya produces over 70 % of sorghum in the country (Mukuru, 1993) but in the acid soils ($pH \leq 5.5$) that are generally low in the available P (2 to 5mg/kg soil) compared to optimal levels of 10 to 15mg P/kg soil (Okalebo *et al*., 2004; Obura, 2008). This poses a major challenge and is therefore among the primary factors responsible for the low grain yields (1.0t/ha) in Kenya compared to 4.3 t/ ha obtained in developed worlds.

Depending on the site, Phosphorus (P) deficiency has been found to reduce maize grain yields in soils by between 28-55% and by almost 100% in most commercially available cultivars (Ligeyo, 2007; Kisinyo, 2011, Maina *et al.*, 2013 unpublished results). The recommended P supplementation rate of 60kg P2O5/ha is largely unaffordable to most resource poor farmers in the region. In addition, the utilization efficiency of P by plants especially in acid soils is often low, ranging only from 10 to 30% in the year of application (Zhul *et al*., 2001; Syers *et al*., 2008). The low utilization efficiency is due to the fact that most of the P gets fixed by clay minerals making it unavailable to the crop (Kaeppler *et al*., 2000; Obura, 2008). Clearly therefore, application of inorganic P alone does not solve the problem. A more sustainable alternative is to develop crop plants that can efficiently extract P even under limiting P levels in the soil. However, such cultivars are yet to be developed for

all sorghum agro-ecologies in Africa. Some efforts have been initiated in Brazil identifying lines with higher Phosphorus use efficiency (Schaffert *et al*., 2001).

Another challenge to sorghum production is the foliar disease anthracnose (*Colletotrichum sublineolum*). The pathogen is highly variable, wide spread and known to infect all the above ground tissues of the plant. Anthracnose can cause yield losses of up to 70% in susceptible cultivars under severe epidemics through defoliation and tissue death (Thakur and Mathur, 2000). Although anthracnose resistant varieties are available, only a limited number of smallholder farmers can easily access such resistant varieties in part due to a weak seed system in most parts of the continent. The difference in taste and end-use attributes of these cultivars also reduces their adoption further limiting spread of such material. Yet deployment of crop resistance remains the most cost effective approach for the management of cereal diseases especially for highly variable pathogens such as *Colletotrichum sublineolum* (Souza-Paccola *et al*., 2003). This is also due to the low return to investments per unit area of inorganic chemicals used. Incorporating resistance genes to endemic pathogens into improved cultivars before they are released would be of great importance.

Although sorghum cultivars tolerant to anthracnose or efficient in phosphorus use could be found, these are tolerant to single stresses thus may not provide good solution because in the farmers' fields, a combination of more than one of these factors is normally present. Currently there are efforts to develop sorghum genotypes with improved phosphorus use efficiency (PUE) and/or anthracnose resistance. Since both challenges occur concomitantly, deployment of disease resistance and PUE is essential. This was the focus of this study.

1.2 Statement of the problem

Sorghum production in western Kenya and indeed most of sub-Saharan Africa occurs generally in marginal soils prone to low soil fertility, pests and diseases. For sorghum, the most debilitating factors that account for low productivity is the low P availability largely due to fixation in the soil, as well as susceptibility to folia diseases such as anthracnose and other market drivers along the value chain. These compound factors are particularly important for resource-constrained farmers who cannot afford pesticides and inorganic fertilizers, the fastest way to remedy these challenges and improve production. Moreover, even the little inorganic P applied in the soil may not be easily accessed by plants due to further fixation by soil clay minerals. A number of breeding lines that combine P efficiency and good yield have been developed by the sorghum breeding project of Moi University. However, some of them are highly susceptible to anthracnose. Breeding sorghum varieties that combine resistance to

C. sublineolum and are efficient in P use, provide suitable alternatives for improvement of sorghum production where both factors limit sorghum productivity. In local sorghum however, only limited studies have been done to improve either P use efficiency or tolerance to anthracnose through exploitation of the genetic potential of the crop. This study therefore sought to understand the mode of gene action governing anthracnose resistance and phosphorus use efficiency in selected sorghum accessions to inform breeding programmes that will generate multiple stress tolerant sorghum as part of the solution to combat these stresses and improve productivity.

1.3 General objective

The overall objective of this study was to contribute to the development of phosphorus use efficient and anthracnose resistant sorghum varieties for use in the acid soils of western Kenya.

1.3.1 Specific objectives

1. To determine the mode of gene action governing the inheritance of phosphorus use efficiency in crosses of selected sorghum genotypes grown in the acid soils of western Kenya.
2. To determine the mode of gene action governing the inheritance of resistance to anthracnose in crosses of selected sorghum genotypes grown in the acid soils of western Kenya.

1.3.2 Hypotheses

H_{O1}: Phosphorus use efficiency or Phosphorus efficiency is inherited as a quantitative trait, with a small or large additive gene action component compared to the non-additive gene action and/ or environmental effects.

H_A: PUE or PE is **not heritable** and cannot be selected for in sorghum genotypes.

H_{O2}: Resistance to anthracnose is conditioned quantitatively, with a small or large additive gene action component compared to non-additive gene action and/ or environmental effects.

H_A: Inheritance of resistance to anthracnose is not controlled by one or a few major genes in selected crosses.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Acid soils and their effects on plant mineral nutrition

2.1.1 The soil mineral nutrition complex challenge to agriculture

It is estimated that 30-40% of the world's arable soils and up to 70% of potentially arable land are acidic. Many of Africa's arable lands occur on old heavily leached soils (Acrisols, Ferralsols and Luvisols) that are associated with acidity, aluminum toxicity and its attendant high levels of P fixation. Most acid soils in Kenya are found in the highlands east of the Rift valley and Western Kenya (Kanyanjua *et al*., 2002). Acid soils (pH 5.5 & below) are commonly associated with Al, Fe, H and Mn^{4+} toxicities and P deficiency. In such soils, P deficiency is attributed to its fixation by Fe^{3+} and Al^{3+} ions (Buresh *et al*., 1997; Obura, 2008; Kisinyo *et al*., 2013). Generally, soil P is slightly more available in a pH range of 6 to 7.5. At pH levels above 7.5, calcium and magnesium may react with phosphorus, reducing its availability to plants. In acidic soils, Fe^{3+} and Al^{3+} increase, either fixing or removing P from the soil solution by forming insoluble complexes limiting the availability of P to plants (Tisdale *et al*., 1990). Since phosphorus is needed for root development, because of its unavailability at this pH, poor root development occurs and as a result low water and mineral salts absorptions from the soil thus affecting normal plant growth (Tisdale *et al*., 1990; Neil,1991).

The detrimental effects of hydrogen ions is not as distinct as that of Al^{3+}cations, but excess hydrogen ions in acid soils affect plant root membrane permeability and ion transport (Kochian *et al*., 2005). The acrisols and ferralsols of western Kenya where sorghum is an important cereal crop are highly weathered, overused and acidic with very low available phosphorus, ranging between 2-5mg P/kg soil compared to 10-15mg P/kg soil required for optimal crop productivity (Okalebo *et al*., 2004*).* Low P availability is therefore among the primary factors responsible for the low and declining sorghum grain yields in western Kenya. A feasible way of improving sorghum productivity in this area is to develop varieties that can perform well under low P conditions, that is, P-use efficient varieties. Such varieties have the ability to solubilize and use fixed P (Kochian *et al*., 2005).

2.1.2 Interaction of moisture stress and phosphorus acquisition in acid soils

Phosphorus concentration in the soil is directly proportional to the water content of the soil and as such phosphorus uptake by plants is often reduced during water stress (Novais *et al*.,

1990). This is of particular concern in the acid soils and sub-soils of the tropical regions where intervals of moisture stress are common throughout the growing season. Plant adaptation to acid soils is linked to better development of the plant's root system in tolerant genotypes that in general are able to grow below the 15 to 20 cm of acidic top soil. Additionally, adapted plants can absorb phosphorus from acid soils when soil moisture is high. Adaptation to Al^{3+} stress, low P availability and short periods of drought appear to be controlled by related metabolic pathway. Cultivars generally efficient in P uptake and also tolerant to Al^{3+} toxicity, have greater yield stability and have better agronomic performance over many growing seasons (Oliveira *et al.,* 2006).

2.2 Role of phosphorus in plant development

Phosphorus (P) is one of the six essential macronutrients required by plants (Hammond *et al*., 2004). It is a component of key molecules such as nucleic acids, phospholipids and ATP, and consequently, plants cannot thrive without a reliable supply of this nutrient (Tisdale *et al*., 1990). In many agricultural systems characterized by application of inorganic P, recovery of applied P by crop plants is very low, because more than 80% of it is fixed and unavailable for plants (Keerthisinghe *et al*., 2001). In the soil, P is found in different pools, such as organic and mineral P with 20 to 80% found in the organic form (Richardson, 1994). Since the rate of diffusion of P in the soil is slow, high plant uptake rates create a zone around the root that is depleted of P. Inorganic phosphate (Pi), the fully oxidized form of phosphorus has unique properties that place it at the centre of metabolism. In plants, Pi plays a pivotal structural and regulatory role at the nexus of photosynthesis, carbon metabolism and energy conservation (Abel *et al*., 2002). The availability of Pi therefore has profound consequences for plant growth and physiology.

2.2.1 Mechanisms of low P tolerance in plants

Adaptations of plants to grow under low P is related to higher phosphorus acquisition efficiency (PAE) and / or higher phosphorus internal utilization efficiency (PUtE) with PAE in plants being dependent on the development of extensive root systems either in association with mycorrhiza, the formation of proteoid roots or root hairs and changes in root physiology by secretion of acid phosphatase allowing the plant to explore a larger volume of soil, improving access to P available in the soil (Gaume *et al*., 2001).

Plants have evolved a number of mechanisms to cope with low inorganic phosphate (Pi) availability. Some of these mechanisms of P efficiency include changes to conserve plant

tissue P, changes in root morphology and changes aimed at increasing the acquisition of P. Low P deficiency tolerant plants therefore may use one or some of these mechanisms for its adaptation to acid soils.

2.2.1.1 Changes to conserve plant tissue P

To optimize the internal use of P, plants recycle P from old to new tissues. Plants have also been noted to mobilize P from non-essential uses to essential uses under P-limiting conditions for example, the substitution and recycling of phospholipids during P deficiency releases Pi from phospholipid head groups. The proportion of mono- and di-galactolipids and sulpholipids has also been noted to increase in the thylakoid membranes under P deficiency thus allowing photosynthesis to continue despite reduction in phospholipid content (Hammond *et al*., 2004). Additionally, under low P conditions, the conversion of sucrose to hexose-P during photosynthesis can proceed via an alternative pyrophosphate (PPi) dependent pathway that requires UDP-glucose pyrophosphorylase as has been observed under P-deficiency in Arabidopsis.

2.2.1.2 Changes in root morphology

When subjected to P deficiency, plants maximize the volume of soil exploited, and thereby, the amount of Pi availability by increasing effective root area (Abel *et al*., 2002; Hammond *et al*., 2004). In response to low P, enhanced root hair formation has been reported in groundnuts (Wissuwa and Ae, 2001), Arabidopsis (Abel *et al*., 2002), pearl millet (Faye *et al*., 2006) and sorghum (Camacho *et al*., 2002). The formation of symbiotic association of roots with mycorrhiza is another related widespread response to low P availability (Abel *et al*., 2002). Mycorrhiza colonizes the root cortex to obtain carbon from their host while assisting the plant in Pi acquisition.

2.2.1.3 Increasing the acquisition of P using organic acids and enzymes

Some plants are able to modify their rhizosphere to improve access to Pi in the soil solution by increasing exudation of organic acids in response to P deficiency to release Pi from the insoluble inorganic salts. Organic acids such as malate and oxalate are involved in many processes operating in the rhizosphere, including nutrient acquisition and metal detoxification (Hammond *et al*., 2004).In sorghum, plants exposed to low P had higher amounts of citrate and malate than those supplied with P. Some dicotyledonous plant roots, especially those of non-mycorrhiza plants such as *Brassica napus* release large amounts of organic acids by up to 4 fold into the rhizosphere within the root apex in response to P deficiency with malate and

citrate as the primary components (Jones, 1998). In *Lupinus albus* and other species with cluster roots, under P deficiency, development of short branched tertiary lateral roots, also known as proteiod roots is induced, accounting for a 13-40 fold increase in malate and citrate exudation (Jones, 1998). In maize, citrate secretion by roots is an important adaptation to low P tolerance (Corrales *et al*., 2007).

Enzymes also play a role in the adaptation of plants to low P conditions. Acid phosphatase is believed to be important for phosphorus scavenging and remobilization in plants (Hammond *et al*., 2004). Similar reports have been made in common beans (Yan *et al*., 2001). In *Brachiaria ruziziensis*, a tropical forage grass, high productivity without P application even in P deficient soil has been reported with no P deficiency symptoms observed (Masuda *et al*., 1997). The ability of *B. ruziziensis* to thrive under low P is attributed to an enzyme whose sequence is homologus to endochitinases of several plants. These enzymes could be used in enhancing availability of P in acid soils.

2.2.2 Inheritance of low P tolerance in plants

Tolerance to low Pi is a heritable trait with the mode of inheritance in a number of crops known. 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 maize, tolerance to low available P is largely conditioned by additive gene effects although dominance was also important (Da Silva *et al*., 1992). In forage sorghum, another gramineae, tolerance to low P has been associated with higher specific combining ability (Gorz *et al*., 1987). In legumes, both additive and dominance effects are important with dominance being prominent in cowpea (Vigna unguiculata), (Ojo *et al*., 2006) and additive effects in beans (Kimani *et al*., 2007). The understanding of the mode of inheritance of low P tolerance is important in any given crop. Such information is useful in guiding the breeding strategy to be used for the breeding of crops that are tolerant to low P.

In a field evaluation on low P soils, sorghum genotypes efficient in P acquisition and responsive to applied P have been identified (Schaffert *et al*., 2001). These results show that it is possible to identify material among African accessions that could be used to improve commercially available genotypes with low PUE. In forage sorghum, improved tolerance to low P has been shown to be due to specific combining ability effects. The implications of these studies for grain sorghum are still unknown. 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 (Yan *et al*., 2001; Presterl *et al*., 2002; Chen *et al*., 2009); The scope to improve local sorghums for both these remains a gap that requires further elucidation of the genetics behind these complex traits. That is the focus of this study.

2.3 Sorghum anthracnose

2.3.1 Importance of the disease

Sorghum anthracnose (Colletotrichum sublineolum) is widely distributed throughout the sorghum growing areas of the world (Wharton and Julian, 1996). It is the most destructive disease of sorghum globally and in Eastern Africa (Ngugi *et al*., 2002; Chala *et al*., 2010). Anthracnose occurs in many African nations and has been reported as a major constraint to sorghum production in West and East Africa, in countries such as Uganda, Mali, Burkina Faso and Kenya (Neya and Le Normand, 1998; Ngugi *et al*., 2002). Yield losses due to anthracnose are often difficult to estimate (Ngugi *et al*., 2000). However, losses greater than 50% have been reported (Pande *et al*., 1991; Ngugi *et al*., 2000; Thakur and Mathur, 2000). In 2000, Frederiksen reported losses of between 30-50% to stalk rot.

Anthracnose affects all above ground parts of the plant including stems, leaves, peduncle, inflorescence and seeds (Thakur and Mathur, 2000; Casela *et al*., 2001).Depending on the area on infection, the disease may be called leaf anthracnose, stalk rot anthracnose, panicle or grain anthracnose, with the foliar phase of the disease being the most frequently observed with clear symptoms at the booting stage (Hess *et al*., 2002). Infected leaves develop circular to elliptical red spots with few to numerous acervuli on lamina with wide margins that vary in colour from red, purple, orange or tan with straw coloured centres (Thakur and Mathur, 2000; TeBeest *et al*., 2004). As the disease progresses, the spots increase in number and coalesce, covering most of the leaf surface (Erpelding and Prom, 2004). The foliar phase of the disease is however rare in the arid regions (Frederiksen, 2000). Infection of the panicle results in death of the florets within the panicle while peduncle infection contributes to panicle breakage prior to harvest due to rotting of the interior of the stalk (Erpelding and Prom, 2004). Panicle and grain infection can result in sterility, grain abortion, premature grain ripening, reduced seed size and poor head development. During severe epidemics, premature defoliation occurs, resulting into retarded plant growth and extensive necrosis. Foliar anthracnose though the most prevalent form of the disease, stalk rot, panicle and grain anthracnose are equally important. The significance of damage caused by this pathogen

necessitates development of appropriate control strategies to manage the disease, so as to reduce crop loss and to avert an epidemic.

2.3.2. Anthracnose epidemics

In the sorghum-anthracnose pathosystem, pathogen variability, host resistance and environment, appear to play the most crucial roles in epidemics. The pathogen, *Colletotrichum sublineolum* is highly variable, existing in many different pathotypes able to affect different sorghum genotypes (Cardwell *et al*., 1989). Although, sorghum lines resistant to anthracnose exist, this high pathogen variability creates problems and leads to low resistance or breakdown of resistance. The primary host is *Sorghum bicolor* (common sorghum) whereas the secondary hosts include Poaceae (cereals) and *Zizania aquatic* (annual wild rice). Generally, the rate of development of any disease epidemics is driven by the initial amount of inoculum, the rate of within season pathogen perpetuation and the proportion of healthy tissue remaining to be infected that interact in time and space (Campbell and Madden, 1990).The primary sources of inoculums are from sporation on the leaves, infected seeds, stalk residues left in the field and/or adjacent growing crops and weeds (Casela and Frederiksen, 1993; Misra and Sinha, 1996). Anthracnose is most severe on mature plants after the formation of the panicle. In the case of the other species of *Colletotrichum*, extensive periods of rainfall are essential for the development of epidemics. The sequential senescence of sorghum leaves also plays an important role in the disease development. Anthracnose is presumed to be spread by conidia exclusively because the teleomorphic state is not known. Conidia can be dispersed over longer distances as dry spore masses while within smaller regions, splash dispersal of moist conidia is largely responsible for the development of epidemics. The disease can also be seed borne (Cardwell *et al*., 1989).

The extent of damage or yield loss due to anthracnose is related to the degree of host susceptibility, the aggressiveness of the pathogen, the physiological status of the host and the environment. Sorghum varieties developed in one environment may succumb to the disease in another environment (Pastor- Corrales and Frederiksen, 1979). Severe epidemics can even occur in sub-optimal conditions, where highly pathogenic strains infect susceptible host cultivars (Pande *et al*., 1991). According to Snyder and Nicholson (1990), young sorghum plants of both genetically resistant and susceptible cultivars were resistant to anthracnose but the susceptible cultivars became progressively more susceptible as they matured.

2.3.3 Genetics of resistance to sorghum anthracnose

Inheritance of resistance to sorghum anthracnose has been reported to be conditioned by both qualitative and quantitative mechanisms (Rooney *et al*., 2006). Single dominant or recessive genes conferring resistance to foliar anthracnose in sorghum have been reported (Boora *et al*., 1998; Singh *et al*., 2006; Erpelding, 2007). In contrast, Warren (1986) and Mohan *et al*. (2011) have reported resistance to foliar anthracnose as being quantitatively inherited. Various studies have reported anthracnose resistance as being conditioned by different numbers of genes with different modes of inheritance. In 1954, Coleman and Stokes reported that resistance to anthracnose was conferred by two closely linked dominant genes each conferring resistance to different phases of the disease. Later it was reported that resistance to anthracnose is controlled by single genetic locus with multiple allelic forms (Jones 1979; Tenkouano, 1993). More recently, a single recessive resistance gene has been implicated (Boora *et al*., 1998) and confirmed to be inherited in qualitative manner (Biruma, 2013).

2.3.4 Sources of resistance to anthracnose

The use of resistant cultivars is the most economical control method for sorghum anthracnose (Pastor-Corrales and Frederiksen, 1980; Thakur and Mathur, 2000; Marley, 2004). Host plant resistance is an important component for the integrated management of foliar anthracnose, and can help reduce quantitative losses in sorghum grain yield (Hess *et al*., 2002). The availability of stable sources of resistance with high yield and good grain quality appears to be very important in the maintenance of sustainable sorghum productivity, as well as reducing the risk of lower sorghum production because of severe anthracnose epidemics (Pande *et al*., 1994; Neya and Le Normand, 1998; Thakur and Mathur, 2000; Hess *et al*., 2002).

Most sources of anthracnose resistance in sorghum are considered to be conditioned by multiple genes (Thakur and Mathur, 2000). However, limited information is available on the genetics of host plant resistance and few sources have been used in sorghum improvement programs. This type of resistance is expected to be inherited in a multigenic manner and significantly influenced by the environmental conditions and can be expressed as a reduced infection frequency, a slower rate of development in the host, and a slower rate of spore production over a shorter period of time (Casela *et al*., 1993). This type of resistance has limited use in hybrid production due to incompatibility with the breeding process. Therefore, additional sources of resistance are essential for hybrid development. Plant breeders have used landraces as the source for specific characteristics in the development of the modern

high yielding varieties (Sleper and Poehlman, 2006). Many improved varieties have a narrow genetic base and, as a consequence, are almost uniformly vulnerable to a host of environmental risks, such as diseases, pests and extreme weather conditions. A number of resistant sorghum lines are available, and several have been used to produce commercial hybrid cultivars in India and the United States, but sorghum varieties grown in the United States represent only a small fraction of those known in the world (Thakur and Mathur, 2000).

Even though Marley and Ajayi (2002) indicated that many local landraces in West Africa lack satisfactory resistance, western Kenya may provide good sources of anthracnose resistant sorghum germplasm due to high disease pressure. Favorable climatic conditions that occur could also contribute to the evolution of host plant resistance (Hess *et al*., 2002). Therefore, Kenyan sorghum germplasm may be a source of genetic variation for anthracnose resistance and the evaluation of these germplasm could result in the identification of new sources of resistance.

2.4 Sectional conclusion

The literature reviewed shows that whereas sorghum is an important staple crop in semi-arid and arid areas of eastern Africa and in the case of this study western Kenya, productivity stubbornly remains low. The low productivity is attributed to a number of biotic and abiotic stresses that are common in these marginal environments. The acrisols and ferralsols of Western Kenya where sorghum is an important cereal crop are highly weathered, overused and acidic with very low available phosphorus, ranging between 2-5mg P/kg soil, compared to 10-15mg P/kg soil required for optimal crop productivity (Okalebo *et al*., 2004*).* Low soil P availability is therefore among the primary factors responsible for the low and declining sorghum grain yields in Kenya. Equally important is the destructive disease anthracnose that is ubiquitous in the region causing severe losses. For these constraints, deployment of resistant material remains the most cost effective manner in which the productivity can be increased. To do so would however require a clear picture of the nature of resistance in these materials to both traits (PUE and resistance to various forms of anthracnose). Inheritance of resistance to either challenge has been done before but limited studies have been done on the same traits in similar background. The purpose of this study was to investigate the nature of gene action that would account for tolerance to both traits.

CHAPTER THREE

3.0 INHERITANCE OF PHOSPHORUS USE EFFICIENCY IN SELECTED SORGHUM GENOTYPES

3.1 Introduction

The acid soils of Western Kenya where most of the Kenyan sorghum is grown have very low available phosphorus (2-5mg P/kg soil, Kisinyo *et al*. 2009) compared to the recommended levels of 10-15mg P/ kg soil for optimal productivity (Okalebo *et al*., 2004; Obura, 2008). These areas are also reported to experience high aluminum toxicity (Obura *et al*., 2010). The net effect of these production challenges is low productivity with sorghum grain yields reported being as low as 1 ton per hectare. Inorganic phosphorus fertilizer supplementation is not economically viable for the resource constrained farmers who are the majority in these agroecologies. In addition, only between 10-30% of the applied P is actually available to the crop while the rest get fixed by clay minerals common in these acrisols and ferralsols. As such the development and subsequent deployment of improved materials that can efficiently harness Pi provides the most cost effective way to address this challenge. Studies in Brazil which also has similar challenges has shown the presence of genetic variability for P Use efficiency in sorghums. Kenya is located within the secondary center of diversity of sorghum and therefore it is not improbable to have genetic variation for PUE given that most African soils are old, highly weathered with high rates of Pi fixation. The objective of this study was to determine the mode of gene action conditioning phosphorus use efficiency (PUE) in selected sorghum accessions to inform sorghum breeding activities for this very important trait.

3.2 Materials and Methods

3.2.1 Experimental site

Field experiments were conducted in Sega village located in Siaya county of Nyanza province in Western Kenya, located at 0˚03'N, 34˚25'E, at an altitude of 1400 m above the sea level. This site is known to be low in available P (2 to 5mg/kg soil).The area receives 800-2000 mm of rainfall annually distributed in two seasons; long rains from March to June and the short rains from September to November. Predominant soil is red loam with a mean temperature of about 24˚C. Prior to experimentation, soil analysis for available phosphorus, total nitrogen and pH, critical study treatments but essential elements needed for sorghum production, was performed on samples collected and analyzed (Okalebo *et al*., 2002).

3.2.2 Plant material used in the study

Six stable inbred lines (C1, G2, K5e, L6, O2 and P5) selected on the basis of their response to P and reactions to anthracnose disease were used in this study (Table 1).

Table 1: Materials used for the study

Cultivars	Source	Grain color	Anthracnose reaction	Phosphorus response
G2	MOI	White	Resistant	Inefficient
K5e	MOI	Red	Susceptible	Inefficient
L6	MOI	Reddish brown	Tolerant	Efficient
P5	MOI	Brown	Highly susceptible	Efficient
C1	ICRISAT	Intermediate; white &cream	Resistant	Efficient
O2 (Seredo)	MOI	Brown	Resistant	Efficient

3.2.3 Population development for genetic studies

A six-parent half diallel cross was used to generate F1 seed using the hot emasculation technique at Kibos, Western Kenya (House, 1985). F1 seed were then planted along with the parents to help identify the true $F1_S$ which were subsequently selfed to F2. The parents and their F2 progeny were then planted in Sega, a low P site in western Kenya where genetic studies on PUE was done.

Pollen transfer at Kibos

Sorghum heads protected from bird damage at Kibos

Plate 1: Pollen transfer and sorghum heads covered to offer protection from bird damage

3.2.4 Experimental layout and field management

The experiment was laid down following a split plot design with two replications. Each replication had two blocks i.e. a P-deficient and P supplemented. The P supplementation was done at the rate of 26kg P/ha with fertilizer levels as the main plot and the genotypes as the sub plot. Each block was sown with 18entries (6 parents and 12 progeny crosses) in two row plots of each entry in 3m long furrows at a spacing of 75 x 15cm and contained following

thinning about 20 plants. A border row of the P-deficient cultivar, K5e was planted to provide the first line of defense especially against livestock. At planting, nitrogen in the form of Calcium Ammonium Nitrate was applied to the P-deficient blocks (to match the 18% N usually contained in DAP) and as side dressing 6 weeks later to a total of 39 kg of N /ha. The plants were both rain fed and irrigated where necessary, insect pests and other diseases were controlled using appropriate chemicals. Weeding was done manually.

3.2.5 Total P assays and phosphorus use efficiency determination

Destructive sampling was done at the plant booting stage (7 weeks after planting) on ten plants per entry. Roots were cut off from the whole plants, washed, fresh weights taken then oven dried to a constant weight. Fresh weights of other plant parts were also taken, chopped into small pieces and then oven dried to a constant weight. Analysis of phosphorus in plant parts (roots, shoots, grains) was performed according to Okalebo *et al.*(2002).The results from the P assays were used to compute phosphorus use efficiency based on total plant dry weight in relation to total plant P (Corrales *et al.,* 2007) as shown below.

$$PUE = \frac{\text{Total plant dry weight}}{\text{Total plant P}}$$

The PUE data was used to compute GCA and SCA effects which were subsequently used to infer inheritance.

3.2 6 Determination of GCA and SCA

GCA (general combining ability) refers to the effects of the parent in the phenotypic mean of its cross. High GCA effects imply the predominance of additive genes over the non additive genes and vice versa.

SCA refers to the phenotypic value of a specific cross compared to the value predicted from the parental GCA values. It is obtained from the linear equation,

Yij =Grand mean +GCAi +GCAj +SCAij where;

Yij = Observed mean of the cross; GCAi = GCA of the first parent, i; GCAj = GCA of the second parent, j and SCAij = Specific combining ability of a cross between parents i and j.

A high SCA effect implies more of the non-additive gene effects (dominance and epistasis). Using the genotypic PUE means from the split plot ANOVA, regression analysis was carried out to obtain GCA (Regression) and SCA (Residual) as shown by the skeletal ANOVA below.

Table 2: Phosphorus use efficiency GCA and SCA skeletal ANOVA

SOV	DF	Ms	VR	F.prob	Exp Ms	VC	VCx2
Genotypes	12	Ms.Gen	VR.Gen				
Regression	5	Ms.Reg	VR.Reg	Fp.Reg	h*(p+2)gca+ σ2e	VC.Reg	VC.Regx2
Residual	7	Ms.Res	VR.Res	Fp.Res	sca+σ2e	VC.Res	
Pooled error	21	Ms.Err			σ2e	VC.Err	

Regression = general combining ability of the parents, Residual = specific combining ability of the cross, SOV= sources of variation, DF = degrees of freedom, Ms = mean squares, F. prob = the probability values, Exp MS = Expected mean square values; δ^2 = variance and VC = variance components.

The variance ratios, probability values, Expected mean square values and variance components were all determined using the Griffing's methods for Diallel analysis (Model 1{fixed parents}, Method 2) where;

a) GCA variance ratio = GCA mean square/pooled error mean square

b) SCA variance ratio = SCA mean square/pooled error mean square

c) GCA variance component =(GCA variance-pooled error variance)/Coefficient

And the coefficient = h x (p+2)

d) SCA variance component = SCA variance-pooled error variance /1

These variance components were calculated using the expected mean square values.

3.2.7 Determination of Baker's Ratio, NSCGD and BSCGD

Fehr, (1993) defined heritability as the ratio of genotypic variance (δ^2_g) to phenotypic variance (δ^2_p). The most important function of heritability to a breeder in a genetic study is its predictive role in expressing the reliability of the phenotypic values as a guide to the breeding value (Falconer and Mackay, 1996). Thus the knowledge of heritability of a trait guides a plant breeder to predict behavior of succeeding generation and helps to predict the response to selection (Waquar-UH-Hag *et al.,* 2008). There are two types of heritability namely; the broad-sense heritability (H) which reflects all the genetic contribution to phenotypic variances including additive and non-additive effects, whereas the narrow-sense heritability (h^2), estimates additive genetic contribution to phenotypic variance. These two types of heritability apply to randomly mated populations. However, in fixed populations as was the case in this study, narrow sense coefficient of genetic determination is used to estimate narrow sense heritability whereas broad sense coefficient of genetic determination is used to estimate the broad sense heritability.

There are three approaches used in the estimation of heritability. These are the use of F2 generation variances, parent-offspring regressions and thirdly, using the variance components. In this study, the variance components approach was used in the calculation of the heritability estimates (Baker's Ratio, Narrow Sense Coefficient of Genetic Determination and Broad Sense Coefficient of Genetic Determination) as follows;

Baker's Ratio = $(\delta^2_{GCA}) / (\delta^2_{GCA} + \delta^2_{SCA})$ i.e. Additive variance / total genetic variability.

NSCGD = $(\delta^2_{GCA}) / (\delta^2_{GCA} + \delta^2_{SCA} + \delta^2_{error})$ i.e. Additive variance / phenotypic variance

BSCGD = $(\delta^2_{GCA} + \delta^2_{SCA}) / (\delta^2_{GCA} + \delta^2_{SCA} + \delta^2_{error})$ i.e. Total genetic variance/ phenotypic variance.

3.3 Other data collected

Twenty plants per plot were randomly tagged for data collection. Data was collected on days to 50% flowering, total plant height, number of effective tillers, number of leaves and on grain yield. Plant height, leaf number (Camacho *et al*. 2002), number of effective tillers and grain yield (Castillon, 2001), which are always reduced by P deficiency, were used as indicators of PUE. The data was subjected to analysis of variance and means compared using Fishers Protected Least Significant Difference Test, LSD at $P \leq 0.05$. All computations were performed using the Genstat statistical software, 14th edition (Lawes Experimental Trust UK).

3.4 Results

3.4.1 Soil sample analysis of experimental sites

The acidity classifications were according to Kanyanjua *et al*. (2002), percent N according to Okalebo *et al*. (2002) and the available P classifications according to Marx *et al*. (1999). These results show that the soils in Kibos were moderately acidic whereas those in Sega were strongly acidic (Table 3).

Table 3: Soil properties for the experimental sites before input applications

Site	Soil pH-H_2O	Total N (%)	Available P(Mg P/kg)
Kibos	5.8	0.20	12.86
Sega	5.2	0.25	8.71

Note: Soil pH was measured in 1: 2.5 (soil: distilled water)

The percent N was moderately low in both sites while for the available P, Kibos had medium P levels whereas Sega had low P levels below ten (8.71) making it a P deficient site.

3.4.2 Phosphorus use efficiency among Parents and their F2 progeny

Analysis of variance on phosphorus use efficiency revealed no significant difference ($P>0.05$) between the tests genotypes used in this experiment. Similarly, the interaction between the

fertilizer and the genotypes were not significant with respect to phosphorus use efficiency. Although non significant differences were observed for PUE in general, a means comparison was performed to get a better picture of individual parent and progeny reactions.

The parental lines, G2 had the highest PUE value (639) followed by O2 (632.2) then C1 (619.2).P5 had the lowest PUE value (529). Among the crosses, G2xL6 had the highest PUE value (764.4) followed by G2xO2 (684.2) then L6xC1 (663.1). P5xC1 had the lowest PUE value among the crosses (Table 4).

Table 4: Means of phosphorus use efficiency of the parents and F2 progenies in Sega

Genotypes	PUE
C1	619.2
G2	639.0
K5e	616.6
L6	580.3
O2	632.2
P5	529.0
G2xL6	764.4
G2xO2	684.2
G2xP5	618.0
K5exL6	625.6
L6xC1	663.1
O2xK5e	652.1
P5xC1	604.8
Grand mean	632.2
LSD	76.5
CV%	11.3

3.4.3 Effect of P on key quantitative traits of the sorghum parents and their F_2 progenies

Significant variations ($P<0.05$) were found in a number of quantitative traits among the sorghum genotypes (Table 5).Tillering ability varied significantly ($P \leq 0.001$) among the F_2 sorghum families and their parents but there was no significant P x genotype interaction for this trait. The mean tiller number per plant among the F_2 sorghum families and their parents under low P was 1. However, under adequate P supply, the mean tiller number per plant among the F_2 sorghum families and the parents was 2. Compared to the parents, most of the F_2 sorghum families had either more than or the same number of tillers as their parental genotypes under low soil P and adequate P. Within each family and parental line, sorghum plants grown under adequate P had more tillers than those grown under low P. Test genotypes showed significant differences for days to 50% flowering ($P \leq 0.05$) but there was no significant P x genotype interaction for this trait. Most of the F_2 sorghum families flowered

earlier than either parent both under low P and adequate P. Most plants grown under adequate P flowered earlier than those plants grown under low available soil P. With respect to yields, test genotypes showed no significant differences among themselves and in their interaction with P. Sorghum plants within each family and parental lines supplied with P had more yields than those plants grown under low P. The number of leaves per plant was highly significantly different ($P \leq 0.001$) among the test genotypes and was also influenced by the P treatment ($P \leq 0.05$). Most of the F_2 sorghum families had more leaves per plant than their parents under low P and adequate P. Sorghum plants within each family and parental lines supplied with P had more leaves than those plants grown under low P. Total plant height was highly significant ($P \leq 0.001$) among the test genotypes. However, the interaction with P was not significant. Within each sorghum family and parental line, plants grown under adequate P were taller than those grown under low available soil P.

Table 5: Effect of P treatment on key quantitative traits of the sorghum parents and their F2 progenies grown in Sega during the short rainy season.

	D F		P H (cm)		No.of leaves		No.of tillers	
	L. P	Adq. P	L. P	Adq. P	L. P	Adq. P	L. P	Adq. P
C1	87	81	77	105	7	7	1	2
G2	92	85	23	71	4	7	1	1
K5e	98	92	102	115	6	8	2	3
L6	95	92	115	132	7	9	2	2
O2	104	81	97	119	8	9	2	3
P5	103	92	123	155	8	10	1	1
G2xL6	96	83	75	145	8	11	1	3
G2xO2	88	84	77	103	8	10	2	3
G2xP5	96	91	116	153	9	12	1	3
K5exL6	102	91	141	153	9	11	2	3
L6xC1	85	81	121	132	9	11	2	3
O2xK5e	89	79	126	160	9	10	2	3
P5xC1	91	89	92	155	8	11	2	3
G. Mean	94	87	102	136	8	10	1	2
LSD	19	6	40	25	1	1	1	1
CV %	8	7	37	16	13	8	39	20

DF=Days to 50% flowering; P H=Plant heights; L.P=Low phosphorus; Adq.P = adequate phosphorus supplies.

	
Performance of sorghum plants under low P at Sega	Performance of sorghum plants under adequate P at Sega

Plate 2: Effects of P on sorghum growth

	
Low plant vigor in low P at Sega	High plant vigor in adequate P at Sega

Plate 3: Performance of P5 × C1 F2 progenies in low and adequate P at Sega

3.4.4 Inheritance of phosphorus use efficiency (PUE)

GCA and SCA mean squares for PUE were both significant (Table 6). SCA variance components were higher than GCA values for PUE. Baker's Ratio (46%) and Narrow sense coefficient of genetic determination (36%) for PUE were low. The Broad sense coefficient of genetic determination value was 78%.

Table 6: Mean squares of combining ability, variance components and heritability estimates for PUE for the parents and F2 progenies in Sega

SOV	DF	PUE	VR	F.prob	ExpMs	VC	VCx2
Genotypes	12	3027	3.444				
Regression	5	3635**	4.135	0.009	h*(p+2)gca+ σ2e	738.161	1476.323
Residual	7	2592*	2.949	0.026	sca+σ2e	1713	
Pooled err	21	879			σ2e	879	
Baker's Ratio						0.46	
NSCGD						0.36	

BSCGD	0.78

* Significant at P≤0.05, ** Significant at P≤0.01, *** Significant at P≤0.001**,** BR = Baker's ratio, NSCGD = Narrow sense coefficient of genetic determination, BSCGD = Broad sense coefficient of genetic determination.

3.4.5 GCA and SCA effects for phosphorus use efficiency

3.4.5.1 GCA effects

C1, L6 and O2 had positive but non-significant GCA effects for PUE, whereas G2 had a highly significant (P≤0.01) positive GCA effects (Table 7).The sorghum genotype K5e had a negative but non-significant GCA effects whereas P5 showed a highly significant (P≤ 0.001) negative GCA effects for PUE.

Table 7: GCA effects for PUE of the parental genotypes grown in Sega

Genotypes	GCA values
C1	3.15ns
G2	31.05**
K5e	-5.15ns
L6	2.85ns
O2	7.15ns
P5	-47.55***
SE.GCA	11.26

** Significant at P≤0.01, *** Significant at P≤0.001**,** ns= non-significant.

3.4.5.2 SCA effects

SCA effects indicate the difference of the observed from the predicted. On the basis of the means, G2xL6 had the highest mean for PUE with a highly significant positive SCA effects (Table 8). The progeny from the cross G2xL6 obtained its PUE from both parents and as such was the best cross predicted from the parental GCA values. The progeny from the cross G2xO2 was the second best cross on the basis of means with a positive non significant SCA effects. The progeny from the cross L6xC1 was the third best in terms of means followed by O2xK5e. K5exL6 came fifth followed by G2xP5 and finally P5XC1. All these crosses had positive but non-significant SCA effects with the exception of K5exL6 which had a negative non significant SCA effects for PUE.

Table 8: SCA effects for PUE for the F2 progenies in Sega

Genotypes	SCA values
G2xL6	97.56**
G2xO2	13.03ns
G2xP5	1.54ns

K5exL6	-5.04ns
L6xC1	24.11ns
O2XK5e	17.13ns
P5xC1	16.19ns
SE. SCA	26.28

** Significant at P≤0.01**,** ns= non-significant.

3.5 Discussions

Phosphorus use efficiency (PUE) refers to the ability of a given genotype to produce biomass or grains under certain nutritional supplementation (Parentoni; Souza Junior, 2008; Whang *et al*., 2010).Inheritance of PUE varies, depending on the crop species, the selection criteria used, the growth stage of the crop and the experimental conditions (field trials, green house experiment or nutrient solution).In this study, ANOVA On PUE revealed no significant difference among the tests genotypes evaluated in this experiment. The lack of significant differences among the test genotypes for this trait could be attributed to the extremely low P availability at this site ((8.71mgP/kg soil) which could have compounded detection of genetic variation at such low levels (Coque and Gallais, 2006).

The parental line G2 had the highest PUE value (639) followed by O2 (632.2) then C1 (619.2) making them the most phosphorus efficient lines. This could be attributed to these lines having high P acquisition and internal utilization efficiencies. P5 which had the lowest PUE value (529) could be assumed to have had low P acquisition and internal utilization efficiencies and as such considered to be a P inefficient line in this study, contrary to its earlier classification. The high PUE parents thus provide a suitable set of parents that could be used to improve potentially high yielding and farmer preferred varieties. The PUE traits of these lines were manifested in their crosses with a cross between the most efficient line and a moderately efficient line, G2xL6 having the highest PUE value (764.4) making it the most efficient cross. The second most efficient cross was G2xO2 (684.2). The presence of some F_2 families with characteristics of the parents indicates that the parental genome conditioning in PUE donor parents were successfully transferred to their progenies. This clearly shows the potential to breed for this trait providing opportunity to improve local P inefficient varieties. Parental lines and their F_2 sorghum families exhibited significant morphological variations when grown under varied P treatments. The F_2 sorghum families segregated for the following traits, days to 50 % flowering, leaf numbers, tillering ability, total plant heights and grain yields in comparison with their parents. Most of the F_2 sorghum families exhibited heterosis (Falconer, 1989), being taller, had higher yields, produced more leaves and tillers than their

parents. Regardless of the sorghum test genotype involved, parental line or F_2 family, most genotypes grown under low P flowered later, had lower yields, and were shorter with fewer leaves and fewer numbers of effective tillers as compared to those supplied with P. Similar results have been reported in other studies which reported significant reduction in tillering and grain yield even among high PUE plants (Castillon, 2001; Camacho *et al*., 2002).

Significant differences ($P<0.05$) for combining ability (SCA and GCA) for PUE were found in this study indicating that both additive and non-additive types of genetic variances condition PUE (Table 6).Even though both additive and non-additive genetic factors were important, higher SCA variance components (1713) than GCA variance components (1476) and the relatively lower values for Baker's ratio (46%) and narrow sense coefficient of genetic determination (36%) observed in this study suggest the predominance of non-additive components over the additive components for the inheritance of phosphorus use efficiency in sorghum. These findings are similar to those reported in maize (Parentoni *et al*., 2006; Chen *et al*., 2009). In forage sorghum, similar results have been reported in female parents whereby additive gene effects were less than the non-additive gene effects in the inheritance of P nutrition (Gorz *et al*., 1987). In other studies in cereals, additive, dominance and epistatic effects have been reported in maize (Duncan, 1994), with additive effects being reported as more important (Silva *et al*., 1992; Furlani *et al*., 1998); non-additive effects (dominance and /or epistasis) being more important than additive effects (Coltman *et al*., 1987; Parentoni *et al*., 2006; Chen *et al*., 2009) and with both additive and dominance effects being important (Chaubey *et al*., 1994).These results however contrast with the findings of Silva *et al*. (1992) and Furlani *et al*. (1998) who reported additive effects as more important in maize for PUE.

Negative values for GCA and SCA are an indication of a contribution towards inefficiency for PUE thus undesired. Positive values on the other hand show a contribution towards efficiency. Among the test genotypes, G2 had a highly significant ($P\leq0.01$**)** positive GCA effects for PUE. C1, L6 and O2 showed positive but non significant GCA effects for PUE. K5e had negative non-significant GCA effects whereas P5 showed a highly significant ($P\leq 0.001$) negative GCA effects for PUE (Table7).This shows that parental lines G2, C1, L6 and O2 with positive GCA effects are good general combiners for PUE and as such have the ability to transmit their efficiency to their progenies. They are therefore very useful in breeding since they can be used in the development of varieties efficient in phosphorus use for farmers' use in the low P soils of Western Kenya.

SCA effects indicate the difference of the observed from the predicted. They cannot therefore be interpreted by themselves and as such must be interpreted from the means of the cross versus the predicted values based on the GCA effects of the two parents. For PUE, positive SCA values are desired while negative ones are undesirable. On the basis of the means, G2xL6 had the highest mean for PUE with a highly significant positive SCA effects making it exceptionally good in efficiency, even far much better than predicted from the two good parental GCA effects. G2xL6 picked up its efficiency from both parents and as such was the best cross predicted from the parental GCA values. This cross could have some of the best progenies when advanced. G2 is thus efficient in P use and in this study contributed its efficiency to its hybrid. It is therefore not inefficient in P use even though earlier classified as being inefficient. G2xO2 was the second best cross on the basis of means. It had a positive non significant SCA effects. The non-significant SCA effects imply that the effects were minor and as such this cross would give results matching those predicted from the parental GCA effects. L6xC1, O2xK5e, G2xP5 and P5XC1 had positive non significant SCA effects suggesting that these crosses would give results matching those predicted from the parental GCA effects. However, they had very low means for PUE thus not good crosses. P5xC1 had the least mean for PUE thus was the worst cross with reference to PUE. K5exL6 had a negative but non-significant SCA effects for PUE and thus performed worse than predicted from the parental GCA effects. This coupled with its low mean for PUE make it undesirable.

3.6 Conclusions

This study shows that indeed there exists genetic variability for PUE among the test genotypes with some parents and their progeny clearly exhibiting superiority for PUE as conditioned by the genetic background. Four parental genotypes (G2, C1, O2 and L6) were identified as good general combiners for P use efficiency and two crosses (G2xL6 and G2xO2) predicted to be good for PUE. These should be used in the development of genotypes efficient in phosphorus use for farmers' use in the low P soils of Western Kenya.

This study further showed that PUE is governed by both additive and non-additive genetic effects suggesting that both are involved in the inheritance of phosphorus use efficiency in the six sorghum genotypes evaluated. However, non-additive genetic effects were shown to be the major factor in phosphorus use efficiency in sorghum. This was indicated by the low values of Baker's ratio and narrow sense coefficient of genetic determination. Efficiency level of the progenies cannot therefore be accurately predicted on the basis of their parental GCA values. Under such a situation therefore, larger genetic gains should be obtained

through the use of inter-population improvement strategies, where one relies more on the selection of the best hybrids rather than the preliminary selection of lines for their general combining abilities. These results are major contributions to the breeding of sorghum genotypes that are PUE compliant for the acid and low P soils of western Kenya and the rest of such similar agroecologies. Thus the work if completed can provide a sustainable way to develop and deploy P use efficient cultivars capable of high productivity even under limiting P levels in the soil.

CHAPTER FOUR

4.0 INHERITANCE OF RESISTANCE TO FOLIAR AND STEM ANTHRACNOSE IN SELECTED SORGHUM GENOTYPES

4.1 Introduction

Western Kenya produces over 70 % of sorghum in the country but with low yields of 1 ton per ha. The low yields are occasioned by many abiotic and biotic stresses. The major biotic stress are foliar diseases notably anthracnose (*Colletotrichum sublineolum*) which causes yield loss of up to 70% through defoliation and tissue death under severe epidemics (Thakur and Mathur, 2000). The disease is endemic in Western Kenya and in addition to the high acidity and low P in most farm lands, thus contributes to lower productivity of the crop. Foliar infection is so far the most frequent form of the disease causing greatest reduction in yield. The disease can however affect all above ground parts of the plant. Development and use of cultivars resistant to *C. sublineolum* remains the most cost effective and sustainable approach to manage the disease (Erpelding and Prom, 2004).Thus the use of anthracnose resistant sorghum varieties would be of paramount importance in Kenya. This would entail introgression of resistance into popular but susceptible cultivars followed by their release and popularization. The development and use of cultivars with stable resistance and understanding of the nature of host resistance is needed to match a hyper variable pathogen such as *Colletotrichum sublineolum.* The objective of this study was to determine the mode gene action governing resistance to both foliar and stem anthracnose to inform resistance-breeding programs for sorghum cultivars targeted for western Kenya.

4.2 Materials and Methods

4.2.1 Experimental site and plant materials

Field trials on the F2 progeny and their parents were conducted in Kibos, an anthracnose hot spot in Kisumu County in western Kenya (Bio Earn, 2009). Kibos is located 0^{o} $3^{'}$ (0.05^{o}) south latitude, 34^{o} 51’ (34.85^{o}) east longitude with an average elevation of 1169m above the sea level and receives 1000-1400mm of rainfall twice annually; long rains between March and June whereas the short rains between August and November. The predominant soil type is clay. Materials used in this study were the same as those used in the phosphorus study. Their selection was on the basis of their response to P use and their reaction to anthracnose disease (Table 1).

4.2.2 Experimental Layout and management

The experiment was laid down following a randomized complete block design with four replications. Each replication had 18entries comprising of 6 parents and 12 progeny crosses. Each entry was planted as two rows each 3m long, sown at a spacing of 60 x 15cm and thinned to about 20 plants per row. The entire experiment was then surrounded by border rows of a highly susceptible anthracnose cultivar-P5, planted earlier as a disease spreader. In this particular site, sorghum crop that was severely affected by anthracnose had been previously grown thus known to be naturally inoculated with Colletotrichum species (Bio Earn, 2009) and as a result screening was done against natural inoculation. At planting, Di-ammonium phosphate fertilizer was applied to the planting holes and Calcium Ammonium Nitrate fertilizer as side dressing 6 weeks later to a total of 39 kg of N /ha. The plants were both rain fed and irrigated where necessary; insect pests and other diseases controlled using appropriate chemicals. Weeding was done manually. Twenty plants were randomly tagged per plot. Plants were then observed frequently to note the first appearance of anthracnose on leaves. Disease progress was followed on the leaves beginning at first disease appearance (four to six weeks after sowing) with data taken at two-week intervals up to flowering and weekly intervals thereafter for five weeks.

4.3 Data Collection and Analysis

Disease severity was assessed on individual tagged plants at 60, 70 and 80 days after planting until physiological maturity on 20 tagged plants per plot, with disease assessment based on percentage leaf area affected (PLAA) using a 1-9 visual qualitative severity rating (Thakur *et al.*, 1998). In this scale, 1=No symptoms of disease on plant part; 2=1-5% leaf area damaged by the disease; 3=6-10% damaged by the disease; 4=11-20% plant part damaged by the disease; 5=21-30%, 6=31-40%; 7=41-50%; 8=51-75% and 9= above 75% leaf area damaged by the disease. Genotypes rated < 3 were considered to be resistant; 3-4, moderately resistant; 4-6, moderately susceptible while 6-9, highly susceptible. Besides the disease severity, data was also collected on grain yield. For each plot, severity data was averaged to give a single severity value based on a mean of the 20 plants evaluated. Plot means were subjected to Griffin's diallel analysis, Model 1(fixed parents), Method 2. Severity data was used to compute the relative area under the disease progress curve (AUDPC) for each genotype as described by Madden *et al*. (2008) where:

$$\text{AUDPC} = \sum_{i}^{n-1} \left[\left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i) \right]^n$$

Where n is total duration of epidemics, y_i is the initial disease at time t_i and y_{i+1} is the final disease assessment at time t_{i+1}.

The initial (1st week data from booting stage) and final (fifth week data from booting stage) disease severities data from field evaluations and area under disease progress curves data were then subjected to the analysis of variance using Genstat statistical software, 14th edition and means compared using Fishers Protected Least significant difference test (LSD) at P≤ 0.05. The importance of GCA and SCA was compared and the inheritance among the crosses interpreted.

4.4 Results

4.4.1 Reaction to anthracnose by the parents and their progeny

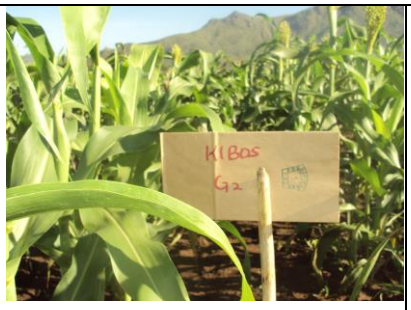
Analysis of variance on initial severity ratings, final severity ratings and AUDPC revealed highly significant differences (P ≤ 0.001) among the test genotypes on their response to the disease for both foliar and stem anthracnose (Table 9).

Table 9: Mean squares for initial severity scores, final severity scores and AUDPC for foliar and stem anthracnose of the parents and F2 progenies in Kibos.

		Foliar anthracnose			Stem anthracnose		
SOV	Df	Initial severity	Final severity	AUDPC	Initial severity	Final severity	AUDPC
Reps.	3	0.27	1.27	412.30	0.61	1.55	699.00
Gen.	12	25.36***	24.96***	20348.4***	8.46***	21.53***	10192.1***
Reps x Gen.	20	0.26	0.19	112.80	0.26	0.50	111.90
Residual	36	0.26	0.33	162.60	0.11	0.26	53.80

AUDPC= Area Under Disease Progress Curve; *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001

Anthracnose susceptible parent, P5.



Anthracnose resistant parent, G2.

Anthracnose resistant parent, C1.

Plate 4: Anthracnose susceptible and anthracnose resistant parents in Kibos

Anthracnose susceptible cross, P5 x C1.	Anthracnose resistant cross, G2 x O2.	Anthracnose resistant cross, K5e x L6.

Plate 5: Anthracnose susceptible and anthracnose resistant crosses at Kibos

Parental genotypes, lines C1 and G2 had the lowest AUDPC values of 28, lowest initial and final severity scores of 1 for both foliar and stem anthracnose respectively (Table 10). Parent P5 had the highest AUDPC values; highest initial and final severities scores for both foliar and stem anthracnose. The progeny, G2xO2 had the lowest AUDPC values, lowest initial and final severity scores for both foliar and stem anthracnose whereas P5xC1 had the highest AUDPC values, highest initial and final severity scores for both foliar and stem anthracnose (Table 10). Overally, the mean AUDPC values and mean final severity scores for foliar anthracnose were higher than those for stem anthracnose.

Parent L6 had the highest yield (2,231 Kg Ha^{-1}) then K5e (1,668Kg Ha-1); whilst P5 was the least yielder (786 Kg Ha^{-1}). In the case of the crosses, K5exL6 had the highest yields whereas P5xC1 had the lowest yields (Table10).

Table 10: Means of foliar and stem anthracnose severity, disease progress and yield of sorghum parents and F2 progenies in Kibos

	Foliar anthracnose			**Stem anthracnose**			**Yields(Kg Ha^{-1})**
Genotypes	Initial Severity	Final Severity	AUDPC	Initial Severity	Final Severity	AUDPC	
C1	1.0	1.0	28.0	1.0	1.0	28.0	1493.0
G2	1.0	1.0	28.0	1.0	1.0	28.0	1232.0
K5e	2.3	3.3	73.5	1.3	3.5	69.1	1668.0
L6	2.0	4.3	86.6	1.5	6.3	112.9	2231.0
O2	1.0	3.0	52.5	1.0	4.0	68.2	1428.0
P5	7.1	7.8	207.2	5.8	7.5	185.5	786.0
G2xL6	2.3	4.0	95.4	1.0	3.8	67.4	1697.0
G2xO2	1.1	3.1	57.8	1.0	1.9	39.8	1132.0
G2xP5	5.3	6.5	169.1	1.5	4.1	84.3	1325.0
K5exL6	2.0	3.6	80.1	1.0	3.8	73.5	2364.0
L6xC1	2.3	4.3	99.8	1.0	3.8	77.9	1735.0
O2xK5e	1.3	4.3	80.5	1.3	4.5	83.1	1839.0
P5xC1	5.9	7.6	194.3	3.1	7.1	148.3	976.0
Grand mean	3.0	4.5	106.8	1.6	4.1	83.1	1502.0

LSD	0.6	0.7	14.9	0.4	0.6	8.6	555.2
CV%	16.9	9.7	9.9	31.0	17.4	12.7	32.6

Severity ratings based on a scale of 1-9 with 1= zero disease symptoms and 9 = above 75% plant tissue damaged by the disease.

Initial severity was taken 55 days after planting; Final severity was taken 83days after planting

AUDPC = Area Under Disease Progress Curve computed according to Madden *et al.* (2008).

4.4.2 Inheritance of resistance to anthracnose disease

GCA and SCA mean squares for initial severity scores, final severity scores and AUDPC were highly significant ($P \leq 0.001$) for both foliar and stem anthracnose (Table 11). GCA mean square values for initial severity scores, final severity scores and AUDPC were higher than SCA mean square values for initial severity scores, final severity scores and AUDPC for both foliar and stem anthracnose (Table 11). Baker's Ratio for the initial severity scores was higher than that for the final severity scores for both foliar and stem anthracnose. This trend was also observed for NSCGD.

Table 11: Mean squares of combining ability, variance components and heritability estimates for AUDPC, initial and final severities scores for foliar and stem anthracnose for the parents and F2 progenies in Kibos

		Foliar anthracnose			Stem Anthracnose		
SOV	DF	Initial severity	Final Severity	AUDPC	Initial Severity	Final Severity	AUDPC
Genotypes	12	4.27	4.44	3423.40	1.86	4.19	2028.30
Regression	5	9.59***	8.41***	6937.1***	3.95***	8.49***	4424.9***
Residual	7	0.46***	1.60***	913.6***	0.37***	1.12***	316.4***
Pooled error	36	0.06	0.08	40.65	0.03	0.07	13.45
σ^2_{GCA}	5	5.10	4.46	3694.26	2.10	4.51	2363.11
σ^2_{SCA}	7	0.40	1.52	872.95	0.34	1.05	302.95
BR		93%	75%	81%	86%	81%	89%
NSCGD		92%	74%	80%	85%	80%	88%
BSCGD		99%	99%	99%	99%	99%	99%

AUDPC=Area Under Disease Progress Curve, BR = Bakers' ratio, NSCGD = Narrow sense coefficient of genetic determination, BSCGD = Broad sense coefficient of genetic determination.

The GCA mean squares for yields were highly significant ($P \leq 0.001$) whereas the SCA mean squares were not significant. Baker's Ratio, narrow sense coefficient of genetic determination and broad sense coefficient of genetic determination were all high.GCA variance components were higher than the SCA variance components for this trait (Appendix 1).

4.4.3 GCA and SCA effects

4.4.3.1 GCA effects

The parental genotypes C1, G2, K5e and O2 had highly significant ($P \leq 0.001$) negative AUDPC GCA effects for both foliar and stem anthracnose, whereas P5 showed highly significant ($P \leq 0.001$) positive AUDPC GCA effects for both foliar and stem anthracnose (Table12). L6 showed positive but non-significant AUDPC GCA effects for foliar anthracnose whereas it had a highly significant ($P \leq 0.001$) positive AUDPC GCA effects for stem anthracnose. G2 had the highest negative AUDPC GCA effects for both foliar and stem anthracnose (Table 12). P5 had the highest positive AUDPC GCA effects for both foliar and stem anthracnose.

Table 12: GCA effects for initial severity scores, final severity scores and AUDPC for foliar and stem anthracnose of the parental genotypes

Disease	Foliar anthracnose			Stem anthracnose		
Genotypes	Initial severity	Final severity	AUDPC	Initial severity	Final severity	AUDPC
C1	-0.49***	-0.91***	-18.38***	-0.34***	-1.04***	-18.91***
G2	-0.59***	-1.08***	-22.90***	-0.58***	-1.64***	-32.18***
K5e	-0.29**	-0.35**	-10.39***	-0.24**	-0.30**	-6.90***
L6	-0.20*	0.30**	2.62ns	-0.15*	0.96***	13.21***
O2	-0.89***	-0.28*	-18.15***	-0.25***	0.05ns	-4.91**
P5	2.65***	2.51***	72.28***	1.74***	2.14***	54.45***
SE. GCA	0.10	0.11	2.42	0.06	0.10	1.39

The parental material, C1, G2 and O2 had negative but non significant GCA effects for grain yields whereas P5 had a highly significant ($P \leq 0.001$) negative GCA effects. L6 showed positive highly significant ($P \leq 0.001$) GCA effects whereas K5e had positive non significant GCA effects (Appendix 2).

4.4.3.2 SCA effects

The progeny G2xO2 had the best means for both foliar and stem anthracnose (Table 10) albeit with relatively weak SCA effects (Table 13). The progeny G2xO2 inherited resistance from both parents and was therefore the best cross as predicted from the parental GCA effects. In the case of foliar anthracnose, K5exL6 was the second best cross followed by O2xK5e, then G2xL6, then L6xC1 then G2xP5 and finally P5xC1. All these five crosses had either significant or highly significant positive AUDPC SCA effects (Table 13). In the case of stem anthracnose G2xL6 was the second best but with a weak AUDPC SCA effects. It was followed by K5exL6 which had a highly significant but negative AUDPC SCA effects. This

cross was followed by L6xC1 with a very weak AUDPC SCA effects then O2xK5e, then G2xP5 and finally P5xC1 (Table 10). O2xK5e and P5xC1 had highly significant positive AUDPC SCA effects.G2xP5 although had a negative and highly significant AUDPC SCA effects; it had a very high mean for the disease.

Table 13: SCA effects for initial severity scores, final severity scores and AUDPC for foliar and stem anthracnose for the F2 progenies

Disease	**Foliar anthracnose**			**Stem anthracnose**		
Genotypes	Initial severity	Final severity	AUDPC	Initial severity	Final severity	AUDPC
G2xL6	0.39ns	0.66*	19.297**	0.09ns	0.43ns	4.32ns
G2xO2	-0.04ns	0.36ns	2.43ns	0.19ns	-0.54*	-5.09ns
G2xP5	0.58*	0.91***	23.40***	-1.31***	-0.43ns	-19.98***
K5exL6	-0.16ns	-0.44ns	-8.52ns	-0.26ns	-0.92***	-14.81***
L6xC1	0.28ns	0.74**	19.16**	-0.15ns	-0.17ns	1.57ns
O2XK5e	-0.22ns	0.76**	12.68*	0.09ns	0.74**	12.94***
P5xC1	1.06***	1.85***	44.04***	0.08ns	2.02***	30.78***
SE. SCA	0.22	0.25	5.65	0.15	0.23	3.25

The crosses G2xL6, G2xO2, L6xC1 and P5xC1 had negative but non-significant SCA effects for yields whereas G2xP5, K5xL6 and O2x K5e had positive non significant SCA effects (Appendix 3).

4.5 Discussions

The objective of this study was to determine the mode of gene action conditioning resistance to anthracnose in sorghum in order to inform resistance breeding. Although the study was conducted under natural infestation, disease pressure was high enough to identify resistance. The study showed highly significant differences ($P \leq 0.001$) amongst the sorghum test genotypes evaluated (Table 9) indicative of genetic variability for resistance to anthracnose. The progeny (G2xO2) derived from two highly resistant parents to anthracnose had the lowest AUDPC values when compared to other progeny for both foliar and stem anthracnose. A cross between the most susceptible genotype and a highly resistant genotype (P5xC1) had the highest AUDPC value among the crosses, for both foliar and stem anthracnose (Table 10).Two parents (C1 and G2) were completely resistant showing no symptoms of both forms of the disease. The parents O2 and K5e were moderately resistant to both forms of the disease. L6 showed moderate susceptibility to both forms of the disease. P5 had the highest AUDPC values for both forms of the disease and the highest initial and final disease severity scores making it the most susceptible genotype in this study (Table 10). These resistant

materials could be used as good sources of resistance in breeding programs. In this study, the mean AUDPC values and final severity scores for foliar anthracnose were higher than those for stem anthracnose (Table 10). Similar findings have been reported elsewhere with foliar anthracnose appearing early especially on susceptible sorghums (Thomas *et al.*, 1996). This may account for the high scores on the leaves in the susceptible genotypes when an evaluation is done at grain physiological maturity as was the case in this study.

Highly significant differences for combining ability based on initial disease severity scores, final disease severity scores and AUDPC among the parents along with their F2 progenies for both foliar and stem anthracnose were found (Table 11). Significant differences for both SCA and GCA effects were found with respect to AUDPC, initial and final disease severity scores for both forms of the disease indicating that both additive and non-additive types of genetic variances condition AUDPC (Table 11).Even though both additive and non-additive genetic factors were important, higher GCA variances for AUDPC than SCA variances suggests the predominance of additive components over the non-additive components for resistance to the disease. Very high values for Bakers ratio, narrow sense coefficient of genetic determination and broad sense coefficient of genetic determination in this study show that additive effects largely determine resistance to anthracnose disease in sorghum and that improvement for disease resistance should be possible through selection. These results corroborate the findings of Liri (2013) who indicated that relatively higher Bakers ratio was responsible for resistance to anthracnose disease in sorghum. Other studies using different populations have reported inheritance of resistance to sorghum anthracnose as qualitative in nature (Biruma, 2013). Given that both SCA and GCA were highly significant these results are not unexpected.

Grain yield in this study was largely due to GCA effects only suggesting that additive gene action conditioned yield performance (Appendix 1). Very high values for Bakers ratio, narrow sense coefficient of genetic determination and broad sense coefficient of genetic determination in this study show that genes with additive effects determine yield performance in sorghum and that improvement for yields are possible through selection.

A comparison of the GCA and SCA means revealed variation. In general, negative GCA effects are indicative of a contribution towards resistance. Positive values on the other hand show a contribution towards susceptibility. Among the test genotypes, G2 had the highest significant negative GCA effects for AUDPC followed by C1 then O2 and K5e in both forms of the disease. P5 had the highest significant positive GCA effects for AUDPC followed by

L6 for both foliar and stem anthracnose (Table 12). This shows that G2, C1, O2 and K5e showing highly significant negative GCA effects for AUDPC are good general combiners for AUDPC and as such have the ability to transmit their resistance to their progenies albeit in varying proportions. They are therefore very useful in breeding since they can be used in the development of anthracnose resistant varieties for farmers because some of their progeny would have moderate resistances to anthracnose. Crosses with moderate resistance could produce transgressive segregates for resistance in subsequent generations (Poehlman and Sleper, 1995; Rieseberg *et al*., 1999; Acquaah, 2008).

For yields, positive values for GCA effects are usually an indication of good yield performance. Negative values on the other hand suggest poor performance. Among the test genotypes, C1, G2, O2 and P5 had negative GCA effects for yield whereas K5e and L6 had positive GCA effects (Appendix 2). This shows that K5e and L6 which had positive GCA effects for yield are good general combiners for yield performance and as such have the ability to transmit their good yield attributes to their progenies. They are thus very useful in breeding since they can be used in the development of high yielding sorghum varieties.

Negative SCA effects in the crosses are generally preferred in diseases while positive SCA effects are very undesirable. SCA effects indicate the difference of the observed from the predicted. They cannot therefore be interpreted by themselves. They must be interpreted from the means of the cross versus the predicted values based on the GCA effects of the two parents. On the basis of the means, G2xO2 had the best mean for both foliar and stem anthracnose. However, it didn't have a very strong SCA effects. This is because both parents had very strong GCA effects thus this cross was predicted to be very good and as such would have some of the best progenies when advanced. For this cross to have had a strong negative SCA effect, it needed to be exceptionally good, even better than predicted from the two good parental GCA effects. G2xO2 picked up resistance from both parents and was the best cross that was predicted from the parental GCA effects. The non-significant AUDPC SCA effects seen for foliar anthracnose meant that the effects were minor (not significantly different from zero) and as such this cross would give results matching those predicted from the parental GCA effects. In the case of foliar anthracnose, K5exL6 was the second best cross on the basis of means thus a good cross. The crosses K5exL6 (-8.52ns) in the case of foliar anthracnose and G2xO2 (-5.09ns) in the case of stem anthracnose showed non-significant negative AUDPC SCA effects suggesting that the effects of the resistant parents involved in these crosses were in the desirable direction. However, they were minor effects and as such these

crosses would give results matching those predicted from the parental GCA values. In the case of stem anthracnose, the highly significant negative AUDPC SCA effects for K5exL6 were desirable with the cross inheriting resistance from K5e which had good negative AUDPC GCA effects. It was therefore not fully susceptible, even though earlier classified as being susceptible. O2xK5e though the third best in terms of means in the case of foliar anthracnose, it had positive AUDPC SCA effects. As a result of this, it would perform worse than predicted. This was also the case for stem anthracnose for this cross. In addition, it had a very high mean for the disease in the case of stem anthracnose making it undesirable. A similar scenario was seen in all the remaining crosses in the case of foliar anthracnose. In the case of stem anthracnose G2xL6 was the second best performer. Its non significant AUDPC SCA effects was an indication that this cross would give results matching those predicted from the parental GCA values. G2xP5 had negative and highly significant AUDPC SCA effects for stem anthracnose and as a result would perform better than predicted. However, it had a higher mean for the disease thus not a good cross. Crosses G2xP5 (-19.98***) and K5exL6 (-14.81***) for stem anthracnose showing highly significant negative SCA effects for AUDPC indicates their ability to perform better than predicted. L6xC1 with a positive non significant SCA effects would perform worse than predicted. It also had a high mean for the disease thus not a good cross. Non-additive effects appeared to be of greater significance in these specific crosses. The rest of the crosses in the case of stem anthracnose (O2xK5e & P5xC1) had highly significant positive AUDPC SCA effects thus would perform worse than predicted. Besides, their very high means for the disease make them undesirable.

In the case of yields, positive SCA effects in the crosses are generally preferred whereas negative SCA effects are very undesirable. On the basis of mean yield performances, K5exL6 had the highest yield. Its positive but non significant SCA effects suggest that this cross would perform as predicted by the parental GCA values. This coupled with its high mean performance makes it a good cross and as such likely to have some of the best progeny when advanced.K5exL6 inherited its good yield performance from K5e. O2xK5e was the second best cross. Its positive but non-significant SCA effects indicated that this cross would give results matching those predicted by the parental GCA values. However, this cross was susceptible to both foliar and stem anthracnose thus not a good cross.G2xP5 though would perform as predicted; its low mean yield performance makes it a bad cross. The remaining crosses (L6xC1, G2xL6, G2xO2 and P5xC1) with negative SCA effects would perform

worse than predicted from the parental GCA values. In addition, their very low means for yield make them undesirable.

4.6 Conclusions

From this study, analysis for resistance to anthracnose based on AUDPC showed both additive and non-additive genetic effects suggesting that both are involved in resistance to anthracnose disease in the six sorghum genotypes used in this study. However, additive genetic effects were the major factor in resistance to anthracnose in this study. Under such a situation therefore, starting with a good F2 mean, resistance could be improved through the crossing of good x good and selecting the best in early generation. This way better chance of getting a pure line exists. The values of Bakers ratio, narrow sense and broad sense coefficient of genetic determination were high. Resistance level of progenies could therefore be predicted accurately on the basis of their parental GCA values.

The study identified four parental genotypes (G2, C1, O2 and K5e) as good general combiners for resistance and predicted three crosses (G2xO2, K5exL6 and G2xL6) to be good for both foliar and stem anthracnose. With respect to yields, K5exL6 yielded highest among the F2 progenies and performed better than predicted from the parental GCA values. It combined both the good attributes of disease resistance and the ability to yield well thus a very good cross. The other two crosses also had reasonably good yields. These are good for advancement because the parents had the ability to transmit resistance to their progenies, suggesting primarily additive inheritance. These should be used in the development of high yielding anthracnose resistant genotypes for farmer use in the anthracnose endemic soils of western Kenya.

CHAPTER FIVE

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 General discussion

Breeding sorghum varieties which combine resistance to anthracnose and are efficient in P use would be the best way of improving sorghum production in Western Kenya where both constraints limit sorghum productivity. A number of breeding lines that yield reasonably well and at the same time are efficient in P use have been developed by Moi University. However, some of these lines are highly susceptible to anthracnose. Breeding programmes however require that they be informed by clear understanding of inheritance of traits of interest in this case PUE and resistance to anthracnose. The objectives of this thesis study were to determine the mode of gene action governing the inheritance of a). Phosphorus use efficiency and b).resistance to anthracnose disease.

Genetic control of phosphorus use efficiency depends on the crop species investigated, the selection criteria used, the growing stage of the crop studied and the experimental conditions. This study showed that the inheritance of PUE is governed by both additive and non-additive genetic effects. However, the low values of Baker's ratio and narrow sense coefficient of genetic determination suggested the predominance of non-additive genetic effects over additive effects in the inheritance of phosphorus use efficiency in the six sorghum genotypes evaluated. The efficiency level of the progenies cannot therefore be accurately predicted on the basis of their parental GCA values in early generation. In other studies, variability in findings too has been reported. For example in maize, additive effects have been reported (Silva *et al*. 1992; Furlani *et al*. 1998); non-additive effects equally important (Coltman *et al*. 1987; Parentoni *et al*. 2006; Chen *et al*. 2009) and both additive and dominance effects as important (Chaubey *et al*., 1994). In sorghum, inheritance of P nutrition in forage sorghums has been attributed to non-additive gene effects.

Heritability of resistance to anthracnose has been previously reported by various studies to be conditioned by different numbers of genes with different modes of inheritance. According to Coleman and Stokes (1954), resistance to anthracnose is conferred by two closely linked dominant genes each conferring resistance to different phases of the disease. Reports have also indicated resistance to anthracnose to be controlled by single genetic locus with multiple allelic forms (Jones, 1979; Tenkouano, 1993). In 1998, Boora *et al*. reported a single

recessive gene conferring resistance in SC326-6. Most recently, Biruma (2013) reported that inheritance of resistance to sorghum anthracnose is qualitative being monogenically controlled. Inheritance of resistance to anthracnose in the present study was found to be controlled by both additive and non-additive genes with the predominance of additive genetic effects over the non-additive genetic effects. Improvements for disease resistance should thus be possible through selection in early generation since resistance levels of the progenies could be predicted accurately on the basis of their parental GCA values. These different findings are not surprising because of the existence of variations in pathogen races, environments in which these genetic studies were conducted and germplasm studied. This coupled with the fact that different sorghum races occupy a wide range of ecological habitats, having been selected for specific local environments and as such are likely to vary in their response to prevalent pathogen races, different evaluation methods, environment and pathogen interaction, justify these different findings.

Parental genotypes (G2, C1 and O2) and crosses (G2xL6 and G2xO2), identified in this study to be resistant to foliar anthracnose were also found to be efficient in phosphorus use. P5, a highly susceptible genotype to anthracnose and P5xC1, a cross susceptible to anthracnose, were both inefficient in phosphorus use.

5.2 Conclusions

The study on the inheritance of phosphorus use efficiency showed that PUE is governed by both additive and non-additive genetic effects. However, the low values of Baker's ratio and narrow sense coefficient of genetic determination obtained suggested the predominance of non-additive genetic effects over additive effects in the inheritance of phosphorus use efficiency. The efficiency level of the progenies cannot therefore be accurately predicted on the basis of their parental GCA values. In this case, larger genetic gains should be obtained through the use of inter-population improvement strategies, where selection is made on the basis of the performance of the hybrid combinations. Similar results have been reported in maize (Coltman *et al*., 1987; Parentoni *et al*., 2006; Chen *et al*., 2009). These results however contradicts those found in maize by Silva *et al*. (1992) and Furlani *et al*. (1998) in which additive effects were reported to be more important.

The study on the inheritance of resistance to anthracnose revealed that both additive and non-additive genetic effects were involved in resistance to anthracnose disease in the six sorghum genotypes evaluated. However, the very high values of Bakers ratio, narrow sense coefficient

of genetic determination and broad sense coefficient of genetic determination were an indication that genes with additive effects largely determined resistance to anthracnose disease in sorghum and that improvement for disease resistance should be possible through early generation selection since resistance levels of the progenies could be predicted accurately on the basis of their parental GCA values. Under such a situation therefore, starting with a good F2 mean, resistance could be improved through the crossing of good x good and selecting the best. These results are in line with the findings of Liri (2013) indicating that the estimates of GCA/SCA variance ratio showed that the relatively high proportion of GCA was responsible for resistance to anthracnose disease in sorghum. However, they contradict those of Biruma (2013) who reported that the inheritance of resistance to sorghum anthracnose is qualitative being monogenically controlled.

5.3 Recommendations

- Study one carried out in Sega, a low P site in western Kenya, identified four parental genotypes (G2, C1, O2 and L6) as good general combiners for P use efficiency and predicted two crosses (G2xL6 and G2xO2) to be good for PUE. These should be used in the development of genotypes efficient in phosphorus use for farmers' use in the low P soils of western Kenya.

- Study two conducted in Kibos, an anthracnose hotspot in western Kenya, identified four parental genotypes (G2, C1, O2 and K5e) as good general combiners for anthracnose resistance and predicted three crosses (G2xO2, K5exL6 and G2xL6) to be good for both foliar and stem anthracnose. We recommend that these be used in the development of anthracnose resistant genotypes for farmer use in the anthracnose endemic soils of western Kenya.

- The three parents (G2, C1 and O2) and the two crosses (G2xL6 and G2xO2) had high PUE and at the same time were resistant to anthracnose. These should be used in the development of multiple stress tolerant sorghum varieties to improve sorghum productivity.

- Having observed that P-use-efficient genotypes were resistant to foliar anthracnose whereas the P-use-inefficient genotypes were susceptible to the disease, the relationship between phosphorus use efficiency and anthracnose resistance, if any, should be investigated through genetic studies.

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LIST OF APPENDICES

Appendix I: Mean squares of combining ability, variance components and heritability estimates of Yield for the test genotypes grown in Kibos.

SOV	Df	YIELDS	VR	F.prob	Exp. Ms	VC	VCx2
Genotypes	12	211103	3.755				
Regression	5	440532***	7.837	4.5E-05	h*(p+2)gca+ σ2e	102934.7	205869.4
Residual	7	47226ns	0.84	0.561733	1sca+σ2e	-8989	
Pooled error	36	56215			σ2e	56215	
Baker's Ratio						1	
NSCGD						0.79	
BSCGD						0.79	

Appendix II: GCA effects for yield of the test genotypes grown in Kibos

Genotypes	Effects
C1	-78 ns
G2	-146 ns
K5e	182 ns
L6	378***
O2	-56 ns
P5	-338***
SE gca	90.04

Appendix III: SCA effects for yield of the test genotypes grown in Kibos

Genotypes	Effects
G2xL6	-66.05 ns
G2xO2	-197.66 ns
G2xP5	278.37 ns
K5exL6	273.1 ns
L6xC1	-95.58 ns
O2XK5e	181.82 ns
P5xC1	-138.954 ns
SE sca	**210.1641** ns