GENOTYPIC VARIATION OF BAMBARA GROUNDNUT (*Vigna subterranea* L. Verdc) FOR TOLERANCE TO LOW PHOSPHORUS IN ACIDIC SOILS

BY

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DECLARATION

Declaration by the student

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DEDICATION

I dedicate this thesis to God, my loving spouse Gloria Nafula and kids Joshua and Joy, my father-in-lawMr. Ernest Simiyu and his family, my parents Mr. Cleophas Wafula and Mrs. GetrudeWafula and family for your moral support and inspiration.

ABSTRACT

Low available phosphorus (P) in acidic soils limits productivity and growth of Bambara groundnuts.The objectives of this study were to (1) evaluate the growth and yield performance of Bambara genotypes under varying levels of lime and P, (2) determine the efficiency and genotypic variation of bambara groundnut for P accumulation (PA) and (3) investigate traits underlying differential phosphorus use efficiency in bambara genotypes. Twelve genotypes were evaluated at 2 sites in Siaya (0.251^0N, 34.254^0E) and Busia (0.250^0N, 34.253^0E) counties in Randomized Complete Block Design in split-split plot arrangements during long and short rain seasons in 2018. Treatments comprised 2 lime levels (0, 4 tonnesha^{-1}), 2 P levels (0, 35 kgha^{-1}) with genotype as whole plot, lime as sub-plots, and phosphate as sub-sub plots. Genotypes were further screened in pot culture applied with 0 or 160 µM Potassium orthophosphate (KH_2PO_4). Effects due to genotypes, lime and P, and season × site interaction were significant ($P \leq 0.05$) for plant height, number of nodules and number of lateral roots. Significant effects due to season were observed for the number of nodules per plant while site significantly affected number of nodules and lateral roots. Genotypes exhibited variation for plant biomass, seed and biological yield due to lime, P, site, seasons and interaction effect of genotype × lime and genotype × site BAM011, BAM010 and BAM002 had increased number of lateral roots and had high seed yields in the two seasons and sites under low and adequate P in limed and un-limed soils.BAM002, BAM010 and BAM012 were responsive to both P and lime application. Genetic variation in PA and P-use efficiency among the genotypes was observed in this study. Phosphorus accumulation (PA) in biomass, seed and biological yields differed due to genotype, P level and genotype × P level interaction. BAM011, BAM010 and BAM002 had high values of seed yields, phosphorus harvest index, phosphorus physiological efficiency index and PA at low P showing that they are efficient in P use. Cultivars were categorized into in-efficient, medium and efficient as per the total index score. Principal component analysis categorized genotypes BAM011, BAM010 and BAM002together at the contrastinglevels of P.Tap root length (TRL), branching number (BN), branching diameter (BD)root volume (RV), rootand shoot biomassdiffered among the genotypes and were highly correlated to each other indicating that these traits are useful for P acquisition and P utilization efficiency. BAM001, BAM002, BAM010 and BAM011 exhibited high values of TRL, BN, BD, RV and root and shoot biomasses at varying levels of P Bambara groundnut contains genetic variation for tolerance to low P and respond well to applied P in acidic soils.Root traits including TRL, BN, BD and RV are among the mechanisms that underlie differential accumulation of P and phosphorus use efficiency in bambara genotypes.High performing responsive and P-efficient cultivars should be multiplied and distributed to farmers for cultivation in low P soil.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

$Ca(NO_3)_2.4H_2O$	Hydrated Calcium Nitrate
Fe EDTA	Iron Ethylnediaminetetracetic acid
H_2MoO_4	Moledinic acid
H_3BO_3	Boric Acid
HGY	High Grain Yield
HP	High Phosphorus
LGY	Low Grain Yield
LP	Low Phosphorus
MGY	Medium Grain Yield
MP	Medium Phosphorus
PA	Phosphorus accumulation
PBER	Phosphorus biological efficiency ratio
PE	Phosphorus efficiency
PHI	Phosphorus harvest index
PPEI	Phosphorus physiological index
PSF	Phosphorus stress factor
PUE	Phosphorus Use Efficiency
TSP	Triple superphosphate
$ZnSO_4.7H_2O$	Hydrated Zinc Sulphate

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Bambara groundnut (*Vigna subterranea* L.Verdc) is an underutilized grain legume that grows in regions that receive less than 800 mm of rainfall relative to other pulse crops in Sub-Saharan Africa (Mateva et al., 2020). It is the third most important pulse crop in Africa after groundnut (*Arachis hypogaea*) and cowpeas (*Vigna unguiculata*) (Shiyam et al.,2016). This crop associates with a species of rhizobia leading to fixation of between 32 $kgha^{-1}$ and 81$kgha^{-1}$ nitrogen (N) in the soil (Benson et al., 2015; Ibny et al., 2019). Thus, makingbambara groundnut an important crop for use in cereal-legume based cropping systems to enhance N nutrition of soils in Sub-Saharan Africa. Bambara grain contains 55.5-70 % carbohydrate, 4-12 % lipids, 3-5% ash,3-12 %fiber, 17-24 % protein, 4.9-48 mg/100 g iron (Fe), 11.44-19.35 mg100 g^{-1} potassium (K), 2.9-12.0 mg/100 g sodium (Na) and 95.8-99.0 mg100 g^{-1} calcium (Ca)henceprovides a balancedmeal (Feldman et al., 2019; Halimi et al., 2019).

Owing to its drought tolerance and nutritional values, bambara groundnut has the potential to cushion resource-constrained farmers against climate change and enhance nutritional security of people in Sub-Saharan Africa. Despite the usefulness of this crop, its production level is low ranging between 300 and 850 $kgha^{-1}$(Mayes et al., 2019). Several environemtal constraints inlcuding soil acidity, moisture stress, phosphorus (P)

deficiency and diseases limit the production of bambara groundnut. Low P availability in acidic soil is a major contributor of reduced crop yields in agricultural ecosystems (Okalebo et al., 2006). Exclusion of P from NPK fertilizer result in 50% yield reduction compared to 43% when N is omitted in maize (*Zea mays* L.) production (Kihara and Njoroge, 2013).

Soils in western Kenya are characterized by low pH of below 5.0 (Kisinyo et al., 2014) and soil available P of below 5 mg kg^{-1} Olsen or 10 mg kg^{-1} Bray 1 (Kihara and Njoroge, 2013). Low P is due to reduced native P, nutrient depletion due to mining and the increased P-adsorption capacity of aluminium (Al), manganese (Mn) and iron (Fe) oxides in the soils (Kisinyo et al., 2014).Legumes are highly sensitive to P deficiency due to its role in nodulation, biological N_2-fixation and energy reactions (Nziguheba et al., 2016; Bello et al. 2018;Lazali and Drevon, 2021).Application of lime (CaO) increases soil pH and corrects Ca and Mg deficiency and thereby improving overall crop performance. However, the resource-constrained smallholder farmers in Sub-Saharan Africa do not apply lime, mainly due to cost of transporting it from the source to their farms. In the absence of liming, farmers would have to apply high rates of phosphate fertilizer in order to increase P availability in soil and support crop growth (Kisinyo et al., 2014).Unfortunately, P-fertilizer use is low due to its high cost than lime, creating a clear need to identify crop cultivars that yield more under limited P in acidic soils and responsive to P nutrition (Namayanja et al., 2014; Korkmaz and Altıntaş, 2016).

Phosphorus efficiency refers to the ability of a crop plant tothrive and produce in a soil containing low amount of native P (Lynch, 2011). Effective varietal selection and development to confer P-efficiency and responsiveness, requires knowledge on the performance of cultivars in soils varying in P status (Nziguhebaet al., 2016). Variation in P acquisition and utilization efficiencies exists in several legumes including soybean (*Glycine max*) (He et al., 2017) common bean (*Phaseolus vulgaris*) (Tembo et al., 2019), mungbean (Reddy etal., 2020) and cowpea (Adusei et al., 2021). Available evidence indicates that Bambara groundnut cultivars have differential response to P application (Eifediyi et al., 2020). Genetic diversity exists among Kenyan bambara genotypes and also exhibit varied response to Fusarium wilt (Odongo et al., 2015; Wakhungu, 2016).Investigation on genotypic variations in P efficiency in Kenyan bambara genotypes is needed to identify cultivars that yield well under low P status and responds efficiently to P supplementation, is critical for improved crop production by small holder farmers.

1.2 Statement of the problem

Bambara groundnut is an orphaned grain legume that has received minimal research attention in Kenya. This crop is dependent on symbiotic N fixation and therefore has a high P requirement. Acid soils prevalent in western Kenya have a pH of less than 5.0. This low pH increases concentration of aluminium, iron and manganese ions in the soil solution. The metal ions also have a high capacity for P-adsorption leading to P deficiency. Withlow P in acidic soils caused by reduced native P, nutrient depletion due to mining and the increased P-adsorption,low yields ranging from 300 to 850 kg/ha of bambara groundnut has been reported in smallholder. The reduced yield hascontributed to food insecurity, low income, and malnutrition in western Kenya.Liming as remedy to

raise soil pH has potential to enhance P availability and improve the performance of bambara groundnut in acidic soils. Unfortunately, liming is not an attractive strategy as majority of smallholder farmers are resource-constrained. A sustainable strategy that involves identification of bambara groundnut cultivars that have low P requirements and responsive is needed to unlock the potential of this crop in P-deficient soils. Although previous studies have documented genetic diversity among bambara groundnut and varied response to Fusarium wilt in Kenya, investigation on genotypic variation of this crop in low and adequate P has not been carried out. This has left smallholder farmers to continue growing bambara genotypes that have not been selected for adaptation to varying soil P status leading to poor yields.

1.3 Justification of the study

To improve bambara groundnut production in western Kenya, low to high throughput screening of available germplasm in contrasting levels of P in acidic conditions is required. This would lead to identification of novel genotype(s) with inherent genetic potential to thrive and yield well in low P soils with or without soil amendment materials. Cultivation of P-efficient bambara genotypes would culminate into efficient utilization of low P present in acidic soils, reduce over-reliance on costly chemical P fertilizer input and minimize environmental degradation accruing from the use of such fertilizers. Furthermore, use of P-responsive genotypes is likely to improve efficiency use of applied P fertilizer. The selection and identification of P-efficient and responsive genotypes is likely to accelerate breeding programs aimed at development of bambara groundnut that are high yielding and adaptive to varying levels of P in the soil.

1.4 Objectives

1.4.1 General objective

To increase production of bambara groundnut in acid soils through cultivation of P efficient genotypes that perform well in low P conditions and efficiently use moderately applied P.

1.4.2 Specific objectives

1. To evaluate the growth and yield performance of bambara groundnut genotypes under varying levels of lime and phosphorus.
2. To determine genotypic variation of bambara groundnut for phosphorus efficiency in acidic soils under varying levels of P.
3. To investigate traits underlying differential phosphorus use efficiency in bambara genotypes

1.5 Research hypotheses

1. Bambara groundnut genotypesshow variation in grain yield, biomass yield and yield related components under varying levels of lime and phosphorus.
2. Bambara groundnut genotypes harbourdifferential abilities forP acquisitionand utilization efficiency that could be exploited to widen genetic base of this crop and support its cultivation on P deficient soils.
3. Bambara groundnut genotypes display adaptive root and shoot attributes for survival in low and adequate P environments.

CHAPTER TWO

LITERATURE REVIEW

2.1Taxonomy, origin and distribution of Bambara groundnut

Bambara groundnut (*Vigna subterranea* L.Verdc.; 2*x*=2*n* = 22) belongs to the family *Fabaceae* and subfamily *Papilionoideae* (Mayes et al., 2019). It is a geocarpic crop, morphologically fits into the same niche as groundnut, is closely related to cowpea, and its grain composition is similar to chickpea (*Cicer arietinum*) (Halimi et al., 2019). Begemann, (1988) asserts that this crop originated from the region between northern Cameroon and north-eastern Nigeria. Since then, it has spread to other countries including Ghana, Burkina Faso, Chad, Corted'Ivore, Mali, Niger, Madagascar and Eastern Africa. In Kenya, the crop is restricted to coast, eastern, western and nyanza regions (Wasula, 2014). Other than African countries, bambara groundnut is also grown in Oceania and South America while also grown Philippines, Sri Lanka, Indonesia, Malaysia and India in Asian countries (Chai et al., 2016).

2.2 Bambara groundnut production

Bambara groundnut is an underutilized indigenous African legume crop grown mainly by smallholder farmers in Sub-Saharan Africa (Chai et al., 2016). Besides the nutritional and cultural value, bambara groundnut exhibits drought tolerance and this partly explains the continued maintenance by the local population (Mateva et al., 2020).Thevariable and unreliable rainfall patterns being experienced in east Africa needs crop plants and agricultural systems that are resilient. This crop is able to survive well in dry areas that receive a maximum 800 mm rainfall annually than other crops (Mubaiwa et al., 2018).

Therefore, bambara groundnut can be a vital component of the resilient cropping systems that brings additional food security. The annual production of bambara groundnut in Africa stands at 300,000 tonnes of which Nigeria contributes 33.33% (Mayes et al., 2019).The average yield of bambara groundnut depends on landrace and region across Africa. Despite the usefulness of this crop, low yields ranging between 300-850 t/ha are reported in Africa (Mayes et al., 2019). This calls for investigation to select and develop novel crop cultivars and invest in good agronomic practices.

2.3 Importance of bambaragroundnut

Bambara groundnut being a rich source of nutrients essential in balancing nutrient deficiencies in sorghum, maize and rice diets. The fresh pods of bambara groundnut are boiled with salt and pepper, and consumed as a snack (Mubaiwa et al., 2018). The dried seeds are processed to flour to prepare various forms of bambara groundnut products including bread and cakes. Bambara groundnut grain pods are processed and used as livestock andpoultry feeds (Mayes et al., 2019). Milk produced from bambara groundnut contains 15-16% protein unlike 4% of soy milk, and the milk is highly valued due to its flavour and colour (Adu-Dapaah et al., 2016). Solution of bambara groundnut leaf sap and *Lantana trifolia* L. is used as an acaricide and insecticide in western Kenya (Bamshaiye et al., 2011). This crop fixes between 32 and 81 $kgNha^{-1}$making it a useful genetic resource for low input and small-scaleagricultural production systems (Benson et al., 2015; Ibny et al., 2019). Harnessing the benefits of this crop has the potential to support crop diversification and provide extra food sources vital in addressing food and nutritional security concerns.

2.4 Role of phosphorus in legume production

2.4.1 Nitrogen-fixation in legumes

Phosphorus (P) is one of keyrequirements for nitrogen fixing bacteria to reduce atmospheric N to ammonia (NH_3), a form utilizable by plants (Mitran et al., 2018). Nodule formation is influenced byP through its basic energy reactions where sixteen molecules of adenosinetriphosphate (ATP) are transformed to adenosinediphosphate (ADP)for every N molecule converted to NH_3 (Berg, 2009). The conduction of N-containing compounds from nodules to other plant part and photosynthate translocation from leaves to root forms the vital aspect of efficiency in symbiotic systems (Meena et al., 2017).Addition of phosphate to the P-deficient soil has contributed to increased biological N-fixation in legumes (Mitran et al., 2018).P fertilization increased nodule formation in green gram (*Vignaradiata* L.) and black gram (*Vigna mungo* L.) by 26% and 30%, respectively (Hayat et al., 2008). P supplementation coupled with inoculation with *Bradyrhizobium* significantly improved nodulation and symbiotic N fixation of mash bean crop (Meena et al., 2017). Variation in rate of P supply from 25-35 kg P/ha led to enhanced N fixation of up to 48kgha^{-1} from 20 kgha^{-1} in green gram (Mitran et al., 2018). P fertilization to common bean plants significantly improved nodule biomass and improved amount of N fixed atmospheric N_2 (Samago et al., 2018; Chekanai et al., 2018), while supply of 30 kg Pha^{-1} significantly increased nodulation in cowpea (Bello et al. 2018). These studies demonstrate that adequate P nutrition is vital for nodulation and nitrogen fixation functions in grain legumes.Therefore, selection and development of legume genotypes with efficientN fixing capacity in P limited conditions could play an enormous role in enhancing soil health and overall sustainability of low input systems.

2.4.2 Productivity of legumes

Phosphorus performs diversified functions in growth and metabolism leading to increased growth and productivity of legumes (Hernandez et al. 2007; Ndakidemi et al. 2006). P supply at the rate of 30 kgha-improved grainproduction in pigeon pea to 1300 kgha^{-1} (Singh and Ahlawat, 2007).This study also showed that the response of lentil to P nutritionwas based on available P status in the soil as well as geographical location. Morphological attributes includingnumber of leaves, plant height, number of branches and leaf area of cowpea significantly improved due to the supplementation of phosphorus (Nyoki et al., 2013).Ndakidemi and Dakora, (2007) assert that phosphorus requirementsof the shoot and root tips are high due to increased metabolism and cell division occur at high rate.P fertilizers application significantly improved root and shoot P accumulation, shoot biomass and grain yield in common bean (Samago et al., 2018; Chekanai et al., 2018). Significant variation among mungbean and cowpea genotypes has been reported for number of grains per pod, grain yield, straw yield, biological yield, plant height and number of pods per plant at low and adequate P levels (Irfan et al., 2017; Adjei-Nsiah et al., 2018). This varied response to P application demonstrate the usefulness of this nutrient in promoting productivity of legumes as well as the need to grow suitably adapted cultivars to achieve optimal use of the limited resource.

Bambara groundnut cultivars respond variably to P application leading to enhanced yield and yield components including nodule numbers, plant height, number of pods, seed P content and grain yield(Toungos et al., 2009; Temegne et al., 2019). When P is co-applied withN in$N_{30}P_{60}$kgha^{-1}, increased number of pods, plant height and grain yield

compared to the control was observed in bambara groundnut (Hasan et al., 2019). All these studies of bambara groundnut response to varying P levels have been carried out in west Africa. In Kenya, studies have focused on characterization of *Rhizobium* associated with bambara groundnut, genetic diversity among bambara groundnut genotypes and screening for resistance to Fusarium wilt (Benson et al., 2015; Odongo et al., 2015;Wakhungu, 2016). Evaluation of the growth and yield performance of bambara groundnut in low P soils as well as the response to P and lime application in western Kenya is not documented. Prevalence of the unfavourable soil conditions associated with P deficiency, soil acidity coupled with resource limited smallholder farmers' have resulted to low legume yield output (Bakari et al., 2020). There is no recommended P fertilizer application for bambara production in western Kenya soils and as a result, majority of smallholder farmers do not apply P in bambara groundnut production on ground that it can tolerate poor soils. This has resulted to low incomes, reduced quality produce, low crop yields and low food production. The later, in turn has aggravated into food shortages, widespread hunger, and malnutrition. Adoption of P-efficient bambara genotypes adapted to P deficient conditions in acidic soil and highly responsive to soil amendment materials (P-fertilizer and lime) will result to increased nutrient availability; improved soil health for microorganisms' development andbiological nitrogen fixation. Consequently, increased crop yields will alleviate food shortages, widespread hunger, malnutrition and reduce poverty level among small scale farmers of western Kenya.

2.5 Status of phosphorus in the soil in Western Kenya

Thirteen percent of the soils in Kenya are acidic in the order of acrisols, ferralsols, nitisols, cambisols, luvisols and lithosols and are rich in high aluminium of 2 cmol $Alkg^{-1}$

with over 20% Al saturation (Kanyanjua et al., 2002; Kisinyo et al., 2014).Aluminium reacts with inorganic phosphates, leaving less than 5.0 mg Pkg^{-1} soil for plant use, which is far below the optimal range of 10-15 mg Pkg^{-1}that is required for crop production (Kisinyo et al., 2014; Kihara and Njoroge, 2013).P-adsorption capacity of acidic soils rich in Al coupled with little or no use of P fertilizers have contributed to increased P deficiency in arable lands (Bakari et al., 2020).Extensive studies carried out between 1996 and 2009 in western Kenya indicate a 50% decrease in yields when P was omitted from NPK fertilizer unlike 43% yield reduction due to N omission (Kihara and Njoroge, 2013). This clearly demonstrates that low available P is a major component to crop production in the in western Kenya.

2.6 Phosphorus management strategies for sustainable crop productivity

2.6.1 Amendment of soil using Lime and P fertilizers

Liming is known to correct soil acidity. Application of lime containing Mg and/or Ca compounds to acidic soils increase $Mg^{2+}$and/or Ca^{2+}ions and reduces $Fe^{3+,}Al^{3+}$, Mn^{4+} and H^{+} ions in the soil solution (Kisinyo et al., 2014). This raises the soil pH and P available P following reduced P-adsorption(Kanyanjua et al., 2002).Apart from correcting soil pH, lime increases development of roots enhancing water and nutrient acquisition required for plant growth (Bakari et al., 2020). Lime lowers aluminium toxicity, lowers soil acidity, and increases available P, Ca, Mg, acquisition of N and P, increasingcrop performance in Kenyan soils (Kanyanua et al., 2002; Kisinyo et al., 2014).Incorporation of lime containing 21% calcium oxide raised soil pH and P availability in western Kenya acid soils (Opala, 2017).

Different crop species respond variably to the application of lime in acidic soils.The yield, height, branch numbers and leaf number of haricot bean increased with co-application of P and lime at rate of 30 $kgha^{-1}$ and 0.4 tha^{-1}, respectively (Kassa et al., 2014). Liming increases root biomass, shoot biomass, nodule number and biomass of cowpea by 42.5%, 35.3%, 65.6% and 50%, respectively (Bello et al., 2018). Application of lime and inoculums significantly increased nodule effectiveness and N fixation in soybean (Bakari et al., 2020).Non-significant difference between limed and un-limed soils was observed for pod and root weight of bambara groundnut (Nweke, 2020). This was attributed to short period of study and/or the low rate of lime applied (300 $kgha^{-1}$).Few studies have been done to understand the response of bambara groundnut genotypes to liming in terms of growth performance in acidic soils with low available P. Despite limited studies in bambara groundnut, these studies indicate that liming has the potential to increase crop yields in acidic soils.

Application of P fertilizers raises the available P in acid soils (Kisinyo et al., 2014; Nziguheba et al., 2016).Kisinyoet al., (2014) recommend P application with liming effect such as *mijingu* rock phosphate and triple superphosphate. Legume yield increases due to application of P fertilizers (see detailed description in section 2.4.2 above). Application of both P fertilizer and lime has the potential to manage P deficient soils for increased crop yields (Barasa et al., 2013; Kisinyo et al., 2014 and Opala, 2017). The variation in P response results from a combination of soil physic-chemicals, landscape position and husbandry that occur within very short distance (Nziguheba et al., 2016). Deployment of

site-specific phosphorus husbandry strategies cognizant of the varying soil nutrients in western Kenya is a vital step to increasing P utilization efficiency.

2.6.2 Use of P-efficient crops

P-efficient cultivars reduces P requirement and improves P fertilizer utilization efficiency incase such cultivars are supplied with moderate P rates. P-efficiency refers to the ability of a crop genotype to thrive and produce well in soils with limited P (Nziguheba et al., 2016). This indicates that P-efficient genotypes have a low critical P requirement. PE arises from P acquisition and utilization efficiency. Plant employs various mechanisms to enhance P utilization efficiency including reallocation of P from metabolically inactive sites or old parts to active sites, alteration of biochemical pathways and release of P from vacuoles (Wang et al., 2010). Genotypic differences in P acquisition efficiency rely on the root system architecture such as basal root growth, root morphology, symbiosis with mycorrhiza, rhizosphere processes, and P acquisition kinetics (Richardson et al., 2011).

P-efficient genotypes can be effectively selected and developed thorough hybridization of polymorphic parents.Rose et al., (2013) indicates that high-throughput screening procedures and breeding programs are vital in developing cultivars for specific environments. Contrasting P levels or expression of a specific P acquisition enhancing trait have been considered important in screening genotypes for P efficiency (Rose et al. 2013). Genotypic variations in PEtraits including root, shoot biomass and concentration of P in these organs have been observed in cowpea (Krasilnikof et al., 2003), peanut (Kraimat and Bissati, 2017), mungbean (Irfan et al., 2017), wheat (Bilal et al., 2018; Nguyen and Stangoulis, 2019), cotton (Iqbal et al., 2019) and rice (Irfan et al., 2020)

among others.Majority of PE studies have concentrated on cereal crops while legumes have received limited attention. Differential abilities in root-surface phosphatase activity and exudation of organic anions have been observed in soybean genotypes (Jemo et al., 2006). Exudation of protons from roots is higher in P-efficient compared to P-inefficient cowpea genotypes (Alkama et al., 2009). Soybean genotypes showed variation in root hair development which is attributed to P acquisition efficiency (Vandamme et al., 2013). Variation in morphological and/or physiological traits among crop cultivars explains the wide variations in these crops. Exploitation of such variants is likely to accelerate development of P-efficient crops that have the potential to improve P management in agricultural ecosystems.

2.7 Inter and intraspecific variations in plant responses to P

Plants display varied ways in response to limited P availability that increases P mobility and acquisition from the soil(Teng et al., 2013; Vejchasarn etal., 2016). Genotypic variation in root traits that enhances topsoil foraging are useful adaptations to P deficient soils (Mori et al., 2016).P acquisition efficiency involve modification of lateral roots, adventitious rooting, the basal roots, and the plasticity of these processes depending on phosphorus availability contribute to P-efficiency in crop species(Bernardino et al., 2019; Long et al., 2019; Reddy et al., 2020). Other mechanisms like soil P mobilization involving symbiosis with vascular arbuscular mycorrhizas,phosphataseexudate, release of organic acids and protons or areuseful as they enhance P acquisition and efficiency(Campos etal., 2018). All these traits improve the exploration and exploitation of P in soil horizons, making it available for plant use. Bean genotypes are known to alter their geotropic curvatures of roots in response to low P availability(Namayanja etal.,

2014). This is enhanced by formation of large numbers of basal roots in top soil where concentration of P is slightly higher. Bambara being a legume also provides potential variability within its genotypes that could be exploited for low P tolerance and adaptability. Evaluation of available genetic variability among bambara groundnut in western Kenya (Odongo etal., 2015) for adaptation to low P and low pH is essential for genetic improvement in crop plants. Thus, screening bambara genotypes under contrasting P and lime levels is in principle with regaining the initial gains of its production in the region. Root system architecture, plant biomass, grain yields, phosphorus accumulation and P indices have been proved to be key indicators of P efficient cultivars worth exploitation in low and high input agricultural systems in mungbean (Irfan et al., 2017 and Reddy et al., 2020), wheat (Bilal et al., 2018) and cotton (Iqbal et al., 2019). Bambara groundnut is not an exception and the findings will be key in identification and selection of novel genotypes for both low and high input production systems and provide important germplasm for development of low P tolerance bambara genotypes.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Evaluation of the growth and yield performance of bambara genotypes under varying levels of lime and P

3.1.1Experimental location and site characterization

Field trials were carried out at two locations in Ligala (0.251^0N, 34.254^0E) and Umala (0.250^0N, 34.253^0E) in Siaya and Busia Counties in western Kenya during the long and short rain seasons of the year 2018. The sites were chosen because they have different soil types which are acidic and rich in low available. Ligala is located at an altitude of 1293 m above sea level in the Lower Midland Sugar Cane Zone (LM1) with an average annual temperature of 20.9-21.8°C (Jaetzold et al., 2010). On other hand, Umala is positioned at an altitude of 1276 m above sea level in the Lower Midland Sugar Cane Zone (LM2) with a mean annual temp of 20-22^0C (Jaetzold et al., 2010).These agro-ecological zones are characterized by a long cropping season between February and July, followed by a short one with moderate rains from August to December. The rainfall received varies from 750 to 950 mm in the long rain season while short season receives between 600mm and 700 mm, with a reliability of 66%. The growing period during the long and short rain seasons lasts for >190 and 130–150 days, respectively. Acrisols are the predominant soil at Ligalawhile cambisols soil at Umala.

3.1.2Plant materials

Seeds of twelve bambara groundnut genotypes were obtained from a collection maintained at the Department of Biological Sciences, University of Eldoret, Kenya. The

genotypes were identified as BAM001, BAM002, BAM003, BAM004, BAM005, BAM006, BAM007, BAM008, BAM009, BAM010, BAM011, and BAM012. These genotypes represent traditional cultivars collected from farmers in western Kenya. Information on seed colour and site of collection is shown in Table 3.1.

Table 3. 1: Bambara groundnut genotypes used in the study

Serial Number	Genotype Name	Seed colour	Collection point
1	BAM001	Red	Koyonzo
2	BAM002	White with black spots	Bumala
3	BAM003	Red	Bumala
4	BAM004	Maroon	Ligala
5	BAM005	Black	Bumala
6	BAM006	Brown with black spots	Koyonzo
7	BAM007	Black	Ligala
8	BAM008	Black	Sega
9	BAM009	Dark red	Sega
10	BAM010	Dark maroon	Ligala
11	BAM011	Maroon	Bumala
12	BAM012	Dark maroon	Koyonzo

3.1.3Soil sampling and analyses

Prior to setting up the trials, soil sampling and analysis was done to ascertain suitability of the two sites for the study. This was done following a zigzag pattern at a depth of 30cm using a soil auger and mixed well to form a random composite sample (Brus and de Gruijter, 1997). The composite sample was kept in khaki bags and transported to the Department of Soil Science laboratory, University of Eldoret for physical and chemical analysis. The soil sample was placed in cardboard boxes and dried at 50^0C for 24 hrs. A mechanical mortar and pestle was used to grind dried soil samples and passed through a 12-mesh (2mm) screen. This was to lower heterogeneity and increase surface area for physical and chemical reactions.

3.1.3.1 Soil pH determination

Soil pH determination was done in 1:2 (w/v) soil: distilled H_2O suspensions as described by Anderson and Ingram (1993). Approximately 40 ml of distilled $H_2O$was added to 20 g of the dried soil, in 100 ml beaker. The mixture was stirred well with a glass rod for 10 minutes and allowed to stand for 30 minutes. The combined electrodes of the pH meter were dipped into the suspension and reading done after 30 seconds. The glass electrode was then removed from the beaker and rinsed with distilled H_2O.

3.1.3.2 Proportion of organic carbon in the soil

Walkley-Black procedure (Nelson and Sommers, 1982) was used to determine total % organic carbon. Approximately 1 g of oven-dried soil was placed in 500 ml beaker and10 ml 1.0 M potassium dichromate($K_2Cr_2O_7$) solution and 20 ml concentrated sulphuric (VI) acid (H_2SO_4) added. The mixture was allowed to stand for 30minutes to allow the reaction to complete. Distilled water of 200 ml was added to the mixture followed by 10 ml of O-phosphoric acid (H_3PO_4) added and 10 drops of diphenylamine indicator. The mixture was placed on a magnetic stirrer after which Teflon-coated magnetic stirring bar was added and allowed to mix and form a deep violet colour. The mixture was then titrated with 0.5 M $FeSO_4$ solution, until the color changed from violet- blue to green showing an end-point. Ablank titration was carried out in the same way.

3.1.3.3 Total soil nitrogen

Kjeldahl digestion method as described by Jackson, (1962)was used to determined total soil nitrogen. Approximately 0.4 g of oven-dried soil was ground and placed into a digestion tube where about 1.8 g of K_2SO_4 was added to control the digestion temperature. H_2SO_4(digestion reagent) of 7.5 ml was added to the soil and left to stand

for 12 hours. The mixture was then heated on a hot plate at 360^0C for 3 hours after which it was corked and allowed to stand to obtain a clear solution for analysis. To the 0.2 ml of the clear filtrate, 5 ml of reagent 1.0 M (0.12 g sodium nitroprusside, 34 g sodium salicyclate, 25 g sodium tartrate and25 g sodium citrate mixed with distilled water to make 1 L) was added, mixed and left to stand for one hour for color development. Using atomic adsorption spectrophotometer, the sample concentration was read at wavelength 655 nm.

3.1.3.4 Soil extractable P and exchangeable potassium, calcium and magnesium

AB-DTPA extractant solution was used as described by Soltanpour and Workman, (1979). The extraction was done by placing about 10.0 g. of oven-dried soil in a 125 ml Erlenmeyer flask, where 20.0 ml of AB-DTPA extractant was added. The flask was then placed on a reciprocating shaker at 180 rotations per minute for 15 minutes. The resulting mixture was filtered through Whatman No.42 filter paper. The blue method described by Murphy and Riley,(1962) was used for P determination. Calcium was determined by Beckman Model DU Flame Spectrophotometer, magnesium by Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer and potassium by Beckman Model B Flame Spectrophotometer.

3.1.3.4.1 Soil extractable P

Approximately (0.4393) monobasic potassium phosphate (KH_2PO_4) was dissolved in distilled $H_2O$to make 1Lof solution (stock solution). This solution contained 100 mg P L^{-1}. Calibration standards containing 0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg P $L^{-1}$was prepared in AB-DTPA extracting solution. Blue-P chemical analysis was carried out by calibrating a graph. A 0.25 ml of the soil extract was pipetted into 2.5 ml spectrometric

tubes. Then 0.25 ml of 5M NaOH was added into each standard, vortex and allowed to react for 20 minutes. Then 10 ml of distilled water and 2.0 ml of ascorbic acid were added. Readings of absorbance were obtained at 882 nm at 10, 20, 30, 40, 60 and 90 minutes after adding the ascorbic acid. Soil samples extracts were analyzed from the calibration graph using the same preparation procedure done for the standards

3.1.3.4.2 Soil exchangeable calcium

Determined following procedure described by Brupbacher, (1968). A standard stock solution of calcium was prepared by dissolving about 2.4972 g of calcium carbonate in a solution containing about800 ml of distilled water and 8 ml of concentrated HCL and made to 1 L with 0.1 MHCL to give 1,000 mg P L^{-1} of calcium. Standards containing 0, 50, 100, 150, and 200 mg P L^{-1} of calcium was prepared from stock solution. The standards were placed in 500 ml volumetric flasks and diluted to the mark by adding 0.1MHCL. The soil sample concentration was determined by means of a Beckman Model DU Flame Spectrophotometer. Calibration curve was prepared by plotting the percent transmittance against calcium concentration in the five standards and amount of calcium in the soil extract was read from the curve.

3.1.3.4.3 Soil exchangeable magnesium

Determined following procedure described by Brupbacher, (1968). Standard stock solution of magnesium was prepared by dissolving approximately 1 g of magnesium metal in dilute HCL (20 ml HCL+400ml H_2O)and solution made to 1 L with more distilled water. Standards containing 0, 5, 10, 15, and 20 mg P L^{-1}were placed into 500 ml volumetric flasks and diluted to the mark with a 0.1 M HCL. Magnesium concentration in the soil extract was read on a Perkin-Elmer Model 303 Atomic Absorption

Spectrophotometer. A standard curve was plotted using absorbance readings against the magnesium concentration of the standard references from which magnesium concentration in the soil filtrate was determined.

3.1.3.4.4 Soil exchangeable potassium

Determined following procedure described by Brupbacher, (1968). Stock solution of potassium solution was prepared by dissolving about 0.953 g of potassium chloride in 200 ml of distilled water and diluted to 1 liter with a 0.1 MHCL to give 500ppm of K. Standard solutions containing 0, 5, 10, 20, and 25 mg P L^{-1}were pipetted into 500ml and diluted to volume with 0.1 MHCL. A standard curve was plotted using absorbance readings from Beckman Model B Flame Spectrophotometer (Beckman instruments, inc) against the potassium concentration of the standard references from which potassium amount present in the soil filtrate was determined.

3.1.3.5 Mechanical analysis for soil separates

Mechanical analysis for soil separates was performed as described by (Bouyoucos, 1962).Fifty (50) g of dried soil was placed in a 500 ml beaker followed by 150ml of distilled water. This was followed by 10 ml of 30% hydrogen peroxide to destroy soil organic matter. A dispersing agent of 50 ml of 10% sodium hexametaphosphate solutionwas added, mixed well with a stirring rod. The mixture was left to stand for 30 minutes after which it was placed a multimix machine and stirred for another 15 minutes. The soil suspension was then transferred to a 1Lhydrometer jar and made to volume by adding more distilled water. Three drops of amyl alcohol were introduced into the hydrometer jar to remove froth. After 40 seconds, first hydrometer reading was taken and was used to calculate proportion of sand. Three hours later, the second hydrometer

reading was taken and was used to calculating proportion of clay.The percent silt was obtainedby subtracting the sum total of percentclay and sand from 100%.Having determined the proportionof sand, silt, and clay, the soil was assigned a textural class using the USDA textural triangle as described by Estefan etal., (2013). The soil at the experimental sites was a sandy clay loam characterized by low pH, low extractable P and with a history of poor crop production. Other physic-chemical characteristics of the soil at the two experimental sites are presented in Table 3.2.

Table 3. 2: Physic-chemical properties of soils at Ligala and Umala in Siaya and Busia Counties.

Parameter	Unit	Values	
		Ligala	Umala
PH		5.20	5.10
Organic carbon	(%)	1.80	1.92
Nitrogen	(%)	0.34	0.43
Phosphorus	$mgkg^{-1}$	5.43	6.37
Calcium	$mgkg^{-1}$	1513.20	744.00
Potassium	$mgkg^{-1}$	56.65	75.54
Magnesium	$mgkg^{-1}$	241.60	214.25
% Sand	(%)	44.00	46.00
% Clay	(%)	36.00	30.00
%Silt	(%)	20.00	30.00
Textural Class		Sandy Clay Loam	Sandy Clay Loam

3.1.4 Experimental procedure

Ploughing and harrowing of the 2 fields in the 2 locations during long and short rain seasons in the year 2018 was done manually until the soil achieved fine tilth suitable for nuts planting.Triple Super Phosphate (TSP), urea and muriate of potash were mixed and incorporated well in the soil in furrows before sowing. Each genotype was sown in an experimental unit measuring 3 m x 0.33m with seed planted at 15cm apart within the row. Two seeds were sown per hill in order to achieve an equivalent seeding rate of 75kg

ha^{-1}. The study was conducted in Randomized Complete Block Experimental Design (RCBD) in a split-split-plot arrangement in 3 blocks. The experimental block measured 43m x 14m which was subdivided into two measuring 21m x 14m. In this study, genotypes were assigned in a whole plot, while lime and P were assigned to sub-plots and sub-subplots, respectively. One month before planting, agricultural lime (homa lime-koru) was applied during long rain season only at the rate of 4 $tonnesha^{-1}$ in one subplot and mixed well with the soil to allow correction of the pH at the two sites. The other subplot received no lime (0 $tonnesha^{-1}$) at the 2 sites. Each sub-plot (limed and un-limed) was then sub-divided into six sub-sub plots into which P was randomized. Three of the sub-subplots received P at the rate of 35$kgha^{-1}$ and the other 3received no P 0$kgha^{-1}$. P application was done as triple superphosphate (TSP) at sowing time. Basal dose of K and N was applied as muriate of potash and urea at the rate of 30$kgha^{-1}$each to provide source of N and Kin the soil, respectively. Weeding was done 3 times to restrict weeds growth.

3.1.5 Data Collection

In each site during long and short season, 3 plants were randomly harvested from each experimental unit at maturity for determination of plant height, number of nodules, number of lateral roots and yield. Number of nodules and lateral roots were manually counted on every plant sampled. Grain and plant biomass consisting of roots, stems and leaves were air-dried at 25^{o}C and weighed to obtained biomass. Biological yield was estimated as the summation of grain and plant biomass yields (Irfan et al. 2017). Percent increase due to P and lime application was calculated as follow;

$$\text{Percent (\%)yield increase due to Phosphorus addition}$$

$$(\%)\text{yield increase} = \frac{(\text{seed yield} - \text{L}, +\text{P}) - (\text{seed yield} - \text{L}, -\text{P})}{(\text{seed yield} - \text{L}, -\text{P})} \times 100$$

$$(\%)\text{yield increase due to liming}$$

$$(\%)\text{yield increase} = \frac{(\text{seed yield} + \text{L}, -\text{P}) - (\text{seed yield} - \text{L}, -\text{P})}{(\text{seed yield} - \text{L}, -\text{P})} \times 100$$

$$(\%)\text{yield increase due to interaction effect of Phosphorus and lime}$$

$$(\%)\text{yield increase} = \frac{(\text{seed yield} + \text{L}, +\text{P}) - (\text{seed yield} - \text{L}, +\text{P})}{(\text{seed yield} - \text{L}, +\text{P})} \text{X}100$$

Where-P= No phosphorus application, +P=Phosphorus application, -L=No lime application, +L=Lime application.

3.1.6 Data Analysis

Data on plant height, number of nodules, number of lateral roots, plant biomass, grain and biological yields in the 2 experimental sites during long and short rain seasons were subjected to combined analysis of variance to test the significance of variation due to site, seasons, lime levels, P levels and their interactions. Analysis was performed using GenStat for Windows (GenStat 2003). Differences among treatment means were separated using Duncan's multiple range tests at $P \leq .05$. Pearson correlation analysis was done to determine the relationship between grain yield, number of lateral roots and plant biomass at both sites, seasons, P levels and lime levels. The analysis was done using the following statistical equation;

$$Y_{ijklmnp} = \mu + R_i + B_{j(i)} + G_k + L_l + P_m + S_n + St_p + GL_{kl} + GP_{km} + GS_{kn} + GStkp + LP_{lm} + LS_{\ln} + \ldots$$
$$\ldots + LSt_{lp} + PS_{mn} + PSt_{mp} + SSt_{np} + GLP_{klm} + GLS_{k\ln} + GLSt_{klp} + GPS_{kmn} + GPSt_{kmp} + \ldots$$
$$\ldots + GSSt_{knp} + GLPS_{klmn} + GLPSt_{klmp} + GLSSt_{k\ln p} + GPSSt_{kmnp} + LPSSt_{lmnp} + GLPSSt_{klmnp} + \varepsilon_{ijklmnps}$$

Where $Y_{ijklmnp}$ is the observation on the $ijklmnp^{th}$ plot corresponding to genotype k in block j of replicate i lime l phosphorus m in season n and site p, μ = general mean, R_i is the effect due to replication in l^{th} lime, m^{th} phosphorus, n^{th} season and p^{th} site, $B_{j(i)}$ is the effect due to j^{th} block in i^{th} replicate in l^{th} lime, m^{th} phosphorus, n^{th} season and p^{th} site, G_k is the effect due to k^{th} genotype in the j^{th} block, in i^{th} replicate, l^{th} lime, m^{th} phosphorus, n^{th} season and p^{th} site, L_l is the effect due to l^{th} lime in m^{th} phosphorus, n^{th} season and p^{th} site, P_m is the effect due to m^{th} phosphorus in n^{th} season and p^{th} site, $S_n$is the effect due to n^{th} season and p^{th} site while $St_p$is the effect due to p^{th} site. GL_{kl} is the effect due to k^{th} genotype by l^{th} lime interaction, GP_{km} is effect due to k^{th} genotype by m^{th} phosphorus interaction, GS_{kn} is the effect due to k^{th} genotype by n^{th} season interaction, GSt_{kp} is effect due to k^{th} genotype by p^{th} site, LP_{lm} is effect due to l^{th} lime by m^{th} phosphorus interaction, LS_{ln} is the effect due to l^{th} lime by n^{th} season interaction, LSt_{lp} is effect due to l^{th} lime by p^{th}site interaction, PS_{mn} is effect due to m^{th} P by n^{th} season interaction, PSt_{mp} is effect due to m^{th} P by p^{th} site interaction, SSt_{np} is effect due to n^{th} season by p^{th} site interaction,GLP_{klm} is the effect due to k^{th} genotype by l^{th} lime by m^{th} P, GLS_{kln} is the effect due to k^{th} genotype by l^{th} lime by n^{th} season, $GLSt_{klp}$ is the effect due to k^{th} genotype by l^{th} lime by p^{th} site, GPS_{kmn} is the effect due to k^{th} genotype by m^{th} P by n^{th} season, $GPSt_{kmp}$ is the effect due to k^{th} genotype by m^{th} P by p^{th} site, $GSSt_{knp}$ is the effect due to k^{th} genotype by n^{th} season by p^{th} site, $GLPS_{klmn}$ is the effect due to k^{th} genotype by l^{th} lime by m^{th} P by n^{th} season, $GLPSt_{klmp}$ is the effect due to k^{th} genotype by l^{th} lime by

m^{th} P by p^{th} site, $GLSSt_{klnp}$ is the effect due to k^{th} genotype by l^{th} lime by n^{th} season by p^{th} site $GPSSt_{kmnp}$ is the effect due to k^{th} genotype m^{th} P by n^{th} season by p^{th} site, $LPSSt_{lmnp}$ is the effect due to l^{th} lime by m^{th} P by n^{th} season p^{th} site, $GLPSSt_{klmnp}$ is the effect due to k^{th} genotype by l^{th} lime by m^{th} P by n^{th} season p^{th} site and $\varepsilon_{ijklmnps}$ represents the residual for the $ijklmnp^{th}$ plot.

3.2 Determination of the genotypic variation of bambara groundnut for P acquisition and use efficiencies

3.2.1 P acquisition and P-related efficiency indices

Phosphorus accumulation (PA) in the grains and plant biomass was determined from plants grown at Ligala site only during the long rain season. Grains and plant biomass from the 5 individual plants from each experimental unite wasoven-dried at 70°C for 48 hours to constant biomass. Dried grains and plant biomass of individual genotypes were milled to fine powder using Wiley's mill. One gram of milled grain and plant biomass samples were placed in 125 ml Erlenmeyer flask which had been previously washed with hot 1:1 hydrochloric acid: distilled water to remove traces of P adsorbed on the glassware. Pulverized samples were digested in 5:1 nitric-perchloric (HNO_3:$HClO_4$) acid to recover P from the material (Jones and Case,1990). To 1g of each sample in a 125 ml Erlenmeyer flask, 20ml concentrated HNO_3(V) acid and 4 ml perchloric acid were added and boiled to the fuming stage under perchloric acid Fortyml of distilled H_2Oafter cooling and then boiled for another 1minute. The fumeless solution was cooled and filtered through filter paper (Whatman No. 42) into100 ml pyrex flask and filled to the mark by adding more distilled water. Quantification of P was determined by vanadate–molybdate method as described by Chapman and Pratt, (1961). Five ml of the filtrate

were pipetted into 100ml volumetric flask followed by 45 ml distilled water. Within 5 minutes, 20 ml of reagent vanado-molybdate was added, diluted to the mark, mixed well and left for colour development after 10 minutes. Standards were prepared by pipetting 0, 2, 4, 5, 10 15 and 20 ml of the 25 $mgPL^{-1}$stock solution into 100 ml volumetric flasks, followed by 20 ml of reagent vanado-molybdate in each case and allowed to stand for 10 minutes for color development. The blank was also prepared using distilled water with 20 ml of reagent vanado-molybdate and diluted to the mark. Determination of % transmittance was done at wavelength of 410 nm using spectronic-70 Electrophotocolorimeter. A calibration curve was obtained by plotting absorbance against each P concentration of the standards. The amount of P in the samples was estimated by referring to the calibration curve. Based on grain yield of genotypes at low and adequate P, phosphorus stress factor (PSF) was estimated as described by Hunt, (1978). Using P concentrations in the grains and plant biomass, P accumulation and efficiency indices such as phosphorus harvest index (PHI) (Siddiqi and Glass,1981), P physiological efficiency index (PPEI) and phosphorus biological yield efficiency ratio (PBER) (Jones et al. 1992),P accumulation (PA) and total P accumulation (TPA) (Zhang et al., 2007) were determined as shown below:

Phosphorus Accumulation (PA):

$$\text{PA}\left(\frac{\text{mg}}{\text{plant}}\right) = \text{Dry plant biomass/seed} \times \text{Phosphorus Concentration}$$

Total Phosphorus Accumulation (TPA):

$$\text{TPA}\left(\frac{\text{mg}}{\text{plant}}\right) = \text{PA in plant biomass} + \text{PA in Seed}$$

Phosphorus Physiological Efficiency Index (PPEI):

$$\text{PPEI} = \frac{\text{Seed Yield}}{\text{TPA}}$$

Phosphorus Biological Yield Efficiency Ratio (PBER):

$$\text{PBER} = \frac{\text{Biological Yield}}{\text{TPA}}$$

Where biological yield is the sum total of seed and plant biomass yields

Phosphorus harvest index (PHI)

$$\text{PHI}(\%) = \frac{\text{Grain PA}}{\text{TPA}} \times 100$$

Phosphorus Stress Factor (PSF)

$$\text{PSF}\ (\%) = \frac{(\text{Seed Yield at P nutrition}) - (\text{Seed Yield at lowP})}{(\text{Seed Yield at P nutrition})}$$

3.2.2 Categorization of bambara genotypes

Based on plant biomass yield, grain yield, biological yield, plant biomass PA, grain PA, TPA, PHI, PPEI and PBER, bambara groundnut genotypes were classified into low, medium and high P acquisition and use efficiencies using total index score at low and adequate P levels (Irfan et al., 2017). These index scores were summed up per genotype for all the parameters measured to give total index score (TIS). TIS were used to rank the genotypes as efficient, medium or inefficient. Classification was done using absolute values with the standard deviation (SD) and population mean (μ) for each parameter per cultivar (Gill et al., 2004). The values 1, 2 and 3 were used to classify the genotypes into groups: 1 (low) if mean <μ-SD, 2(medium) if μ-SD < mean <μ+SD or 3(high) if the mean is >μ+ SD. The genotypes were further grouped into various categories by calibrating a graph in which seed yield ($gplant^{-1}$) was represented on the x-axis and total PA (mg/ plant) on the y-axis. Each genotype in this graph was represented by a circle.

HGY, MGY, and LGY represented high, medium, and low seed yield while HP, MP and LP indicated high, medium, and low PA of the genotypes tested (Irfan et al., 2017).

3.2.3 Data Analysis

Data on PA, PHI, PPEI and PBER were subjected to analysis of variance to examine significance due to genotype and Plevels and their interaction. Analysis was performed using GenStat for Windows (GenStat 2003). Differences among treatment means were separated using Duncan's multiple range tests at $P \leq .05$. Pearson correlation analysis was done to determine the relationship between grain yield and various phosphorus efficiency parameters at both levels of P. Principal Component Analysis (PCA) and biplots of the PCA were constructed to identify genotypes with suitable traits for effective selection and breeding to improve P-efficiency. The analysis was done using following statistical equation;

$$Y_{ijk} = \mu + R_i + G_j + P_k + GP_{jk} + \varepsilon_{ijkl}$$

Where *Yijk* is the observation on the ijk^{th} plot corresponding to genotype *j* in replicate *i* in the phosphorus *k*, μ is the general mean, R_i is the effect due to i^{th} replicate in the k^{th} P, G_j is the effect due to j^{th}genotype in the i^{th} replicate, k^{th} P, P_k is the effect due to k^{th} P, $GP_{jk}$is the effect due to j^{th}genotype by k^{th} P interaction and ε_{ijkl} represents the residual error for the ijk^{th} plot.

3.3 Morphological traits associated with enhanced P-acquisition and P utilization efficiency

3.3.1 Study Site and Pot Screening System

Pot experiment was conducted under a glasshouse during the months of march-April 2019 at the University of Eldoret located at 0.584^0 N 35.309^0 E and altitude of 2100 m above sea level. The bambara groundnuts were grown in 2-liter pots under greenhouse in a completely randomized (CRD) design in three replications. The pots were perforated on lower part to allow free drainage of excess water and placed on greenhouse benches. Soil used in the experiments was 2.5kg of sand soil that had been pretreated with 0.5 M HCl to remove plant debris and inorganic forms of phosphorus (P) and 0.5 M NaOH to remove humus. The soil was manually packed ensuring a homogenous continuum that exposed the whole soil fractions equally to water.

3.3.2 Crop establishment

Uniform sized seeds of individual genotypes were selected and surface sterilized in a 1% sodium hypochlorite(NaOCl) solution for 2 min on a reciprocating shaker at 150 rotations per minute. The seeds were then rinsed 5 times using distilled water. The sterilized seeds were placed on a wet towel in 100 mm diameter petri dishes for 24 hours to imbibe water at room temperature of 25^0C, in the dark. Three seeds of individual genotype were sown in the pots. After germination, two healthy plants per pot were maintained under the greenhouse. Each pot was supplied with $75cm^3$ of half-strength Hoagland nutrient solution deficient of P every 5days from sowing till harvest at 35 days after emergence (DAE). In addition to Hoagland nutrient solution, two levels of P were established (0µM and 160µM KH_2PO_4) following recommendation by Rose et al., (2013) for screening

cultivars for P acquisition and utilization efficiency based on their performance attributes under contrasting P levels. The pots were irrigated daily until seedlings emerged after 7 days with 200 ml of water. After germination, the plants were irrigated every two days with 250 ml of water until harvest at 35 DAE.

3.3.3 Data Collection

Root harvesting for individual plants was done by inverting the pot upside-down to let its content out which was then washed under running tap water. After washing, the shoots were cut off from roots. The shoot length (SL) and tap root length (TRL) were determined. The branching number (primary lateral roots) (BN) were then counted. The root volume (RV) was determined by water displacement (Pang et al. 2011).Root system was immersed in water and the volume of displaced water taken as an equivalent of the root volume. Branching density (BD) was obtained by dividing BN by TRL. Shoot dry weight (SDW) and root dry weight (RDW) were determined after drying in an oven at70^0C for 48 hours and expressed as gplant^{-1}.

3.3.4 Data analysis

Data on morphological traits including tap root length (TRL), branching number (BN), root volume (RV), branching density (BD), shoot length (SL), RL:SL ratio, root dry weight (RDW), shoot dry weight (SDW), RW:SW ratio, were subjected to analysis of variance to examine significant variation due to genotype, P levels and their interactions. Analysis was performed using GenStat for Windows (GenStat, 2003). Differences among treatment means were separated using Duncan's multiple range tests at $P \leq 0.05$.

The following statistical equation was used;

$$Y_{ijk} = \mu + R_i + G_j + P_k + GP_{jk} + \varepsilon_{ijkl}$$

Where *Yijk* is the observation on the *ijk*th plot corresponding to genotype *j* in replicate *i* in the phosphorus *k*, μ is the general mean, R_i is the effect due to i^{th} replicate in the k^{th} P, G_j is the effect due to j^{th}genotype in the i^{th} replicate, k^{th} P, P_k is the effect due to k^{th} P, $GP_{jk}$is the effect due to j^{th}genotype by k^{th} P interaction and ε_{ijkl} represents the residual error for the ijk^{th} plot.

CHAPTER FOUR

RESULTS

4.1 Evaluation of growth and yield performance of bambara genotypes under varying levels of lime and phosphorus

4.1.1 Analysis of variance of morphological attributes of bambara genotypes grown at varying phosphorus and lime levels during long and short rain seasons at Ligala in Siaya County and Umala in Busia County

Bambara groundnut genotypes differed significantly ($P \leq .05$) in plant height, number of nodules and number of lateral roots per plant due to phosphorus levels, lime levels and season (Appendix 1). Site was only significant for the number of nodules and number of lateral roots per plant. The interaction effects of season x site was significant for all three morphological attributes. The interactions genotype x lime, genotype x season, genotype x site, phosphorus x season, lime x site and season x site showed significant variation for plant height. The interactions including genotype x season, site x season, lime x phosphorus, genotype x site x season, and lime x site x season displayed variation for number of nodules per plant in this study. There was also significant interactions lime x season, phosphorus x site x season and genotype x phosphorus x site x season for the number of lateral roots. The lime x season x site displayed significant variation for the number of nodules only. The interactions lime x season and genotype x site was significant for plant height and number of lateral roots only. On other hand, genotype x lime and lime x site was significant for plant height only. The interaction effect of lime x

phosphrous and genotype x season x site were significant for the number of nodules and lateral roots.

4.1.2 Response of bambara groundnut genotypes to lime application at Ligala

Plant height ranged from 24.00 to 28.96 cm during long rain season in un-limed soil with low P supply (Table 4.1). On addition of P in un-limed soil, plant height ranged from 24.63 to 30.70 cm at Ligala. The genotype BAM007 exhibited the maximum plant height of 28.96 cm while BAM008 grew to the highest plant height of 30.70 cm under low and adequate P during long rain season, respectively. BAM010 achieved the lowest plant height of 24.00 cm and 24.63 cm under low and adequate P supply during long rain season at Ligala, respectively. Plant height of bambara groundnut genotypes at low P varied from 24.40 cm to 29.26 cm in limed soil with low P whereas 25.13-32.26 cm was observed under adequate P in the long rain season at Ligala (Table 4.1). BAM001 with 24.40 cm and 25.13 cm was the shortest in limed soils under low and adequate P during long rain season, respectively. BAM007 was the tallest exhibiting 29.26 cm and 32.26 cm when grown under low and adequate P in limed soil. On average, plant height increased by 3.3% at both un-limed and limed soil under P application during long rain season.

Table 4. 1: Mean values of plant height, number of nodules and lateral roots of12 bambara genotypes grown at varying phosphorus and lime levels during short rain season at Ligala.

	Plant Height (cm)				No. of Nodules/plant				Number of lateral roots/plant			
	Lime 0tonnesha^{-1}		Lime 4tonnesha^{-1}		Lime 0tonnesha^{-1}		Lime 4tonnesha^{-1}		Lime 0tonnesha^{-1}		Lime 4tonnesha^{-1}	
Genotype	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P
BAM001	25.00bc	26.63ab	24.40c	25.13d	5.44cd	6.77cd	8.55ac	6.33d	21.88a	27.33a	24.22a	28.88ab
BAM002	26.40bc	30.46a	27.36bc	26.53cd	4.77d	9.55cd	7.22bc	11.44ac	22.00a	22.11bf	22.88ab	26.88ad
BAM003	27.06ab	26.93ab	25.63c	27.53bd	6.33bd	8.00cd	6.55bc	9.55bd	20.77ac	21.66cf	19.22cd	23.88de
BAM004	26.70bc	26.33ab	26.76bc	26.40cd	4.88d	6.33cd	5.55c	7.00cd	17.11cd	18.88ef	17.33d	21.44e
BAM005	24.63c	27.30ab	27.26bc	27.30bd	6.22bd	7.33cd	5.22c	6.88cd	20.77ac	18.44f	19.55bd	21.11e
BAM006	27.26ab	28.83ab	27.83ab	28.70ad	7.22bd	8.33cd	8.44ac	9.22bd	20.66ac	21.11cf	20.11bd	21.44e
BAM007	28.96a	28.70ab	29.26a	32.26a	5.88cd	6.33cd	7.44bc	7.66bd	16.44d	20.44df	20.55bd	25.33bd
BAM008	28.00a	30.70a	27.70ab	30.76ac	5.44cd	7.66cd	6.66bc	7.66bd	18.77ad	23.77ad	19.88bd	25.55bd
BAM009	26.90ac	27.70ab	27.80ab	28.56ad	6.77bd	5.66d	8.22ac	9.66bd	17.77bd	23.33ad	19.33cd	27.11ad
BAM010	24.00c	24.63b	26.30bc	26.46cd	9.33ab	13.22ab	8.22ac	11.22ac	22.22a	24.77ac	24.77a	28.44ac
BAM011	27.83ab	30.50a	27.50bc	31.16ab	8.33ac	10.00bc	10.33ab	11.66ab	21.00ab	26.22ab	24.88a	29.55a
BAM012	24.20c	29.90ab	26.36bc	30.80ac	10.33a	15.66a	11.77a	14.11a	19.00ad	23.00be	21.66ac	25.00cd
Mean	26.41	27.51	27.27	28.42	6.75	8.74	7.85	9.37	19.87	22.59	21.2	25.39
SE	0.44	0.38	0.49	0.48	0.31	0.44	0.40	0.44	0.37	0.44	0.37	0.41

Notes: Means of individual treatment with different letter(s) in a column significantly vary as per Duncan's multiple Range test P≤0.05,–P=0kg/ha P, +P=35kg/ha P, P=Phosphorus, SEM=Standard error of mean,

Mean number of nodules per plant varied from 4.77 to 10.33 and 5.66 to 15.66 under low and adequate P application in un-limed soil at Ligala during long rain season (Table 4.1). BAM002 and BAM009 had the lowest number of nodulesplant^{-1} of 4.77 and 5.66 at low and adequate P, respectively. The genotype BAM012 had the maximum number of nodules/plants of 10.33 and 15.66 at low and adequate P supply, respectively. On limed plots, the number of nodules plant^{-1} varied from 5.22- 11.77 and 6.33-14.11 in low and adequate P supply, respectively. Genotype BAM012 recorded the maximum number of nodules of 11.77 and 14.11 at both low and adequate P in limed soil, respectively. On the contrary, BAM004and BAM001 recorded the least number of nodules of 55.22 and 6.33 at limited and adequate P, respectively. Overall, the addition of P increased number of nodules plant^{-1} by 29.5% and 19.4% in un-limed and limed soil, respectively. Lime application averagely increased number of nodules by 16.3% and 7.2%under low and adequate P supply, respectively

Mean lateral roots genotype^{-1} varied from 16.44 – 22.22 to 18.44 – 27.33 under low and adequate P fertilization during long rain season at Ligala (Table 4.1).The maximum number of lateral roots of 22.22 and 27.33 was observed for genotypes BAM010 and BAM001 at low and adequate during the long rain season, respectively. BAM007 had the lowest number of lateral roots of 16.44 while BAM005 recorded the least number of lateral roots of 18.44 at low and adequate P supply during long rain season. Number of lateral roots ranged from 17.33-24.88 cm and 21.11-29.55 cm under low and adequate P nutrition during long rain season in limed plots at Ligala (Table 4.1). A maximum number of lateral roots of 24.11and 29.55 was achieved in BAM011 under both low and

adequate P during long rain season. The lowest number of lateral roots of 17.33 and 21.11 was observed in BAM004 and BAM005at 0kg/ha and 35kg/ha P, respectively. The application of P on overage increased the number of lateral roots by 13.7% and 19.8% in un-limed and limed plots, respectively. Overall, the application of lime improved number of lateral roots/plants by 6.7% and 12.4% in low and adequate P supply, respectively.

4.1.3 Morphological traits of bambara groundnut genotypes in un-limed and limed soil during short rain season at Ligala

The plant height of bambara cultivars varied from 24.23 cm to 27.76 cm and 25.43 cm to 28.93 cm at low and adequate P supply in un-limed soil during short rain season (Table 4.2). BAM008 and BAM009 achieved the highest plant height of 27.76cm and 28.93 cm under low and adequate P supply in un-limed soil, respectively. The genotypes BAM001and BAM003 exhibited the lowest plant height of 24.23 cm and 25.43 cm at low and adequate P. In limed soil, plant height varied from 24.63-28.46 and 24.63-30.73 during short rain season at low and adequate P. The shortest genotype was observed in BAM004 with 24.63 cm at both low and adequate P fertilization. BAM006 and BAM011 with 28.46 cm and 30.73 cm were the tallest at limited and adequate P, respectively. The application of P enhanced plant height by 1.8% and 5.3% in un-limed and limed soils, respectively. The short season reduced the plant height by 0.5% and 1.9% under low and adequate P fertilization in un-limed plots, respectively. The short season also reduced plant height by 1.9% and 0.9% at low and adequate P in limed plots, respectively.

Table 4. 2: Mean values of plant height, number of nodules and lateral roots of 12 bambara genotypes grown at varying phosphorus and lime levels during short rain season at Ligala.

	Plant Height (cm)				No. of Nodules/plant				Number of Lateral roots/plant			
	Lime 0tonnesha^{-1}		Lime 4tonnesha^{-1}		Lime 0tonnesha^{-1}		Lime 4tonnesha^{-1}		Lime 0tonnesha^{-1}		Lime 4tonnesha^{-1}	
Genotype	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P
BAM001	24.23e	25.66b	25.36bc	25.43b	10.66ab	9.55ac	8.55ac	8.66b	20.00ad	22.00de	25.88a	25.77ad
BAM002	24.40e	25.60b	26.56bc	26.30b	6.44ce	6.88bd	5.88c	9.11b	23.00a	23.01de	26.22a	27.66ab
BAM003	24.76de	25.43b	25.90bc	25.56b	5.44e	5.77cd	6.11c	8.11b	20.77ac	21.77de	20.77c	26.44ad
BAM004	25.70be	26.53ab	24.63c	24.63b	5.22e	6.33bd	6.55bc	8.33b	16.88d	21.11e	19.55c	23.55d
BAM005	27.53ab	26.93ab	25.06bc	26.90b	5.66de	6.66bd	6.00c	8.22b	17.22cd	22.77de	20.33c	24.33cd
BAM006	24.96ce	27.06ab	28.46a	27.63ab	7.33ce	8.22bd	7.00bc	9.77b	18.77bd	25.55ac	20.33c	24.88bd
BAM007	26.36ae	27.93ab	26.20bc	27.10ab	5.88de	5.33d	9.00ac	9.44b	17.44cd	22.77de	19.33c	25.66ad
BAM008	27.76a	25.70b	24.70c	25.76b	6.22ce	7.55bd	7.66bc	7.33b	19.11bd	24.11bd	20.33c	26.88ac
BAM009	27.16ad	28.93a	27.56b	26.46b	5.22e	4.88d	8.44ac	12.11a	18.66bd	22.11de	19.88c	25.33bd
BAM010	25.66be	25.90b	24.70c	25.43b	8.66ac	9.77ab	11.22a	9.66b	20.22ad	27.00a	21.77bc	27.88ab
BAM011	26.43ae	27.90ab	28.06a	30.73a	8.22bd	9.55ac	10.55ab	12.22a	21.22ab	26.33ab	24.88ab	28.55a
BAM012	27.33ab	28.4a	26.60bc	27.10ab	10.88a	11.88a	10.00ab	10.00ab	18.88bd	23.44ce	21.33c	27.66ab
Mean	26.28	26.74	26.74	28.16	7.16	7.94	8.42	9.42	19.35	23.50	21.72	26.22
SE	0.30	0.33	0.45	0.48	0.30	0.38	0.47	0.49	0.35	0.29	0.39	0.30

Notes: Means of individual treatment with different letter(s) in a column significantly vary as per Duncan's multiple Range test P≤0.05,–P=0kg/ha P, +P=35kg/ha P, P=Phosphorus, SEM=Standard error of mean,

The mean number of nodulesplant^{-1}varied from 5.22-10.88 and 5.33-11.88 in un-limed plots during short rain season under low and adequate P fertilization (Table 4.2). The highest number of nodulesplant^{-1}was observed in BAM012 with 10.88 and 11.88 at limited and adequate P supply, respectively. The least number of nodulesplant^{-1}of 5.22 at low P was achieved in BAM004 and BAM009 while BAM007 had the lowest of 5.33 at adequate P supply in un-limed soil. The mean number of nodulesplant^{-1}varied from 5.88-11.22 and 7.33-12.22 at adequate P in limed soil. BAM010 with 11.22 at low P and BAM011 with 12.22 at low and adequate P in limed plots. BAM002 and BAM008 had the least number of nodulesplant^{-1}of 5.88 and 7.33at low and adequate P, respectively. Overall, the mean number of nodulesplant^{-1}increased by 6.1% at low P while it reduced by 9.1% under adequate P fertilization in un-limed soil during short season.The short season howeverrealized an increased number of nodulesplant^{-1}by 7.3% and 0.5% under low and adequate P supply in limed soil, respectively.

The mean number of lateral roots varied from 16.88-23.00 to 21.11-27.00 at low and adequate P in un-limed soil in Ligala during the short rain season (Table 4.2). BAM004 registered the lowest number of lateral roots of 16.88 and 21.11at both low and adequate P fertilization, respectively. On other hand, BAM002 and BAM010 had the maximum number of lateral roots of 23.00 and 27.00 at low and adequate P in un-limed soil, respectively. The mean number of lateral roots ranged from 19.33-26.22 to 23.55-28.55 under low and adequate P fertilization in limed plots respectively (Table 4.2). The lowest number of lateral roots of 19.33 and 23.55 were recorded by genotypes BAM007 and BAM004 at deficient and adequate P, respectively. BAM002 and BAM011 exhibited the

highest number of lateral roots of 26.22 under low P and 28.55 at adequate P supply, respectively. The addition of P improved the mean number of lateral roots by 21.4% and 20.6% in un-limed and limed soil, respectively. The short season decreased the number of lateral roots by 2.6% and increased by 4.0% under low and adequate P fertilization in un-limed soil conditions, respectively. In limed conditions, the short season recorded an increased number of lateral roots by 2.5% and 3.3% in low and adequate P, respectively.

4.1.4 Response of bambara groundnut genotypes to amendment of soil with lime at Umala during long rain season.

The average plant height of bambara genotypes varied from 23.96-26.93cmto 24.06 – 29.53cm under low and adequate P nutrition in un-limed soil during long rain season (Table 4.3). The tallest genotypes in plant height were observed in BAM008 and BAM011with 26.93 cm and 29.53 cm at low and adequate P in un-limed soil, respectively.BAM001 and BAM004 exhibited the lowest plant height of 23.96 cm and 24.06 cm at low and adequate P in un-limed soil, respectively. The response of plant height to contrasting levels of P ranged from 24.93-28.56 cm to 25.93-33.10 cm under low and adequate P application in limed soil. Maximum plant height of 28.56 and 33.10 cm was achievedby genotype BAM007 at both low and adequate P in limed soil during long rain season, respectively. On the contrary, BAM001 and BAM003 recorded the minimum plant height of 24.93 and 25.93 cm at low Pandadequate P supply in limed plots. The umala location exhibited the highest overall mean plant height than Ligala in both un-limed and limed plots during long rain season.

Table 4. 3: Mean values of plant height, number of nodulesandlateral roots of 12 bambara genotypes grown at varying levels of phosphorus and limeduring long rain season at Umala.

	Plant Height (cm)				No. of Nodules/plant				Number of Lateral roots/plant			
	Lime 0tonnesha^{-1}		Lime 4tonnesha^{-1}		Lime 0tonnesha^{-1}		Lime 4tonnesha^{-1}		Lime 0tonnesha^{-1}		Lime 4tonnesha^{-1}	
Genotype	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P
BAM001	23.96b	25.40ac	24.93b	26.23d	11.00ab	9.77bd	12.22ab	11.44ad	24.33a	21.66ac	23.00bd	22.33bc
BAM002	26.03bc	26.96ac	26.23ab	27.30bd	5.33c	7.44d	9.33bc	9.22cd	23.22ab	22.22ac	23.44bd	24.22ab
BAM003	25.33bc	27.10ac	25.43b	25.93d	5.88c	9.55bd	6.11c	10.66bc	22.77ac	23.33ab	19.55d	24.88ab
BAM004	21.76c	24.06c	25.46b	27.20cd	6.22c	7.11d	6.88bc	6.66d	18.33cd	20.22bc	19.33d	21.00c
BAM005	25.60bc	27.10ac	27.43ab	29.03bd	9.00ac	7.00d	7.44bc	14.66ac	18.00d	22.33ac	20.66cd	23.77ab
BAM006	25.03bc	27.70ac	26.30ab	28.63bd	9.00ac	7.44d	8.00bc	9.44cd	22.00ad	22.22ac	20.77cd	23.55ac
BAM007	26.06bc	28.63ab	28.56a	33.10a	6.88bc	5.77d	8.77bc	12.77ad	19.44bd	19.66c	21.44cd	24.00ab
BAM008	26.93a	28.43ac	26.90ab	30.33ac	9.55ac	7.88cd	8.55bc	10.22cd	19.77ad	21.55ac	24.44ac	23.88ab
BAM009	26.46a	26.40ac	27.26ab	30.33ac	6.88bc	6.55d	9.22bc	11.44ad	20.11ad	23.44ab	22.00cd	23.22ac
BAM010	26.73a	27.86ac	27.70ab	27.90bd	9.88ac	12.88ab	11.33ac	12.77ad	23.11ab	22.44ac	26.11a	25.66a
BAM011	26.66a	29.53a	27.70ab	31.23ab	9.44ac	16.66a	11.88ac	17.11ab	22.11ad	24.00a	22.66bd	24.33ab
BAM012	26.36a	24.95bc	25.53b	26.29d	12.55a	12.55ac	16.22a	17.88a	23.66ab	20.77ac	23.33bd	23.77ab
Mean	25.58	27.04	26.87	28.60	8.47	9.22	9.67	12.03	21.41	21.99	22.56	23.72
SE	0.29	0.39	0.29	0.46	0.44	0.52	0.56	0.64	0.44	0.32	0.42	0.26

Notes: Means of individual treatment with different letter(s) in a column significantly vary as per Duncan's multiple Range test P≤0.05,–P=0kg/ha P, +P=35kg/ha P, P=Phosphorus, SEM=Standard error of mean,

Mean number of nodulesplant^{-1}of the bambara genotypes varied from 5.33-12.55 to 5.77-16.66 under low and adequate P supply during the long rain season, respectively (Table 4.3). BAM012 and BAM011 exhibited the maximum number of nodules of 12.55and 16.66 at low and adequate P in un-limed plots during long rain season, respectively. On the other hand, the least number of nodules of 5.33 and 5.77 was observed in BAM002 and BAM007at low and adequate P in un-limed soil, respectively. On liming, the number of nodules ranged between 6.11-16.22 to 6.66-17.88 under low and adequate P supply during long rain season, respectively (Table 4.3). Genotype BAM012 with 16.22 and 17.88 numbers of nodules at low and adequate P was observed in limed soil during long rain season. The lowest value of 6.11 was achieved by BAM003 at low P while BAM004 with 6.66 exhibited the minimum value at adequate P. Averaged across the genotypes, the number of nodules increased by 8.9% and 24.4% in un-limed and limed soil due to P application. The mean overall number of nodules was higher in Umala than Ligala in both un-limed and limed plots during long rain season.

Number of lateral roots of the genotypes ranged from 18.00-24.33 to 19.66-24.00 at low and adequate P during the long rain season (Table 4.3). Genotypes BAM001 and BAM011 with 24.33 and 24.00 exhibited the maximum number of lateral roots at low and adequate P supply during long rain season in un-limed soil, respectively. BAM005 and BAM007 exhibited the lowest number of lateral roots of 18.00 and 19.66 at low and adequate P, respectively. In limed soil, mean number of lateral roots of the tested genotypes varied from 19.33-26.11 to 21.00 – 25.66 under low and adequate P nutrition during long rain season (Table 4.3). The maximum number of lateral roots of 26.11 and

25.66 was achieved in BAM010 at both low and adequate P during long rain season, respectively. BAM004 with 19.33 and 21.00 had the lowest number of lateral roots at both low and adequate P during long rain season in limed soil, respectively. An increase of 2.7% and 5.1% was observed for the mean number of lateral roots in un-limed and limed soil upon application of P, respectively. The umala location realized higher overall mean number of lateral roots than Ligala at low P in both un-limed and limed plots. On the other hand, Ligala location had higher overall mean number of lateral roots than observed at Umala at adequate P supply in both un-limed and limed plots during the long rain season.

4.1.5 Response of bambara groundnut genotypes to lime application in Umala during short rain season at Umala

Plant height of bambara genotypes varied from24.83 -29.16 to 24.10 - 29.70 cm in un-limed soil during short rain season (Table 4.4). BAM011with 29.16 and 29.70 cm recorded the maximum plant height at both low and adequate P during short rain season, respectively. On the other hand, genotype BAM001 had the minimum plant height of 24.83 cm at low P while BAM004 with 24.10 cm was the shortest at adequate P. The plant height varied from 24.73–29.96 to 24.63–32. 23 cm in limed plots in the short rain season (Table 4.4).BAM007 with 29.96 cm and 32.23 cm exhibited the maximum plant height at both low and adequate P in limed soil, respectively. Conversely, genotype BAM001 under low and adequate P supply had minimum plant height of 24.73 cm and 24.63 cm during short rain season, respectively. The overall mean plant height increased by 2.4% and 3.8% due to P supply in un-limed and limed soil during short rain season, respectively.

Table 4. 4: Mean values of plant height, number of nodules and lateral roots of 12 bambara genotypes grown at varying levels of phosphorus and lime during short rain season at Umala.

	Plant Height (cm)				No. of Nodules				Number of Lateral roots			
	Lime 0tonnesha^{-1}		Lime 4tonnesha^{-1}		Lime 0tonnesha^{-1}		Lime 4tonnesha^{-1}		Lime 0tonnesha^{-1}		Lime 4tonnesha^{-1}	
Genotype	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P
BAM001	24.83c	25.73ab	24.73b	24.63d	11.00a	8.11ab	9.00ab	10.44b	19.88ac	22.66a	23.55ab	22.66ae
BAM002	28.86ab	27.26ab	25.63bc	26.46cd	6.88ac	8.44ab	6.11ab	9.22b	22.55a	23.00a	25.66a	25.33ab
BAM003	25.43bc	26.80ab	25.20bc	25.50cd	7.44ac	10.44ab	6.11ab	11.33ab	20.77ac	21.44ab	22.88ac	23.22ad
BAM004	25.73ac	24.10b	26.60ac	27.66bd	5.77c	8.22ab	6.77ab	7.55b	20.00ac	20.88ac	21.77bc	21.77cd
BAM005	26.50ac	27.86ab	26.50ac	29.56ac	8.88ac	8.00ab	5.44b	11.88ab	19.55ac	21.11ac	22.66ac	22.22bd
BAM006	25.20bc	27.26ab	26.93ac	27.63bd	9.55ac	8.55ab	10.22a	8.66b	19.66ac	19.33ac	21.55c	21.33c
BAM007	27.30ac	28.76ab	29.96a	32.23a	9.33ac	8.11ab	8.33ab	11.00b	17.55c	18.00bc	21.33c	21.00d
BAM008	27.10ac	28.30ab	28.13ac	29.33ac	7.77ac	8.33ab	7.77ab	8.77b	18.11bc	17.88bc	21.88bc	21.44c
BAM009	27.90ac	27.16ab	26.43ac	29.70ac	6.55bc	6.44b	9.33ab	9.33b	18.22bc	17.22bc	21.22c	23.00bd
BAM010	26.20ac	27.86ab	29.00ab	26.00cd	7.44ac	8.11ab	9.11ab	9.66b	22.11ab	22.66a	24.44ab	25.66a
BAM011	29.16a	29.70a	29.20ab	31.46ab	8.11ac	9.44ab	10.55a	10.77b	21.11ac	23.00a	24.77ab	25.66a
BAM012	27.20ac	28.95ab	27.86ac	28.60ad	10.55ab	11.33a	10.11a	15.77a	20.44ac	22.22a	21.11c	23.55ac
Mean	26.79	27.43	27.18	28.22	8.08	8.63	8.24	10.37	20.00	20.79	22.74	23.07
SE	0.34	0.43	0.38	0.50	0.38	0.37	0.41	0.46	0.36	0.40	0.28	0.36

Notes: Means of individual treatment with different letter(s) in a column significantly vary as per Duncan's multiple Range test

P≤0.05,–P=0kg/ha P, +P=35kg/ha P, P=Phosphorus, SEM=Standard error of mean,

The mean number of nodules ranged from 5.77– 11.00 to 6.44 – 11.33 under low and adequate P nutrition during the short rain season in un-limed soil, respectively (Table 4.4). BAM001and BAM012 had the maximum number of nodules of 11.00 and 11.33 at low and adequate P in un-limed soil during the short rain season. Genotypes BAM004 and BAM009 displayed the lowest number of nodules of 5.77 and 6.44 at low and adequate P in un-limed soils during short rain season. The mean number of nodules/plant ranged from 5.44 – 10.55 to 7.55 – 15.77 under low and adequate P supply in limed soil during short rain season (Table 4.4). BAM011 and BAM012 exhibited the highest number of nodules of 10.55 and15.77 at deficient and adequate P in limed conditions, respectively. The lowest number of nodules per plant of 5.44 and 7.55 was observed in BAM005 and BAM004 at low and adequate P in limed plots during short rain season, respectively. On average, the number of nodules increased by 6.8% and 25.8% in un-limed and limed soils due to addition of P during short rain season. Umala had higher number of nodules than Ligala in un-limed soil at both low and adequate P supply during short season. Ligala achieved relatively high number of nodules at low P in limed soil compared to Umala. On other hand, Umala exhibited a higher number of nodules than Ligala at adequate P in limed plots.

Mean number of lateral roots of bambara genotypes varied from 17.55-22.55 to 17.22 - 23.00 under low and adequate P in un-limed soil, respectively (Table 4.4). Genotypes BAM002 and BAM011with 22.55 and 23.00 had maximum number of lateral roots under low and adequate P nutrition in un-limed soil, respectively. The least number of lateral roots of 17.55 and 17.22 was observed in BAM007 and BAM009 at low and adequate P in un-limed soil, respectively. The mean number of lateral roots varied from 21.11to

21.00 - 25.66 at low and adequate P in limed soil during short rain season, respectively (Table 4.4). BAM002 with 25.66 exhibited the highest number of lateral roots at low P whileBAM010 and BAM011 had the maximum number of 25.66 at adequate P in limed conditions. The lowest number of lateral roots of 21.11 and 21.00 was observed in BAM012 and BAM007 at low and adequate P in limed soil during short rain season, respectively. Overall, the overall mean number of lateral roots increased by 4.0% and 1.5% in un-limed and limed soils upon addition of P. The Umala location had relatively higher number of lateral roots than Ligala at low P in both un-limed and limed soil conditions during short rain season. On other hand, Ligala recorded a higher number of lateral roots than Umala at adequate P supply in both un-limed and limed soil conditions during short rain season.

4.1.6 Analysis of variance of yield and yield components at Ligala in Siaya County and Umala in Busia County

Bambara groundnut cultivars significantly ($P \leq .05$) differed in plant biomass, grain yield and biological yield due to lime, phosphorus, season and site (Appendix 1).The interaction effects of genotype x lime, genotype x season, lime x season, phosphorus x season, genotype x site, phosphorus x site and season x site showed significant variation for plant biomass, grain yield and biological yield. The interactions genotype x lime x season and genotype x phosphorus x season were significant ($P \leq .05$) only for plant biomass. Interactions of genotype x phosphorus, lime x phosphorus, genotype x phosphorus x season, lime x phosphorus x season, genotype x season x site and phosphorus x season x site displayed significant ($P \leq .05$) variation for grain yield only.

On other hand, genotype x lime x season and genotype x phosphorus x season interactions were significant for biological yield only.

4.1.7 Yield attributes of bambara groundnut genotypes in un-limed and limed soil during long rain season at Ligala

The plant biomass varied from 10.65 – 18.39 gplant^{-1}to 12.79 – 20.37 gplant^{-1} under low and adequate P fertilization in un-limed soil in the long rain season at Ligala, respectively (table 4.5). The genotype BAM011 exhibited the maximum plant biomass of 18.39 gplant^{-1}at low P whereas BAM010 achieved the highest plant biomass of 20.37 gplant^{-1}at adequate P in un-limed soil during long rain season. The lowest plant biomass of 10.65 gplant^{-1}and 12.79 gplant^{-1}was achieved in BAM007 at limited and P supply, respectively. The response of bambara genotypes to liming varied from 11.19 – 18.72 g/plant to 12.11 – 22.70 gplant^{-1}at low and adequate P (Table 4.5). BAM011 exhibited the maximum plant biomass of 18.72 and 22.70 gplant^{-1}at both low and adequate P in un-limed soil, respectively. The lowest plant biomass of 11.91 and 12.11 gplant^{-1}was exhibited by BAM007 at both low and adequate P in un-limed soil, respectively. Other genotypes including BAM001, BAM002 and BAM005 also had relatively high plant biomass at low and adequate P in both un-limed and limed soil. On average, P addition increased plant biomass by 14.7% and 14.3% in un-limed and limed soil, respectively.

Table 4. 5: Mean values of plant biomass, grain and biological yields of 12 bambara genotypes grown at varying phosphorus and lime levels during long rain season at Ligala.

	Plant Biomass (gplant^{-1})				Grain Yield (gplant^{-1})				Biological Yield (gplant^{-1})			
	Lime 0tha^{-1}		Lime 4tha^{-1}		Lime 0tha^{-1}		Lime 4tha^{-1}		Lime 0tha^{-1}		Lime 4tha^{-1}	
Genotypes	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P
BAM001	15.04bd(2)	16.32ce(2)	15.22b	17.41bd	2.70ac(2)	3.33ac(3)	3.32a	4.03ab	17.68bc(2)	19.62cd(2)	18.52ab	21.42bc
BAM002	16.61ab(3)	18.60ac(3)	18.61a	19.82ab	3.01ab(3)	4.02a(3)	3.43a	4.34a	19.72ab(3)	22.53ac(3)	22.01a	24.00ab
BAM003	15.33bd(2)	17.61bd(2)	16.13ab	17.70bc	2.31bc(2)	3.14ac(2)	2.61bc	3.42ac	17.60bc(2)	20.70bd(2)	18.73ab	21.10bc
BAM004	14.38bd(2)	17.10ce(2)	13.90bc	17.30bd	2.20bc(2)	2.82bc(2)	2.42bc	3.21ac	16.63bd(2)	19.89cd(2)	16.20bc	19.73cd
BAM005	13.50de(2)	14.67eg(2)	13.58bc	16.00cd	2.52ac(2)	3.20ac(2)	2.72bc	3.41ad	16.00ce(2)	17.91de(2)	16.10bc	19.11cd
BAM006	13.62ce(2)	15.23dg(2)	13.36bc	15.33ce	2.21bc(2)	2.39c(2)	2.33bc	2.82cd	15.81ce(2)	17.60de(2)	15.61bc	18.21ce
BAM007	10.65f(1)	12.79g(1)	11.19c	12.11e	2.20bc(2)	2.53c(2)	2.31bc	3.03bd	12.78e(1)	15.28e(1)	13.36c	15.09e
BAM008	11.71ef(1)	13.50fg(1)	13.20bc	14.00de	2.22bc(2)	2.50c(2)	2.54bc	2.84cd	13.92de(1)	16.00e(1)	15.53bc	16.82de
BAM009	11.70ef(1)	13.31fg(1)	18.64a	21.78a	2.31bc(2)	2.54c(2)	2.42bc	3.38ac	14.00de(1)	16.03e(1)	20.80a	25.00ab
BAM010	16.20ac(2)	20.37a(3)	18.58a	22.62a	2.52ac(2)	3.53ab(3)	3.41a	3.94ac	18.73ac(2)	23.91a(3)	21.90a	26.51a
BAM011	18.39a(3)	19.72ab(3)	18.72a	22.70a	3.19a(3)	3.54ab(3)	3.49a	4.14ab	21.59a(3)	23.22ab(3)	22.12a	26.80a
BAM012	13.51de(1)	15.70df(2)	13.92bc	15.01ce	2.10c(1)	2.93bc(2)	2.75bc	3.29ac	15.63ce(2)	18.52de(2)	16.81bc	18.10ce
Mean	14.21	16.3	15.4	17.61	2.46	3.04	2.81	3.49	16.7	19.3	18.1	21
SEM	0.41	0.45	0.75	1	0.06	0.09	0.15	0.17	0.46	0.52	0.86	1.13

Notes: Means of individual treatment with different letter(s) in a column significantly vary as per Duncan's multiple Range test P≤0.05,–P=0kg/ha P, +P=35kg/ha P, P=Phosphorus, SEM=Standard error of mean. The indices presented in parenthesis were scored as 1 (inefficient) if the genotype had mean of <μ-SD, 2 (medium) with genotype mean of between μ−SD to μ+SD and 3 (efficient) if genotype had mean of >μ+SD, (Gill et al. 2004). The indices were used to compute total index score that could classify genotypes as efficient, medium or inefficient for Ligala site during long rain season in un-limed soil only. μ= population mean, SD= standard deviation.

Grain yield response of the bambara groundnut genotypes to P varied from 2.10–3.19 gplant^{-1} to 2.39–4.02 gplant^{-1} at low and adequate P in un-limed soil (Table 4.5). The maximum grain yield of 3.19 and 4.02 gplant^{-1}at low and adequate P supply was recorded for genotypes BAM011 and BAM002 in non-limed soil during long rain season, respectively. BAM012 had the lowest grain yield of 2.10 gplant^{-1}on the plants that receivedlow P while the lowest grain yield of 2.39 gplant^{-1}under P nutrition was detected on BAM006in un-limed soil during long rain season at Ligala. High grain yields of 2.70, 3.01 and 2.52 gplant^{-1}at low P in un-limed soil were observed BAM001, BAM002, BAM005 and BAM010, respectively. Similarly, genotypes BAM001, BAM005, BAM010 and BAM011 also produced 3.20 gplant^{-1}under adequate P fertilization in soil that was not amended with lime. The mean grain yield of the bambara groundnut genotypes ranged from 2.31-3.49 gplant^{-1} to 2.82 - 4.34 gplant^{-1} at low and adequate P in limed soil during long rain season (Table 4.5). BAM011 and BAM002 exhibited the maximum mean grain yield of 3.49 and 4.34 gplant^{-1} at low and adequate P supply during long rain season in non-limed soil, respectively. In contrast, BAM007 producedthe lowest grain yield of 2.31 gplant^{-1} at low P while lowest grain yield of 2.82 gplant-1 was observed on BAM006 grown with adequate P during long rain season. Other genotypes including BAM001, BAM005, BAM010 and BAM012 posted relatively high mean grain yield under low and adequate P fertilization in both un-limed and limed soil during long rain season in Ligala. BAM003, BAM009 and BAM012 produced grain yieldgreater than 3.29 gplant^{-1} under adequate P in limed soil. The application of P increased mean grain yield by 23.6% and 24.2% in un-limed and limed soil during long rain season.

Biological yield of bambara genotypes ranged from 12.78–21.59 gplant^{-1}to 15.28–23.91 gplant^{-1}under low and adequate P supply during the long rain season in un-limed (Table 4.5). BAM011 and BAM010 posted the highest biological yield of 21.59 and 23.91gplant^{-1}at limited and P supplementation, respectively. The least biological yield of 12.78 and 15.28 gplant^{-1}was observed in BAM007 at both low and adequate P. The average biological yield of genotypes in limed soil ranged from 13.36 – 22.12 gplant^{-1}to 15.09 – 26.80 gplant^{-1}at low and adequate P during the long rain season (Table 4.5). BAM011 had the highest biological yield of 22.12 and 26.80 g/plant at low and adequate P, respectively. BAM007 had the lowest biological yield of 13.36 and 15.09 gplant^{-1}at both low and P supply respectively. The mean overall biological yield of genotypes increased by 15.6% and 16.0% in un-limed and limed soil on addition of P.

4.1.8 Response of bambara groundnut genotypes to lime in short rain season at Ligala

Response of genotypes to P for Plant biomass in non-amended soil ranged from 10.23-13.96 to 11.93 – 15.36 gplant^{-1} under low and adequate P supply during short rain season (Table 4.6). The study revealed that genotype BAM002 and BAM011 accumulated highest biomass of 13.96 and 15.36gplant^{-1}plant biomass at low and adequate P in un-limed soil. The lowest biomass of 10.23 and 11.93 gplant^{-1} at low and adequate P was observed on genotypeBAM007 in the plots that did not received lime. Upon liming, BAM011 and BAM002 with 13.86 gplant^{-1}at low P and 16.30 gplant^{-1} at adequate P showed the highest plant biomass during short rain season, respectively (Table 4.6). Genotype BAM007 displayed the lowest biomass of 10.73 and 11.66 gplant^{-1} at both low and on soil that received adequate P and in limed soil, respectively. Application of P in

un-limed and limed soil across the genotypes increased mean biomass by 12.7% and 13.1%, respectively. The short rain season recorded the lowest plant biomass than the long rain season under low and adequate P supply in un-limed and limed soils.

The grain yield of the genotypes ranged from 1.36–2.03 gplant^{-1}to 1.63–2.53 gplant^{-1}at low and adequate P in un-limed soil during the short rain season (Table 4.6). BAM011 had the highest grain yield of 2.03 and 2.53 gplant^{-1}at low and adequate P, respectively. On the other hand, genotype BAM007 and BAM012had the lowest grain yield of 1.36 gplant^{-1}at low P whereas BAM004 achieved the lowest grain yield of1.63 gplant^{-1}atadequate P. In limed soil, the grain yield of bambara genotypes ranged from 1.56–2.10 and 1.83 – 2.72 gplant^{-1}at low and adequate P during short rain season (Table 4.6). The maximum grain yield of 2.10 and 2.72 gplant^{-1}at low and adequate P was produced by BAM002 and BAM011 in limed soil during short rain season, respectively. BAM007 and BAM008 had the lowest grain yield of 1.56 gplant^{-1} at low P while BAM004 had the lowest grain yield of 1.83 gplant^{-1} under P supply. Across the genotypes, P nutrition increased grain yield by 13.7% in short rain season in limed soil. The short season witnessed a significant reduction in grain yield compared to long rain season under low and adequate P fertilization and in both un-limed and limed soil at Ligala (Table 4.5 and 4.6).

P nutrition enhanced grain yield of the genotypes by 23.7 and 23.6% during long rain and short rain seasons at Ligala (Table 4.5 and 4.6). Phosphorus application increased seed yield per plant in all the genotypes tested. However, the degree of increase due to P levels varied depending on the genotype. P application gave the highest grain yield increase in

the genotypes BAM010 (40.1%), BAM012 (39.5%), BAM003 (35.9%) and BAM002 (33.6%) during long rain season (Table 4.5). The lowest response to P was observed for the genotypes BAM006 (8.1%), BAM009 (10.0%) and BAM008 (12.6%) during long rain season at Ligala (Table 4.6). During short rain season, Genotypes BAM001 and BAM012 gave the highest increase of 39.9 and 36.8 % during short rain season at Ligala, respectively.

Biological yield of the genotypes varied from 11.60-15.96 gplant^{-1}to 13.66-17.90 gplant^{-1}at low and adequate P in un-limed soil during short rain season (Table 4.6). BAM002 and BAM011 realized the maximum biological yield of 15.96 and 17.90 gplant^{-1}at low and adequate P in un-limed soil, respectively. The lowest biological yield of 11.60 and 13.66 gplant^{-1}were observed in BAM007at both low and P supply, respectively. The addition of P improved biological yield of the bambara genotypes from 14.08 gplant^{-1}at low P to 16.04 gplant^{-1}during short rain season. The biological yield of the genotypes ranged from 12.29 – 15.86 to 13.66 – 18.40 gplant^{-1}at low and adequate P in limed soil during short rain season, respectively (Table 4.6). BAM010 and BAM002 exhibited the maximum biological yield of 15.86 gplant^{-1}and 18.40 gplant^{-1}at low and adequate P, respectively. BAM006 and BAM007 with 12.29 and 13.66 gplant^{-1}at low and adequate P exhibited the lowest biological yield. Addition of P increased biological yield of the bambara genotypes by 12.4% in limed soil. The genotypes recorded the lower biological yield in the short rain season than the long rain season under low and adequate P fertilization in both un-limed and limed soils.

Table 4. 6: Mean values of plant biomass, yield and biological yields of 12 bambara genotypes grown at varying levels of phosphorus and lime during short rain season at Ligala.

	Plant Biomass (gplant^{-1})				Grain Yield (gplant^{-1})				Biological Yield (gplant^{-1})			
	Lime 0tha^{-1}		Lime 4tha^{-1}		Lime 0tha^{-1}		Lime 4tha^{-1}		Lime 0tha^{-1}		Lime 4tha^{-1}	
Genotypes	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P
BAM001	13.53ab	14.83ab	12.60ad	14.86ac	1.43c	2.00bd	1.69bc	2.31ab	14.96ab	16.83ac	15.36ab	16.80ab
BAM002	13.96a	14.96ab	13.26ab	16.30a	2.00a	2.33ab	2.10a	2.68a	15.96a	17.30ab	15.20ab	18.40a
BAM003	13.73ab	14.73ab	13.56ab	14.60bd	1.70ac	2.20ac	1.89ac	2.32ab	15.43ab	16.9ac	14.86ac	16.40bc
BAM004	12.76ac	14.80ab	13.16ac	14.23bd	1.50bc	1.63d	1.69bc	1.83bc	14.26bc	16.43ac	13.43ce	16.36bc
BAM005	12.73ac	13.66bd	11.80ce	13.70cd	1.56bc	1.80cd	1.63bc	1.86bc	14.30bc	15.46cd	13.92bd	15.73bc
BAM006	12.56bc	14.00ac	12.30bd	13.53cd	1.50bc	1.83cd	1.62bc	1.92ac	14.06bc	15.83bc	12.29e	15.50bc
BAM007	10.23e	11.93e	10.73e	11.66e	1.36c	1.73d	1.56c	1.89ac	11.60e	13.66e	13.23de	13.66d
BAM008	10.50e	12.20de	11.66de	13.00de	1.46bc	1.80cd	1.56c	2.03ac	11.96de	14.00de	15.36ab	15.03cd
BAM009	11.13de	12.56ce	13.53ab	14.60bd	1.53bc	1.76d	1.82ac	1.86bc	12.66de	14.33de	15.09ab	16.46bc
BAM010	13.16ac	15.03ab	13.00ad	15.63ab	1.86ab	2.36ab	2.09a	2.55ab	15.03ab	17.40ab	15.86a	16.96ab
BAM011	13.50ab	15.36a	13.86a	15.46ab	2.03a	2.53a	1.99ab	2.72a	15.53ab	17.90a	14.66ac	17.23ab
BAM012	11.90cd	14.60ab	13.03ad	14.85ab	1.36c	1.86cd	1.63bc	1.95ac	13.26cd	16.46ac	14.66ac	16.30bc
Mean	12.47	14.05	12.71	14.38	1.61	1.99	1.77	2.16	14.08	16.04	14.46	16.26
SEM	0.23	0.23	0.18	0.23	0.05	0.06	0.04	0.04	0.25	0.26	0.20	0.22

Notes: Means of individual treatment with different letter(s) in a column significantly vary as per Duncan's multiple Range test P≤0.05,–P=0kg/ha P, +P=35kg/ha P, P=Phosphorus, SEM=Standard error of mean.

4.1.9 Growth of bambara groundnut genotypes in un-limed and limed soil during long rain season at Umala

Bambara groundnut genotypes response to phosphorus in un-limed soil ranged from 10.73-17.90 gplant^{-1}to 12.76-20.40 gplant^{-1}at low and adequate P in the long rain season (Table 4.7). The lowest plant biomass of 10.73 and 12.76 gplant^{-1}at low and adequate Pwas observed by BAM007 in un-limed soil, respectively. The genotype BAM003 exhibited the maximum plant biomass of 17.90 gplant^{-1}at low P whereas BAM011 recorded the highest plant biomass of 20.40 gplant^{-1}at adequate P in un-limed soil. The response of genotypes to Pin limed soil varied from 11.66-18.60 to 12.36 – 22.83 gplant^{-1}under low and adequate P fertilization (Table 4.7). The genotype BAM009 had the highest plant biomass value of 18.60 gplant^{-1}at low P while BAM011 with 22.83 gplant^{-1}had the maximum value at adequate P in limed conditions. The lowest plant biomass of 11.66 and 12.36 gplant^{-1}at low and adequate P was achieved by BAM007 in limed soil, respectively.

Grain yield the bambara groundnut genotypes ranged from 2.08 –3.60 gplant^{-1}to 2.50–4.21gplant^{-1} at low and adequate P in un-limed during long reason (Table 4.7). The maximum grain yield of 3.60 and 4.24 gplant^{-1}under low and adequate P supply was achieved by genotype BAM002 in long rain season at Umala. Genotype BAM012 and BAM006 had the lowest grain yield of 2.10 and 2.50 gplant^{-1}at low and adequate P in un-limed soil, respectively. Apart from BAM002, other genotypes including BAM001, BAM010 and BAM011achieved high grain yields greater than 2.50 gplant^{-1}at low P and adequate P in un-limed soil at Umala. The grain yields of the genotypes oscillated from 2.29 – 3.85 gplant^{-1}to 2.93 - 4.79 gplant^{-1}under low and adequate P fertilization in limed

soil during long rain season at Umala (Table 4.7). BAM002 and BAM011 exhibited the maximum grain yield of 3.85 and 4.79 gplant^{-1}at low and adequate P supply in limed soil during long rain season, respectively. The least grain yield of 2.25 gplant^{-1}and 2.93 gplant^{-1}was observed in BAM007 and BAM006 at low and adequate P during long rain season. Aside from BAM002, other genotypes BAM001, BAM003, BAM010 and BAM011 accumulated high grain yield of over 3.13 gplant^{-1}at low P in limed soil. All genotypes with exception of BAM006 responded well to both phosphorus and lime application recording greater than 3.40 gplant^{-1}at Umala during long rain season. On average, P application increased grain yield of the genotypes by 25.9% and 28.6% in both un-limed and limed soil, respectively.

The biological yield of bambara genotypes varied from 12.83-20.23 to 15.26-23.93 gplant^{-1}under low and adequate P in un-limed soil during the long rain season at Umala (Table 4.7). Genotypes BAM002 and BAM011 exhibited the highest biological yield of 20.23 gplant^{-1}at low P and 23.93 gplant^{-1}at P supply, respectively. The lowest biological yield of 12.83 and 15.36 gplant^{-1}at low and adequate P was observed in BAM007. Biological yield of genotypes upon application of lime ranged from 13.82-22.53 to 15.46-27.43 gplant^{-1}at low and adequate P supply during the long rain season (Table 4.7). BAM010 posted maximum biological yield of 22.53 and 27.43 gplant^{-1}at both low and adequate P. The lowest biological yield of 13.82 and 15.46gplant^{-1}at both low and adequate P was realized by BAM007 in limed conditions, respectively. Overall, the application of P increased biological yield by 14.3% and 15.7% in un-limed and limed soil.

Table 4. 7: Mean values of plant biomass, grain and biological yields of 12 bambara genotypes grown at varying levels of phosphorus and lime during long rain season at Umala.

	Plant biomass (gplant^{-1})				Grain yield (gplant^{-1})				Biological yield (gplant^{-1})			
	Lime 0tha^{-1}		Lime 4tha^{-1}		Lime 0tha^{-1}		Lime 4tha^{-1}		Lime 0tha^{-1}		Lime 4tha^{-1}	
Genotypes	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P
BAM001	15.66bc	15.96bc	15.23bc	17.36bc	2.93b	3.52ab	3.36b	3.82b	18.60ab	19.13bc	18.60bc	20.93b
BAM002	16.63ab	18.56ab	16.96ab	18.23b	3.60a	4.21a	3.85a	4.70a	20.23a	22.10ab	20.82ab	21.40b
BAM003	17.90a	19.83a	15.30bc	17.56bc	2.30cd	3.13bd	3.13b	3.76b	20.20a	23.60a	18.43bd	20.70b
BAM004	16.10bc	17.73ab	14.40bd	17.13bc	2.16d	2.80cd	2.60c	3.36bc	18.26bc	21.10ab	17.00cd	19.93b
BAM005	14.76cd	14.50cd	13.63cd	15.96bc	2.16d	2.86cd	2.59c	3.49bc	16.93cd	17.70cd	16.23cd	19.13bc
BAM006	13.80d	15.06cd	13.13cd	15.66bc	2.11d	2.50d	2.29c	2.93c	15.91de	17.56cd	15.42de	18.60bc
BAM007	10.73f	12.76d	11.66d	12.36d	2.10d	2.72cd	2.25c	3.34bc	12.83g	15.26d	13.82e	15.46c
BAM008	11.86ef	13.76cd	13.06cd	15.60bc	2.16d	2.56d	2.32c	3.29bc	14.02fg	16.33cd	15.39de	18.56bc
BAM009	11.96ef	14.00cd	18.60a	21.46a	2.13d	2.82bd	2.36c	3.56bc	14.10fg	16.43cd	20.86ab	25.56a
BAM010	16.23bc	20.10a	18.56a	22.66a	2.69bc	3.43ac	3.75a	4.76a	18.92ab	23.53a	22.53a	27.43a
BAM011	16.16bc	20.40a	18.26a	22.83a	2.83b	3.53ab	3.77a	4.79a	19.00ab	23.93a	22.04a	26.23a
BAM012	13.26de	14.35cd	14.06cd	14.70cd	2.08d	2.94bd	2.53c	3.34bc	15.20ef	17.05cd	16.60ce	17.85bc
Mean	14.59	16.44	15.24	17.63	2.44	3.11	2.81	3.76	17.02	19.46	18.14	20.98
SEM	0.38	0.49	0.43	0.56	0.09	0.08	0.11	0.11	0.43	0.58	0.51	0.64

Notes: Means of individual treatment with different letter(s) in a column significantly vary as per Duncan's multiple Range test P≤0.05–P=0kg/ha P, +P=35kg/ha P and SEM=Standard error of mean

4.1.10 Performance of bambara groundnut genotypes in un-limed and limed soil during short rain season at Umala

Genotypes accumulated biomass ranging from 10.56 – 13.20 to 11.73 – 14.26 gplant^{-1} under low and adequate P fertilization in un-limed soil at Umala in the short rain season (Table 4.8). The genotypes BAM010 and BAM011 accumulated the highest plant biomass of 13.20 and 14.26 gplant^{-1}at low and adequate P in the short rain season, respectively. The lowest plant biomass of 10.56 and 11.73 gplant^{-1}at both low and adequate P was achieved by BAM007. The lowest plant biomass of 11.16 and 11.60 gplant^{-1}under low and adequate P was achieved in BAM007 in limed soil at Umala during short rain season (Table 4.8). Genotype BAM002 with 13.43 gplant^{-1}had the maximum plant biomass at low P whereas BAM011 posted the lowest plant biomass of 15.83 gplant^{-1}at adequate P. The short rain season produced the lowest plant biomass than long rain season at Umala (Table 4.8). High plant biomass of 14.05 gplant^{-1}was observed at Umala compared to 13.97 gplant^{-1}at Ligala across all treatments. On average, the number of lateral roots significantly and positively ($r=0.74^{*}, P\leq0.05$) correlated with plant biomass.

Table 4. 8: Mean values of plant biomass, grain and biological yieldsof 12 bambara genotypes grown at varying levels of phosphorus and lime during short rain season at Umala.

	Plant biomass (gplant^{-1})				Grain yield (gplant^{-1})				Biological yield (gplant^{-1})			
	Lime 0tha^{-1}		Lime 4tha^{-1}		Lime 0tha^{-1}		Lime 4tha^{-1}		Lime 0tha^{-1}		Lime 4tha^{-1}	
Genotypes	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P
BAM001	12.20ac	12.36bc	11.76cd	12.20cd	1.80bc	2.17a	1.96ab	2.33ac	14.00bd	14.33ab	13.73bc	14.33bc
BAM002	11.90ac	13.40ac	13.43a	13.63bc	2.20a	2.56a	2.29a	2.78a	14.10ad	15.40a	15.62a	15.86b
BAM003	13.10ab	13.93ab	12.36ac	12.73bd	1.60cd	2.06b	1.78bc	2.41ae	14.80ac	16.00a	14.04bc	14.83bc
BAM004	12.13ac	13.00ac	13.26ab	13.20bc	1.33df	1.80bd	1.39c	2.02be	13.46de	14.96ab	14.66ab	15.13b
BAM005	12.43ac	12.90ac	12.56ac	12.96bd	1.39df	1.82bc	1.50c	2.11ae	13.82bd	14.83ab	14.06bc	15.16b
BAM006	12.70ab	12.83ac	12.06cd	13.23bc	1.23ef	1.73cd	1.32c	2.12ae	13.93bd	14.90ab	13.39cd	15.53b
BAM007	10.56d	11.73c	11.16d	11.60d	1.29df	1.62cd	1.43c	1.86ce	11.85f	13.46b	12.60d	13.46c
BAM008	11.86bc	12.63ac	11.93cd	13.36bc	1.17f	1.40d	1.30c	1.72e	13.03de	14.70ab	13.53cd	15.50b
BAM009	11.26cd	12.43bc	12.50ac	13.86b	1.26ef	1.53cd	1.30c	1.76e	12.52ef	14.30ab	13.80bc	15.63b
BAM010	13.20a	13.93ab	13.26ab	14.10b	2.06ab	2.43ab	2.23ab	2.27ad	15.16a	15.73a	15.30a	15.83b
BAM011	12.90ab	14.26a	13.20ab	15.83a	2.03ab	2.51a	2.21a	2.79a	14.93ab	16.06a	15.33a	18.26a
BAM012	12.16ac	13.05ac	12.26bd	13.30bc	1.53ce	1.74bd	1.60bc	2.10ae	13.70cd	14.95ab	13.86bc	15.20b
Mean	12.2	13.06	12.48	13.36	1.57	1.90	1.70	2.19	13.77	15.00	14.16	15.40
SEM	0.15	0.17	0.14	0.20	0.06	0.05	0.06	0.05	0.17	0.18	0.16	0.22

Notes: Means of individual treatment with different letter(s) in a column significantly vary as per Duncan's multiple Range test P≤0.05,–P=0kg/ha P, +P=35kg/ha P, P=Phosphorus, SEM=Standard error of mean.

The yields of the genotypes ranged from 1.17–2.20to 1.40-2.56 gplant^{-1}at low and adequate P in un-limed soil during short rain season (Table 4.8). BAM002 had the highest grain yield of 2.20 g/plant and 2.56 gplant^{-1}at both low and adequate P in the short rain season. The lowest grain yield of 1.17 and 1.40 gplant^{-1}at both low and adequate P was observed in BAM008. Four genotypes BAM001, BAM003, BAM010 and BAM011 also posted high grain yields at both low and adequate P in un-limed soil. The genotypes BAM002 and BAM011 produced maximum grain yield of 2.29 gplant^{-1}and 2.79 gplant^{-1}at low and adequate P in limed soil during short rain season, respectively. The least grain yield of 1.30 and 1.72 gplant^{-1}was reported in BAM009and BAM008 under low and adequate P fertilization in limed soil during short rain season at Umala, respectively. Apart from BAM002, other genotypes that performed well under low P in limed soil were BAM001, BAM003, BAM010 and BAM011. All genotypes with exception of BAM007, BAM008 and BAM009 performed well under phosphorus and lime application during short rain season at Umala. The short season significantly ($P \leq 0.05$) reduced grain yields of the genotypes than the long rain season in both un-limed and limed soil at Umala. Across all treatments, Umala had the highest grain yields than Ligala. The number of lateral roots on average significantly (*r=0.91**) correlated with grain yields. Plant biomass also significantly (*r=0.79**) correlated with grain yield During long and short rain seasons at Umala Average grain yield increased by 27.5 and 21.0%, respectively (Table 4.7 and 4.8). Genotypes BAM012 and BAM003 gave maximum increase in grain yield of 41.2% and 36.1% in long rain season (Table 4.7). On the other hand, genotype BAM002 with 16.9% increase in grain yield was the least during long rain season. BAM006 and BAM010 had the maximum and minimum grain

yield increase due to P fertilization (Table 4.7). Overall, the response of BAM006, BAM007 and BAM008 to P improved in short rain season compared to long rain season. Genotypes BAM010, BAM012, BAM003, BAM002 and BAM001were more responsive to the added P in the soil.

Biological yield of the genotypes ranged from 11.85-15.16 to 13.46-16.06 gplant^{-1}at low and adequate P during short rain season (Table 4.8). The maximum biological yield of 15.16 and 16.06 gplant^{-1}was observed in BAM010 and BAM011 at low and adequate P in un-limed soil, respectively. The lowest biological yield of 11.85 and 13.46 gplant^{-1}was realized in BAM007 at both low and adequate P in un-limed soil. BAM002 had highest biological yield of 15.62 g/plant at low P while BAM011 recorded maximum value of 18.26 gplant^{-1}at adequate P in limed soil (Table 4.8). The lowest biological yield of 12.60 and 13.46 gplant^{-1}was exhibited by BAM007 at both low and adequate P.

4.1.11 Response of bambara genotypes to lime application at different sites during long and short rain seasons

Liming improved grain yield of the genotypes across the sites and seasons. At Ligala, Grain yield increased by 14.2 and 9.9% due to amelioration of soil with lime during long and short rain seasons at Ligala, respectively. Genotype BAM010 and BAM012 exhibited highest increase in grain yield of 35.3% and 31.0% when grown in limed soil compared to un-limed soil during long rain season at Ligala (Table 4.5). BAM009 had the lowest increase of 4.8% under lime conditions during long rain season. BAM012, BAM009 and BAM001 showed high increase of 19.9%, 19.0% and 18.2% respectively (Table 4.6) in the short rain season. The minimum grain yield increase of 4.5% was

observed in BAM005 during short rain season at Ligala (Table 4.6). An overall increase of 15.2% and 8.3% was observed during long and short rain seasons due to liming effect at Umala, respectively. Genotypes BAM010, BAM003 and BAM011 had the highest grain yield increase of 39.4%, 36.1% and 33.2% owing to amendment of soil with lime, respectively (Table 4.7). The least grain yield increase due to liming was observed on genotype BAM007 (7.1%), BAM008 (7.4%) and BAM006 (8.5%) during long rain season (Table 4.7). Genotypes BAM003 (11.3%) and BAM009 (3.2%) had the maximum and minimum grain yield increase due to liming during short rain season (Table 4.8).

4.12 Effects of lime and phosphorus for grain yield of bambaragenotypes

Average grain yield increase of 14.8% and 8.5% was observed during long and short rain seasons at Ligala, respectively. Genotypes BAM009, showed a high grain yield increase due to P and lime interaction of 33.1% (Table 4.5). The lowest increase of 6.6% in seed yield was observed in BAM005. In short rain season, BAM001 and BAM005 with 15.5% and 3.3% posted the highest and lowest increase in seed yield due to P and lime interaction. An average of 20.9% and 15.3% increase in grain yield was observed due P and lime interaction in long and short rain seasons at Umala, respectively. Genotypes BAM011 and BAM001 produced the maximum and minimum grain yield increase of 35.7% and 8.5%during long rain season, respectively. Similarly, BAM008 and BAM001 had the highest and lowest grain yield increases due to lime and P interaction during short rain season. Overall, BAM009, BAM006, BAM007 and BAM008 benefited more when grown in limed soil with P supplementation compared to growing either under lime or P alone.

4.2: Determination of P acquisition and use efficiencies of the Bambara genotypes

4.2.1: Phosphorus accumulation in plant biomass and Grain yield

Phosphorus accumulation (PA) in the plant biomass, grain yield and biological yield was significantly ($P \leq 0.05$) influenced by genotype at different level of P (Table 4.9). Significant ($P \leq 0.05$) genotypes x phosphorus interaction effects were observed for PA in biomass, grain and TPA. PA in the plant biomass ranged from 27.17-46.5 mgplant^{-1}at low P to 49.49-78.93mgplant^{-1}at adequate P. The highest PA at limited and adequate P supply was observed by BAM002 and BAM011 respectively. On other hand, genotype BAM008 with 27.17 mgplant^{-1}and genotype BAM012 with 49.40 mgplant^{-1}had the lowest PA in the biomass at low and adequate P respectively. P application resulted to an increasing trend in the plant biomass P accumulation. Plant biomass accumulated more phosphorus compared to grain P. The highest amount of PA of 18.02mg/plan was exhibited by BAM002 while BAM006 had the least amount of 8.91 mgplant^{-1}at low P. Under P nutrition, BAM002 exhibited the maximum PA in the grain of 23.78mgplant^{-1}whereas BAM004 had the least grain PA of 13.01 mgplant^{-1}.

Across genotypes, P accumulation in the grain increased from 10.9 mgplant^{-1}in low P to 16.8 mg/plant at adequate P, translating to 53.66% increase in grain P upon application. Total P accumulation in biomass (TPA) ranged from 37.03-64.52 mgplant^{-1}at low P to 62.98-99.11 mgplant^{-1}when adequate P was supplied. Genotype BAM002 exhibited the highest TPA of 64.52mgplant^{-1}at low P while BAM011 registered the highest TPA of 99.11mg/plant in adequate P. BAM008 and BAM004 produced the lowest TPA of 37.03 mgplant^{-1}and 62.98 mgplant^{-1}at limited and adequate P, respectively. Overall, the TPA

increased from 46.6 mgplant^{-1}in low P to 74.49 mgplant^{-1}at adequate P. Further analysis revealed a strong positive correlation ($P \leq 0.05$) between total phosphorus accumulation with grain yield, plant biomass and biological yield at both 0 kgha^{-1}and 35 kgha^{-1} P (Table 4.10). Genotypes BAM011, BAM010 and BAM002 that accumulated high amount of P at both levels of P also had high grain yield. A significant positive correlation was also observed between plant biomass (r = 0.80*) and grain yield (r = 0.99*) at 0kgha^{-1} and 35kgha^{-1} P levels, respectively.

4.2.2 Phosphorus stress factor

Bambara genotypes significantly ($P \leq 0.05$) differed at low P for relative suppression in grain yield (Table 4.9). Phosphorus stress factor (PSF) varied from 7.53 to 28.61% among the tested genotypes at low P. All tested genotypes were responsive to P supplementation recording PSF greater than7.53%. Genotypes BAM010 and BAM012 were the most responsive genotypes showed PSF of 28.61% and 28.33%, respectively. The least reduction in grain yield was observed in BAM006 with PSF value of 7.53%.

Table 4. 9: Mean values of phosphorus accumulation (mgplant^{-1}) in plant biomass, grain and index scores of 12 Bambara genotypes grown at low and adequate P without lime application during long rain season at Ligala in Siaya.

Genotype	PA Plant biomass		PA Grain		TPA		PSF (%)
	-P	+P	-P	+P	-P	+P	
BAM001	37.22bc(2)	54.04c(2)	10.01c(2)	18.48bd(2)	47.23de(2)	72.53b(2)	18.92ad(2)
BAM002	46.50a(3)	67.55b(2)	18.02a(3)	23.78a(2)	64.52a(3)	91.34a(3)	25.12ac(2)
BAM003	39.75b(2)	56.23c(2)	10.49bc(2)	15.03ef(2)	50.24cd(2)	71.27bc(2)	26.43ab(3)
BAM004	33.62ce(2)	49.96c(2)	9.29c(2)	13.01f(1)	42.91ef(2)	62.98c(2)	21.99ad(2)
BAM005	31.10de(2)	50.72c(2)	10.83bc(2)	15.06ef(2)	41.95f(2)	65.79bc(2)	21.58ad(2)
BAM006	31.83de(2)	52.32c(2)	8.91c(2)	15.89df(2)	40.75fg(2)	68.22bc(2)	7.53d(1)
BAM007	34.71cd(2)	52.74c(2)	9.81c(2)	14.93ef(2)	44.52ef(2)	67.68bc(2)	13.04ad(2)
BAM008	27.17f(2)	50.55c(2)	9.86c(2)	14.45ef(2)	37.03g(1)	65.03bc(2)	11.20bd(2)
BAM009	31.95de(2)	54.95c(2)	9.90c(2)	16.76ce(2)	41.86f(2)	71.72bc(2)	9.06cd(1)
BAM010	39.96b(2)	74.59a(3)	12.33b(2)	19.18bc(2)	52.29bc(2)	93.77a(3)	28.61a(3)
BAM011	43.77a(2)	78.93a(3)	11.19bc(2)	20.18b(2)	54.96b(3)	99.11a(3)	9.89cd(1)
BAM012	30.35ef(2)	49.49c(2)	10.58bc(2)	14.94ef(2)	40.93fg(2)	64.43bc(2)	28.33a(3)
Mean	35.66	57.68	10.94	16.81	46.60	74.49	18.44
SE	1.68	2.93	0.69	0.88	2.23	3.66	2.00
F-values							
G	33.59*		21.96*		41.88*		131.36*
P	837.62*		334.47*		1018.02*		
G×P	5.99*		2.44*		6.21*		

Notes: Means of individual treatment with different letter(s) in a column significantly vary as per Duncan's multiple Range test $P \leq 0.05$,–P=0kg/ha P, +P=35kg/ha P, SEM=Standard error of mean, G = genotype, P = phosphorus levels, *= Significant at $P \leq 0.05$, NS=Non-significant at $P \leq 0.05$Index scores are represented in parenthesis

Table 4.10: Pearson correlation matrix showing relationship between total phosphorus accumulation and grain yield, plant biomass and biological yield for 12 Bambara genotypes at low and adequate P without lime applicationduring long rain season at Ligala in Siaya.

Un-limed									
	PB,-P	BY,-P	TPA,-P,	GY,-P,		PB,+P,	BY,+P,	TPA,+P,	GY,+P,
PB,-P	1				PB,+P,	1			
BY,-P,	0.99*	1			BY,+P,	0.99*	1		
TPA,-P	0.77*	0.79*	1		TPA,+P	0.79*	0.80*	1	
GY,-P,	0.80*	0.85*	0.75*	1	GY,+P,	0.80*	0.85*	0.76*	1

*Correlation is significant at the $P \leq 0.05$; PB=Plant Biomass BY=Biological yield, GY=Grain yield, TPA=Total Phosphorus Accumulation, -P=0kgha^{-1} P, +P=35kgha^{-1} P.

4.2.3 Phosphorus efficiency parameters

Phosphorus harvest index (PHI) was significantly ($P \leq 0.05$) influenced by genotype and genotype xPlevel while P level alone had no effect (Table 4.11). Genotypes showed variation in abilities to convert accumulated P to grain P. The PHI increased with application of P application and ranged from 15.01%in BAM004 to 24.03% in BAM001 at low P. Under P nutrition, PHI varied from 20.42% in BAM011 to 26.01% in BAM002. Genotypes BAM010 and BAM011 showed higher PHI at low P compared to adequate P level. On average, genotypes with a high PHI produced more grain yield than those having low PHI.

Table 4. 11: Mean values of various phosphorus efficiency indices and index scores of 12 Bambara genotypes grown at low and adequate P without lime application during long rain season at Ligala in Siaya.

Genotype	PHI (%)		**PPEI (**g of grain mg^{-1} P)		**PBER (**g biomass mg^{-1} P)	
	P-	P+	P-	P+	P-	P+
BAM001	24.03a(2)	25.50ab(3)	52.22ac(2)	53.26a(3)	346.49cd(2)	273.91d(2)
BAM002	22.61ab(3)	26.01a(3)	50.36bd(2)	45.59bd(2)	299.11i(1)	236.45j(2)
BAM003	20.00bc(2)	21.10c(2)	45.73cd(2)	43.98bd(2)	350.21c(2)	265.15f(2)
BAM004	15.01cd(2)	20.80c(2)	50.46bd(2)	44.69bd(2)	342.58de(2)	286.72b(2)
BAM005	20.12bc(2)	22.89ab(2)	54.24ac(2)	42.37d(2)	368.94b(3)	293.89a(2)
BAM006	19.20bc(2)	23.04ab(2)	52.68ac(2)	48.93ac(2)	335.46fg(2)	259.37h(2)
BAM007	17.73cd(2)	22.10bc(2)	46.94cd(2)	46.21ad(2)	337.76ef(2)	270.42e(2)
BAM008	18.52bd(2)	22.20bc(2)	42.50d(2)	39.06d(1)	376.15a(3)	262.40g(2)
BAM009	17.71cd(2)	23.31ab(2)	51.09bd(2)	46.81ac(2)	329.56gh(2)	258.43h(2)
BAM010	21.80ab(2)	20.52c(2)	56.11a(3)	48.06ac(2)	348.60cd(2)	262.31g(2)
BAM011	21.71ab(2)	20.42c(2)	56.75ab(3)	50.26ab(3)	327.43h(2)	244.82i(1)
BAM012	18.10cd(2)	23.21ab(2)	61.11a(3)	46.15ad(2)	369.80b(3)	277.76c(2)
Mean	19.71bc	22.59bc	51.70ac	46.28ad	344.35de	266.00f
SE	0.74	0.53	1.43	1.06	6.15	4.66
F-values						
G	5.34*		5.53*		259.98*	
P	3.20NS		28.23*		15119.60*	
G×P	2.12*		1.68NS		33.76*	

Notes: Means of individual treatment with different letter(s) in a column significantly vary as per Duncan's multiple Range test $P \leq 0.05$,–P=0kg/ha P, +P=35kg/ha P, SEM=Standard error of mean, G = genotype, P = phosphorus levels, *= Significant at $P \leq 0.05$, NS=Non-significant at $P \leq 0.05$Index scores are represented in parenthesis
PHI=Phosphorus Harvest Index, PPEI=Phosphorus Physiological Efficiency Index, PBER=Phosphorus Biological Efficiency Ratio

Phosphorus physiological index in grain (PPEI) varied significantly among genotypes at each level of P (Table 4.11). The genotypes BAM001and BAM012 exhibited the highest PPEI value of 53.26g of grain mg^{-1}P and 61.1 g of grain mg^{-1}P under adequate and low P, respectively. The lowest PPEI of 39.06 g grain mg^{-1}P and 42.5g grain mg^{-1}P were recorded by BAM008 in adequate and low P, respectively. With the exception of

BAM001, the rest of the genotypes showed higher values of PPEI at limited P compared to adequate P. From the results, PPEI in grain was higher at limited P than adequate P supply. The mean PPEI increased from 51.7 g of grain mg^{-1} P in plants grown at low P to 46.28 g of grain mg^{-1} P under adequate P application.

Phosphorus biological yield efficiency ratio (PBER) differed significantly ($P \leq 0.05$) between the two levels of P (Table 4.11). Interaction between genotypes and phosphorus levels for PBER varied significantly ($P \leq 0.05$). Bambara genotypes recorded high PBER values at low P, ranging from 299.11-376.15g biomass mg^{-1}P. With supplemental P, PBER values decreased to 236.45-293.89g biomass mg^{-1}P (Table 4.11). The maximum PBER value of 376.15 g and 293.89g biomass mg^{-1}P was observed in genotypes BAM008 and BAM005 at limited and adequate P, respectively. Conversely, BAM002 recorded the least PBER values of 299.1 and 236.5 g biomass mg^{-1}P in both low and adequate P. BAM008 and BAM012 recorded the highest PBER values but registered low biological yield at low P.

4.2.5 Grouping of Bambara groundnut genotypes

Bambara genotypes showed variation with respect to each trait assessed in this study. Based on total index score (TIS) derived from plant biomass, grain yield, biological yield, PA in plant biomass, PA in the grain, TPA, PSF, PHI, PPEI and PBER (Table 4.12), bambara genotypes were classified into efficient, medium and in-efficient. BAM002 and BAM011 exhibited the highest index score of 26 and 24, respectively at low P (Table 4.12) and were on this basis classified as P-efficient at low P. Three genotypes: BAM007, BAM008 and BAM009 with an index score of 18, 18 and 17 respectively, were grouped as P inefficient at low P conditions. The remaininggenotypes were classified as medium

at low P. On addition of P, BAM002, BAM010 and BAM011 recorded the highest total index score of 23 and subsequently were classified as P efficient. BAM008 recorded the lowest total index score of 15 and was classified as P inefficient at adequate P. The remaining 8 genotypes were grouped as medium in performance at adequate P.

Table 4. 12: Classification of 12 Bambara genotypes within three performance levels based on total index scores grown at low and adequate P without lime application during long rain season at Ligala in Siaya.

Genotype	Low P		Adequate P	
	TIS	Grading	TIS	Grading
BAM001	20	M	21	M
BAM002	26	E	23	E
BAM003	21	M	18	M
BAM004	20	M	17	M
BAM005	21	M	18	M
BAM006	19	M	18	M
BAM007	18	I	16	M
BAM008	18	I	15	I
BAM009	17	I	16	M
BAM010	22	M	23	E
BAM011	24	E	23	E
BAM012	21	M	18	M

Notes: The genotypes were classified as efficient if they had mean of >μ+SD, medium with mean of between μ−SD to μ+SD and inefficient if having mean of <μ-SD (Gill et al. 2004). Classification was based on total index score (TIS) derived from the following traits: phosphorus stress factor, plant biomass, grain yield, biological yield, Phosphorus accumulation in plant biomass, grain phosphorus accumulation, total phosphorus accumulation, phosphorus biological efficiency ratio (PBER), phosphorus harvest index (PHI) and physiological phosphorus efficiency index (PPEI).E=efficient, M=Medium and I=In-efficient in P use efficiency with respect to the parameters measured.

The relationship between TPA and grain yield was used to classify Bambara genotypes into four groups; high grain yield-high phosphorus acquisition (HGY-HP), medium grain yield-medium phosphorus acquisition (MGY-MP), medium grain yield-low Phosphorus acquisition (MGY-LP) and low grain yield-medium phosphorus acquisition (LGY-MP)(Figure 4.1). BAM002 and BAM011 were grouped as HGY-HP while BAM008 was placed in MGY-LP at low P supply. Similarly, BAM012 was grouped as LGY-MP.BAM008 in group MGY-LP is P-inefficient based on the acquisition of native P. The rest of the genotypes (8) were grouped into MGY-MP showing a medium potential in both P acquisition and utilization in producing grains under low P conditions. With adequate P, BAM002, BAM010 and BAM011 were placed in HGY-HP. Four genotypes BAM006, BAM007, BAM008 and BAM009 were classified in LGY-MP. Five genotypes were grouped in MGY-MP showing their medium potential in both P accumulation and grain yield at adequate P (Figure 4.2).

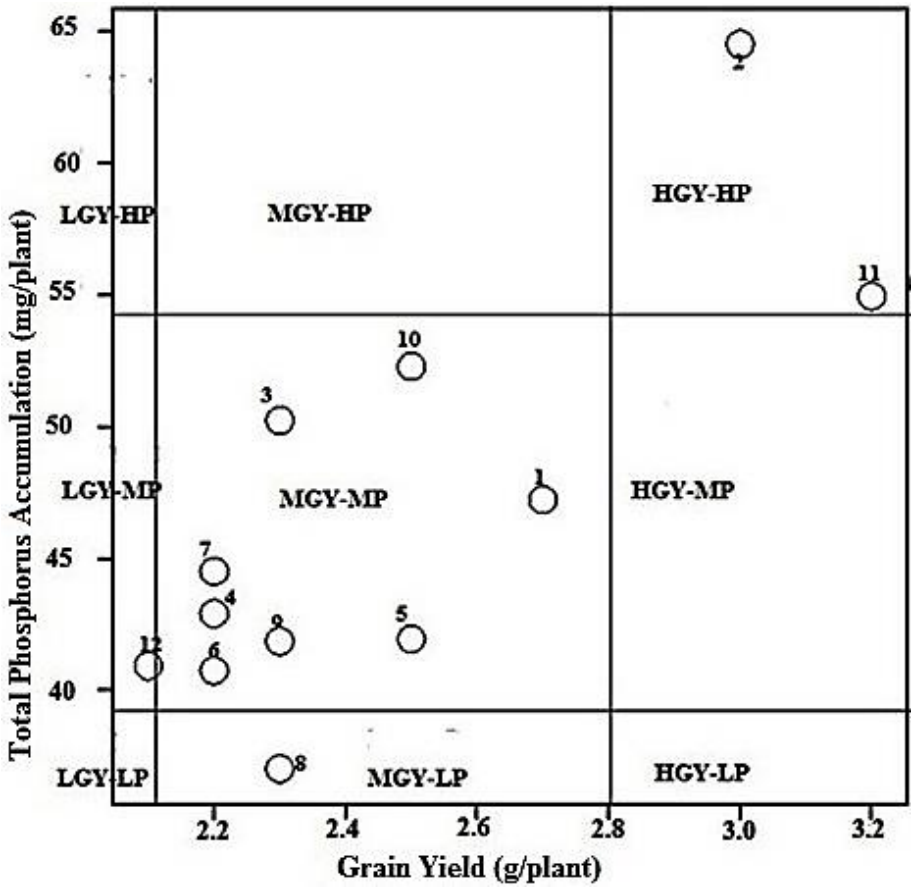

Figure 4. 1: **Distribution of 12 Bambara genotypes with respect to their grain yield and total P accumulation at low P.**

LGY=Low grain yield, MGY=Medium grain yield, HGY=High grain yield LP=Low Phosphorus, MP=Medium Phosphorus, HP=High Phosphorus. Numbers in the figure represent names of genotypes studied in the experiment as 1=BAM001, 2=BAM002, 3=BAM003, BAM=004, 5=BAM005, 6=BAM006, 7=BAM007, 8=BAM008, 9=BAM010, 10=BAM010, 11=BAM011, 12=BAM012

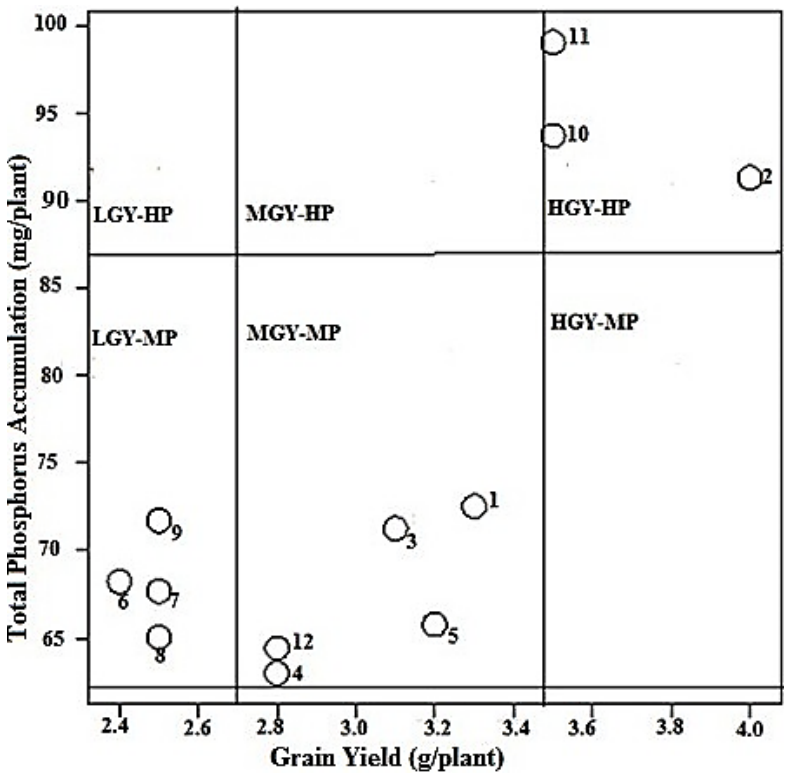

Figure 4. 2: **Distribution of 12 Bambara genotypes with respect to their grain yield and total P accumulation at adequate P.**
LGY=Low grain yield, MGY=Medium grain yield, HGY=High grain yield LP=Low Phosphorus, MP=Medium Phosphorus, HP=High Phosphorus. Numbers in figure represent names of genotypes studied in the experiment as 1=BAM001, 2=BAM002, 3=BAM003, BAM=004, 5=BAM005, 6=BAM006, 7=BAM007, 8=BAM008, 9=BAM010, 10=BAM010, 11=BAM011, 12=BAM012

Principal component analysis (PCA) of the 12 Bambara cultivars under low P showed 85.08% variation in PC1 and PC2 (Table 4.13). Grain yield, PA in the grain and TPA contributed the most to variation in 67.71% of PC1 at low P. PC2 correlated positively with PPEI and negatively with PA for plant biomass at low P (Table 4.13). PCA bi-plot groupings of Bambara genotypes and parameters at low P is shown in Figure 4.3. Bambara genotypes BAM002, BAM010 and BAM011 were grouped together based on grain yield, PA in the grain, TPA, biological yield, plant biomass, and PA in plant biomass. BAM003, BAM004, BAM005, BAM006, BAM007, BAM008 and BAM009

were ranked together based on PBER. On other hand, BAM001 was grouped alone based on PHI and PPEI. PCA of the 12 Bambara genotypes under adequate P exhibited79.04% variation in PC1 and PC2 (Table 4.13, Figure 4.4). PA in plant biomass and TPA contributed the most to variation in PC1 at adequate P, whereas, PC2 correlated positively with PBER and negatively with PHI (Table 4.13). PCA bi-plot groupings placed BAM002, BAM010 and BAM011 again clustered together at adequate P based on plant biomass, biological yield, PA in plant biomass, TPA, grain yield and PA in the grain. BAM004, BAM005, BAM006, BAM007, BAM008 and BAM009 were categorized together based on PBER at adequate P. BAM001 and BAM003 were grouped alone based on PPEI and PHI at adequate P, respectively.

Table 4. 13: Principal component analysis (PCA) of yield, phosphorus accumulation and phosphorus efficiency indices of 12 bambara genotypes grown under low and adequate P without lime application during long rain season at Ligala in Siaya.

.	Principal Components			
	PC-1	PC-2	PC-1	PC-2
Parameters	Low phosphorus		Adequate phosphorus	
Plant Biomass	0.35818	-0.1414	0.076	0.154
Grain Yield	0.38537	0.15368	0.140	0.027
Biological Yield	0.37073	-0.0985	0.090	0.181
PA in Plant Biomass	0.38621	-0.1596	0.431	0.446
PA in Grain	0.39067	0.09479	0.124	0.024
TPA	0.39602	-0.0864	0.554	0.470
PBER	-0.2513	0.40583	-0.689	0.711
PHI	0.22545	0.47034	0.001	-0.082
PPEI	0.11479	0.72031	0.063	0.106
Eigen values	6.094	1.563	5.597	1.516
% of Variance	67.71	17.37	62.19	16.849
Cumulative %	67.71	85.08	62.19	79.039

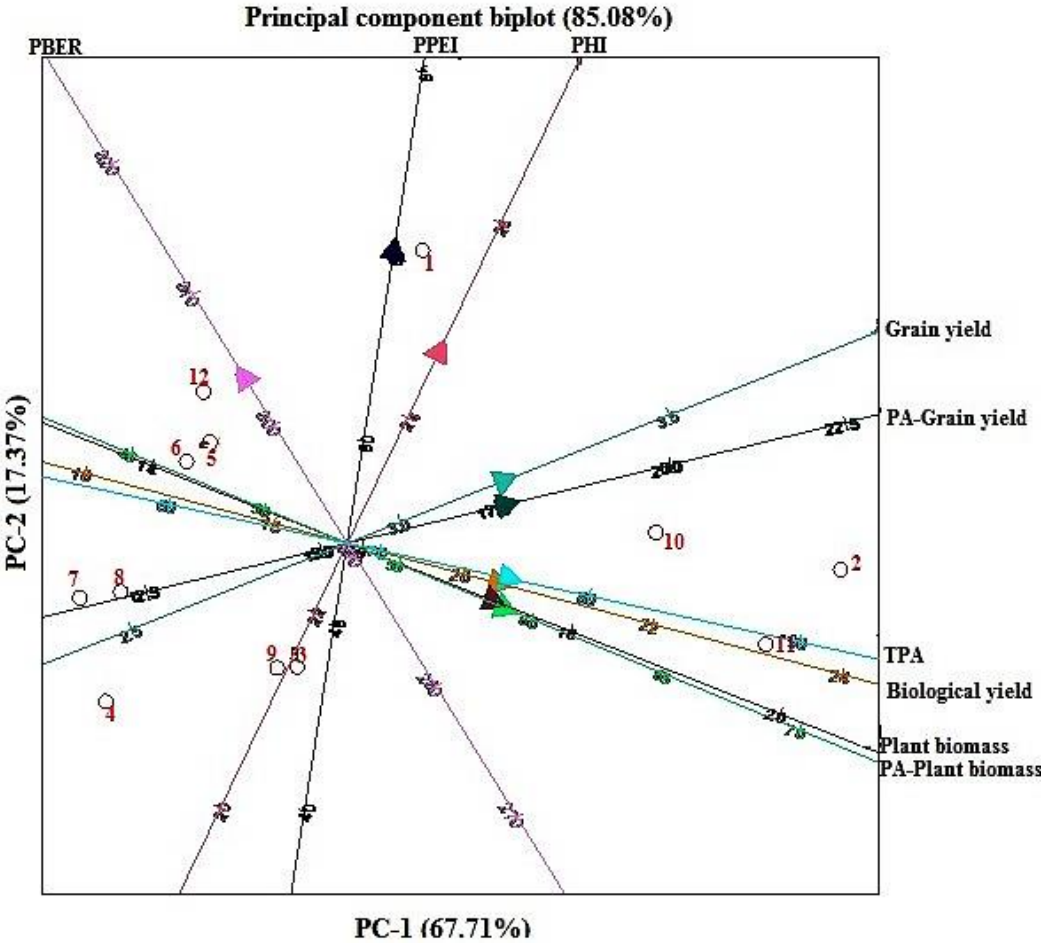

Figure 4. 3: Principal component analysis biplot of yield, phosphorus accumulation and phosphorus efficiency indices of 12 bambara genotypes grown at low P.
PHI=Phosphorus Harvest Index, PPEI=Phosphorus Physiological Efficiency Index, PBER=Phosphorus Biological Efficiency Ratio. Numbers in figure represent genotypes as follows: 1=BAM001, 2=BAM002, 3=BAM003, BAM=004, 5=BAM005, 6=BAM006, 7=BAM007, 8=BAM008, 9=BAM010, 10=BAM010, 11=BAM011, 12=BAM012.

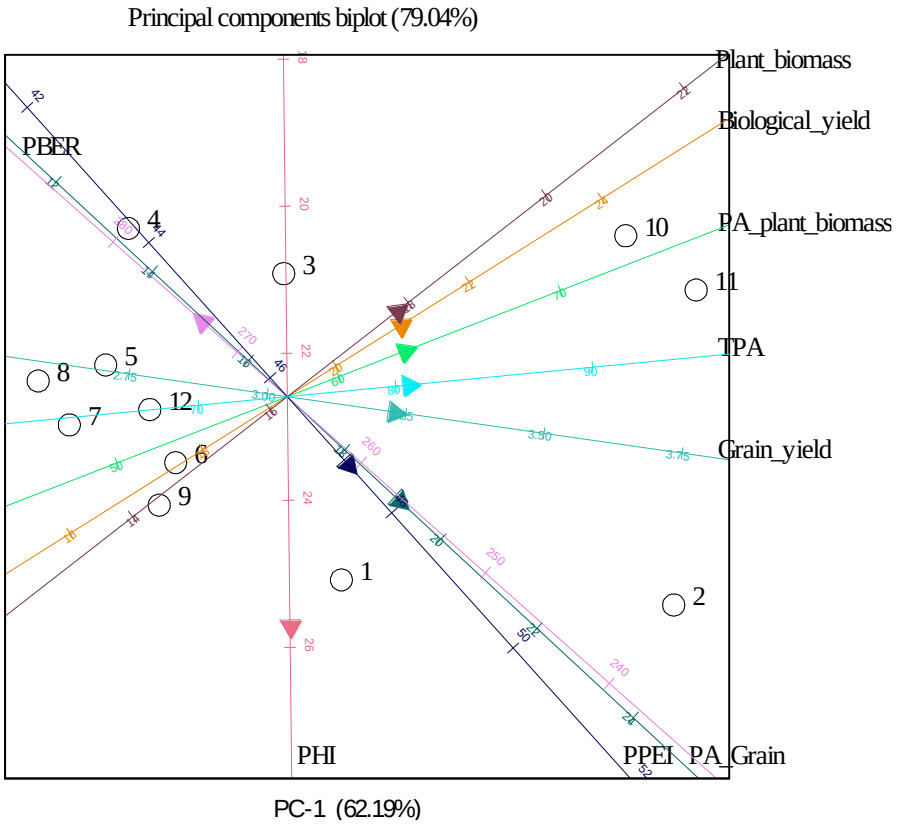

Figure 4. 4: **Principal component analysis biplot of yield, phosphorus accumulation and phosphorus efficiency indices of 12 bambara genotypes grown at adequate P. PHI=Phosphorus Harvest Index, PPEI=Phosphorus Physiological Efficiency Index, PBER=Phosphorus Biological Efficiency Ratio. Numbers in figure represent genotypes as follows: 1=BAM001, 2=BAM002, 3=BAM003, BAM=004, 5=BAM005, 6=BAM006, 7=BAM007, 8=BAM008, 9=BAM010, 10=BAM010, 11=BAM011, 12=BAM012.**

4.3 Traits underlying differential phosphorus use efficiency in bambara genotypes

4.3.1 Rooting depth, branching number, branching density and volume

Bambara groundnut genotypes showed significant (*P≤0.05*) variation for tap root length (TRL) under varying levels of P at 35 days after emergence (DAE) (Table 4.14). The TRL of the genotypes increased when P was applied the interaction effect of genotype and phosphorus was not significant).TRL ranged from 35.66-53.00 cm at P0µMto 42.66-53.50 cm at P160µM, respectively. Genotype BAM010 with 53.00 cm had the longest TRL while BAM005 was shortest with 35.66 cm at P0µM. BAM002 and BAM005with 53.50 and 42.66 cm had the longest and shortest TRL at P160µM, respectively. Overall, across the tested genotypes; the root depth was limited to a depth of 30-60 cm.

The bambara groundnut genotypes root branching numbers and branching density was exclusively done for first-order lateral roots. Branching numbers (BN) of the bambara genotypes varied significantly (*P≤0.05*) due to varying levels of P. However, the interaction between genotypeandphosphorusfor BN was not significant (Table 4.14). The highest BN per plant of 98.00 and 110.33was observed in BAM002 and BAM011at P0µM and P160µM respectively. The lowest BN of 8.00 in P0µM and 67.00 was achieved inBAM006 under P0µM and P160µM, respectively. Overall, BN increased from 80.26-87.88 across the genotypes, representing 9.5% upon P nutrition.

Table 4. 14: Mean values of tap root length (TRL), branching number (BN), branching density (BD), root volume (RV), shoot length (SL), and tap root length: shoot length (RL: SL) ratio of bambara genotypes grown at P0µM and P160µM in sandculture at 35 days after emergence (DAE).

	TRL(cm)		BN		BD		RV		SL		TRL:SL Ratio	
Genotypes	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P
BAM001	52.00a	52.50a	85.00ab	92.66b	2.08ab	1.62ac	4.60ab	4.06b	24.66c	28.50a	2.11a	1.85a
BAM002	49.33ab	53.50a	98.00a	108.83a	2.23a	1.83ab	5.13a	5.41a	28.50ac	29.66a	1.73b	1.78ab
BAM003	43.83c	46.66bc	75.00bd	80.0bd	1.83cd	1.82ab	3.81bc	3.76b	26.66bc	29.83a	1.77b	1.50bd
BAM004	39.33cd	46.16bc	81.33bc	87.16bc	1.80bc	1.89a	3.68bc	4.13b	29.83ab	31.00a	1.27de	1.59ad
BAM005	35.66d	42.66c	65.00de	78.33bd	1.81bc	1.80ab	3.40bc	3.76b	30.33a	31.66a	1.12e	1.40d
BAM006	42.16bd	43.16c	58.00e	67.50cd	1.39d	1.56bc	3.53bc	3.83b	30.33a	31.83a	1.34de	1.41cd
BAM007	41.16bd	48.33ac	69.00ce	84.00bc	1.66cd	1.73ac	3.25c	4.11b	30.33a	30.50a	1.36ce	1.47cd
BAM008	38.83cd	50.00ab	74.66bd	76.00cd	1.95ac	1.49c	3.28c	3.76b	30.66a	31.83a	1.21de	1.69ad
BAM009	39.66cd	44.00bc	80.16bd	82.00bd	2.06ab	1.84ab	3.51bc	3.68b	30.66a	31.16a	1.29de	1.46cd
BAM010	53.00a	52.66a	97.66a	108.66a	2.06ab	1.86ab	5.43a	5.95a	32.00a	31.33a	1.65bc	1.69ad
BAM011	51.16a	52.50a	97.33a	110.33a	2.20a	1.86ab	5.58a	6.01a	29.66ac	31.50a	1.72b	1.73ac
BAM012	39.66cd	44.16bc	79.00bd	82.00bc	2.07ab	1.78ac	3.91bc	4.51b	27.00bc	32.50a	1.47bd	1.44cd
Mean	44.06	47.79	80.26	87.88	1.92	1.77	4.1	4.42	29.65	30.51	1.51	1.59
SE	0.99	0.7	1.96	2.06	0.04	0.03	0.14	0.13	0.41	0.35	0.04	0.03
F values												
G	7.71*		12.44*		4.79*		9.62*		3.12*		8.55*	
P	16.39*		12.29*		12.82*		4.61*		4.04*		3.53NS	
G X P	1.28NS		0.39NS		2.15*		0.45NS		1.59NS		2.36*	

Notes: Means of individual treatment with different letter(s) in a column significantly vary as per Duncan's multiple Range test *P≤0.05*, SEM=Standard error of mean, G = genotype, P = phosphorus levels, *= Significant at $P \leq 0.05$, NS=Non-significant at $P \leq 0.05$, TRL=Tap Root Length, BN=Branching Number of Lateral,BD=Branching density Roots, RV=Root Volume, SL=Shoot Length, RL: SL=Root Length: Shoot Length.

Significant (*P≤0.05*) variation for branching density (BD) was observed among the bambara genotypes under varying levels of P (Table 4.14).The interaction between genotype and phosphorus for BD was also significant. Bambara genotypes grown under P160µMshowed significant (*P≤0.05*) decrease in BD than those at P0µM. The average BD reduced from 1.92 cm^{-1} at P0µM to 1.77 cm^{-1} at P160µM (Table 4.14). BD ranged from 1.39 cm^{-1} in BAM006 to 2.23 cm^{-1} in BAM002 at P0µM and 1.49 cm^{-1} in BAM008 to 1.89 cm^{-1} in BAM004 at P160µM.BAM002, BAM004, BAM010 and BAM011 had the highest number of BD at P0µM. All genotypes with the exception of BAM006 and BAM007, showed a reduction in BD at P160µM compared to P0µM.

The root volume (RV) of the bambara genotypes significantly (*P≤0.05*) varied at both P levels (Table 4.14). BAM011 achieved the maximum RV of 5.58 ml and 6.01 ml at both P0µM and P160µM, respectively. The least RV of 3.25 ml and 3.68 ml was observed in BAM007 and BAM009 at P0µMandP160µM, respectively. On average, RV of the tested genotypes increased from 3.91-4.51 ml, translating to 15.3% increase due to P supplementation.

4.3.2 Shoot length and Tap root length: shoot length ratio

The shoot length (SL) of the bambara genotypes displayed significant (*P≤0.05*) variation at different levels of P levels. Addition of P increased SL across the genotypes. The maximum SL of 32.00 cm and 32.50 cm was observed in BAM010 and BAM012, atP0µM and P160µM respectively. Conversely, BAM001 produced a minimum SL of 24.66 cm and 28.50 cm at both P0µM and P160µM.

TRL: SL showed significant (*P≤0.05*) variation for genotype but not for phosphorus. The interaction effect of genotype and P levels was significant for TRL: SL. Averaged across the 12 genotypes, TRL: SL increased by 5.3% when the genotypes were grown at P160µM compared to P0µM. BAM001 with 2.11 and 1.85 had the maximum ratios at both P0µM and P160µM, respectively. Conversely, the lowest ratio of 1.12and 1.40 was displayed by BAM006 and BAM005 atP0µM and P160µM, respectively.

4.3.3 Plant biomass (root and shoot) production and Root: shoot dry weights ratio

The bambara genotypes significantly (*P≤0.05*) varied at P levels for root dry weight (RDW), shoot dry weight (SDW), total dry weight and root dry weight: shoot dry weight (RDW: SDW) ratio (Table 4.15). The interaction between genotype and phosphorus for these traits was not significant. RDW ranged from 0.98-1.24 $gplant^{-1}$at P0µM to 1.03-1.27 $gplant^{-1}$under P160µM nutrition. Genotype BAM010 exhibited the highest RDW of 1.24 $gplant^{-1}$at P0µM whereas BAM002 with 1.27 $gplant^{-1}$had the maximum RDWatP160µM. The least RDW of 0.98 and 1.03 $gplant^{-1}$was found in BAM009 at P0µM and P160µM, respectively (Table 4.15).

Shoot dry weight (SDW) of bambara genotypes was 2-fold that of RDW at both P0µM and P160µM for the tested genotypes (Table 4.15). The SDW across all genotypes increased by 12.2% upon addition of P. The maximum SDW of 2.80 $gplant^{-1}$and 3.09 $gplant^{-1}$was achieved in BAM002 at P0µMP and 160µM, respectively. The least SDW of 1.97 $gplant^{-1}$and 2.22 $gplant^{-1}$was reported in BAM008 and BAM009 at P0µMand P160µM, respectively. The genotypes BAM011, BAM010 and BAM002 with high SDW also had high values of root traits. A positive and significant correlation was observed

between SDW with different root traits including *TRL (r=0.74*, P≤0.05),* BN *(r=0.81*, P≤0.05),* BD *(r=0.56, P≤0.05)* and RV *(r=0.90*, P≤0.05)* at P0µM (Table 4.16).

Table 4. 15: Mean values of root dry weight (RDW), shoot dry weight (SDW), total dry weight (TDW) and root weight : shoot weight (RW: SW) ratio of bambara genotypes at 35 DAE grown in pot culture containing P0µM and P160µM.

	RDW(gplant^{-1})		SDW(gplant^{-1})		TDW(gplant^{-1})		RW:SW Ratio	
Genotypes	-P	+P	-P	+P	-P	+P	-P	+P
BAM001	1.03cd	1.08bc	2.25cd	2.73cd	3.28bd	3.81cd	0.46c	0.39d
BAM002	1.16ac	1.27a	2.80a	3.09a	3.97a	4.37a	0.43d	0.41cd
BAM003	1.04cd	1.13ad	2.40c	2.51e	3.44b	3.65ce	0.45c	0.45ad
BAM004	1.12ad	1.09bc	2.13de	2.39ef	3.27bd	3.49eg	0.51a	0.45ac
BAM005	1.08bd	1.13ad	2.21d	2.41ef	3.30bc	3.59df	0.49b	0.46ac
BAM006	1.03cd	1.14ad	2.02e	2.35ef	3.05ce	3.49eg	0.50ab	0.48ab
BAM007	1.01d	1.05bc	2.01e	2.28fh	3.02de	3.33fg	0.50ab	0.46ac
BAM008	1.00d	1.11bc	1.97e	2.24gh	2.97e	3.35fg	0.51a	0.49a
BAM009	0.98d	1.03e	2.09de	2.22h	3.07ce	3.25g	0.47bc	0.46ac
BAM010	1.24a	1.26a	2.61b	2.88bc	3.86a	4.14ab	0.47bc	0.43bd
BAM011	1.21ab	1.23ab	2.68ab	2.95ab	3.90a	4.19a	0.45c	0.41cd
BAM012	1.06bd	1.20ac	2.25cd	2.70d	3.31bc	3.90bc	0.47bc	0.44ad
Mean	1.09	1.15	2.29	2.57	3.37	3.72	0.48	0.45
SE	0.02	0.02	0.04	0.04	0.05	0.05	0.01	0.01
F values								
G	6.02*		49.04*		33.53*		4.77*	
P	11.90*		141.64*		93.92*		12.44*	
G X P	0.63NS		1.73NS		1.09NS		0.93NS	

Notes: Means of individual treatment with different letter(s) in a column significantly vary as per Duncan's multiple Range test *P≤0.05*, SEM=Standard error of mean, G = genotype, P = phosphorus levels, *= Significant at *P ≤ 0.05*, NS=Non-significant at *P ≤ 0.05*

Total dry weight (TDW) across all the genotypes improved by 10.4%, for plants grown at P160µM compared to those grown at P0µM. BAM002 produced the maximum TDW of 3.97 gplant^{-1}and 4.37 gplant^{-1} atP0µM and P160µM(Table 4.15). On the other hand,

BAM008 with 2.97 gplant^{-1}and BAM009 with 3.25 gplant^{-1} achieved the lowest TDW at P0µM and P160µM, respectively.

A significant (*P≤0.05*) drop by 6.3% in RW: SW ratio of the genotypes was observed under P160µM condition compared to P0µM condition (Table 4.15). Genotype BAM008 had the highest ratio of 0.51 and 0.49at P0µM and P160µM, respectively. The lowest ratio of 0.43 and 0.39 was recorded in BAM002 and BAM001 at P0µM and P160µM, respectively. Increase in RW: SW led to significant reduction in SDW and TDW. This was observed by significant (*P≤0.05*) and negative correlation between RW: SW and SDW (*r=-0.83*),* and TDW (*r=-0.75*)* (Table 4.15).

Table 4. 16:Pearson correlation matrix showing relationship between root, shoot traits and plant biomass of Bambara genotypes grown at P0µM and P160µM.

P0µM	TRL	BN	BD	RV	SL	TRL:SL	RDW	SDW	TDW	RW:SW
TRL	1									
BN	0.72**	1								
BD	0.21	0.83*	1							
RV	0.88*	0.87*	0.55	1						
SL	-0.34	-0.09	0.17	-0.1	1					
RL:SL	0.89*	0.55	0.06	0.68*	-0.72*	1				
RDW	0.59*	0.72*	0.55	0.84*	0.26	0.3	1			
SDW	0.74*	0.81*	0.56	0.90*	-0.14	0.60*	0.82*	1		
TDW	0.72*	0.82*	0.58*	0.91*	-0.04	0.54	0.89*	0.98*	1	
RW:SW	-0.69*	-0.66*	-0.39	-0.71*	0.52	-0.74*	-0.39	-0.83*	-0.75*	1
P160µM										
TRL	1									
BN	0.82*	1								
BD	-0.02	0.51	1							
RV	0.70*	0.89*	0.44	1						
SL	-0.33	-0.15	0.19	0.15	1					
TRL:SL	0.92*	0.72*	-0.07	0.5	-0.64*	1				
RDW	0.46	0.63*	0.29	0.83*	0.11	0.34	1			
SDW	0.67*	0.83*	0.37	0.85*	-0.26	0.64*	0.85*	1		
TDW	0.62*	0.81*	0.37	0.86*	-0.18	0.58*	0.90*	0.99*	1	
RW:SW	-0.64*	-0.78*	-0.37	-0.61*	0.53	-0.71*	-0.43	-0.83*	-0.76*	1

*Correlation is significant at the $P \leq 0.05$.TRL =Tap root length, BN=branching number, BD= branching density, RV=root volume, SL=shoot length , TRL :SL=tap root length : shoot length ratio, RDW=root dry weight, SDW=shoot dry weight, TDW=total dry weight

CHAPTER FIVE

DISCUSSION

5.1 Evaluation of morphological and yield performance of bambara genotypes

Low phosphorus is predominant in agricultural ecosystems and limits the growth of crops. Plant heights of bambara groundnut genotypes differed significantly ($P \leq 0.05$) due to phosphorus and lime levels as shown in Appendix 1.Significant effects due to genotype x lime, genotype x season, genotype x site, phosphorus x season, lime x site and season x site for plant heightshows that plant height of the genotypes responded variably to the application of lime and P at the two sites during long and short seasons. Variation in plant height under contrasting phosphorus levels has been observed among bambara groundnut varieties (Hasan et al., 2019; Temegne et al., 2019; Eifideyi et al., 2020). Liming coupled with P application makes this nutrient to be more available for enhanced plant nourishment, growth and development. This could explain the difference in plant height among genotypes in this study. Lime and phosphorus application have contributed to differences in plant height in haricot bean varieties (Kassa et al., 2014) and rice bean (Kumar et al., 2014).

Number of nodules per plant varied significantly among genotypes due to lime levels, phosphorus levels, season and site (Appendix 1). In this study, significant genotype x season, site x season, lime x phosphorus, genotype x site x season, and lime x site xseason for number of nodulesplant^{-1}. This depicts that number of nodules across the

genotypes was depended on season, site, lime and P. Liming increased the number of nodules at both sites during the short and long rain seasons. Soils from Ligala and Umala where the experiments were conductedhad low pH of 5.20 and 5.10, respectively as shown in Table 3.2. Therefore, liming at 4 tonnesha^{-1} raised the soil pH by reducing $Al^{3+,}$ H^{+}, $Mn^{4+,}$ and Fe^{3+} ions concentration in soil solution. Consequently, the fixed P is made available resulting to enhanced formation of nodules by the native *Rhizobia* in the soil (Kisinyo et al., 2014; Bello et al., 2018). The application of lime has been found to increase the nodule number in soybean and rice bean(Bekere et al., 2013; Kumar et al., 2014).

In this study, there was significant response to P application, in terms of number of nodules per plant. The application of P in un-limed plots at the two sites during long and short rain season increased the number of nodules among the genotypes. Phosphorus influences activities such as nodulation, N_2 fixation, and specific nodule activity due to its role in energy reactions (Nziguheba et al., 2016).Bambara groundnut cultivars respond variably to P application leading to enhanced nodule numbers(Toungos et al., 2009; Temegne et al., 2019). Application of P increased nodule number in green gram and black gram (Hayat et al. 2008), mash bean crop (Meena et al., 2017) and common bean(Samago et al., 2018; Chekanaiet al., 2018).These results demonstrate the need to apply P to the soils in Western Kenya to improve nodulation of the legumes in this region. This will in turn improve soil health through symbiotic biological nitrogen fixation (BNF) that will help reduce cost of crop production (Benson et al., 2015; Ibny et al., 2019).

The highest number of nodules was observed when the bambara genotypes were co-applied with lime and phosphorus. This shows that combined application of lime and phosphorus is an attractive strategy for enhancing nodulation in bambara genotypes. Farmers in areas having phosphorus deficient acid soils are encouraged to apply lime since it influences release of adsorbed P, increasing availability of phosphorus for plant use and creates an ecosystem suitable for *Rhizobia* to thrive in. Nodulation occurs when a root hair of the legume plant is infected by *Rhizobia*. Plant responds by forming more cells around the area infection, resulting into a nodule which forms a site of bacterial growthand fixation of nitrogen from the atmosphere (Mitran et al., 2018). Co-application of lime with phosphorus improved number of nodules/plant in cowpea and rice bean (Kumar et al., 2014; Bello et al., 2018).

The short rain season recorded a lower number of nodules per plant among the genotypes than long rain season under low and adequate P in both un-limed and limed soils at Ligala and Umala locations. There was also a significant interaction between site and season for number of nodules per plant among the tested genotypes. The short rain season in both sites receives a lower amount of rainfall than the long rain season (Jaetzold et al., 2010), which explains the observed differences in the number of nodules per plant in this study. Long rain season in both sites appears to have provided favorable conditions for the bambara genotypes to form nodules. A marked variation in the number of nodules per plant was reported in *Pongamia piñata* following seasonal effects (Chaukiyal et al., 2000).Gei and Powers, (2015) reported high nodulation in *Gliricidiasepium* and

Leptolobium panamense during dry and wet seasons. The genotypes produced a higher number of nodules at Umala compared to Ligala. The variation at the two sites could be attributed to variations in soil nutrients and textures as shown in Table 3.2. Keino et al., (2015) observed that poor growth of soybean was due to limitation of nutrients such as potassium and magnesium.

The number of lateral roots per plant of the bambara genotypes varied significantly due to lime levels, phosphorus levels and site as shown in appendix 1. There was also significant interactions genotype x season, lime x season, genotype x site, lime x site, season x site, genotype x phosphorus x site, genotype x season x site, phosphorus x site x season and genotype x phosphorus x site x season for the number of lateral roots. This demonstrates differential ability of bambara genotypes exhibited in lateral roots under varying levels of lime and phosphorus at different sites.BAM001, BAM002, BAM010 and BAM011 consistently recorded the maximum number of lateral roots under low and adequate P in un-limed and limed soils during long and short seasons at Ligala and Umala, indicating that they have potential to scavenge for P. These genotypes should be promoted for cultivation in soils with low P owing to their inherent ability to produce high number of lateral roots at low P soil P status that can be achieved by applying lower rates of mineral P fertilizer and lime. Variation in the number of lateral roots under varying levels of P has been observed in native Australian legumes (Adams et al., 2002), and *Lupinus angustifolius* (Chen et al., 2013).

In this study, a high number of lateral roots were observed in phosphorus fertilized plots in both un-limed and limed soils. Liming corrects the soil pH making P available, and

enhances root development for water and nutrient acquisition in crops (Bakari et al., 2020). An increase in number of lateral roots under P supply is attributed to the role it plays in energy reactions that are essential in metabolically active sites of root and shoot development (Ndakidemi and Dakora, 2007). At Ligala the Bambara genotypes had more lateral roots compared to Umala. This could be partly explained by a lower native P value of 5.43 $mgPkg^{-1}$ of soil at Ligala and thus increased number of lateral roots compensates in sourcing P from the soil. Hong & Long, (2000) and Miguel et al., (2013) describe extensive lateral roots as essential in increasing surface area in contact with soil for P acquisition.

Plant biomass, grain yield and biological yield of bambara genotypes varied significantly ($P \leq 0.05$) in response to lime levels, P levels, season and site as shown in appendix 1. Significant interactions lime x season, phosphorus x season, and phosphorus x site was significant for plant biomass, grain and biological yields. This implies that bambara genotypes contain useful genetic variation for response to soil phosphorus availability that could be exploited to improve their ability to extract P from the soil with varying levels of phosphorus and lime. Genetic differences among mungbean(Irfan et al., 2017), cowpea (Adjei-Nsiah et al. 2018; Bello et al. 2018) common bean (Samago et al., 2018; Chekanai et al., 2018) and bambara (Temegne et al., 2019) genotypes contributes to variation in grain yields and plant biomass under contrasting phosphorus and lime application (Irfan et al., 2017; Temegne et al., 2019).

The high grain yields under low and adequate phosphorus during long rain and short seasons at Ligala and Umala was observed in BAM001, BAM002, BAM010 and

BAM011 as indicated in Table 4.5-Table 4.8.Thisdepicts that these genotypes have high efficiency in P acquisition and utilization while at the same time they respond well to P application across seasons and sites. A significant correlation between seed yield and number of lateral roots, plant biomass and number of lateral roots and grain yield and plant biomass supports the assertion that crop genotypes that have high number of lateral roots as well as plant biomass tend to realize high grain yields (Olusanya and Moninuola, 2017). These genotypes are desirable for cultivation in a wide range of P environments as well as different seasons with variable rainfall amount without compromising the yield. High yielding genotypes at low P indicates that they have a low critical P requirement and have enhanced P acquisition efficiency characterized by high number of lateral roots (Richardson et al., 2011).The remaining genotypes had lower yields at low P than adequate P in un-limed soil across the sites and seasons, demonstrating that they are inefficient and responsive genotypes. The productivity of inefficient responsive genotypes can be sustained when supplied with little amount of phosphorus or via liming the acidic soils to release fixed P. Crop cultivars have been previously categorized into inefficient nonresponsive, efficient non-responsive, inefficient responsive and efficient responsive (Nziguheba et al., 2016; Irfan et al., 2017).

Significant genotype x lime, genotype x site, phosphorus x site and season x site was only for plant biomass, grain yield and biological yield in this study, shows that bambara genotypes varied in plant biomass, grain and biological yield under the two sites, seasons and varying levels of lime and phosphorus application. Thus, the favourable weather conditions with high amount of rain received during long rain season explains the high

yields exhibited across the genotypes (Jaetzold et al., 2010). Similarly, the variation in soil properties (Table 3.2) could also account for differences in the yield across the sites. More so, liming of the acidic soils coupled with addition of small quantity of inorganic P fertilizer is likely to increase plant biomass and grain yields of the genotypes tested in this study. Barasa et al., (2013) notes that incorporation of phosphorus and lime in the soil has significant increase in pulse yield. All the bambara genotypes showed a positive response to P under contrasting levels of lime. Genotypes BAM006, BAM007, BAM008 and BAM009 showed improved response to P under limed condition compared to P or lime alone. This underlines the effect of soil acidity that limits these genotypes from accessing nutrients for yield production. Thus, productivity of these genotypes can only be maintained under soil amelioration with both P and lime. Genotypes BAM002, BAM001, BAM010 and BAM011, were efficient in grain yield under contrasting P and lime conditions indicating that these genotypes fit well to varied agricultural systems ranging from low to high input systems.

Significant improvement in seed yield of 11.9% across the genotypes, sites and seasons was attributed to liming. Agricultural lime has high content of Ca^{2+} and/or Mg^{2+} ions. Its application to soil, lowers $Al^{3+,}$ H^{+}, $Mn^{4+,}$ and Fe^{3+} ions concentration in soil solution and thus increase in soil pH (Barasa etal, 2013; Kisinyo et al., 2014; Opala, 2017). The increased soil pH best fit legume growth and yield production which is usually affected by low pH. With improved soil pH due to liming, the fixed P is made available and accessible to plants for crop production (Okalebo et al., 2005; Kisinyo et al., 2014).This also improves microbial activity benefiting from increased available P and pH hence

enhanced biological nitrogen fixation (BNF). The improved plant biomass and seed yield across the genotypes at low P could be attributed to liming effect. Legumes utilize calcium for structural support of plant cells and as asecondary messenger when plant is physically or biochemically stressed. On the other hand,Mg is a component of chlorophyll, enzyme activator and nucleic acids stabilizer (Barasa et al., 2013). Therefore, application of agricultural lime partly explains the increase in grain yield of bambara genotypes. Genotypes BAM001, BAM002, BAM003, BAM010, BAM011 and BAM012 were efficient in grain yield under lime conditions. Such genotypes should be promoted for production due to their ability to yield under low P in a lime amended soil. The yield of common bean was significantly enhanced through correction of soil pH via application of lime (Hirpa etal., 2015).

Umala site recorded the highest grain yields than Ligala. The two sites different physic-chemical characteristics as shown in Table 3.2 and this could explain the differences in plant biomass, grain and biological yield observed at the two sites (Keino et al., 2015). The short rain season at the two sites receives a lower amount of rainfall than the long rain season (Jaetzold et al., 2010), which partly explains the observed differences in crop performance in this study. Environmental variations observed in different sites and seasons contribute to differences in plant biomass and seed yield of crop genotypes (Gei and Powers, 2015; Samago et al., 2018).

5.2 Variation in P acquisition and use efficiencies of the Bambara cultivars

Phosphorus accumulation (PA) in plant tissues depicts how efficient the genotype acquires and accumulates P. PA in the plant biomass, grain and biological yields was significantly ($P \leq 0.05$) influenced by genotype at different levels of P application.

Interaction between genotypes x phosphorus application was highly significant (Table 4.9) indicating that genotypes tested had differential ability to acquire and accumulate P from the soil. A highly positive and significant correlation between seed PA and seed yield ($r = 0.63^*, P \leq 0.05$) was achieved under low P. This demonstrates that the bambara cultivars that concentrates relatively more phosphorus in their seeds realized higher seed yield (Yaseen and Malhi, 2009; Bilal et al., 2018). P application significantly increased grain P concentration indicating that PA is dependent on P availability. Genotypes BAM002, BAM010 and BAM011 (Table 4.5) produced high grain yields and accumulated high amount of P in both biomass and grain at adequate and low P (Table 4.9).These genotypes were apparently considered to be P-efficient of absorbed P for biomass and grain production at both levels of P. Genotypes with high acquisition of P are suitable genetic resources that should be promoted for cultivation owing to their inherent ability to produce high yield at relatively low soil P status that can be reached by applying lower rates of chemical P fertilizer than would be recommended.

Phosphorus stress factor (PSF) differentiates between responsive and non-responsive genotypes to P. It also shows the inherent ability of a cultivar to yield biomass and/or yield upon P nutrition (Irfan et al., 2020). Bambara genotypes tested varied significantly for PSF (Table 4.9). These genotypes responded to P application but also showed a decrease in grain yields due to low P. PSF varied from7.53% to 28.61% among genotypes demonstrating the differential potential to withstand low P conditions in the soil. Genotypic variation in PSF have been previously reported in other crops including wheat (Yaseen and Malhi, 2009), mungbean (Irfan et al., 2017) and rice (Irfan et al.,

2020). BAM002, BAM003, BAM010 and BAM012 were the most P responsive genotypes depicting that increased productivity of these genotypes can be sustained under P supply. Genotype BAM011 had a lower PSF compared to BAM002 and BAM010. However, its high seedyield at both low and adequate P levels (Table 4.5) indicates that that it is a low-P tolerant genotype that yields well under varying P status in the soil.

Phosphorus harvest index (PHI) describes the portion of accumulated P in grains (Irfan et al., 2017). PHI differed due to genotype and genotype x phosphorus interaction. This indicates differential ability of the cultivars to partition part of the accumulated plant P to grain P under different levels of P. PHI of Bambara genotypes linearly increased with P nutrition but non-significantly implying that reallocation of P from the biomass to grain was similar at both low and adequate P supply conditions. A positive and significant correlation ($r = 0.75^*$, $P < 0.05$) between grain yield and PHI at limited P revealed that the Bambara genotypes were able to use internal P efficiently. The genotypes BAM001, BAM002, BAM010 and BAM011 with high PHI, retained high P in the grain and produced more grain yield. These genotypes with high PHI values should be investigated for traits underlying P translocation from different plant parts to the grain to provide an insight into the underlying P-efficiency mechanisms. Genotypic variation for PHI at contrasting levels of P has been observed in dry bean and mungbean (Fageria et al., 2013; Irfan et al., 2017) and is a useful selection criterion for P-efficiency as depicted by the findings of this study.

Phosphorus physiological efficiency index (PPEI) describes the efficiency of a genotype to utilize P and produce seed yield or plant biomass per unit of absorbed P in plant at maturity (Irfan et al., 2017). Bambara genotypes varied significantly for PPEI at both low

and adequate P supply (Table 4.11). This indicates that the tested genotypes have differential utilization efficiency of absorbed P. Variation of bambara genotypes for P requirements demonstrates that the genotypes that accumulate more P under low P conditions can tolerate P-deficiency stress. Differential abilities for PPEI under contrasting P nutrition has also been reported in other crops including wheat and mungbean (Yaseen and Malhli, 2009; Irfan et al., 2017). Genotypes BAM002, BAM010 and BAM011 with high values of PPEI at low P, and low values afterP nutrition are P-efficient (Table 4.11). These cultivars best fit in limited P environments since they can accumulate and utilize P more efficiently for high seed production. Phosphorus biological efficiency ratio (PBER) measures the ability of a cultivar to produce biomass per unit of absorbed P (Irfan et al., 2017). PBER significantly differed at varying levels of P and reduced with P application (Table 4.11). This depicts differential ability among the genotypes to produce biomass for every unit of absorbed P from the soil. Genotypes BAM002, BAM010 and BAM011 that had high yield at both adequate and low P, also had lower values of PBER. Similar results was observed in wheat and mungbean genotypes, where higher PPEI and PBER values were recorded at low P than adequate P (Yaseen and Malhli, 2009; Fageria et al., 2013; Irfan et al., 2017).

The bambaracultivars were grouped into four categories; HGY-HP, MGY-MP, LGY-MP and MGY-LP at low P supply (Figure 4.1). Genotypes BAM002 and BAM011 in the HGY-HP group were considered P-efficientin acquisition and utilization of the absorbed P to yield high seed under low P. Nonetheless, BAM011 accumulated less P (Table 4.9), and thus appears to be more efficient in P utilization compared to BAM002. BAM010 in

MGY-MP, was medium in acquisition ofP and efficient in seed yield production demonstrating an ability to partition available nutrient to grain production under low P supply.BAM012 categorized as LGY-MP, indicated that this genotype has low utilization efficiency of the absorbed P. Under P application, the bambara genotypes were categorized into three groups: HGY-HP, MGY-MP and LGY-MP as shown in Figure 4.2. Genotypes BAM002, BAM010 and BAM011 in HGY-HP were efficient in P acquisition responsive to P application (Table 4.12; Figure 4.2). P nutrition moved BAM010 from MGY-MP at low P to HGY-HP under adequate supply of P. Likewise, BAM008 in MGY-LP at low P shifted to LGY-MP at adequate P supply together withBAM006, BAM007 and BAM009. The results are consistent with results reported from previous studies on other crops (Irfan et al., 2017; Bilal et al., 2018). The finding has shown that some genotypes can perform well at both low and adequate P. Similarly, other genotypes could only yield well under P supply compared to P deficiency. Thus, confirming the need to screen genotypes for P efficiency at both limiting and adequate P conditions to gauge their responsiveness to P supplementation. BAM002, BAM010 and BAM011 in the present study consistently performed well under both P levels hence are desirable. Balemi and Negisho, (2012) asserts that P utilization efficient cultivars have low internal P requirements for normal physiological activities and thus could perform wellfor every unit of absorbed P. In light of that, such genotypes produce high yields with minimum incorporation of P in the soil. Thus, P acquisition and utilization efficient genotypes should be promoted for cultivation for both low and high input agricultural systems (Kruse et al., 2015).

Principal component analysis (PCA) indicated that the first two principal components contributed a total variation of 85.05% and 79.04% at low and adequate P, respectively (Table 4.13). Genotypes BAM002, BAM010 and BAM011 were categorized together based on grain yield, PA in the grain, TPA, biological yield, plant biomass, and PA in plant biomass at both low and adequate P supply as shown in Figure 4.3 and 4.4. This implies that these traits are important in breeding for high yields. These genotypes scored highly for the discriminative traits and hence are most P efficient and responsive in the current study. Biomass and grain yield production coupled with phosphorus acquisition at limited P nutrition have been identified as significant traits in the directional selection of P efficient crop genotypes (Bilal et al., 2018; Iqbal et al., 2019; Irfan et al., 2020). Promotion of these genotypes in present study in a strategic recombination program of the P-efficient genotypes may improve gains for these traits in the progeny under low P supply. Besides contributing to breeding, the information on bambara genotype characterization may be applied in low-input cropping systems to improve productivity of the crop.

5.3 Root and shoot traits underlying differential phosphorus acquisition and utilization efficiency in Bambara genotypes

Tap root length (TRL), branching number of lateral roots (BN), branching density (BD), root volume (RV), shoot length (SL), root dry weight (RDW), shoot dry weight (SDW), total dry weight (TDW) and root weight to shoot weight ratio of the bambara groundnut genotypes varied significantly at the contrasting P levels (Table 4.14; Table 4.15).This shows that bambara genotypes harbor genetic variation in root traits and plant biomasses at varying levels of P. Differential root traits and plant biomasses at the contrasting levels

of P has been observed in cowpea (Krasilnikoff et al,. 2003), chick pea (Ramamoorthyet al., 2017), *Trifolium subterraneum* (McLachlan et al,. 2020), and mungbean (Reddy et al., 2020).Genetic variation in tap root length, total root length, branching density, branching diameter, root volume, root dry weight, shoot dry weight and shoot length was observed in different bambara genotypes under rainout shelter (Mateva et al., 2020). A significant and positive correlation between tap root length and branching number ($r=0.72^{*}, P\leq0.05$), and root volume ($r=0.88^{*}, P\leq0.05$) in this study, confirms the inherent and differential ability of bambara genotypes for extensive root system development for acquisition of nutrients and water absorption at varying soil depth. Reddy et al., (2020) reported that total root length was positively and significantly correlated with total root surface area, total root volume, total root tips and root forks under both low and optimum P.BAM002, BAM010 and BAM011 had the highest TRL, BN, BD, RV, RDW and SDW at low P. These genotypes also recorded high grain yields and accumulated high amount of P at low P. This demonstrates that improved root traits at low P could have been responsible for enhanced P accumulation leading to high yields of these genotypes. This shows that TRL, BN, BD and RV could be mechanisms underlying the variation in phosphorus efficiency in the bambara genotypes in this study. Therefore, these root traits could be exploited for screening of P efficiency in bambara genotypes at low P.

The variation in TRL, BN, BD, RV and shoot traits upon addition of P was genotype dependent. All genotypes recorded high TRL, BN, RV, SL, RDW and SDW on addition of P. The available P at the rooting zone was used by the plant for plant photosynthesis

leading to increased growth in root traits and plant biomasses (Hasan et al., 2018). The reduced tap root length, branching number and root volume at low P can be attributed to lack of P that plays an important role in energy reactions that is essential in metabolically active sites of root and shoot development (Ndakidemi and Dakora, 2007). Thus, with few cells forming at root tips, plants experience reduced absorption of nitrogen and magnesium resulting to eventual growth retardation (Iqbal etal., 2019). Genotypes BAM001, BAM002, BAM010 and BAM011 consistently recorded high BN, BD, and RV atP160µM P demonstrating that they are P responsive.

All genotypes with the exception of BAM004, BAM006 and BAM007 had reduced BD on addition of P160µMcompared to P0µM.The genotypes with high BD at P0µM P indicate that they are well adapted to thrive in P-deficient ecosystem. Due to high available P content of top soil, root system architecture that often results to increase in root surface area in top soilenhances P acquisition efficiency. This is effected by increased root length in the upper part of soil through shallowgrowth angle of axial roots (Van de Wiel et al., 2016).The reduced TRL with enhanced BD at limited P demonstrates the effect of localized soil available P on root proliferation. These changes promote a shallow root system with increased branching which explains a higher BDatP0µMthan P160µMP. Hence, these traits help genotypes to scavenge for relatively immobile P in shallow soils suggesting a foraging ability. Induced P-deficiency modifications has been reported in other crops root architecture as adaptations for topsoil foraging including common bean (Lynch and Brown, 2001) and *Arabidopsis* (Williamson et al., 2001).Therefore, root system architecture including lateral branching, branching density and root hair density are advantageous for P acquisition efficiency.

Root and shoot dry weights increased linearly with P application. This shows the responsiveness of bambara genotypes to applied P.P nutrition is essential in plant physiological processes that contribute to improved plant root system facilitating more nutrient acquisition and water absorption that led to high biomass production (Abbas et al., 2018; Iqbal et al., 2019). This is attributed to increased photosynthesis process and partitioning of photosynthates to roots and shoots (Irfan et al., 2018). This explains why reduced biomass production was observed at P0µM compared to P160µM. At limited P environment, plants partition large portion of their resources to the root system (Sandaña& Pinochet, 2014). This consequently results to reduced growth of the above ground parts and/or including decline in yield. Studies have suggested that limited P environment restricts expansion of leaves due to reduced carbon assimilation resulting into low shoot length and biomass (Teng et al., 2013). This can also be linked to reduced absorption of nitrogen and magnesium resulting to eventual growth retardation (Iqbal etal., 2019).

Root: shoot weight ratio for the tested genotypes differed significantly and reduced when the genotypes were grown under P supply. This implies that bambara genotypes genetically differ in allocation of accumulated biomass at both roots and shoots at varying P levels.BAM001, BAM002, BAM010 and BAM011 had low root weight: shoot weight at both P0µM and P160µM. These genotypes are efficient in accumulating above ground part biomass. On other hand, BAM004, BAM006, BAM007 and BAM008 had high root: shoot weight ratio and are P inefficient. Plants respond to low P by partitioning

more of their photosyhates to the production of heterotrophic tissues rather than autotrophic tissues(Sandaña& Pinochet, 2014; Vejchasarn et al., 2016; Iqbal etal., 2019). Increased heterotrophic tissue production favours root development at the expense of shoot, consequently resulting to an increase in root: shoot dry weight ratio (Mašková &Herben, 2018).

Genotypes which scored low values of RDW and SDW, recorded high root: shoot ratios at both P levels in this study. These genotypes are considered inefficient in P utilization (Iqbal et al., 2019). Increased shoot biomass production as observed in BAM001, BAM002, BAM010 and BAM011 at P0µM is a desirable trait for cultivar selection and varietal development. This shows an enhanced photosynthetic activity that ensures enough assimilates to the above ground tissues and an indicator of P utilization efficiency (Irfan et al., 2017). In the present study, genotypes BAM001, BAM002, BAM010 and BAM011 gave relatively higher shoot dry weight compared to the rest of genotypes at P0µM. Interestingly, these genotypes also registered improved root traits. A significant and positive correlation between shoot biomass with TRL *(r=0.67*),* BN (*r=0.83*),* and RV (*r=0.85**) at P0µM and TRL (*r=0.74**), BN (*r=0.81**), and RV (*r=0.90**) under P160µM confirms the importance of these root architecture traits in above ground biomass production. Ramamoorthy et al., (2017) reported similar results where root traits had great influence in conferring biomass and seed yield advantages at terminal growth in chickpea (*Cicer arietinum* L.). Thus,bambara genotypes with high values of root system architecture, produced high root and shoot biomass and are apparently efficient in both P acquisition and utilization.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The bambara groundnut genotypes showed variation in plant height, number of nodules, number of lateral roots, plant biomass, grain and biological yields under varying levels of phosphorus and lime during long and short seasons at Ligala and Umala sites. BAM001, BAM002, BAM010 and BAM011 performed well under low and adequate P in both un-limed and limed soils.
2. Bambara groundnutgenotypes harbour genetic variation for P accumulation and utilization efficiency. BAM002, BAM010 and BAM011 genotypes showed a higher potential inP acquisition and utilization and also responded well to P supplementation. P efficient and responsive bambara genotypes would be multiplied and promoted for cultivation in low and adequate P fertilized soils.
3. Bambara groundnut genotypes displayed variation in root traits including TRL, BN, BD and RV. These traits could be the mechanisms underlying the variation in phosphorus efficiency in the bambara genotypes observed in this study.

6.2 Recommendations

1. The genotypes that are efficient in P and responsive to applied P including BAM001, BAM002, BAM010 and BAM011 should be multiplied and promoted for cultivation in low and adequate P fertilized soils.

2. Bambara genotypes varied in P utilization efficiency, and should be considered as breeding material for future development of bambara varieties that are high yielding and adaptive to varying levels of P.
3. The root traits identified in this study should be exploited for screening of bambara genotypes for P efficiency.Genotypes BAM001, BAM002, BAM010 and BAM011 showed an enhanced root systemand should be promoted for cultivation in soils with varying levels of P. Physiological and genetic markers linked to P efficiency in bambara groundnuts should be investigated.

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APPENDICES

Appendix I: Analysis of varianceof plant height, number of nodules, number of lateral roots, plant biomass, grain yield and biological yield of 12 bambara genotypes grown at varying levels of phosphorus and lime during long and short rain seasons atLigala and Umala.

Source of variation	d.f.	Plant height	No of nodules	No.lateral roots	Plant biomass	Grain yield	Biological yield
Replication stratum	2	1.57	0.03	0.13	4.16	3.6	6.53
Replication.Genotype stratum							
Genotype	11	9.67*	4.91*	13.11*	72.00*	46.17*	99.18*
Residual	22	1.71	2.26	2.11	1.28	26.26	1.4
Replication.Genotype.LimeLevel stratum							
LimeLevel	1	11.88*	20.46*	156.89*	55.47*	2768.14*	127.47*
Genotype.LimeLevel	11	3.54*	1.07NS	2.1NS	16.84*	3.28*	17.3*
Residual	24	0.62	0.95	0.77	0.43	0.39	0.41
Replication.Genotype.LimeLevel.Plevel stratum							
Plevel	1	24.16*	19.09*	130.5*	137.63*	2244.49*	224.33*
Genotype.Plevel	11	0.82NS	1.14NS	1.58NS	1.12NS	4.03*	1.31NS
LimeLevel.Plevel	1	0.11NS	2.08NS	0.44NS	0.58NS	17.45*	1.11NS
Genotype.LimeLevel.Plevel	11	0.79NS	0.55NS	0.81NS	0.43NS	0.18NS	0.41NS
Residual	48	1.62	2.36	1.43	2.88	0.39	2.72
Replication.Genotype.LimeLevel.Plevel.*Units* stratum							
Season	1	3.42NS	6.51*	1.55NS	1117.15*	4408.65*	1999.45*
Site	1	1.55NS	38.44*	8.87*	10.74*	39.64	18.86*
Genotype.Season	11	1.23NS	2.76*	2.93*	30.8*	26.43	36.48*

LimeLevel.Season	1	4.97*	0.36NS	4.96*	24.54*	63.25*	39.23*
Plevel.Season	1	9.54*	3.22NS	0.03NS	27.7*	126.09*	51.7*
Genotype.Site	11	1.88*	1.4NS	3.05*	2.64*	6.14*	2.37*
LimeLevel.Site	1	5.4*	0.25NS	1.48NS	1.69NS	0.58NS	1.19NS
Plevel.Site	1	2.7NS	0.17NS	112.96*	6.19*	7.14*	8.35*
Season.Site	1	15.27*	5.69*	15.7*	16.67*	36.92*	7.43*
Genotype.LimeLevel.Season	11	0.52NS	0.75NS	1.1NS	8.39*	0.79NS	7.81*
Genotype.Plevel.Season	11	0.46NS	0.64NS	1.93NS	1.8NS	2.59*	2.11*
LimeLevel.Plevel.Season	1	0.97NS	0.7NS	5.69NS	1.25NS	8.93*	2.71NS
Genotype.LimeLevel.Site	11	1.3NS	1.17NS	0.98NS	1.35NS	0.28NS	1.23NS
Genotype.Plevel.Site	11	0.52NS	1.08NS	2.08*	1.11NS	0.68NS	1.04NS
LimeLevel.Plevel.Site	1	0.19NS	4.42*	4.16*	0.06NS	0.81NS	0.16NS
Genotype.Season.Site	11	1.58NS	1.88*	2.32*	1.43NS	5.32*	1.68NS
LimeLevel.Season.Site	1	0.02NS	5.41*	0.52NS	1.48NS	0.65NS	1.01NS
Plevel.Season.Site	1	0.52NS	0.98NS	6.55*	4.29*	6.02*	2.27NS
Genotype.LimeLevel.Plevel.Season	11	1.03NS	0.62NS	0.52NS	0.56NS	0.32NS	0.5NS
Genotype.LimeLevel.Plevel.Site	11	0.9NS	1.5NS	0.83NS	0.43NS	0.09NS	0.42NS
Genotype.LimeLevel.Season.Site	11	0.78NS	0.79NS	0.62NS	0.76NS	0.26NS	0.74NS
Genotype.Plevel.Season.Site	11	1.13NS	1.22NS	3.59*	0.21NS	0.49NS	0.21NS
LimeLevel.Plevel.Season.Site	1	0.54NS	0.39NS	0.42NS	0.2NS	0.67NS	0.35NS
Genotype.LimeLevel.Plevel.Season.Site	11	0.35NS	0.24NS	0.61NS	0.68NS	0.16NS	0.58NS
Residual	1440	4.232	16.6	11.07	1.048	0.04275	1.149
Total	1727	3857.47	39510.9	30321.9	4478.83	383.74	6838.69

Notes: *=Significant at P≤0.05, NS=Non-significant at P≤0.05

Appendix II: Reagents and apparatus for preparation of stock solutions

Soil P, Ca, Mg and K extraction using AB-DTPA extractant

Reagents

DTPA Solution 0.005 M: Dissolve 9.85 g. of DTPA in about 4.5 L of distilled water in a polyethylene container. Stir overnight for total dissolution, dilute to 5.0 L with pure water. This solution is stable with regard to pH.

NH_4HCO_3 1M-DTPA 0.005 M Extracting Solution at pH = 7.60: Accurately weigh and dissolve 79.06 g. of ammonium bicarbonate (NH_4HCO_3) in about 700 ml of 0.005 M DTPA. Adjust the pH to 7.60 with slow agitation while diluting to 1 L with more DTPA solution. Prepare this solution fresh daily as needed.

5M Sodium Hydroxide: Dissolve 200 g of NaOH in about 800 ml of distilled water in a 1 liter volumetric flask and dilute to volume with distilled water.

Nitric acid:Perchloric acid digestion (wet-oxidation) of plant materials for total P extraction

Apparatus:

1. Erlenmeyer flask, 125 ml.
2. Whatman No. 4.2 filter paper

Reagents:

1. Concentrated nitric (V) acid
2. 60% perchloric acid
3. Concentrated sulphuric (VI) acid

Determination of phosphorus in plant tissue(Venado-Molybdate Method)

Reagents:

1.Vanado-Molybdate reagent-Dissolve 20 g NH4-Molybdate, $(NH)_6M0_70_{24}.4H_2O$, in 200 ml hot water and cool. Dissolve separately 1 g of NH_4-metavanadate in 120 ml hot water, cool and add 140 ml conc. $HN0_3$ under a fume hood. Gradually add molybdate solution to the vanadate solution and dilute to 1 L.

2. P standard solution, stock, 100 ppm P. - Dissolve 0.4394 g dry anhydrous KH_2PO_4 in distilled water and dilute to 1liter. Store the solution in a dark pyrex glass bottle at a cool place.

3. P standard solution, 25 ppm P - Dilute the 100 ppm P stock solution 4 times. Fresh solution should be prepared periodically to insure high acruracy.

Determination of percent organic carbon in soil (walkey-black method)

Apparatus;

Burette of 50 ml

Reagents:

1. Potassium dichromate ($K_2Cr_2O_7$) 1N, prepared by dissolving 49.04 g of $K_2Cr_2O_7$ in 500 ml distilled water and diluting to 1 liter.
2. Concentrated H_2SO_4
3. Concentrated O-phosphoric acid (H_3PO_4)
4. diphenyl amine sulfonate (10%)
5. Ferrous sulphate (O.5M) –prepared by dissolving 139 g of $FeSO_4.7H_2O$ in water 500 ml distilled water followed by 15ml conc. H_2SO_4 and diluted to 1 liter.

SOIL MECHANICAL ANALYSIS BY (HYDROMETER METHOD)

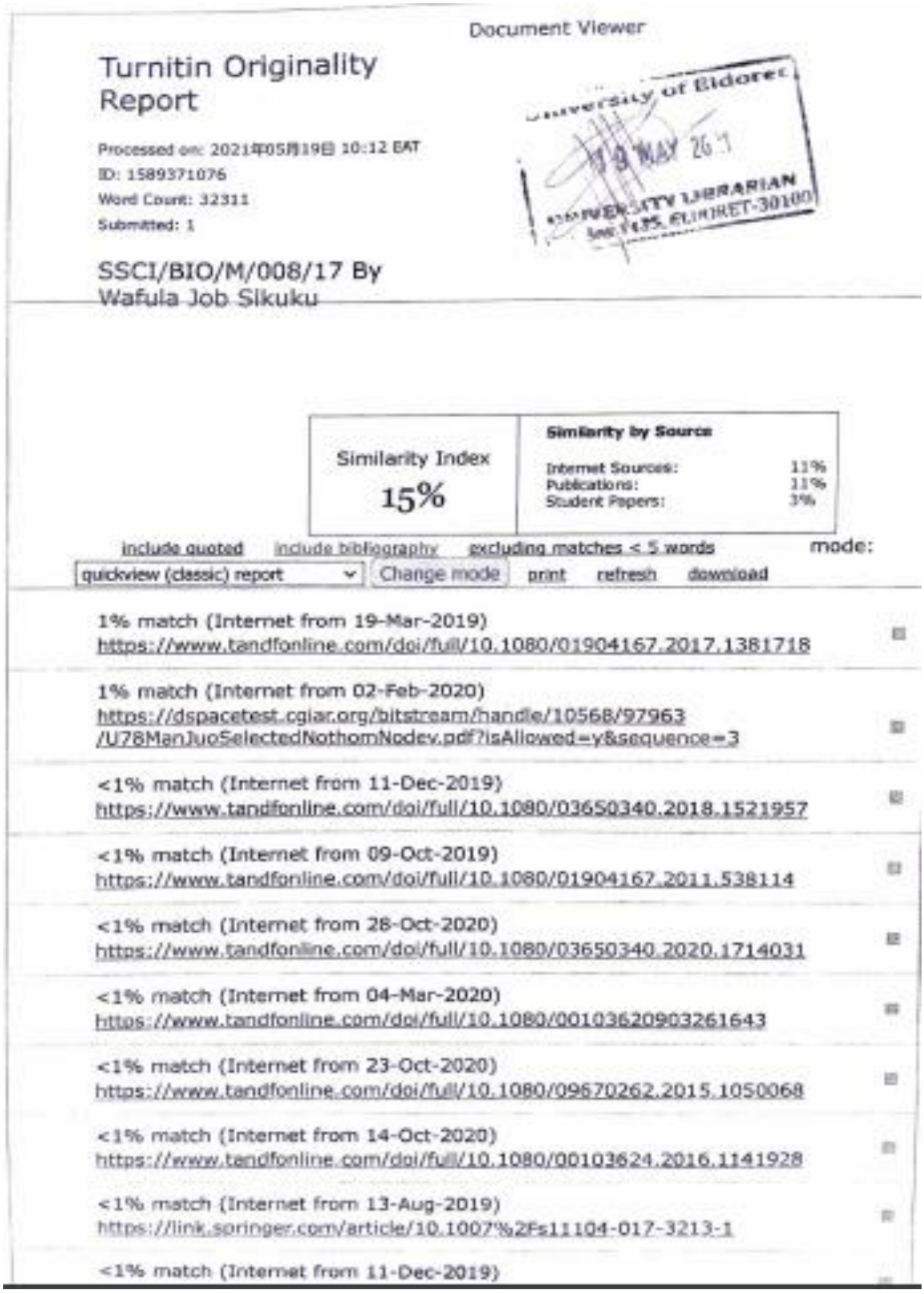
Apparatus and *Reagen*ts:

1. Multimix machine with baffled -milkshake' cups
2. One liter glass cylinders
3. Hydrometers for measuring soil suspension density
4. Thermometers for measuring soil suspension temperature
5. Sodium hexametaphosphate
6. A 2mm mesh sieve

Preparation of stock solutions and a half-strengthHoagland solution

Component	Stock Solution	ml Stock Solution L^{-1}
Macronutrients		
2M KNO_3	202 g/L	1.25
2M $Ca(NO_3)_2 \cdot 4H_2O$	236 g/0.5 L	1.25
Iron (Sprint 138 iron chelate)	15 g/L	0.75
2M $MgSO_4 \cdot 7H_2O$	493 g/L	0.5
Micronutrients		
H_3BO_3	2.86 g/L	0.5
$MnCl_2 \cdot 4H_2O$	1.81 g/L	0.5
$ZnSO_4 \cdot 7H_2O$	0.22 g/L	0.5
$CuSO_4 \cdot 5H_2O$	0.08 g/L	0.5
$H_2MoO_4 \cdot H_2O$ or	0.09 g/L	0.5
$Na_2MoO_4 \cdot 2H_2O$	0.12 g/L	0.5
Phosphate		
1M KH_2PO_4	136 g/L	0.5

Appendix III: Turnitin originality report



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