GENETIC IMPROVEMENT OF COWPEA (*Vigna unguiculata* (L.) WALP) FOR PHOSPHORUS USE EFFICIENCY

By

SABA BABA MOHAMMED

(10512766)

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DECLARATION

I hereby declare that except for references to works of other researchers, which have been duly cited, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

....
Saba Baba Mohammed
(Student)

....
Prof. Pangirayi B. Tongoona
(Supervisor)

....
Prof. Frank. K. Kumaga
(Supervisor)

....
Dr Daniel K. DZIDZIENYO
(Supervisor)

.......20th May, 2019..................
Prof. Muhammad F. Ishiyaku
(Supervisor)

ABSTRACT

Cowpea is an important grain legume crop for millions of humans, fodder for livestock and source of income for all the value chain actors. Its productivity is constrained by several biotic and abiotic stresses such as drought and poor soil fertility. Aligned with poor soil fertility in most growing areas, this thesis describes phosphorus (P) use and acquisition of elite cowpea lines from different breeding programmes. In the first chapter, a brief overview was provided about cowpea as an important multi-purpose legume, constraints to its production including P deficiency and knowledge of farmers on using P based fertilizers. A participatory survey of farmers was conducted across 36 villages of cowpea growing areas in northern Nigeria, using a semi-structured questionnaire and focus group discussions to determine farmers' perceptions on phosphorus fertilization, prevailing cowpea cropping systems, use of improved varieties and farmers' perceived production constraints and preferred traits. Results showed that farmers were aware of fertilizers as important for growth and increased yield but did not know the major need of P for cowpeas. Intercropping with cereals was the most popular cropping system. A little above 20% used improved varieties of cowpea. Farmers identified insects and yield as the major constraint and preferred trait, respectively. Screening experiments, both in the screenhouse and low P field, to identify and group elite cowpea lines based on performance under low P and high soil P conditions using shoot dry weight and other parameters were conducted. There was significant diversity among the elite lines for adaptation to low P and response to applied P fertilizer. A few cowpea lines such as IT97K-556-6, IT84S-2246-4 and IT89KD-288 produced above average yield under sub-optimal and high P conditions. The relative reduction in yield as a result of soil P deficiency compared to high P performance was over 50%. Cowpea lines with above average performance under the contrasting P soils were grouped as efficient and responsive lines and are suitable for cultivation under limited and optimum P input systems. There was a significant reduction in days to flowering and maturity among cowpea lines under high

P conditions relative to low P. To understand the genetics underlying P use and uptake efficiency, a quantitative trait loci (QTLs) mapping study was undertaken through marker-trait analysis with a biparental recombinant inbred lines (RIL) mapping population. A total of 27 QTLs were detected across 7 of 11 linkage groups of cowpea. These QTLs were different from the previously identified ones, indicating they are new QTLs under varying P conditions. These genomic regions were associated with flowering time, yield components and P use efficiency traits under low and high P conditions. In addition, a genome-wide association mapping study (GWAS) using DArTseq derived SNP markers on 400 diverse cowpea lines to identify QTLs and SNP markers based on historical recombination events underlying the genetics of tolerance to low P and response to applied mineral P fertilizer was conducted. The GWAS mapping resulted in the identification of over 60 SNP markers significantly associated with adaptation to low P conditions and response to P application as measured by differences in shoot dry weight, P use and uptake efficiency under two soil P conditions. In conclusion, this research revealed that farmers did not use the recommended fertilizer types and rates for cowpea production, use of mixed intercropping was the popular cropping system and cultivation of landraces was most prominent over improved varieties. The screening of cowpea lines under different P concentrations showed varied performance, and lines were grouped into efficient responsive, efficient non-responsive, inefficient responsive and inefficient non-responsive classes based on their performance in low and high P growth media. Marker-trait analysis with a biparental RIL population led to the identification of QTLs and SNPs that will lay the foundation for marker-assisted selection, that will fast-track the development of P efficient varieties. The study reported for the first time on cowpea use of high-density SNP markers to identify QTLs and markers associated with P traits under field conditions using genetic materials relevant to sub-Saharan African breeding programmes. The validation of SNP markers identified from this study before their use in marker-assisted selection is highly recommended.

DEDICATION

This Thesis is dedicated to the memory of late Professor Balarabe Tanimu, the former Director (2008-2012), Institute for Agricultural Research Ahmadu Bello University, Zaria Nigeria for his sincere counselling that led me to Plant Breeding.

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LIST OF ABBREVIATIONS

ABU: Ahmad Bello University

APUE: Agronomic Phosphorus Use Efficiency

DArT: Diversity Array Technology

FAO: Food and Agricultural Organization

GAPIT: Genomic Analysis and Prediction Integrated Tool

GBS: Genotyping By Sequencing

IAR: Institute for Agricultural Research

ICRISAT: International Crop Research Institute for the Semi-Arid Tropics

IITA: International Institute for Tropical Agriculture

LME: Linear Mixed Effect

MLM: Mixed Linear Model

PCA: Principal Component Analysis

P: Phosphorus

PRA: Participatory Rural Appraisal

PUE: Phosphorus Use Efficiency

PuPE: Phosphorus Uptake Efficiency

SNP: Single Nucleotide Polymorphisms

UCR: University of California Riverside

WAAPP: West Africa Agricultural Productivity Programme

WACCI: West Africa Centre for Crop Improvement

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is a grain legume that belongs to the family Fabaceae (Ehlers & Hall, 1997; Singh *et al.*, 2002). It is a diploid ($2n = 2x = 22$) species with a genome size of 620 Mbp and a self-pollinated species (Boukar *et al.*, 2018). It is reported to have divergent domestication with two major gene pools distributed across Western and Southern Africa with each pool related to wild species found in those geographic regions (Huynh *et al.*, 2013). Nigeria is the world's largest producer and consumer of cowpea grains (Coulibaly & Lowenberg-Deboer, 2002) and accounts for over 65% of global production (Abate *et al.*, 2012).

It is a multi-purpose grain legume rich in protein and provides food for humans and fodder for livestock in sub-Saharan Africa (SSA). The crop contributes considerably to food security and poverty reduction in the SSA. It has a potential of up to 3, 000 kg ha^{-1} (AATF, 2012). Cowpea is an important component of farming systems in areas where soil fertility is limiting, where it is cultivated as an intercrop with major cereals (Olufajo & Singh, 2002; Singh & Ajeigbe, 2007).

The crop's yields in West and Central Africa are low (< 450 kg ha^{-1}) (Abate *et al.*, 2012), due to series of biotic stresses like pests, diseases and parasitic weeds, and abiotic stresses such as drought, heat stress, and poor soil fertility especially low nitrogen and phosphorus. Phosphorus (P) is a crucial macronutrient required by cowpea for optimum growth and development. Legumes including cowpea are only able to fix substantial amounts of N in the presence of sufficient available soil P (Armstrong & Griffin, 1991; Hussain, 2017; Krasilnikoff *et al.*, 2003). Unfortunately, soils of most cowpea growing areas in West Africa are deficient in available P (Gyan-Ansah *et al.*, 2016; Hussain, 2017), where soils are mostly acidic (low pH values) and sandy. Sandy soils are generally poor, with low organic matter content, and deficient in major nutrients like N and P (Saidou *et al.*, 2012; Sanginga *et al.*, 2000). P plays an important role in early root formation, crop quality, enhanced disease tolerance, seed formation and several biochemical processes such as photosynthesis, respiration, energy storage, and transfer, cell division & enlargement (Griffith, 2013; Johnston & Seyers, 2009; Maharajan *et al.*, 2017).

The use of P-formulated fertilizers appears to be a quick and easy fix for P deficiency of soils, but that option has not been largely adopted by most smallholder cowpea growers due to several reasons. The production of inorganic P-based fertilizers from rock phosphate reserves is expensive in most developing countries, making P fertilizers not readily available especially in rural markets, and relatively expensive for rural farmers when available (Mullins & Thomason, 2009; Olufowote & Barnes-Mcconnell, 2002; Rothe *et al.*, 2013).

Many local farmers also are unaware of the critical role played by P in plant nutrition and its resultant effect on crop yield, so they apply low to no P fertilizers in cowpea fields (Nkaa *et al.*, 2014; Karikari *et al.*, 2015). In addition, most of the P applied in the form of fertilizers and manure is easily bound and becomes unavailable in the soil by reacting with complexes of Fe and Al in acidic soils, and Ca in alkaline soils, making the amount of available P for plant use extremely low (Ho *et al.*, 2005; Lynch, 2011; Mullins & Thomason, 2009). Therefore, the most sustainable solution is to develop cowpea varieties that yield well in low soil P conditions, and produce higher yield when P is applied (Adusei *et al.*, 2016; Nkaa *et al.*, 2014; Wang *et al.*, 2010).

Even though P is important for increased yield of cowpea, there is limited information about perceptions of its use in cowpea production and possible reasons for low to no use of mineral P fertilizers in growing areas by smallholder farmers. Earlier reports have indicated the existence of genetic variability of P uptake and have suggested exploiting this variation to develop superior high yielding varieties with ability to fix N under sub-optimal soil P (Sanginga *et al.*, 2000). This aligned well with major goals of cowpea improvement programmes that include pyramiding genes for tolerance to biotic and abiotic traits like low P soil conditions (Timko *et al.*, 2008). Despite numerous reports of genetic variability for P use and uptake among cowpea lines, there has been no effort to identify parental lines for P use and acquisition efficiency among biparental and multi-parent advanced generation inter-cross (MAGIC) recombinant inbred lines (RIL) populations genotyped by the Cowpea Team at the University of California, Riverside USA as part of outputs of the Tropical Legume II Project (Huynh *et al.*, 2013).

Conventional breeding programmes have made substantial progress in the past few decades, but progress has been limited in improving certain quantitative traits. Marker-assisted breeding could increase efficiency in developing low P tolerant varieties. However, the number of useful QTLs and other markers identified for important quantitative traits in cowpea are limited (Timko *et al.*, 2008; Agbicodo, 2009) when compared to crops like maize, soybean, and wheat.

There are few studies on QTLs and markers for P efficiency traits in cowpea using biparental mapping populations (Fonji, 2015; Rothe, 2014) or genome-wide association mapping studies of molecular markers for low P tolerance and response to applied mineral P fertilizers (Ravelombola *et al.*, 2017). Therefore, there is a need to identify additional QTLs and markers associated with genomic regions controlling the inheritance of P efficiency traits for cowpea improvement. Absence of significant QTLs and information on useful markers on P traits have limited ability to adapt molecular breeding to improve the locally adapted farmer preferred lines, which are low yielding and cultivated under low soil fertility conditions. The low yield levels of cowpea lead to deficits in places like Nigeria (AATF, 2012) and because of such deficits, over 518, 400 metric tons of cowpea grains have to be imported into Nigeria annually from the neighbouring countries (Coulibaly & Lowenberg-Deboer, 2002). More recent statistics are not available. However, considering Nigeria (with a population of over 180 million people) and as the largest consumer of cowpea in the world, this figure is likely to have increased substantially over the last 17 years.

Aligned with these above-mentioned challenges, this research was designed to initiate the genetic improvement of cowpea for adaptation to low soil phosphorus tolerance and efficient use of applied phosphorus. The specific objectives were to:

- assess smallholder cowpea farmers' knowledge, perception on P fertilization of cowpea, perceived constraints and preference traits in major growing areas in Nigeria,
- phenotype parents of biparental and multi-parent advanced generation inter-cross recombinant inbred lines populations for P use and acquisition efficiency,
- map cowpea genomic regions and identify SNP markers associated with P use and acquisition efficiency, and
- conduct a genome-wide association mapping for adaptation to low P tolerance and response to applied P fertilizer.

CHAPTER TWO

2.0 REVIEW OF LITERATURE

2.1 Biology, origin and distribution of cowpea

Cowpea is a diploid and self-pollinating crop with 11 chromosomes and genome size of about 620 million base pairs. The crop is commonly known as beans in Nigeria and black-eye pea in the United States (Ehlers & Hall, 1997; Xu *et al.*, 2011). It is an herbaceous plant that belongs to the family *Fabaceae* and the only cultivated subspecies of the genus *Vigna* while other subspecies namely; *dekindtiana, stenophylla, and tenuis* are wild types (Padulosi & Ng, 1997). The cultivated cowpea is sub-divided into five cultivar groups; *Unguiculata*, *Sesquipedalis* (yard-long-bean), *Textilis*, *Biflora*, and *Melanophthalmus* (Timko & Singh, 2008a).

Cultivated cowpeas are believed to have originated from Africa, as its wild relatives are only found in the continent (Padulosi & Ng, 1997). There are several speculations as to the precise centre of origin of the crop in Africa. However, West and Central Africa encompassing Nigeria, southern parts of Republic of Niger, northern Benin, Togo, northwestern Cameroon and parts of Burkina Faso are the areas with most diversity of cultivated cowpeas, while wild species are mostly found in southern Africa encompassing Namibia, Zambia, and Zimbabwe (Padulosi & Ng, 1997). Recent molecular studies have supported these conclusions, as two gene pools were attributed to Western and Southern Africa regions (Huynh *et al.*, 2013). Cowpeas are grown across tropics and sub-tropic areas of the world (Olajide & Ilori, 2017). The main production areas of the crop are in sub-Saharan Africa, with Nigeria being the world's largest producer followed by the Niger Republic. Cowpea was introduced to the Indian subcontinent from Africa approximately over 3000 years ago and moved from Asia to southern Europe. It is believed that cowpea reached the USA in the 1700 BC from West Indies (Ehlers & Hall, 1997).

2.2 Cowpea – a food and nutrition security crop

Cowpea is a multi-functional, important grain legume with diverse uses and benefits (Ehlers & Hall, 1997; Singh & Singh, 2016; Timko *et al.,* 2007) as food, feed and source of income when sold raw or as processed products for millions of its value chain actors (Boukar *et al.,* 2018; Coulibaly & Lowenberg-Deboer, 2002; ICRISAT, 2017a; Langyintuo *et al.,* 2003). The crop is an integral component of traditional cropping systems in the semi-arid tropics where it is mainly cultivated in mixed intercrops of cereals like sorghum, pearl millet and maize in some areas (Ewansiha *et al.,* 2014; Olufajo & Singh, 2002; Singh & Ajeigbe, 2007; Singh *et al.*, 2003). The crop is relatively tolerant to limited soil moisture conditions making it suitable for cultivation in marginal areas of Sahelian agro-ecologies of arid and semi-arid areas known to be prone to drought (Agbicodo *et al*., 2009; Ehlers & Hall, 1997; Muchero *et al.*, 2013; Muchero *et al.*, 2009) where cereals like maize are unable to thrive or are less resilient.

Cowpea haulms serve as a nutritious fodder for livestock due to their high content of lysine and tryptophan, the two most limiting amino acids in cereals, thereby supporting integration of crop-livestock farming in the main producing areas like northern parts of Nigeria (Ehlers & Hall, 1997; Ewansiha *et al.*, 2014; Kingley & Vernon, 2015; Samireddypalle *et al.*, 2017; Singh *et al.*, 2003; Tipilda *et al.*, 2005). Its ability to fix atmospheric nitrogen through biological nitrogen fixation with *Bradyrhizobium* spp. makes it a good soil fertility replenisher, contributing as much as in 50 - 100 kg N ha^{-1} (Beaver *et al.*, 2003; Kyei-Boahen *et al.*, 2017; Sanginga *et al.*, 2000; Santos *et al.*, 2011). The spreading types of cowpea varieties with semi-determinate to indeterminate growth habit have been used as a weed control tool due to their weed suppressive effects and in the control of *Striga hermonthica* of cereals through a phenomenon known as suicidal germination (Ehlers & Hall, 1997; Hall *et al.*, 2003; Matsui & Singh, 2003).

It is a cheaper source of quality protein especially for households that cannot afford animal-based proteins, thereby making it an essential resource for addressing nutritional insecurity in poor nations by complementing calorie rich cereals, roots and tuber crops (Boukar *et al.*, 2018; Philips *et al.*, 2003; Ojiewo *et al.*, 2018; Singh *et al.*, 2003). Improved productivity, development and deployment of high yielding varieties with tolerance to biotic and abiotic stresses would enable cowpea growers to produce more grains for consumption and sell, thereby contributing to food security and a better standard of living.

2.3 Genetic gains and constraints to cowpea production in Nigeria

The global area under cowpea cultivation is over 12 million hectares with an approximate production of 7 million tonnes of grain (FAOSTAT, 2016), with Africa accounting for 95% of the global production and Nigeria, Niger and Burkina Faso being leading producers in that order. The average yield of the crop is less than 600 kg ha^{-1} compared with genetic potential of 2,500 - 3,000 kg ha^{-1} (AATF, 2010; ICRISAT, 2017a) due to several biotic and abiotic factors including insect pests, diseases, parasitic weeds, drought, heat, poor soil fertility and poor agronomic practices (Boukar *et al.*, 2018; Ehlers & Hall, 1997; ICRISAT, 2017b; Ojiewo *et al.*, 2018; Timko *et al.*, 2007). The cultivation is largely under rainfed conditions (Hall *et al.*, 2003; Olufajo & Singh, 2002) with little off-season cultivation under mixed intercrop of major cereals using limited to no inputs like improved seeds, fertilizers and chemicals (Ajeigbe *et al.*, 2010; Ewansiha *et al.*, 2014; Singh & Ajeigbe, 2007).

Cowpea research began in the early 1960s in Nigeria and many other countries in sub-Saharan Africa with most starting cowpea research programmes around 1980. International research organizations became involved in cowpea research in the 1970s with the involvement of Canada's International Development Research Centre and the International Institute of Tropical Agriculture (IITA) - Semi-Arid Food Grains Research and Development (SAFGRAD) in 1977 (Boukar *et al.*, 2018). A major goal in breeding programmes for the crop is stacking of resistance to biotic and abiotic stresses. Research organizations both at the national and international level are at the forefront of genetic improvement of cowpea for biotic and abiotic stress tolerance (Timko *et al.*, 2008). Over the past six decades, considerable progress has been made towards genetic improvement of cowpea for biotic and abiotic stresses that constrained its productivity (Ehlers & Hall, 1997; Singh *et al.*, 2002; Timko *et al.*, 2007) by the IITA and African National Agricultural Systems (NARS) largely using conventional approaches (Huynh *et al.*, 2013). Detailed review on milestones in different areas of breeding cowpea has been reported (Blade *et al.*, 1997; Ehlers & Hall, 1997) and more recently (Boukar *et al.*, 2018).

Early breeding was focused on the collection of germplasm, screening for resistance to insect pests, diseases, early maturity, plant architecture, and seed quality. These procedures have led to the varietal release in the past, they are, however, relatively time consuming and expensive, with low efficiency as it takes over 8 years to develop varieties (Ehlers & Hall, 1997; Huynh *et al.*, 2013). In addition, most of the earlier efforts have studied above-ground shoot traits (Hammond *et al.*, 2009; Lynch, 2011; Lynch & Brown, 2012; Lynch, 2013) while root traits have received little attention in most breeding programmes due to the underground nature and limited high-throughput phenotyping tools for root systems (Burridge *et al.*, 2016; Canto *et al.*, 2018; Lynch, 2013; Paez-Garcia *et al.*, 2015). Selection based on genomic loci in tight linkage with molecular markers and traits of interest is expected to increase progress, precision and reduce the duration of cycles of phenotypic evaluation. Marker-assisted selection is expected to accelerate the rate of genetic gain and potentially reduce the cost of phenotypic evaluations (Boopathi, 2013; Collard *et al.*, 2005; Batieno *et al.*, 2016; Kelly *et al.*, 2003; Maharajan *et al.*, 2017). The success of MAS in breeding is dependent on the quality of phenotypic and genotypic data for marker-trait association studies.

2.4 Breeding phosphorous efficient cowpea: constraints, accomplishments, and prospects

A serious challenge in this century is providing enough food and energy for the world, with nearly a billion people facing hunger and malnutrition. Current projections show that the trend is expected to worsen with the prediction of the world population reaching 9 billion by the year 2050 (Godfray *et al.*, 2010; Nelson *et al.*, 2012). Explosive population growth, varying climates especially for moisture deficit and poor soil fertility are challenges for global agriculture. There is an urgent need for increased food production to meet the increased demand of a growing world, and this must come from efficient input use such as fertilizer and water due to the impact of climate change on the available lands. The development of crop varieties with high yield under low nutrient conditions and limited water supply has become a priority for breeding programmes (Vinod & Heuer, 2012).

Soils of sub-Saharan are inherently low in organic matter and nutrients like nitrogen and phosphorus, the two most essential macronutrients for plants (Bishopp & Lynch, 2015; Ho *et al.*, 2005; Lynch, 2011; York *et al.*, 2013). P is an integral part of all cellular activity and required as a component of cell bio-energy (in the form of ATP), nucleic acids (DNA & RNA), NADPH, phospholipids, and phosphoproteins (Vinod & Heuer, 2012).

Cowpeas are mostly cultivated on small plots by smallholder farmers with limited access to credit facilities, mechanization and synthetic fertilizer. They rely on P in the soil for efficient N fixation, early maturity, tolerance to pests and diseases, and optimum yield (Ankomah *et al.*, 1996; Hussain, 2017; Sanginga *et al.*, 2000; Santos *et al.*, 2011). Therefore, improving the genetic make-up of cowpea for P adaptation and response to P fertilization is the best and most sustainable strategy for low and high inputs systems because P is a problem in all inputs system today. In high input agriculture in developed nations, excessive P fertilization leads to acidification, and eutrophication via P runoff to water bodies, while low P in low input systems has resulted in low yield and reduced income of farmers.

2.4.1 Phosphorus and cowpea production

P deficiency is a major yield-limiting constraint in most agro-ecosystems in the world especially in West Africa (MacDonald *et al.*, 2011). Most of the world's agricultural production occurs on low P soils which affect over 50% of global arable land and about 75% of cultivable land in Africa (Lynch, 2011). Rock phosphate, a major resource for producing inorganic P fertilizers, is a non-renewable natural resource and pure grade of P deposits are limited and being depleted rapidly (Cordell *et al.*, 2009). The distribution of P reserves globally is uneven, with the majority of the world rock phosphate being in Morocco, USA and China, in that order. There are concerns in certain quarters that the global reserves of rock phosphate would be exhausted in the next 3 - 4 centuries (Kauwenbergh, 2010).

Cowpea productivity is highly associated with the amount of P fertilization, as several reports have shown increased yield and performance of the crop as P input increases (Adusei *et al.*, 2016; Gyan-Ansah *et al.*, 2016; Karikari *et al.*, 2015; Kolawole *et al.*, 2008; Kyei-Boahen *et al.*, 2017; Oladiran *et al.*, 2012; Saidou *et al.*, 2012). Although empirical evidence has shown strong cowpea response to P fertilization, the use of synthetic P fertilizer is not well adopted among smallholder farmers due to poor availability especially in rural areas, low awareness on need for P fertilizer and high cost (Adusei *et al.*, 2015; FanWay, 2015; Mahamane, 2008). Therefore, development and deployment of varieties that are efficient in uptake and use of P will improve the productivity of the crop and benefit varied cropping systems, ensure cleaner environment that would come from reduced P application and increased yield in low P soils (Mahamane *et al.*, 2006; Rothe *et al.*, 2013).

P is the main limiting nutrient for cowpea cultivation in tropical soils of West Africa due to the acidic and high sand content of the soils (Abdou, 2018; Mahamane, 2008; Saidou *et al.,* 2011). This is due to P fixing properties of those soils leading to formation of complexes with Fe and Al oxides that immobilize P, thereby making it unavailable for plants even when supplied in sufficient quantity (Vinod & Heuer, 2012), and low to no application of P as inorganic fertilizer (Adusei *et al.,* 2016; Singh & Ajeigbe, 2007) is another challenge. P uptake and efficient use from growth media in crop plants are governed by a combination of factors such as soil properties, genotypic differences of crop plants and exudation of chemical compounds (Richardson *et al.,* 2011; Johnson *et al.,* 1996).

The level of nutrients beyond which an increase in such nutrient does not result in yield increase is referred to as a critical level (Mullins & Thomason, 2009). Critical level of soil P for grain legumes have been reported as 10 mg kg^{-1} (Adeoye & Agboola, 1985), conversely the rate of P depletion in soils of Africa is above 10 kg ha^{-1} year^{-1} and this shows that tropical soils are low in available P (Henao & Baanante, 1999; FAO, 2000). Therefore, ability of genotypes to give optimal yield under limiting P conditions calls for concerted efforts to develop cowpea varieties with enhanced ability in acquiring limited soil P and efficient utilization of P applied. Effective use of nutrients especially available soil P, tolerance to drought stress, and micronutrient deficiency will increase adaptation and yield of cowpea in low input farming systems on marginal lands. There is a difference between elemental P and phosphate (P_2O_5) in fertilizers. Most soil test outputs are elemental P, while commercial fertilizers are formulated as phosphate. One unit of elemental P (e.g. 1 lb) is little more than two units of phosphate (2.29 Ibs) and fertilizers are recommended as phosphate. P is available for plant uptake in soil solution (dissolved in soil water) as negatively charged orthophosphate anions ($H_2PO_4^-$, HPO_4^{2-}) (Mullins & Thomason, 2009).

Application of nitrogen fertilizer is not necessary on cowpea fields which are efficiently nodulated, even on infertile soil. However, most growers do not inoculate cowpea seeds before planting. Moisture deficit and high temperature could hinder the efficiency of inoculation (Mullen *et al.*, 2003). In growing areas in Nigeria, suitable strains of *Rhizobia* species for cowpea have not been identified to produce commercial *Rhizobia* inoculant, hence; growers do not inoculate cowpea seeds before planting in most areas in Nigeria.

Due to lack of availability and the high cost of inorganic fertilizers, the direct use of indigenous rock phosphate has been shown as an alternative to the water soluble fertilizer (Adusei *et al.*, 2015), but this has not been adopted by most growers of cowpea. In soils deficient in available P, P should be provided at the rate of atleast 10 kg P ha^{-1}. This is the same as applying about 120 kg single super phosphate (SSP) ha^{-1} and providing 12 kg of sulfur ha^{-1}. Application of molybdenum may be required for cowpea on some acid soils while deficiency of Zinc can occur in alkaline clay soils. Liming of acid soils may be used to improve the availability of fixed soil P in soils with P fixing properties (Mullen *et al.*, 2003).

2.4.2 Screening cowpea for adaption to low P soils and response to mineral P addition

Several procedures have been employed in phenotyping crop plants for P use. The use of hydroponic solutions supplemented with different P concentrations coupled with imaging of root system architecture has been used for cowpea (Rothe *et al.*, 2013). Plants in pots in sandy soils and natural low P sandy field soils have been used (Saidou *et al.*, 2007). Digital imaging of root traits has been demonstrated for dicots including cowpea and monocots root phenotyping (Das *et al.*, 2015). A review of high-throughput imaging technique including visible imaging, imaging spectroscopy, thermal infrared imaging, fluorescence imaging, 3D imaging and tomographic imaging suitable for phenotyping plants for biotic or abiotic stress (disease, insects, drought and salinity) have been documented (Li *et al.* 2014). More recently, manual excavation followed by visual scoring of root numbers and angles, termed *Shovelomics*, have been used for crops like soybean, maize, common bean and cowpea (Burridge *et al.*, 2016; Colombi *et al.*, 2015; Trachsel *et al.*, 2011) and this has enabled high throughput phenotyping of maize root architecture under field environments (Abiven *et al.*, 2015).

Using these screening approaches, genetic diversity for P uptake and use have been found to exist in the cowpea gene pool, such as Sanginga *et al.* (2000) who reported differences in P absorption of cowpea genotypes as a result variation in the root system architecture. Using low P field soils in screening in pots, two popular lines from Nigeria; IT89KD-288 and Danila were identified as good performers for grain yield under no or minimal P addition (Adusei *et al.*, 2015). In another screening work with white silica sand supplemented with Hoagland nutrient solution varied in P concentration, four cowpea genotypes with tolerance to low P (Big John, IT97K-1069-6, IT98K-476-8, and TX2028-1-3-1), and (CB46, and Golden-Eye Cream) with partial low P tolerance via high seed P content were reported (Rothe *et al.*, 2013). Contrary to the report of Adusei *et al.* (2015) and Saidou *et al.* (2012), a cowpea landrace (Danila), earlier found to be adapted to low P conditions was reported to have poor tolerance to low soil P (Rothe, 2014). This discrepancy could be due to using different genetic materials bearing the same name, a problem widespread with landraces among farmers. Several other workers have reported similar findings (Kolawole *et al.*, 2008; Mahamane, 2008; Ojo *et al.*, 2006; Oladiran *et al.*, 2012; Saidou *et al.*, 2011).

Different traits have been used as determinants of low P tolerance in cowpea; for example, tolerance to low soil P was determined at harvest, using plant height, shoot and root dry weights, and shoot P-content, and shoot-to-root ratios as indices (Kakamega *et al.*,, 2011; Mahamane, 2008; Rothe *et al.*, 2013; Saidou *et al.*, 2012). Seed P concentration and grain yield per plant as indices for P tolerance in cowpea from rock phosphate fertilization (Gyan-Ansah, 2012; Ojo *et al.*, 2006) and shoot biomass yield has been extensively used (Kugblenu *et al.*, 2014; Rothe *et al.*, 2013; Sanginga *et al.*, 2000). The dry shoot biomass appears to be the most potent criteria for assessing tolerance to low P, since P deficiency slows down utilization of carbohydrate by plants (IAEA, 2013).

2.4.3 Management of phosphorus deficiency and mechanism of low P adaptation in cowpea

Phosphorus use efficiency (PUE) refers to the ability of crop plants to grow and yield substantially in soils with sub-optimal available P (Hammond *et al.*, 2009; Leiser *et al.*, 2015; Pask *et al.*, 2012). Developing PUE crops will improve food sufficiency of poor nations and ensures the sustainability of agriculture in high input systems of rich nations (Bishopp & Lynch, 2015). For soils with low available P or depleted P from continuous cultivation without replenishment, the use of P fertilizers to supplement low soil P is recommended to avoid soil mining. However, application of P is not a common practice in low input cropping systems due to the high cost of P fertilizer and inadequate supply (Agwu, 2004; Bationo *et al.*, 2002; FanWay, 2015; Horn *et al.*, 2014).

Indeed, the use of inorganic P fertilizers as long term solution to low soil P has both economic and environmental trade-offs, since the global reserves of rock phosphate, a critical ingredient for making P fertilizer are unevenly distributed and fast depleting (Kauwenbergh, 2010; Wrage *et al.*, 2010). There is also an imbalance in P use with some rich nations using too much, while others using too little P (MacDonald *et al.*, 2011; Provin & Pitt, 2002). Excessive use of P is associated with eutrophication of lakes and water bodies resulting from leaching and P runoff (Delgado-Baquerizo *et al.*, 2013).

Deficiency of P needs to be recognized before applying management practices. Symptoms of P stress in most plants appear on leaves (stunted, reduced expansion, surface area, and numbers), as delayed flowering & maturity, reduced growth, and purplish colouration along the margin of young leaves especially in maize and tomatoes (Beegle & Durst, 2002) and on some cowpea genotypes (Mahamane, 2008). Discolouration symptoms are not common phenomena on all plants and seldom appears under field conditions. Reduced fruit size and quality, decreased tolerance to diseases and low yield (Armstrong & Griffin, 1991) are also prevalent. Symptoms are often more pronounced on the shoot than the root system, thereby leading to low shoot to root dry biomass and on older lower leaves than young active meristematic ones because of mobilization and subsequent translocation from older tissues to active growing ones (Armstrong, 1999). P deficiency impacts root growth and development, as rooting depth and vigour are greatly reduced. Certain plants produced dark green leaves from P deficiency due to the accumulation of carbohydrate resulting from continuous production from photosynthesis and slow utilization. P deficiency may be a secondary problem in some acid soils, such as those with high levels of iron and aluminium, which could restrict root development (Ismail *et al.,* 2007).

The effectiveness of P applied as fertilizer or manure is usually low ranging from 5 - 10% (Beegle & Durst, 2002; Lynch, 2011), due to P fixation in soils. Response to soil P by crop plants is dependent on its availability in the soil solution and genetic potential of the crop to take it up. P uptake from soil solution depends on a number of factors such as enhanced root system architecture, since P moves largely by diffusion in the root zone, this makes root traits like root hairs, and lateral branching very important for P acquisition (Bates & Lynch, 2001; Bishopp & Lynch, 2015; Chimungu *et al.*, 2014; Krasilnikoff *et al.*, 2003). Soil resources are stratified with P reserves concentrated mainly in the top layer (0 - 15cm), while water and nitrate tend to be more available in the lower strata, hence genotypes with topsoil foraging ability via expanded lateral roots are better equipped to access limited soil P (Burridge *et al.*, 2016; Lynch, 2013; Paez-Garcia *et al.*, 2015; Ramaekers *et al.*, 2010), while nitrate and water are better accessed by deep rooting genotypes (Ho *et al.*, 2005; Miguel *et al.*, 2015; Trachsel *et al.*, 2013).

Strategies for the management of P fixations include application of high quality P fertilizers to provide enough plant available P, good timing and method of application, conducting soil test (Mullen *et al.*, 2003), application of rock phosphate in soils with high P fixation (Mahamane, 2008) and breeding varieties that can access P reserves in these soils, especially those with well-developed root architecture (Mehra *et al.*, 2015; Nnadi & Mohammed-Saleem, 1996; Snapp *et al.*, 2018).

Plants have adapted to P deficiency using various strategies. Root exudates such as sugars, oligosaccharides, organic acids are secreted by some roots, and these are capable of inducing soil microbial activity within the rhizosphere to release immobilized P fixed in complex forms (Simpson *et al.*, 2011; Wu *et al.*, 2015). Interestingly, a major QTL identified in rice called *phosphorus uptake* (Pup1) for tolerance to low P deficiency has been associated with root growth (Gamuyao *et al.*, 2012). The larger root system has been identified in maize for low P tolerance (Li *et al.*, 2009).

High seed P content and large root surface area have been found in cowpea to be responsible for tolerance to low P conditions (Rothe, 2014). Rothe (2014) also author reported inheritance of P tolerance to be governed by additive genes with high narrow-sense heritability. P uptake is enhanced in crops with large root surface area as a result of long root hairs and branched root systems which permit the plant to explore wider topsoil areas and thereby provide better access to P reserves (Bishopp & Lynch, 2015; Lynch & Brown, 2012). The transport of P to plants is believed to be via root plasma membrane by P transporters, therefore, larger root systems would be beneficial for plants in gaining access to limited P reserves (Paszkowski *et al.*, 2002). Furthermore, the use of soil amendments with Biochar has been associated with increased uptake of immobile soil nutrients like

P in unfertile soils and drought prone areas (Abiven *et al.*, 2015). Association formed between soil mycorrhizal fungi such as arbuscular and vesicular mycorrhizal and cowpea root system in some genotypes is known to enhance uptake of nutrients and water (Mullen *et al.*, 2003; Saidou *et al.*, 2012) in poor soils. The fungal hyphae serve as extensions of the root architecture that extends the volume of soil exploration and thereby increasing efficient soil resource of plants (Islam *et al.*, 1980). Certain soil bacterial genera such as *Pseudomonas*, *Enterobacter*, and *Bacillus* are able to dissolve the insoluble P forms in the soil (FAO, 2000), thereby making more P available for plant use.

2.5 Prospects in breeding P efficient cowpea varieties using genomic resources

For a successful implementation of modern breeding programmes in cowpea, certain genomic tools and resources are required. These include high throughput genotyping facilities for accurate fingerprinting of parents and progenies resulting from hybridization. SNP based genotyping platforms such as Illumina GoldenGate (Muchero *et al.*, 2009), and 60K Iselect SNP Infinium (Justin, 2014) are now available for cowpea programmes. The cowpea GoldenGate Assay can genotype effectively 96 DNA samples over 1,536 SNP loci, and the genotypic data is usually provided via 'GenomeStudio' software by Illumina *Inc*, which provides data visualization and primary summarization (Boukar *et al.*, 2016; Muñoz-Amatriaín *et al.*, 2017).

Different mapping populations have been developed for cowpea; such as biparental recombinant inbred lines (Andargie *et al.*, 2013; Andargie *et al.*, 2011, 2014; Huynh *et al.*, 2013; 2016; Lucas *et al.*, 2012; Muchero *et al.*, 2013; Omo-Ikerodah *et al.*, 2008), and more recently an eight-parent multi-parent advanced generation intercross population (MAGIC) recombinant inbred lines (Huynh *et al.*, 2018). Molecular markers are used in the construction of genetic linkage maps used for the detection of genomic regions containing genes controlling the expression of agronomic traits (Sánchez-Sevilla *et al.*, 2015).

Markers that are tightly linked to important genes can be deployed in marker-assisted breeding (MAB). Until recently, the number of molecular markers for cowpea has been limited. In addition, consensus genetic maps formed from merging multiple individual linkage maps have been constructed. Such maps are very useful for breeders as they serve as an important resource in analyzing traits inheritance and marker-trait associations (Muchero *et al.*, 2009; Muñoz-Amatriaín *et al.*, 2017; Lucas *et al.*, 2011).

Several QTLs have been mapped using different marker systems, such as AFLP; QTLs for cowpea golden mosaic virus (Rodrigues *et al.*, 2012), S*triga* resistance (Boukar *et al.*, 2004), drought-induced senescence (Muchero *et al.*, 2009), charcoal rot resistance (Muchero *et al.*, 2011) and flower bud thrip resistance (Omo-Ikerodah *et al.*, 2008) were mapped. SCAR markers were used by Boukar *et al.*, (2004) to map S*triga* resistance QTLs. QTLs for seed weight were mapped by Fatokun *et al.*, (1992) using RFLP markers. Linkage and QTL mapping with SSRs have been conducted for the following traits; pod fibre layer thickness (Andargie *et al.*, 2011), pod length and domestication-related traits (Kongjaimun *et al.*, 2012), time of flower opening and days to flowering (Andargie *et al.*, 2013), pod number per plant (Xu *et al.*, 2013), floral scent compounds (Andargie *et al.*, 2014), and pod tenderness (Kongjaimun *et al.*, 2012).

Furthermore, SNP markers were employed by several workers to map QTLs for cowpea traits. These include; cowpea bacterial blight resistance (Agbicodo *et al.*, 2010), foliar thrip tolerance (Lucas *et al.*, 2012), hastate leaf shape (Pottorff *et al.*, 2012), charcoal rot resistance (Muchero *et al.*, 2011), flower and seed coat colour (Xu *et al.*, 2011), days to flower, nodes to first flower, leaf senescence (Xu *et al.*, 2013), heat tolerance and seed size (Lucas *et al.*, 2013), *Fusarium* wilt resistance (*Fot* race 3) (Pottorff *et al.*, 2012), and *Fusarium* wilt resistance (*Fot* race 4) (Pottorff *et al.*, 2014).

Understanding the genetic architecture of quantitative traits variation is one of the major foci of modern biology (Mackay *et al.,* 2009). QTLs and molecular markers are potential candidates for marker-assisted selection in cowpea. An excellent review on available cowpea molecular markers ranging from AFLP, RFLP, SCAR, SSR, and SNP has been reported (Boukar *et al.,* 2016; Huynh *et al.,* 2013; Muñoz-Amatriaín *et al.,* 2017; Ndeve, 2017; Varshney *et al.,* 2009; Varshney *et al.,* 2013).

2.6 Farmers' Preferences and Knowledge of P deficiency

Low phosphorus (P) is a wide-spread abiotic factor constraining productivity of cowpea in farmers' field. This is aggravated by continuous cultivation of land without application of recommended fertilization and unsustainable practices such as removal of crop residues to feed livestock and bush burning that continuously deplete soil nutrients and organic matter content (Abdou, 2018). Fertilizer application among farmers has been little to none due to several reasons, such as lack of awareness on the importance of P for cowpeas, while high cost and poor accessibility to phosphate fertilizers has hindered those with awareness (Bationo *et al.,* 2002).

It is important to understand farmers' perception on phosphate fertilization on cowpea fields especially that there is widespread misinformation among some farmers that cowpeas do not need fertilizers, such information will help in designing programmes targeting increase productivity of cowpeas and soil management practices (Marenya *et al.,* 2008). Knowledge of farmers on soil types and using it to identify soils with poor fertility has been reported (Kome *et al.,* 2018). Many farmers are aware that low crop yield, the prevalence of *Striga* and yellowing of leaves are indicators of poor soil fertility especially for major cereals (Souhore *et al.,* 2017). Recent studies have documented farmers preferred traits and perceptions on different constraints militating cowpea productivity (Lawan, 2014). There are little to no documentation on knowledge and perceptions of farmers on recommended fertilizers for cowpea especially in the major growing areas in Nigeria.

CHAPTER THREE

3.0 Assessing Farmers' Knowledge, Perceptions and Use of Phosphorus Fertilization for Cowpea Production in Northern Guinea Savannah of Nigeria

3.1 Introduction

Cowpea is a popular leguminous crop in Nigeria and other countries in sub-Saharan Africa and provides food for over two hundred million people (AATF, 2010). Cowpea supplies a substantial amount of daily protein needs of most people (Lowenberg-DeBoer & Ibro, 2008) in the growing areas. The yield of cowpeas grown by local farmers is low, less than 600 kg ha^{-1}, compared to the yield potential of 1,500 – 2,500 kg ha^{-1} (AATF, 2012; ICRISAT, 2017a). The low yield is due to many biotic (insect pests, diseases, parasitic weeds) and abiotic constraints (low soil fertility especially phosphorus, drought, heat) (ICRISAT, 2017a). Other important factors resulting in low yield are low plant density due to intercropping and wide intra-plant spacing (Olufajo & Singh, 2002; Ewansiha *et al.,* 2014). There is also a major price fluctuation due to the volatility of markets, lack of quality control and standards (Abate *et al.*, 2012) such that prices are low at harvest and go up when most farmers have finished selling their stocks. Such price instability hinders farmers from adopting improved technologies.

Among abiotic factors responsible for low yield of cowpea are poor soil fertility, especially nitrogen (N) and phosphorus (P) (Bationo *et al.*, 2002). Cowpeas can fix a considerable amount of N in the presence of adequate P, however, it is poor in accessing soil available P (Bationo *et al.,* 2002). For soils very low in P and those with P fixing properties, it is desirable that P be applied as inorganic fertilizer or manure (Bationo *et al.*, 2002; Mahamane, 2008; Saidou *et al.*, 2012). Most farmers in poor nations are unaware of P as a yield-boosting factor that needs to be applied (Horn *et al.,* 2014). The non-use of synthetic P fertilizer by many smallholder farmers are compounded by the high cost of fertilizers (Bationo *et al.*, 2002), and non-availability (Olufowote & Barnes-Mcconnell, 2002). There is limited information on the level and practice of P fertilization in cowpea growing areas, especially in northern Nigeria. The low level of adoption of improved management practices such as (phosphate fertilization, improved cropping systems, improved seeds, planting spacings, and insect control measures) by smallholder farmers (Horn *et al.*, 2014), who account for most food production in developing nations, has been partly due to lack of involvement of product end-users in planning and design of such practices, thereby leading to low levels of adoption, since these technologies are mostly results of researchers' conceived problems and were developed under optimum conditions of research institutions.

3.2 Farmers' perception of cowpea productivity and yield constraints

Cowpeas are mainly produced in the northern parts of Nigeria, while the southern part of the country provides a market for grains from the north (Agwu, 2004; Boukar *et al.*, 2018; Lowenberg-DeBoer & Ibro, 2008). For a new technology such as improved variety to have a high level of adoption by farmers, it must incorporate inputs and thinking of all stakeholders in the product development plan (Persley & Anthony, 2017). Understanding the knowledge and preferences of farmers and consumers and taking them into account when designing a new product is critical to the successful adoption of the product (Kushwaha *et al*, 2004). For instance, using a "person-on-the-street" approach, the level of awareness and acceptance of genetically modified (GM) cowpea was investigated in northern Nigerian States of Gombe, Adamawa, Jigawa, and Kano, and it was revealed that 90% of consumers had knowledge of biotechnology-derived crops and showed willingness to adopt such products when commercialized in Nigeria, while 10% expressed certain ethical concerns and disapproved of the technology (Kushwaha *et al.*, 2004).

Appropriate understanding of end-users' perception and needs is important before release of new products, since adoption of new products and technologies does not guarantee increase in productivity (Chambers, 1994), as was clearly demonstrated in the early 2000s in some famine-hit Southern Africa countries, especially Zambia, when GM corn grains donated by the US were rejected by the recipients due to poor perception and ethical concerns for GM crops (http://news.bbc.co.uk/2/hi/africa/2371675.stm). Studies on consumers' preferred traits like grain size, texture, seed coat colour, cooking time, ease of hilum and testa removal are important for market development but are often not taken into account with high priority in breeding programmes. Consumers surveyed in Ghana, Mali, and Nigeria would pay high prices for large cowpea grains and rejected grains with exit holes created by storage weevils (Mishili *et al.*, 2007). Traits like these are crucial for key stakeholders in the cowpea value chain and should be considered in designing and deploying products that fit the needs of the end-users (Mishili *et al.*, 2007).

Cultivation of landraces, especially photoperiod sensitive types with low yield potential, is still very popular with cowpea growers despite years of research by national, international research institutions and development partners (Mishili *et al.*, 2007). The popularity of landraces among farmers is partly due to their large grains preferred by many consumers (Huynh *et al.*, 2013; Lucas *et al.*, 2015; Lo *et al.*, 2018). Knowledge of farmers and consumers preferences will rapidly drive demand-led breeding and facilitate uptake and use of improved varieties, thereby leading to increased income for farmers and other cowpea value-chain actors such as grain merchants, and processors (Lowenberg-DeBoer & Ibro, 2008). Understanding farmers knowledge of production technologies and farming systems is critical to increasing the level of crop productivity and adoption of new farming technologies (Hoffmann *et al.*, 2007). In Nigeria, especially in the northern parts where there is a substantial amount of cowpea production, farmers cultivate cowpea in intercrops with maize, sorghum and pearl millet (Agwu, 2004). In areas where research organizations like IITA and NARS have been in touch with farming communities, sole cropping of cowpea and other improved cereal-legume intercropping has been advocated but the level of adoption has not been well documented. Farmers' knowledge and perception of phosphorus and other inorganic fertilizers in cowpea fields have not been investigated. Thus, there is little information about farmers' knowledge of P fertilization, level of adoption of sole cropping, perceived production constraints, and preferences of cowpea traits in northern parts of Nigeria.

The objectives of this research were to assess farmers knowledge of phosphorus fertilization, identify prevailing popular cowpea cropping systems, determine farmers' perceived production constraints, preferred varieties, reasons for preference of cultivated varieties, and management strategies for production constraints.

3.3 Materials and Methods

3.3.1. Description of study areas

The study was carried out across 36 villages in twelve local government areas (LGA) of three States in the northern guinea savannah agro-ecological zone of Nigeria (Fig. 3.1). These States; Kano, Kaduna, and Katsina, are known for a significant level of cowpea production in the region and have been previously surveyed (Kormawa *et al.,* 2002; Langyintuo *et al.*, 2003; Lowenberg-DeBoer & Ibro, 2008; USAID, 2015). These States fall under "*Northern Guinea Savannah*", one of the three agro-vegetational regions of Nigerian savannah zones. Kano State is the second most populous State in Nigeria after Lagos, with a population of over 11 million (NBS, 2012). It lies mostly in the Sudan savannah with pockets of other types of savannah ecologies giving it a wider range of growing period with an annual rainfall of 500 -1000 mm. Cowpea is grown by most farmers in Kano State and serves as an important source of income and food (Kormawa *et al.*, 2002; Lowenberg-DeBoer & Ibro, 2008).

Kano is home to the biggest international cowpea grain market (*Dawanau-Market*) in West Africa, serving as the main importing and exporting terminal for cowpea grains (Lowenberg-DeBoer & Ibro, 2008). The four districts surveyed in Kano were Albasu, Bunkure, Tsanyawa and Minjibir LGAs. The second State for the survey was Kaduna State. It is in the central part of northern Nigeria, with a population of over 7 million (NBS, 2012) and constitutes one of the main cowpea producing and consuming areas in Nigeria. Kaduna's annual rainfall is 1000 - 1500 mm (NAERLS *et al.,* 2017). Four LGAs were visited and surveyed; Birnin-Gwari, Giwa, Kajuru, and Makarfi. The third surveyed State was Katsina State; located in northwestern Nigeria, with a population of over 6 million. It is also a major producing zone of the crop and has three main agro-ecologies; Sudan, Sahel and northern Guinea savannah. The four LGAs surveyed were; Matazu, Kaita, Danja and Dandume. The annual rainfall of the State is within 1000 – 1600 mm (NAERLS *et al.,* 2017).

3.3.2 Sampling procedure

A two-step sampling procedure was undertaken to select cowpea farmers for the study. The first was to identify major production areas in Nigeria from review of literature (Kormawa *et al.,* 2002; Lowenberg-DeBoer & Ibro, 2008; USAID, 2015) and random selection of three States in the northern 'cowpea belt', a term used to describe major cowpea production zones (Lowenberg-DeBoer & Ibro, 2008). The second step comprised a random selection of 420 households involved in cowpea cultivation across the selected study sites with the help of village extension agents for the various studied areas. Each of the randomly selected farmers was based on criteria, that she/he have grown cowpea in the previous season.

3.3.3 Data acquisition and approach

Semi-structured questionnaires were administrated to an average of 10 farmers per site in each of the LGAs visited using stratified random sampling procedure and data were collected from a total of 420 farmers in these villages (an average of 35 farmers from 4 sites in a LGA, that is 35*4 LGA*3 States). Data was collected on socio-economic characteristics, the experience of cowpea production, use of fertilizers, knowledge of phosphorus-based fertilizers in cowpea fields, cropping system, varietal preferences, preferred traits, constraints of cowpea production and mitigation strategies being used. The questionnaires were administered by trained enumerators while household data, cropping system, varietal use, production constraints, and preference traits were validated from information obtained via focus group discussion sessions with key informants and personal observations during the survey.

Focus group discussion (FGD) sessions were conducted between January through March 2016. One group per every site, with each comprising average of 8 - 10 participants, was interviewed for a detailed insight into some of the questions stated in the questionnaire. The criteria for inclusion in the FGD was having grown cowpea in the previous season. The groups included young and old male and female growers except for Kano State, where separate groups of men (young and old) and women (young and old) were formed due to cultural norms. The assistance of village extension agents from the Agricultural Development Programme (ADPs) of the target States facilitated the formation and discussion sessions. The FGD was managed by the principal researcher and in some areas facilitators who usually introduced the topics for discussion, and guided members of the group towards effective participation. Pair-wise comparison charts were used to rank constraints and preferences identified by farmers from the FGD exercise.

To further understand the thinking of farmers on the use of phosphorus specific fertilizers on cowpea fields, a five-point Likert scale was used with seven questions to investigate the perception of the respondents. Responses to Likert items were coded on a five-point scale, where 1 = strongly disagree, 2 = disagree, 3 = undecided, 4 = agree and 5 = strongly agree. Factor analysis was used to help determine important variables influencing farmers' perceptions of P-based fertilizers. Factor analysis was executed on (SPSS v.20.0). It is important to test the adequacy of sampled respondents for factor analysis to be valid, therefore Kaiser Meyer-Olkin measure of sampling adequacy (KMO - MSA) was estimated. The strength of the relationship between perception variables was measured using Bartlett's test of sphericity.

A pair-wise comparison chart (PCC) was used during the FGD held in each of the 36 sites to identify cowpea production constraints and preferred traits in varieties. Constraints and preferences were identified through FGD and ranked per site. PCC helped to identify the most important constraint faced by farmers. Identified constraints were arranged in a matrix of rows and columns on a whiteboard and ranked in a pairwise manner, such that when two constraints were ranked, the most important receives 1 and the less important gets 0.

3.3.4 Data analysis:

Data collected from questionnaires and FGD were coded and analyzed using SPSS v.20.0 and Nlogit 4.0 software. Results were summarized and presented with descriptive statistics and factor analysis outputs, while binary logit model was used to study farmers' choices of using synthetic fertilizers on cowpea or not, as two mutually exclusive events such that when a farmer used synthetic P fertilizers as a chosen option, the other is by default not taken (Horn *et al.*, 2014). The use of P containing fertilizer for cowpea was modelled as a dependent variable with a binary choice taking 1 if the farmer uses P based fertilizer in cowpea fields and 0 as otherwise. The logit model was appropriate for a response (s) with two possible outcomes such as 1 or 0, yes or no, or present and absent (Mendesil *et al.*, 2016; Naziri *et al.*, 2014). The use of P containing fertilizers by cowpea farmers (use and non-use) served as dependent variable while sex, marital status, age, household size, level of formal education, experience in growing cowpea, ability to detect nutrient deficiency symptoms, cropping systems, attendance of field days, and contacts with extension agents served as independent (explanatory) variables (Table 3.1) for factor analysis in Nlogit software.

The original data set included information on different levels of formal education (primary, secondary and tertiary) but these levels of formal education were categorized as formal vs no formal education to arrive at a simple distinction between formal education and informal education where formal is 1 and the latter is 0, thereby measured as a dummy variate. The main aim of this analysis was to describe the way in which the use of P or P containing fertilizers was influenced by explanatory variables mentioned above. Empirically, the model for estimating the determinants of the probability of farmers' using P containing fertilizer was as described as follows (Verbeek, 2004):

$$\ln\left[\frac{P_x}{1-P_x}\right] = \beta_o + \sum \beta i \ Xi$$

where P_x is the probability of an event occurring (1) if the farmer uses P containing fertilizer and (0) is otherwise; βo is a constant term; βi is a coefficient associated with the independent variate x_i, and x_i is the independent variate. Several independent variables likely to influence the choice of farmers using synthetic P based fertilizers on cowpea were identified and used in the model. These are presented in the table below;

Table 3.1: List and description of variables used for binary logit model

Variable	**Description**	**Variable type**	**Units**
Dependent variable			
Use P Fertilizers	Farmers' use of P on cowpea	Dummy	1= yes, 0 = no
Independent variable			
Sex	Sex of the farmer	Dummy	1= male, 0 = female
Status	Marital status of the farmer	Dummy	1 = married, 0 = single
Age	Age of the farmer	Years	Continuous
Household-size	Size of farmers' household	Persons	Continuous
Education	Formal education of a farmer	Dummy	1 = formal, 0 = informal
Experience	Experience in cowpea cultivation	Years	Continuous
Def-Symptoms	Ability to know nutrient deficiency symptoms	Dummy	1 = yes, 0 = no
Cropping-System	System of cowpea cultivation	Dummy	1 = sole, 0 = mixed
Field-day	Attendance of cowpea field day	Dummy	1 = yes, 0 = no
Extension-Contact	Access to cowpea production information from extension agents	Dummy	1 = yes, 0 = no

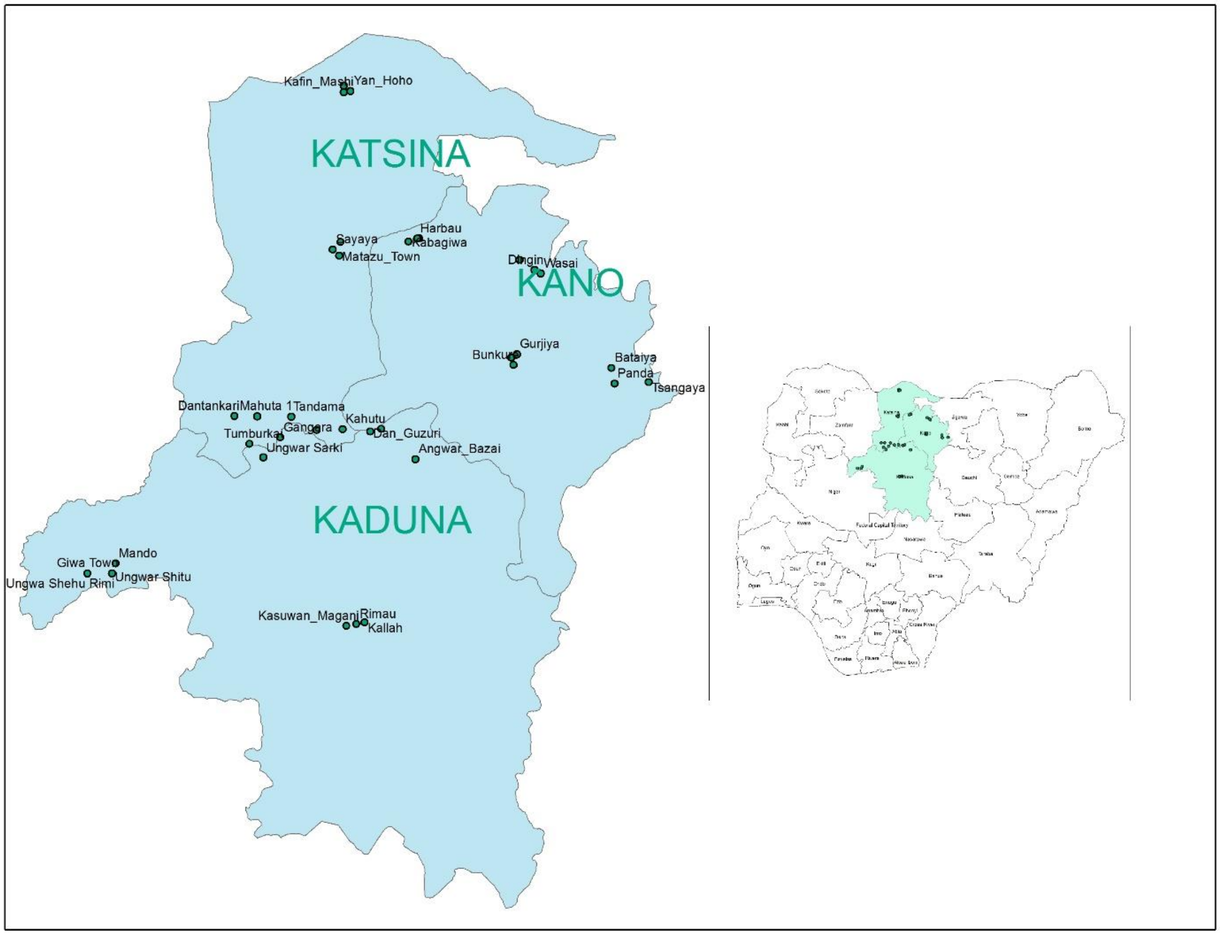


Figure 3.1: Map of study sites across three northern Nigerian States. **Inset**: Map of Nigerian States

3.4 Results

3.4.1 Socio-Economic Metrics of the Respondents

The majority (85%) of cowpea farmers across the three States of the study were male (Table 3.2). There was an interesting trend in certain areas with a good representation of female cowpea producers: Birnin-Gwari, Bunkure, Kajuru, Makarfi, and Tsanyawa (Table 3.2). In these areas, female cowpea farmers had good contacts with extension agents and were involved in cooperative activities. At Tsanyawa, most of the women farmers had post-secondary education and used hired labour for the management of their farms. Table 3.3 shows that most of the farmers were married (96%), indicating the establishment of the family is an important cultural norm of these farming communities.

Table 3.2: Sex distribution of respondents

	Gender of Responder			Percent by gender	
Local Government Areas	Male	Female	Total interviewed	Male	Female
Albasu	35	3	38	92	8
Birnin-Gwari	30	5	35	86	14
Bunkure	25	10	35	71	29
Dandume	29	0	29	100	0
Danja	36	0	36	100	0
Giwa	37	5	42	88	12
Kaita	30	0	30	100	0
Kajuru	24	17	41	59	41
Makarfi	19	11	30	63	37
Matazu	32	3	35	91	9
Minjibir	35	0	35	100	0
Tsanyawa	24	10	34	71	29
Total	356	64	420	85	15

The age of farmers cultivating cowpea varied widely: most farmers were between 30 - 50 years old across all sites. Our surveys showed that the level of formal education among the respondents was generally low, as farmers without any form of formal education constituted over 37% (Table 3.3). Approximately, 21% of the respondents had received some formal education: at least the six years of primary, secondary education. The post-secondary qualification - mostly two-year certificate courses were reported in areas close to urban centres like Albasu, Dandume, Matazu, and Tsanyawa. Most farmers in some areas had informal (Qur'anic) education (37%) and were able to read and write literature in the local language (Hausa) using Arabic alphabets, a system known as "*Ajami*".

Half of the cowpea farmers interviewed (50%) had more than ten years' experience of cowpea cultivation, 27% had up to 20 years' experience, while approximately 2% have been growing cowpeas for up to 40 years. The various years of experience in growing cowpeas imply that these farmers should be aware of the traditional cowpea production practices (Table 3.3).

Table 3.3: Socio-economics attributes of cowpea farmers surveyed across study areas

Attributes of the Respondents		**Frequency (420)**	**Percent (%)**
Marital Status of Responder			
	Married	402	95.7
	Single	18	4.3
Age distribution of Responder	11 - 20	9	2.1
	21 - 30	56	13.3
	31 - 40	127	30.2
	41 - 50	108	25.7
	51 - 60	91	21.7
	> 60	29	6.9
Level of Responder's Education	Primary	88	21.0
	Secondary	89	21.2
	Tertiary	88	21.0
	Informal	155	36.9
Years of cowpea production	1-10 years	211	50.2
	11-20 years	113	26.9
	21-30 years	66	15.7
	31-40 years	23	5.5
	Above 40 years	7	1.7

3.4.2 Cowpea cropping systems and varieties under cultivation by farmers

There were varied cropping systems depending on the aim of the grower, type of variety planted (early or late maturing), level of education, contact with extension agents and sex of the farmer. Intercropping of cowpea with major cereals like maize, sorghum, and pearl millet was the major cropping system in most areas. On the average for the 12 LGAs studied, 42% of respondents planted cowpea in a mixed intercropping fashion (Table 3.4). Sole cropping of cowpea was adopted by 25% of the farmers and was more popular in Albasu, Bunkure, Kajuru, Minjibr, and Tsanyawa over other studied areas. In-depth discussions with these farmers revealed that sole cropping in these areas was popular among farmers with basic formal education, contacts with extension, and those that participated in the demonstration programmes such as the USAID-supported cowpea upscaling project, and IITA’s varietal demonstration programmes in these areas.

Relay cropping was another system found in a few localities (9%), where cowpea cultivation followed the harvest of main cereals like maize. Usually, such farmers would grow early maturing varieties of maize and follow it with cowpea varieties, mostly medium to late maturing types. A good number of farmers (23%) had both sole and mixed-intercropped fields (Table 3.4). Farmers grew cowpea in mixed intercrop to maximize the use of available land and resources especially as they believe cowpeas benefit from residual fertilization of maize fields. In most mixed intercropped fields, separate application of fertilizer was usually not carried out for cowpea. Farmers planted the cowpeas late so that most crops would have been harvested by the time cowpeas were ready for harvesting, thereby making more labour available and also that gave the crop sufficient time to dry in the field reducing the stress of sun drying fresh or undried pods associated with early maturing varieties that mature and get harvested in the middle of the rainy season.

Cultivation of landraces vis-a-vis improved varieties was found to be still very popular among farmers. These landraces are characterized by low yield potential compared to improved cowpea varieties. On average across the study areas, 79% of the respondents' planted landraces and only 6% planted improved varieties. Improved varieties were used more in Albasu, Minjibir, Bunkure, and Tsanyawa and a good number of farmers cultivated both landraces and improved varieties (15%) on different plots (Table 3.4), thereby making total cultivation of improved varieties 21%.

Some of the landraces being used might be improved varieties introduced to farmers by different intervention programmes and projects, whose names were changed to local names over time, making it difficult to distinguish them from local ones. For instance; some of these varieties were given names of the projects or programmes that introduced them like Dan-Project (translated as son of project), Dan Research (son of research), Kwankwaso (introduced by former administration of Kano State Governor called Kwankwaso), Dan-OC (son of officer-in-charge), Dan-KATARDA, and Dan-KNARDA among others. Other local cultivars with suspicious names were Dan-Acre, Dan-Gombe, Dan-Sokoto, and Dan-Arbain. These might all be improved varieties that were introduced to farmers at different times. No efforts were made to clearly ascertain these varieties as improved ones and they were grouped as landraces. So, any study aimed at determining the level of adoption of improved cowpea varieties should ask farmers when and where these varieties were obtained originally, and will need to use more precise tools to identify improved varieties from landraces.

Table 3.4: Percentage of farmers reporting different cropping cowpea systems and use of local vis-a-vis improved varieties

Cropping Systems	**Local government areas (LGAs)**												
	Albasu	Birnin_Gwari	Bunkure	Dandume	Danja	Giwa	Kaita	Kajuru	Makarfi	Matazu	Minjibir	Tsanyawa	Mean
Sole cowpea	42	17	51	0	0	19	3	71	10	9	51	26	**25**
Mixed cropping	50	17	14	90	61	50	57	27	43	46	31	24	**42**
Relay intercrop	0	54	3	0	14	19	0	0	7	3	0	9	**9**
Sole & Mixed plots	8	11	31	10	25	12	40	2	40	43	17	41	**23**
Varieties In-Use													
Landraces	45	100	94	97	97	86	93	100	90	71	46	29	**79**
Improved Varieties	3	0	0	3	0	0	0	0	3	3	49	6	**6**
Local & Improved	53	0	6	0	3	14	7	0	7	26	6	65	**15**

3.4.3 Cowpea farmers' knowledge and use of phosphorus-based fertilizers

Most farmers used some forms of fertilization indicating that farmers were aware of the importance of fertilizers in crop productivity. Most (82%) farmers across all studied areas (Figure 3.2) used some forms of fertilizers on their fields. Several kinds of fertilizers and combinations were used including nitrogen phosphorus potassium (NPK), single super phosphate (SSP), Urea, farmyard manure (FYM), NPK + Urea, SSP + NPK, NPK + FYM, FYM + SSP, FYM + Urea, NPK, Urea + FYM (Figure 3.3). FYM is a combination of animal droppings like poultry, cow dung, ashes, and refuse dumps. Most farmers did not use phosphorus specific fertilization to grow cowpea. Only 10% used SSP; a pure phosphorus-based commercial fertilizer, 11% used a combination of SSP+NPK, 0.5% used FYM plus SSP and 17% added no fertilizers. Many farmers believed cowpea does not require fertilizers and hence did not systematically add recommended fertilizers to cowpea fields.

Over 81% of farmers knew when cowpea plants were suffering from inadequate nutrient in the soil (Table 3.5). Most farmers (88.2%) reported having noticed the presence of nodules on roots (Table 3.5). The identification of root nodules was facilitated during the interview with photographs of cowpea roots with nodules attached. However, the majority were unaware (72%) of roles played by nodules (Table 3.5) and 6% perceived nodules to be harmful (perceived as *Striga* attachments) to the health of the plant.

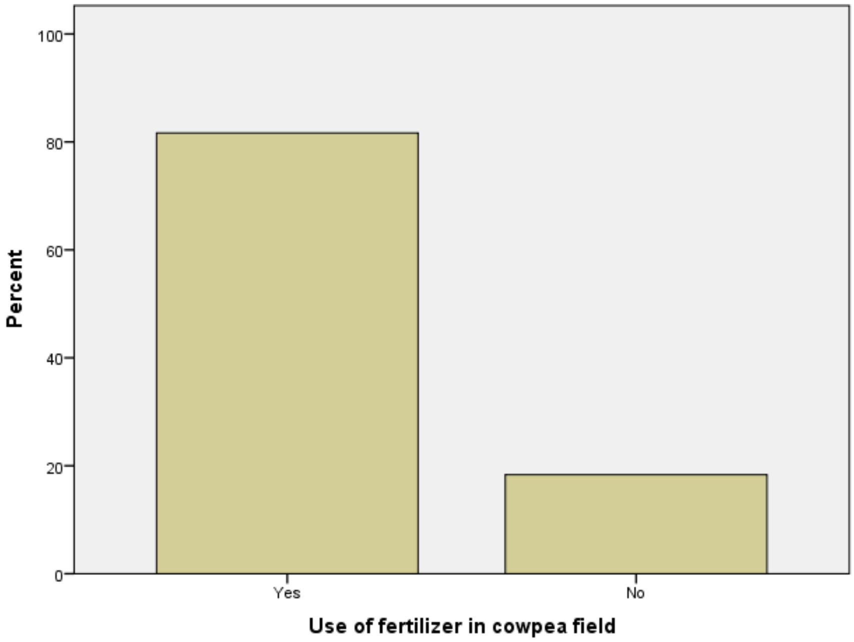


Figure 3.2: Survey responses on fertilizer use in cowpea fields, X-axis is survey responses and Y-axis is a percent of farmers using fertilizers

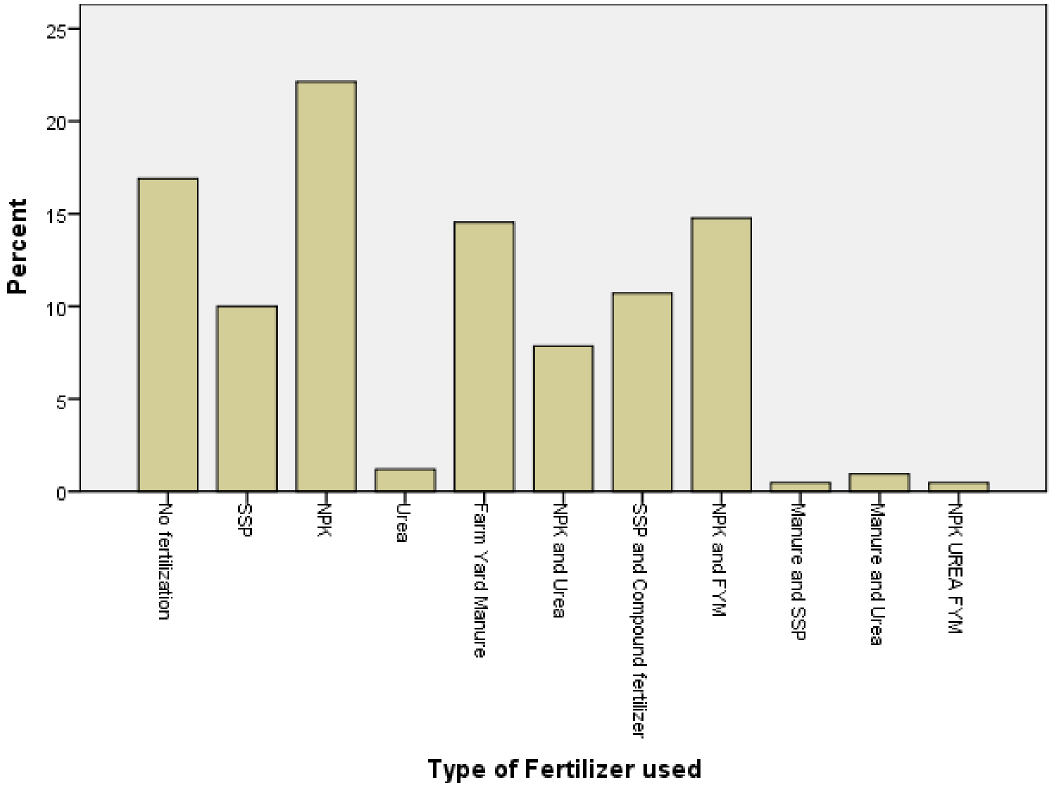


Figure 3.3: Percentage of cowpea farmers using different types of fertilizers, X-axis is fertilizer types being used by farmers and Y-axis is a percent of farmers using fertilizers

Table 3.5: Percent of farmers recognizing nutrient deficiency on cowpea, presence of nodules on cowpea roots and knowledge of the role played by nodules in cowpea health

	Frequency	Percent
Deficiency Recognition		
Yes	337	80.2
No	83	19.8
Knowledge of Nodules		
Yes	370	88.2
No	50	11.8
Percentage reporting role of nodules for cowpea		
Yes	92	22
No	302	72
Harmful	26	6

3.4.4 Farmers' perceptions of phosphorus fertilization on cowpea plants

Descriptive statistics of the Likert items showed that the Likert item "Phosphorus increases cowpea yield", accounted for most of the variation with the highest mean of 4.4. The result of KMO test showed a value of 0.620 (Table 3.6), which falls within the range of acceptable values for satisfactory factor analysis (Kaiser & Rice, 1974). The Bartlett's test of sphericity was highly significant (Table 3.6) and therefore, the null hypothesis was rejected. Several Likert items were influential in determining farmers' perception on the use of P-based fertilizers (Table 3.7). The first three principal components were retained for further interpretation since they explained over half of the variation (61.1%) in the dataset (Table 3.7).

Table 3.6: Descriptive statistics on Likert items for the perception of farmers on cowpea phosphorus fertilization, and sampling adequacy test

Variables (Perception)		Mean	Std. Deviation
Phosphorus reduces growth & vigour		1.9	1.3
Phosphorus increases cowpea yield		4.4	1.1
Phosphorus use increases the cost of production		3.0	2.9
Phosphorus use is labour intensive and time-consuming		2.7	1.3
I do not use P because of no prior knowledge on its use		2.9	1.5
I do not use P because it is expensive to purchase		2.8	1.4
I do not use P because it is unavailable in the market		2.9	1.5
Sampling adequacy and strength of relationship among variables			
Kaiser-Meyer-Olkin Measure of Sampling Adequacy		0.620	
Bartlett's test of sphericity	Approx. Chi-Square	268.093	
DF		21	
Significance.		0.000	

Table 3.7: Variables and their contribution to the total variance of the principal components

	Principal Components (PC)		
Variables (Factors)	**1**	**2**	**3**
Phosphorus reduces growth & vigour	NA	0.790	NA
Phosphorus increases cowpea yield	NA	-0.799	NA
Phosphorus use increases the cost of production	0.713	NA	NA
Phosphorus use is labour intensive and time-consuming	0.648	NA	NA
I do not use P because of no prior knowledge on its use	NA	NA	NA
I do not use P because it is expensive to purchase	0.775	NA	NA
I do not use P because it is unavailable in the market	NA	NA	0.869
Variance (%) explained by PC	24.6	21.1	15.2
Cumulative variance (%) explained by PC	24.6	45.9	**61.1**

NA = no contribution to the component by the variable.

3.4.5 Determinants for use and non-use of phosphorus-based fertilizers among cowpea farmers

A binary logit model was used to model factors influencing use and no-use of phosphorus fertilizers among cowpea farmers, taking use or no-use as the dependent variable. The model showed the positive and significant prediction of use of P on cowpea by farmers being influenced by three independent variables; knowledge of nutrient deficiency symptoms, attendance of field day and contacts with extension agents (Table 3.8).

Table 3.8: Binary logit outputs on variable influencing use of phosphorus fertilizers

Variable[1]	**Coefficient**	**SE**	**b/St. Er.**	**P[|Z|>z]**	**Mean of X**
Constant	2.895**	1.041	2.772	0.007	NA
Sex	-0.741	0.312	-2.374	0.018	1.15
Status	-0.370	0.539	-0.686	0.493	1.04
Age	0.066	0.118	0.557	0.577	4.72
Household-size	0.006	0.014	0.408	0.683	13.49
Education	-0.162	0.982	-1.649	0.099	2.74
Experience-cowpea	-0.282	0.133	0.212	0.832	1.81
Def-symptoms	-0.986**	0.274	-3.598	0.0003	1.19
Cropping-system	-0.105	0.107	-0.977	0.329	2.28
Field-day	0.453*	0.206	2.193	0.028	0.50
Extension-contacts	0.380*	0.164	2.324	0.020	0.78

Definition of variables are given in Table, *P<0.05, **P<0.01, N = 420, log likelihood = -242.6675, LR x^2 (11) = 53.39966, Prob > x2 = 0.0000, McFadden pseudo-R^2 = 0.0991205, NA = not applicable

3.4.6 Farmers' production constraints and preference traits

The following constraints were common to most sites; insect pests, aphid infestation, limited access to improved seeds, *Striga*, *Maruca*, and drought varied across location (Table 3.9). Other common constraints identified were adulterated chemicals, limited access to improved seeds, pod sucking bugs, and fluctuation in market prices. It is interesting to note that, farmers in some areas specifically mentioned aphid, *Maruca*, pod sucking bugs, and termites as their constraints rather than mentioned the generic term of insect pests as was used by some farmers.

Farmers identified and listed different traits as preferred traits in cowpea varieties. Across all locations, farmers ranked high grain yield as the most preferred trait in a variety. Other preferences varied between the studied areas, such as the appealing look of grains termed as good market value and large-seeded were ranked second and third most preferred traits in most of the locations (Table 3.10).

Other qualities identified as preferred traits in most of the sites were resistance to pests, early maturity, and good fodder quality. Interestingly, small-seeded grains were mentioned as an important characteristic in two LGAs, this was preferred by local food vendors for making steam-cowpea paste called "*moi-moi*", while large-seeded types were preferred by those interested in using cowpea grains for direct consumption in local dishes such as rice plus cowpea. Reasons given for early maturity was that they provided quick food and money to take care of other crops in the field while those with good fodder types provided feed for their livestock.

Table 3.9: Farmers' perceived production constraints identified during focus group discussion using pair-wise ranking in Northwestern Nigeria in 2017

	Local Government Areas											
Constraints	**Albasu**	**Bunkure**	**Tsanyawa**	**Minjibir**	**Matazu**	**Kaita**	**Danja**	**Dandume**	**Birnin-Gwari**	**Giwa**	**Kajuru**	**Makarfi**
Adulterated chemicals	-	4	3	-	-	-	-	4	-	3	-	-
Aphid	1	2	-	-	3	2			2	3	2	2
Diseases	-	7	-	-	-	-	-	-	-	-	4	-
Drought	5	-	-	3	2	4		2		1	5	5
Flower abortion	-	10	-	-	-	-	-	-	-	-	3	-
Flower thrips	6	-	-	-	-	-	-	-	-	-	-	-
Fluctuation in price	-	11	6	-	-	-	-	-	-	-	-	-
High cost of chemicals	-	-	5	-	-	-	-	-	-	-	-	-
Insect pests	2	1	-	2	1	1	1	1	5		3	1
Late maturity	9	-	-	-	-	-	-	-	6		-	-
Limited access to seeds	-	3	1	-	-	-	2	3	-	-	-	3
Maruca	4	5	-	-	-	-			1	2	6	4
Pod shattering	8	-	-	-	-	-	-	-	-	-	-	-
Pod sucking bugs	-	9	-	-	-	3	-	-	3	3	6	5
Poor access to fertilizers	-	8	2	-	3	-	3	-	-	-	-	-
Poor soil fertility	-	-	-	4	-	-	-	-	-	-	-	-
Poor storage facility	-	10	4	-	-	-	-	-	-	-	-	-
Seed quality	7	-	-	-	-	-	-	-	-	-	-	-
Seed viability	3	-	-	-	-	-	-	-	-	-	-	-
Striga	2	6	-	1	2	-	-	-	4		1	-
Termites	9	-	-	-	-	-	-	-	6		-	-
Weeds	-	-	-	-	-	-	-	-	6		-	-

*Numbers indicates Ranks
1 = most important constraint,
11 = least important constraint
-denotes constraint was not reported in the area

Table 3.10: Cowpea farmers' preference traits identified during focus group discussion using pair-wise ranking in Northwestern Nigeria in 2017

	Local Government Areas											
Preferences	**Albasu**	**Bunkure**	**Tsanyawa**	**Minjibir**	**Matazu**	**Kaita**	**Danja**	**Dandume**	**Birnin-Gwari**	**Giwa**	**Kajuru**	**Makarfi**
High yield	1	1	1	1	-	1	3	1	1	1	1	1
Good market value	2	2	2	-	-	-	-	-	2	-	2	-
Large-seeded grains	3	3	3	3	-	-	2	-	-	3	4	2
Access to P-fertilizers	-	-	-	-	2	-	-	-	-	-	-	-
Early maturity	-	-	2	5	1	2	-	2	-	-	-	4
Good fodder quality	7	-	3	4	-		3		-	-	-	-
Resistance to pests	4	4	-	2	3	3		3	-	-	-	-
Good taste	8	6	-	-	-	-	-	-	-	5	4	-
Non-shattering	-	-	-	-		-	-	-	-	-	-	-
Pod quality	9	-	-	-	-	-	-	-	-	-	-	-
Purity of seeds	8	-	3	-	-	-	-	-	-	4	4	-
Seed colour	5	-	-	-	-	-	-	-	-	-	-	-
Seed quality	6	5	-	-	-	-	-	-	3	2	3	-
Small-seeded grains	-	-	4		-	-	-	-	-	-	-	3

1 = most important preference,
9 = less important preference
- denotes traits was not reported in the area as a preference

3.4.7 Access of farmers to field-days on cowpea and agricultural extension services

Over half of the farmers (60%) reported no attendance at a field-day on cowpea cultivation and only about 10% attended more than five. The trend was similar with contacts with agricultural extension agents; 44.3% reported no contact, 33.1% had one to five contacts, and only 23% had more than five contacts with extension personnel that educate and guide them on cowpea production practices (Table 3.11). Some of the farmers that reported attendances of field-days were those that participated in the recent USAID-sponsored upscaling project at Matazu, Minjibir, and Albasu areas, whilst others were farmers used by IITA as out growers' programme at Giwa area.

Table 3.11: Percentage of farmers with experience attending field-days and having contacts with extension agents

	Attendance of field day		**Contact with Extension Agents**	
	Frequency	Percentage	Frequency	Percentage
None	251	59.8	186	44.3
1 - 5	129	30.7	139	33.1
> 5	40	9.5	95	22.6
N	420	100	420	100

N = total number of respondents

3.5 Discussion

The study interviewed a total of 420 farmers using semi-structured questionnaires and focus group discussion (FGD) across 36 sites visited. There were more FGD sessions for men because they were more involved in cowpea production in the studied areas. The KMO-MSA value of 0.620 in this study was above the accepted minimum value (0.50), implying the adequacy of sampled respondents for the study. Field (2009) has established that over 300 respondents are adequate for sampling analysis, therefore, the 420-sample size used was more than adequate to investigate research questions at hand.

In the present study, the majority of cowpea growers in the northern parts of Nigeria were men. This is probably because of the religious and cultural background of the respondents, as most women do not engage in direct crop cultivation activities like land preparation, planting, weeding, and field management. Women are mostly responsible for post-harvest processing, threshing, and winnowing. Contrary to the practice in some southern and west African countries like Zambia and Burkina Faso where women have been reported as dominant producers of cowpea (Gómez, 2004; Nkongolo *et al.*, 2009). The results of this study corroborate findings in northern Ghana (Akpalu *et al.*, 2014) and northeast of Nigeria (Iya & Kwaghe, 2007) that more men are involved in cowpea farming than women, and women are involved in post-harvest operations like threshing and winnowing of grains. Most of the respondents did not have a formal education. This is similar to what Akpalu *et al.* (2014) reported that over half (57%) of cowpea farmers in northern Ghana had no formal education. The poor participation in formal education could be among the main limiting factors for the use of improved technologies among these farmers. Farmers' education is very key to the success of any agricultural development programme, thus there is an urgent need to upscale the level of awareness of smallholder farmers to new farming technologies; availability of improved seeds, use of P fertilizers, and other improved technologies.

Results revealed that cowpea farmers were aware of the important roles fertilizers play in having normal and healthy plants in the field, but most did not use P-specific fertilizer formulations. There were several reasons advanced by farmers for non-use of P-based fertilizers on cowpea plants. Most farmers claimed to be unaware of the need to use P specific fertilizers like SSP for cowpea cultivation, while those with knowledge of its use gave reasons like lack of availability in rural markets and high cost as reasons for not using it. FanWay (2015) pointed out that Nigerian farmers were constrained by inadequate technical knowledge, research, and dissemination of new findings. Similarly, it has been noted elsewhere that most farmers in developing countries do not have sufficient access to phosphate fertilizers in their communities (Magani *et al.*, 2009; Chimungu & Lynch, 2014) and similar findings were reported among cowpea growers in Namibia (Horn *et al.*, 2014). In addition, most cowpea farmers believed that cowpeas did not require fertilizers and hence did not systematically apply the required amount of recommended fertilizers, this is in concordance with the perception of cowpea farmers in northern Namibia (Horn *et al.*, 2014).

Most of the Nigerian arable land are poor in soil fertility, especially low organic matter content, severe N deficiency (< 0.1% N), 75% P deficiency (< 10 mg P/kg) and 60% (< 25mg K/kg) (FanWay, 2015), this poor soil fertility is mainly due to low use of fertilizers and other unhealthy practices like bush burning, and removal of plant residues after harvest for feeding animals and roofing/constructing local structures (Bationo *et al.*, 2002). Low usage of fertilizers in the region is primarily due to the high cost of fertilizers, especially in countries like Nigeria, where most of the fertilizers and its raw materials are imported (FanWay, 2015) and untimely availability of the products. This underscores the need to create and sustain platforms to continuously educate, and guide farmers on sustainable agronomic and management practices. From interviews conducted and focus group discussion sessions, it was established that the use of P-based fertilizer like SSP for cowpea production was only common among growers with some levels of formal education and those with contacts with projects like IITA programmes, and USAID-sponsored cowpea upscaling project in some northern Nigeria states of Kano, Katsina, and Sokoto (Adetonah *et al.*, 2016; ICRISAT, 2017b)**.**

The most popular cropping system for cowpea in the study areas was intercropping with major cereals like maize, sorghum, and pearl millet. This is similar to what was reported in northern Namibia (Horn *et al.*, 2014), northern Ghana (Akpalu *et al.*, 2014), and earlier in Nigeria (Olufajo & Singh, 2002; Ewansiha *et al.*, 2014). Despite the huge benefits associated with sole cropping of cowpeas, only about 25% reported cultivation of cowpea as a sole crop. The traditional approach of intercropping cowpea with cereals is associated with low grain and fodder yield due to low plant density per ha, as this practice is associated with wider intra-row spacing, shading of cowpeas by cereals, little or minimal fertilization for cowpea and poor level of pest management (Olufajo & Singh, 2002; Ewansiha *et al.*, 2014)**.**

Farmers seem to be more comfortable with intercropping cowpeas because intercropping provides insurance in case of failure one of the crops, multiple benefits, and ease of pest management (Mawo *et al.*, 2016; Olufajo & Singh, 2002). There is need to create awareness among cowpea growers to adopt intercropping of improved cereal-legume cropping systems such as the 2 rows maize – 4 rows cowpea planting system that produced higher yield per unit area than the traditional practices (Ajeigbe *et al.*, 2010; Singh & Ajeigbe, 2007). Many farmers seem to be unaware of this improved intercropping style (2: 4 maize – cowpea system), as this planting system is rarely seen in farmers' fields. Incorporating farmers in technology design and development, testing and validation will greatly enhance the speed of dissemination and adoption by the end-users. Based on this premise, some researchers have advocated development of cowpea varieties with genetic potential that fit into mixed intercropping systems popular with farmers because the current popular intercropping system is faced with problems of low plant population, low yield, insect and disease incidence, shading of cowpeas, drought and low soil fertility (Olufajo & Singh, 2002). However, others have argued that there is no need for separate breeding programmes for sole and mixed cowpea, since most varieties that do well in the sole system also do well in mixed intercrop (Singh *et al.*, 2002).

Information during FGD showed that the average cowpea yield among the respondents ranged from 300 to 1000 kg/ha compared with the genetic potential of 1500 to 2500 kg ha^{-1} for the pure sole cropping (Nkongolo *et al.*, 2009). Some farmers asserted during one FGD that, improved cowpea varieties were not used mostly because they did not grow well in intercrop scenario while farmers were more interested in planting cowpea as an intercrop. Use of landraces over improved varieties were reported by farmers. This indicates there is poor adoption of improved varieties and this may be due to non-involvement of end-users in the process of development and deployment of cowpea varieties (Nkongolo *et al.*, 2009). Horn *et al.* (2014) reported over 70% cowpea farmers in northern Namibia used local landraces instead of improved varieties, and 76% in northern Ghana used landraces (Akpalu *et al.*, 2014).

The results of binary logit analysis revealed that use of P-specific fertilizer was strongly associated with farmers' ability to determine nutrient deficiency, attendance of field-days and contacts with agricultural extension agents. This is consistent with the opinion that farmers' education is important for successful adoption of farming technologies and recommendations. The model used to understand factors determining the use of P fertilizers for cowpea, has been used previously to estimate factors influencing knowledge of Napier stunt disease (Khan *et al.*, 2014)**,** farmers' knowledge of pea weevils (Mendesil *et al.*, 2016) and decision to use pesticides in vegetable crops (Sharma *et al.*, 2015).

Cowpea farmers in this study had poor exposure to production guidance and did not have enough contacts with extension agents. This is probably due to the low farmer to extension ratio (1:10,000) in Nigeria (Haruna & Abdullahi, 2013; NAN, 2016) as against the recommendation of 1:800 - 1000 extension agents to farm families ratio by the World Bank. This clearly demonstrates the need to provide farmers with training and education on cowpea practices to achieve sustainable cowpea food system. Contacts with extension agents provide an opportunity for agricultural information exchange including better crop management practices and soil fertility improvement strategies, as such information results in farmers having better knowledge about yield increasing factors. Agricultural input use and advisory guides might be important to educate smallholder farmers on knowledge about crop management practices (Belt *et al.*, 2015).

Farmers indicated their major production constraints as insect pests and the most preferred trait they wanted to see in a variety as yield. Most farmers did not know the name of chemicals they used in controlling cowpea pests, while some even used adulterated chemicals and others used non-recommended chemicals or dosages. Some respondents indicated that certain insecticides used did not protect their fields from pest damage. This might be due to various factors as highlighted above. Pesticides application and associated problems for farmers in developing countries have been documented by earlier reports (Khan *et al.*, 2015; Pretty & Bharucha, 2015). Use of non-recommended dosages and adulterated pesticides leads to poor pest control and expose farmers to health hazards and pollution of the environment (Pretty & Bharucha, 2015). These findings underscore the need to educate farmers to avoid problems associated with dosage and unhealthy exposure to chemicals. Based on the market demand, farmers produced cowpeas with different seed coat colours in the surveyed area. These findings corroborate earlier reports that market demand constitutes an important decision making factor for farmers to adopt and produce certain varieties (Coulibaly & Lowenberg-Deboer, 2002; Lowenberg-DeBoer & Ibro, 2008; Mishili *et al.*, 2007).

3.6 Conclusions

Cowpea growers do not use recommended fertilizer types and rates for cowpea, thereby contributing to low grain yield that has characterized African agriculture. Most of the 420 farmers that participated in the study were aware of fertilizers being important for crop growth and healthy development but did not know the appropriate fertilizer recommendations for cowpeas. Those who were aware of the need to use P fertilization on cowpea complained of high cost as reasons for not using P fertilizers. The Use of P was strongly predicted by farmers' knowledge of nutrient deficiency symptoms, exposure to training and guidance from field-day events and interaction with extension agents. Cowpea is still cultivated in the traditional intercropping with cereals. Farmers' practices the intercropping to avoid the risk of crop failure and maximize benefits from the limited land available to them. It is imperative to train farmers in the areas included in this study on the need for P fertilization and advantages of sole cropping of cowpeas and or improved 2: 4 cereal-cowpea planting system to improve yields.

The use of improved varieties was low as most growers used landraces that have low yield potential. Many farmers claimed ignorance of improved cowpea varieties and this calls for the need to increase advocacy and dissemination of available improved technologies. Insect pests, especially (aphids, *Maruca*, pod sucking bugs), weeds (*Striga*), drought and lack of availability of fertilizers at the time needed were the major production constraints identified by farmers. Farmers expressed willingness to adopt improved varieties and use SSP if provided to them or made available in the rural markets at subsidized prices. Most farmers indicated high yield was the most important trait they wanted in new varieties. Breeding for high yield should remain the most important priority of cowpea breeding programmes. It is important to incorporate farmers' knowledge and perception when designing new varieties as this will greatly facilitate the diffusion and adoption of new varieties among farmers.

CHAPTER FOUR

4.0 Phenotyping Cowpea for Phosphorus Efficiency and Response in Low P Environments

4.1 Introduction

Production of inorganic P fertilizers from rock phosphate reserves is costly (Kauwenbergh, 2010) thereby making them not readily available, and expensive in cowpea growing regions (Kolawole *et al.*, 2002). Global rock phosphate reserves, a key ingredient for making inorganic P are limited, unevenly distributed and predicted to end in the next few decades (Cordell & White, 2009b). Applied P could be fixed into forms not readily available for plant use (Korkmaz & Altıntaş, 2016). Furthermore, over 70% of P applied via fertilizers are not utilized in the current year of application, resulting in about 10 - 30% uptake while the uptake in the succeeding years decreases, thereby making the uptake of P by plants below optimum (Reynolds *et al.*, 2012).

Excessive use of P fertilizers as often practised in high inputs system raises the risk of environmental degradation, eutrophication of water bodies and water pollution due to P runoff (Bishopp & Lynch, 2015). In low input systems, most local farmers are not aware of the importance of P as a yield-determining factor for cowpea, and thereby use little to zero inorganic P (ICRISAT, 2017a). Due to the foregoing, the use of P fertilizers cannot be a sustainable option due to its attendant economic, and environmental cost (Lynch & Brown, 2012) and the most sustainable solution is to develop varieties that can give good yield under low soil P, and optimum yield when P is applied (Hammond *et al.*, 2009).

To understand the role played by P in the growth and development of crop plants, several approaches have been advocated; such as the use of nutrient solutions and inert media like sand or gravel cultures (Hoagland & Arnon, 1950). Several modifications of Hoagland and Arnon (1950) have been made and used in hydroponic, and sand culture medium to study nutrient deficiency in plants. An example of such modification was Johnson *et al.* (1994) on the effect of P stress on white lupin and a slight modification to Johnson *et al.* (1994) nutrient formula to investigate the P use efficiency (PUE) of cowpea lines (Rothe, 2014). Genotypic differences, level of P in the growth media and soil type contribute significantly to the P uptake of plants. There is limited information on mechanisms governing the differential response of cowpea under low or high P supply, especially on root traits. An understanding of these mechanisms would help in designing breeding methods and selection of appropriate varieties for specific environments like areas with limited use of P due to cost or high soil fixation of P.

The total soil P pool is in most cases over 100 times more than plant-available soil P, therefore the main idea of P efficient and responsive genotypes is to identify individuals that can access P not usually available to most genotypes under suboptimal soil P (P efficiency), but in addition respond to external P supply (P responsiveness) (Reynolds *et al.*, 2012). As such cowpea lines were classified based on shoot dry matter (DM) yield as efficient responsive (ER), inefficient responsive (IER), efficient non-responsive (ENR) and inefficient non-responsive genotypes (IENR) (Gyan-Ansah, 2012; Fonji, 2015; Hammond *et al.*, 2009; Saidou, 2005; Korkmaz *et al.*, 2009; Mahamane, 2008; Pask *et al.*, 2012; Zapata & Roy, 2004), see Figure 4.1. This classification takes into consideration the performance of genotypes under nutrient stressed (efficient vs inefficient) and optimum nutrient (responsive vs non-responsiveness) conditions (Caradus *et al.*, 1980; Gerloff, 1987). It has been used in CIMMYT wheat breeding (Pask *et al.*, 2012). The ER group produced a higher yield than others under low P and responds positively to the external supply of P. ENR group produced an above-average yield in low P supply and below average yield when P is supplied. IER group produced below average yield in low P conditions but responded positively to P addition while IENR group produced below average yield in low and high P conditions (Pask *et al.*, 2012). Such grouping would permit selection of varieties with adaptation to specific soil nutrient conditions. The ER and ENR classes are the most desirable genotypes for both high and low inputs systems.

Response to added Phosphorus

I = Non-Efficient but P Responsive (IER)	II= Efficient and Responsive to P (ER)
III = Non-Efficient and No Responsive to P (IENR)	IV=Efficient and No-responsive to P (ENR)

Adaptation to low soil available phosphorus

Figure 4.1: Quadrants of genotypes based on performance in low soil P & Response to applied P for any measured traits (Mahamane, 2008).

In the present study, parental lines used for the development of two discovery populations, namely; biparental and multi-parent advanced generation inter-cross population recombinant inbred lines (RIL) were screened for P utilization efficiency (PUE) and P acquisition efficiency (PAE) using nutrient sand culture combination, and under natural low soil P field. Many of the lines used are parents in some biparental RIL populations previously described (Huynh *et al.*, 2018; Huynh *et* *al.*, 2013; Muñoz-Amatriaín *et al.*, 2017). The RILs were developed for use by the international cowpea community, and have been previously phenotyped for several important biotic and abiotic stresses such as tolerance to aphids, drought, *macrophomina*, *Striga*, foliar thrips, heat and other phenological attributes like leaf morphology and maturity (Huynh *et al.*, 2013). PUE is the ability of plants to produce high yield per unit of P in plant or supplied, while P acquisition efficiency (PAE) is the ability to take up more P from low soil P pool (Reynolds *et al.*, 2012). PUE and PAE are important indices for abiotic stress, and these inbred lines have not been previously characterized for these traits, so there was the need to use a high-throughput phenotyping strategy to establish the PUE and PAE of these lines for further use in breeding programmes. The central goal of this research was to determine genetic variability in cowpea for P acquisition and response to P addition. The specific objectives were therefore to investigate;

- the response of cowpea lines to various levels of P fertility,
- the relationship between growth parameters and tissue P concentration,
- the level of genetic diversity of root hair length and density, and
- differences in seedling root architectural traits.

4.2 Materials and Methods

4.2.1 Screenhouse experiment

The experiment was conducted at the Institute for Agricultural Research, Ahmadu Bello University (IAR/ABU) Zaria Nigeria. The aim was to evaluate genetic variability among cowpea lines under various concentrations of P in the growth medium.

4.2.1.1 Screening media

The soil used was river-sand from a local stream called *Rafin Kudungi* at the Ahmadu Bello University (N11^{0}09'49.6'' E007^{0}37'13.8'' on 668m elevation). River-sand has been used in an earlier P-response study (Saidou, 2005), due to its low available P and other physicochemical properties (Table 4.3). The river-sand was sieved with < 2 mm sieve to remove debris, air-dried for 24 overnight and acid-washed by soaking in 1% HCl for 24 hours and rinsed several times with tap water until the pH was between 5.5 to 6.5. The soil physical and chemical properties were determined at the Department of Soil Science, Ahmadu Bello University Zaria – Nigeria. Pots (24 cm x 24 cm diameter by height) were filled with 5 kg of acid-washed river-sand (< 2 mm). Prior to filling the pots with sand, they were lined with a damp-proof membrane cut into 47 cm x 47 cm, to prevent the sandy soil from escaping via the perforated holes of the pots and to reduce the level of water loss from pot drains. The linings were later perforated gently with needles to ensure free flow out of water and nutrients.

4.2.1.2 Plant materials

Plant materials were thirty (30) cowpea lines, of which 20 were parental lines for 12 biparental RILs, and eight - parents MAGIC RILs, and 10 popular Nigerian lines. The RIL parents were from the University of California, Riverside (UCR), USA (Table 4.1).

4.2.1.3 Nutrient media and phosphorus treatments

A modified Hoagland nutrient solution used on white lupin (Johnson *et al.*, 1996) and on cowpea lines with little modifications on P concentration was adapted (Rothe, 2014). Stock solutions were prepared for each of the salts (Table 4.2). Defined quantities of each stock solution were then measured into a 20-litre bucket, and reverse osmosis water (RO) was used to make up the required volume for various P treatments with the pH adjusted to 6.5 with NaOH or HC1. The RO was used as diluent due to its low content of dissolved solutes especially calcium and magnesium since the P source was calcium phosphate. Therefore, using water sources with high Ca could potentially increase Ca content of the growth medium and that could likely make P not readily available for plants uptake. P was supplied as $Ca(H_2PO_4)_2.H_20$ at 0 mg, 1.5 mg and 30 mg for the zero, low and high P treatments, which were equivalent to 0 M, 25.0 µM and 0.5 mM of $Ca(H_2PO_4)_2.H_2O$ in the solution.

4.2.1.4 Experimental procedure

The 30 cowpea lines were planted in a total of 450 pots in a factorial arrangement using 3 P concentrations; 0 mg, 1.5 mg and 30 mg P kg^{-1}, with cowpea lines and P levels as treatments and arranged in a randomized complete block design in five replications. All pots received the following; 3.0 mM KNO_3, 2.5 mM $Ca(NO_3)_2$, 1.0 mM $MgSO_4$, 12.0 µM FeEDTA, 4.0 µM $MnCl_2$, 22.0 µM H_3BO_3, 0.4 µM $ZnSO_4$, 0.05 µM $NaMoO_4$, 1.6 µM $CuSO_4$ except $Ca(H_2PO_4)_2.H_2O$ that was applied to low and high P pots (Johnson *et al.*, 1996; Rothe, 2014).

The average daily temperature during the growth period was ± 27°C and the relative humidity was 70 - 80%, with 13/11 hours of day/night length (monitored with a Digital Thermometer). Seeds were treated with a commercial fungicide *(AllStar* containing 20% w/w thiamethoxam, 20% w/w metalaxyl-M and 2% w/w difenoconazole, Syngenta Crop Protection AG, Basel, Switzerland) at a rate of four kilograms to a sachet of 10 g before planting based on manufacturer's recommendation.

Prior to planting, pots were watered to field capacity with 1000 ml of RO water, two seeds were planted per pot and later thinned to one plant per pot at ten days after sowing (DAS). Pots were watered with the dilute nutrient solution described in **4.2.1.3 Nutrient media and phosphorus treatments** as follows; 300 ml per pot at planting, and subsequently 300 ml was applied at 3 DAS, 6 DAS, 9 DAS, 16 DAS, 23 DAS, 30 DAS, 37 DAS, 44 DAS, and 51 DAS. The appropriate quantities of nutrient solution were dispensed using graduated beakers. Pots were periodically supplied with RO water to prevent wilting and prevent the accumulation of salts in the soil. Plants were protected against insect pests by spraying with Karate (50 g/l lambda-cyhalothrin, Syngenta Crop Protection AG, Basel, Switzerland) and applied at a rate of 1.0 l ha^{-1} as at when due. Figure 4.2 shows the layout of the screening experiment and Fig. 4.3. shows cowpea genotypes with differential responses to P nutrition. **Right pot:** high P, **middle pot**: low P while **left pot**: no-P plant (stunted, and defoliated leaves and poor growth).

Figure 4.2. An overview of the experimental plants in the Screenhouse

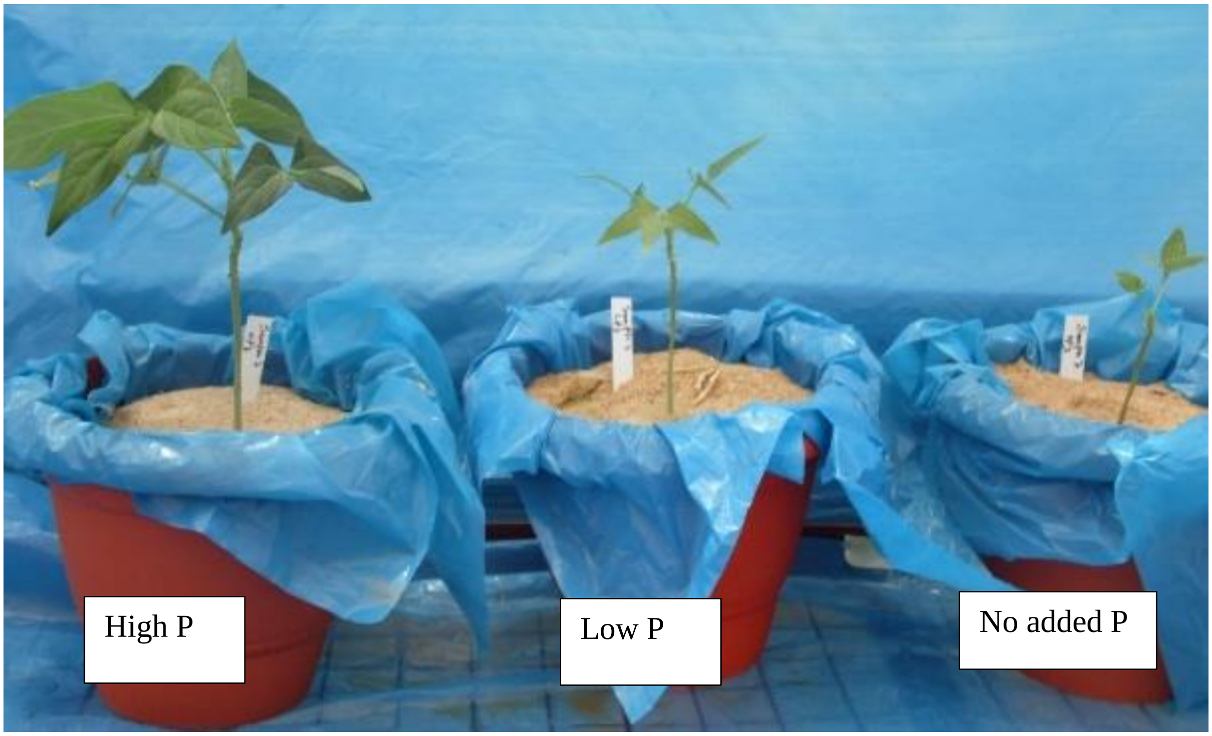


Figure 4.3: Effect of P concentrations on a line (IAR-48). **Left** - High P plant, **Middle**-low P plant & **Right**, No P plant.

Table 4.1: List of cowpea lines screened for both screenhouse and field experiments in 2016 and their seed source

#	Genotype	Source of Seeds	#	Genotype	Source of Seeds
1	UCR 779	UC Riverside, USA	16	Aloka-local	IITA Kano, Nigeria
2	Yacine	UC Riverside, USA	17	B301	IITA Kano, Nigeria
3	58-77	UC Riverside, USA	18	Tvu-14676	IITA Ibadan, Nigeria
4	CB 27	UC Riverside, USA	19	Kanannado	IAR Samaru, Nigeria
5	CB 46	UC Riverside, USA	20	IT86D-1010	IAR Samaru, Nigeria
6	Danila	UC Riverside, USA	21	SuVita2	UC Riverside, USA
7	TVU-7778	UC Riverside, USA	22	IT00K-1263	UC Riverside, USA
8	IT82E-18	UC Riverside, USA	23	24-125B-1	UC Riverside, USA
9	IT97K-556-6	UC Riverside, USA	24	Vita7	UC Riverside, USA
10	IT93K-503-1	UC Riverside, USA	25	524B	UC Riverside, USA
11	IT89KD-288	UC Riverside, USA	26	IAR-48	IAR Samaru, Nigeria
12	IT84S-2246	UC Riverside, USA	27	IT90K-277-2	IAR Samaru, Nigeria
13	IT97K-499-35	UC Riverside, USA	28	DanMisra	IAR Samaru, Nigeria
14	IT84S-2049	UC Riverside, USA	29	SAMPEA-17	IAR Samaru, Nigeria
15	Sanzi	UC Riverside, USA	30	UAM-1055-6	UAM Benue, Nigeria

Table 4.2**:** Nutrient salts, stock and final concentrations applied on cowpea lines in sand culture

#	**Nutrient Salt**	**MW/FW (g/mol)**	**Stock Conc.**	**Stock Mass (g/L)**	**Element Supplied**	**Molar Conc. of Final Solution/L**
1	KNO_3	101.10	1.0M	101.10	K, N	3.0 mM
2	$Ca(NO_3)_2.4H_2O$	236.15	1.0M	236.15	Ca, N	2.5 mM
3	$MgSO_4$	120.37	1.0M	120.37	Mg, S	1.0 mM
4	Fe EDTA	367.05	1.0mM	0.367	Fe	12.0 µM
5	$MnCl_2$	125.84	1mM	0.126	Mn, Cl	4.0 µM
6	H_3BO_3	61.83	1mM	0.062	B	22.0 µM
7	$ZnSO_4.H_2O$	179.47	1mM	0.179	Zn, S	0.4 µM
8	$NaMoO_4.2H_2O$	241.95	1mM	0.242	Na, Mo	0.05 µM
9	$CuSO_4$	159.61	1mM	0.160	Cu, S	1.6 µM
10	$Ca(H_2PO_4)_2.H_2O$ (Low P)	252.07	1mM	0.252	P	25.0 µM
11	$Ca(H_2PO_4)_2.H_2O$ (High P)	252.07	0.5M	126.04	P	0.5 mM

Courtesy: Johnson *et al.*, 1996, modified in the present form by Rothe, 2014

4.2.1.5 Data collection, plant assays and analysis

At eight weeks after sowing (WAS), plant height was measured, and the experiment was terminated. All the lines were uprooted, the shoots were detached from the roots above soil surface using secateurs. Roots were cleaned by repeated washing under a running tap to remove soils under a 1 mm mesh opening. Fresh shoot and root samples were dried for 24 hours in the screenhouse under ambient temperature, and later moved to an incubator (Percival, Boone IOWA 50036) set at 60-65°C for 36 hours until the stable dry weight was attained and weighed using a digital scale (Scout^{tm} pro SPU202, Ohaus Corporation).

The following parameters were recorded; shoot dry biomass (g), root dry biomass (g), and shoot to root biomass ratio computed. Prior to weighing, roots were checked for any adhering soil particles, which were carefully removed when found. Dried shoot and root samples were ground and passed through a 60-mesh size sieve and analyzed for P concentration using Vanadate-molybdate method (Kitson & Mellon, 1944) at the Department of Soil Science, Ahmadu Bello

University Zaria-Nigeria. Data were analyzed for differences in the parameters recorded with the general linear model, and means were generated from SAS Proc GLM (SAS 9.4, *licensed to* *http://www.wacci.ug.edu.gh/*). Graphical representation of the results made with R and XLSTAT packages.

4.2.2 Field experiment

A field experiment was conducted at the research farm of the Institute for Agricultural Research, Ahmadu Bello University (IAR/ABU) Samaru, Nigeria during 2016 growing season. Samaru (N11^0 10'31.7'', E 007^{0}36'43.9'' on 709 m elevation, *(Garmin GPS$_{map}$ 78s*) is in the northern guinea savannah of Nigeria in West Africa and has a unimodal rainfall pattern with an annual rainfall of about 1000 – 1200 mm (NAERLS *et al.*, 2017). The experimental area was 45 m x 13 m (585m^2), the land used had been left fallow for several years, extensive soil sampling was conducted, and samples were tested for available P content, which was consistently found to be low (4 - 6 mg P kg^{-1}) (Table 4.7). The land was then cleared of shrubs, roots and stubble, sprayed with glyphosate (*Round-up*) at the rate of 4 l ha^{-1}, then ploughed, harrowed twice and ridged.

4.2.2.1 Experimental design

A strip-plot design with two replications and two factors (three P levels, and thirty cowpea lines) was used. Table 4.1 shows the list of the lines screened. The cowpea lines were vertical factors on the row while P levels served as horizontal factors in the design. Commercial single super phosphate (SSP) fertilizer was the source of P applied at 0, 10 and 60 kg P ha-^{1} at 5 days after sowing (DAS) for zero, low and high P treatments.

Prior to sowing, seeds were treated with a broad-spectrum commercial fungicide *(AllStar* containing 20% w/w thiamethoxam, 20% w/w metalaxyl-M and 2% w/w difenoconazole, Syngenta Crop Protection AG, Basel, Switzerland) at a rate of 4 kg to a sachet of 10 g based on manufacturer's recommendation. Sowing was by hand at an intra and inter-row spacing of 0.20 m and 0.75 m. Plots were one row of 2 m each with a 1 m unplanted walkway between plots. Plants were protected against insect pests and weeds using recommended insecticides and hoe weeding.

4.2.2.2 Data collection and analysis

Data on plant height, days to flowering and maturity, shoot dry weight, and pod yield were collected at physiological maturity. Two plants were uprooted from each strip plot using shovels for shoot dry weight measurement. Shoot samples were first air-dried in the screenhouse under ambient temperature and later moved to an Incubator (Percival, Boone IOWA 50036) for drying at 60 - 65^{o}C for 2 days until a stable weight was attained and weighed with a digital scale (Kerro BL20001). Dried pods were hand threshed and weighed, while P concentration of shoot samples were determined using Vanadate-molybdate approach (Kitson & Mellon, 1944) at the Department of Soil Science, IAR/ABU Nigeria.

4.2.3 Assessing genetic diversity of root hair and seedling root architecture traits

A total of 50 lines including 20 used for phenotyping under the screenhouse and field environments in this study were used to assess diversity in cowpea's root hair and seedling root architecture traits at the Root Lab, Pennsylvania State University, USA.

4.2.3.1 Experimental design

The cigar-roll method of seedling phenotyping was used (Burridge *et al.*, 2016; Miguel *et al.*, 2015). Cowpea seeds were surface sterilized with 0.5 % sodium hypochlorite (NaOCl) for 1 min and placed onto brown germination paper (Anchor Paper, St. Paul, MN, USA) saturated with 0.5 mM CaSO4 (Plate 1). Further cleaning steps were applied, which involved dipping seeds in a 0.1% copper solution (*Captan*, 50%WP) for 1 min before placing them on germination paper and rolling the paper into a "cigar-roll" configuration to reduce the incidence of fungus growth.

Five seeds of each genotype were placed 2 cm from the top of a 20 cm long piece of germination paper, rolled into a moderately tight cigar-roll configuration and placed in a 2-litre beaker containing 0.5 mM CaSO4. Each roll-up constituted a replicate and was composed of 4 - 5 seedlings in an individual cigar-roll configuration. Five to nine replications were made for each of the genotypes. Each of the beakers was filled with 10 – 16 rolls and later placed in an incubator chamber for 48 - 72 hours set at 32°C and then moved to a light chamber set at 28°C with a photoperiod of 16/8 hours (light/darkness). Three representative seedlings of each genotype from replicated roll sets were taken for data collection.

4.2.3.2 Seedling root architecture phenotyping

Cowpea genotypes were evaluated for primary root length (PRL in cm), basal root number defined as the number of first-order lateral roots within 1 cm of the base of the hypocotyl (BRN), number of first-order lateral roots on the primary root between 2-5 cm from the base of the hypocotyl (TBD5) and number of lateral roots on taproot between 5 and 10 cm from the base of the hypocotyl (TBD10) of the taproot length. Sampled seedlings were spread on a flat tray surface and the measurements defined above were taken (Figure 4.4).

Sterilized seeds on soaked germination paper

Seeds rolled on germination and placed in 2 L container containing CaSO4

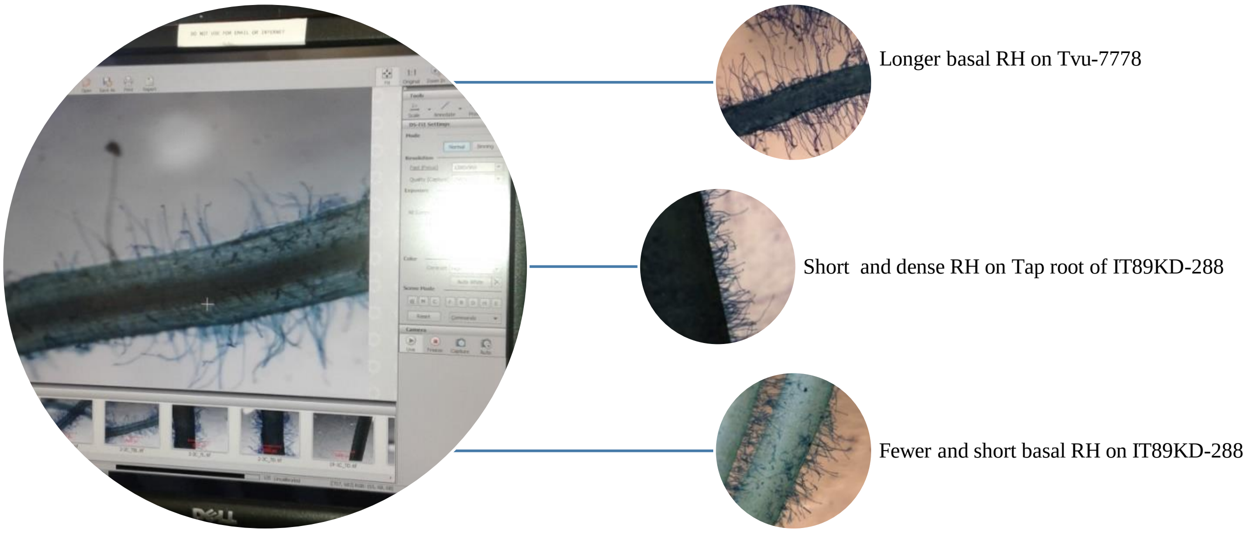
Germinating seedlings prior to assessment

Cowpea seedling being assessed for root architecture traits after 10 days of cigar-roll

Figure 4.4: Pictorial procedure of seed “Roll-Up” on germination paper and assessment of seedling roots

4.2.3.3 Root hair phenotyping

Two cm root sections of basal, taproot and lateral roots on taproot were taken from 14 days old seedlings. In each replicate, root sections with root hairs were imaged for their root hair length and density using a Nikon Camera (Nikon Digital Sight DS-Fi1, Nikon Corporation Japan) mounted on a dissecting microscope (Nikon SMZ 1500, C-DSS115, Nikon Corporation Japan) set at 30x magnification using an imaging software (NIS-Elements F4.30.01.64-bit) (Figure 4.5). A detailed description of this procedure has been provided in Hanlon *et al.* (2018). Root hair lengths and densities were measured using an open-access image processing software *ImageJ* (https://imagej.nih.gov/ij/) to count along the edge for length and middle of the root section for density. The length of 10 root hairs was traced with the *ImageJ* tool per picture, and a total of 3 - 4 pictures per replicate and 12 pictures for each line, making a total of 240 root hairs per line were measured for root length hair and density per mm^2.



Longer basal RH on Tvu-7778

Short and dense RH on Tap root of IT89KD-288

Fewer and short basal RH on IT89KD-288

Figure 4.5: Root hair imaging and representative root hair contrast on some cowpea genotypes studied

4.3 Results

There was significant genetic variation among cowpea lines tested under contrasting P levels both at screenhouse using river-sand-Hoagland nutrient solution and natural low P field environment, where SSP was used as a source of P-fertilizer. Shoot dry biomass, root dry biomass and plant height measured in the screenhouse experiment increased with the increasing concentration of P in the different treatments. The river-sand used had low to very low physical and chemical properties such as pH in H_2O (1:1 water: soil mixture), pH in 0.01M $CaCl_2$ was acidic, with mean available P content of 3.70 mg kg^{-1} (Bray 1 method), total N and organic carbon were very low and as well as other physicochemical properties (Table 4.3).

4.3.1 Dry matter production of shoot, root, total plant biomass and P concentration

There was highly significant genetic variation among the lines for all the parameters measured for the three P levels (Table 4.4). The addition of P in nutrient solution had positive effects on plant height, shoot dry weight (SDW), root dry weight (RDW) and total plant biomass (TPB) and shoot to root ratio (Table 4.5 - 4.6). The effect of P was more pronounced for shoot dry matter and total plant biomass than root dry matter yield. Genotypes TVu-7778, 58-77, B301, *Aloka-local*, IT84S-2049, *Kanannado*, TVu-14676, and *Sanzi* were poor performing for SDW yield while IT97K-556-6, CB46, *Yacine*, CB27, IT93K-503-1, IT89KD-288, IT84S-2246-4 and *Danila* had good performance under no-external P (OP) media. Similar patterns were observed in RDW and TPB for the lines in low P (LP) media.

The parental lines IT84S-2246-4, *Yacine* and TVu-14676, 58-77 which are parents for two RIL populations had contrasting yields in shoot dry weight, root dry weight and total biomass yield. Furthermore, lines with above average performance in their SDW, RDW and TPB in OP media were generally taller in height than lines with poor yields (below average) in OP media, indicating the importance of P in maintaining good plant height. All lines responded positively to the P addition in (LP media), as the SDW, RDW, TPB and PHT were higher compared to the performance of same lines in OP due to the impact of P supplied in the growth media. Genotypes CB46, IT93K-503-1, IT97K-556-6, CB27, IT89KD-288, IT84S-2246, IT86D-1010 and IT97K-499-35 were more efficient in converting P applied in the LP medium to biomass yields (SDW & RDW) than TVu-7778, 58-77, B301, *Aloka-Local*, *Kanannado* and IT82E-18 that had low biomass yield, except that RDW of TVu-14676 was slightly higher as well as Danila gave comparable SDW yield in LP with best-performing lines (Table 4.5).

The effect of P in nutrient solution was more vivid in the HP medium, as the SDW, RDW, TPB, and PHT were higher compared to the performance of the same lines in OP and LP medium. IT93K-503-1, IT97K-556-6, CB27, CB46, and IT82E-18, IT89KD-288, IT84S-2246-4, TVu-14676, were more efficient in translating P in the medium to shoot yield while Tvu-7778, Danila, UCR 779, 58-77, and B301 were less efficient in HP medium. Similar response patterns followed for RDW of the lines. IT93K-503-1 and IT97K-556-6 were the best performing lines in total biomass (SDW + RDW) with Danila and TVu-7778 being the lowest in total biomass production in HP. Effect of HP was also more apparent on plant height, as IT93K-503-1 (37 cm) and IT97K-556-6 (21.4 cm), were tallest in response to HP addition compared to Tvu-7778 (12.10 cm), Danila (14 cm), UCR 779 (about 12 cm) which were not very good in making use of P in growing in height.

The differential response of all lines to varying P rates revealed that total biomass generally increased from OP to HP, with IT89KD-288, IT84S-2246, and IT00K-1263 having the highest biomass while lines such as 58-77, B301 and Tvu-7778 had low total biomass yield in OP. Similarly, Vita7, I89KD-288, IT84S-2246-4, IT00K-1263 had superior performance when P was optimum over Tvu-7778, Danila and B301 (Figure 4.6).

Table 4.3: Physical and chemical properties of the river-sand used for pot experiment

Analysis	**Results (River sand)**				**Unit**	**Rating**
	Composite Sample 1	**Composite Sample 2**	**Composite Sample 3**	**Mean**		
pH (H_2O)	6.4	5.3	6.1	5.9	NA	Acidic
pH (0.01M $CaCl_2$)	5.5	4.9	5.6	5.3	NA	Acidic
ECE (dsm)	1.30	0.06	1.10	0.82	dsm	
Organic Carbon	0.24	0.24	0.24	0.24	g kg soil^{-1}	Very low
Total Nitrogen	0.11	0.07	0.14	0.11	g kg soil^{-1}	Very low
Available P	4.2	3.4	3.3	3.7	mg kg soil^{-1}	Very low
Calcium^{++}	1.84	1.45	1.25	1.51	Cmol/kg	Very low
Magnesium^{++}	0.28	0.32	0.35	0.32	Cmol/kg	Very low
Potassium	0.09	0.09	0.06	0.08	Cmol/kg	Very low
Sodium	1.71	1.59	0.28	1.19	Cmol/kg	Very low
H^+ Al	0.4	0.8	0.6	0.60	Cmol/kg	Very low
CEC	4.32	4.25	2.54	3.70	Cmol/kg)	Low
Clay	12	8	8	9.33	%	
Silt	6	6	4	5.33	%	
Sand	82	86	88	85.33	%	
Texture	Loamy Sand	Loamy Sand	Loamy Sand	Loamy Sand		

Table 4.4: Probabilities ($p < 0.05$) of F-test of the analysis of variance for the shoot, root, total biomass and tissue P content of cowpea lines evaluated in the Screenhouse

Source	**PHT**	**SDW**	**RDW**	**TPB**	**SR**	**ShootPCont**	**RootPCont**
Lines	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Phosphorus	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Line x P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0037	0.189
Mean	16.93	1.04	0.70	1.74	1.33	1230.41	779.95
CV	39.60	50.24	36.46	42.03	27.76	86.05	89.91

PHT = plant height, SDW = shoot dry weight, RDW = root dry weight, SR = shoot to root ratio, ShootPCont = shoot P concentration and RootPCont = root P concentration

Table 4.5: Plant heights, shoot and root dry biomass of different cowpea lines evaluated under three levels of phosphorus in a Screenhouse experiment

	Plant Height (cm)				**Shoot Dry Weight (g)**				**Root Dry Weight (g)**			
Lines	**OP**	**LP**	**HP**	**Mean**	**OP**	**LP**	**HP**	**Mean**	**OP**	**LP**	**HP**	**Mean**
24-125B-1	13.9	11.9	18.7	14.9	0.4	0.5	2.8	1.2	0.4	0.4	1.8	0.9
524B	14.5	14.3	17.3	15.4	0.6	0.6	2.4	1.2	0.7	0.6	1.1	0.8
58-77	11.7	12.8	16.8	13.8	0.2	0.3	1.5	0.7	0.2	0.3	0.8	0.4
Aloka-local	15.2	15.4	24.9	18.5	0.3	0.4	2.2	1.0	0.2	0.3	0.8	0.4
B301	10.7	11.8	41.0	21.2	0.2	0.2	1.4	0.6	0.2	0.2	0.8	0.4
CB27	16.7	16.1	18.0	16.9	0.5	0.5	1.7	0.9	0.5	0.5	0.9	0.6
CB46	15.4	15.6	16.4	15.8	0.5	0.6	1.7	0.9	0.5	0.6	1.0	0.7
Danila	12.6	12.6	13.7	13.0	0.5	0.6	1.1	0.7	0.3	0.4	0.6	0.5
DanMisra	10.0	10.2	13.9	11.4	0.3	0.3	2.2	1.0	0.3	0.3	1.3	0.6
IAR-48	12.7	12.6	16.4	13.9	0.4	0.4	2.5	1.1	0.5	0.4	1.8	0.9
IT00K-1263	14.2	15.4	19.4	16.4	0.6	1.1	3.8	1.8	0.7	0.9	2.1	1.2
IT82E-18	11.9	11.1	13.9	12.3	0.3	0.3	1.6	0.8	0.7	0.5	1.6	1.0
IT84S-2049	19.4	18.3	24.7	20.8	0.3	0.4	2.3	1.0	0.3	0.4	1.0	0.5
IT84S-2246-4	23.2	23.8	25.0	24.0	0.9	0.7	3.6	1.7	0.5	0.5	1.0	0.7
IT86D-1010	16.7	17.0	35.4	23.0	0.3	0.5	2.3	1.0	0.3	0.4	1.0	0.6
IT89KD-288	26.6	25.8	41.3	31.2	1.1	1.1	3.2	1.8	0.6	0.6	1.2	0.8
IT90K-277-2	11.1	9.7	16.5	12.4	0.2	0.2	2.5	1.0	0.3	0.2	1.3	0.6
IT93K-503-1	20.7	20.5	36.6	25.9	0.5	0.5	2.3	1.1	0.4	0.6	1.2	0.7
IT97K-499-35	16.1	15.9	19.3	17.1	0.4	0.5	2.7	1.2	0.4	0.5	1.2	0.7
IT97K-556-6	19.9	14.4	21.4	18.6	0.5	0.5	2.0	1.0	0.7	0.6	1.4	0.9
Kanannado	16.2	15.9	36.6	22.9	0.3	0.4	1.9	0.8	0.2	0.3	1.1	0.6
SAMPEA-17	10.5	12.8	16.3	13.2	0.4	0.5	3.4	1.4	0.5	0.6	1.7	0.9
Sanzi	14.6	15.0	52.6	27.4	0.3	0.4	2.5	1.1	0.3	0.3	0.9	0.5
SuVita2	11.3	12.5	12.8	12.2	0.5	0.5	2.7	1.2	0.4	0.4	1.2	0.7
Tvu-14676	8.5	8.5	16.5	11.2	0.2	0.3	2.1	0.9	0.4	0.4	1.4	0.7
Tvu-7778	11.5	12.1	12.1	11.9	0.1	0.3	1.0	0.5	0.2	0.3	0.7	0.4
UAM-1055-6	12.2	12.0	13.5	12.6	0.4	0.4	1.4	0.7	0.3	0.3	0.9	0.5
UCR-779	9.2	9.7	11.6	10.1	0.3	0.5	1.4	0.7	0.5	0.7	1.5	0.9
Vita7	13.9	11.2	21.4	15.5	0.5	0.7	3.3	1.5	0.7	0.7	2.2	1.2
Yacine	14.4	14.2	16.0	14.9	0.4	0.5	1.4	0.8	0.6	0.6	1.3	0.8
Min	8.5	8.5	11.6	10.1	0.1	0.2	1	0.5	0.2	0.2	0.6	0.4
Max	26.6	25.8	52.6	31.2	1.1	1.1	3.8	1.8	0.7	0.9	2.2	1.2
Mean	14.5	14.3	22.0	16.9	0.4	0.5	2.2	1.0	0.4	0.5	1.2	0.7

OP = no-P application, LP= 1.5 mg P kg^{-1} soil, HP = 30 mg P kg^{-1} River sand

Table 4.6: Total plant biomass and shoot to root ratio of different cowpea lines under three levels of phosphorus in a Screenhouse experiment

	Total Plant Biomass (g)				**Shoot to Root Biomass Ratio**			
Lines	**OP**	**LP**	**HP**	**Mean**	**OP**	**LP**	**HP**	**Mean**
24-125B-1	0.8	0.9	4.6	2.1	0.9	0.9	1.6	1.1
524B	1.2	1.3	3.5	2.0	0.9	1.0	2.0	1.3
58-77	0.4	0.6	2.3	1.1	0.9	1.0	1.8	1.2
Aloka-local	0.5	0.7	3.0	1.4	1.3	1.4	2.8	1.8
B301	0.4	0.4	2.2	1.0	0.9	1.0	1.6	1.1
CB27	0.9	1.0	2.7	1.5	0.9	1.2	1.8	1.3
CB46	1.1	1.3	2.7	1.7	1.0	0.9	1.7	1.2
Danila	0.8	1.0	1.7	1.2	1.4	1.6	1.7	1.6
DanMisra	0.6	0.6	3.6	1.6	1.0	1.3	1.7	1.3
IAR-48	0.9	0.7	4.3	2.0	0.9	1.0	1.4	1.1
IT00K-1263	1.3	1.9	5.9	3.0	1.0	1.3	1.9	1.4
IT82E-18	1.0	0.9	3.3	1.7	0.5	0.6	1.0	0.7
IT84S-2049	0.6	0.8	3.3	1.6	1.2	1.2	2.4	1.6
IT84S-2246-4	1.4	1.2	4.6	2.4	1.8	1.7	3.5	2.3
IT86D-1010	0.7	0.9	3.3	1.6	1.1	1.2	2.2	1.5
IT89KD-288	1.7	1.6	4.5	2.6	1.9	1.9	2.6	2.1
IT90K-277-2	0.5	0.4	3.8	1.6	0.9	1.1	1.9	1.3
IT93K-503-1	0.9	1.1	3.5	1.8	1.3	1.0	1.8	1.4
IT97K-499-35	0.8	0.9	3.9	1.9	1.1	1.1	2.2	1.5
IT97K-556-6	1.2	1.1	3.4	1.9	0.7	0.8	1.5	1.0
Kanannado	0.5	0.7	3.0	1.4	1.4	1.2	1.6	1.4
SAMPEA-17	0.9	1.1	5.0	2.4	0.8	0.8	2.0	1.2
Sanzi	0.6	0.7	3.4	1.6	1.1	1.2	2.7	1.7
SuVita2	0.9	0.9	4.0	1.9	1.3	1.3	2.3	1.6
Tvu-14676	0.6	0.7	3.4	1.6	0.7	0.7	1.4	0.9
Tvu-7778	0.3	0.5	1.7	0.8	0.6	1.2	1.6	1.1
UAM-1055-6	0.7	0.7	2.4	1.2	1.5	1.1	1.4	1.4
UCR-779	0.8	1.2	2.8	1.6	0.6	0.8	1.0	0.8
Vita7	1.1	1.4	5.5	2.7	0.7	0.9	1.5	1.0
Yacine	1.1	1.1	2.6	1.6	0.7	0.9	1.1	0.9
Min	0.3	0.4	1.7	0.8	0.5	0.6	1.0	0.7
Max	1.7	1.9	5.9	3.0	1.9	1.9	3.5	2.3
Mean	0.8	0.9	3.5	1.8	1.0	1.1	1.9	1.3

OP = no-P application, LP= 1.5 mg P kg^{-1} soil, HP= 30 mg P kg^{-1} River sand

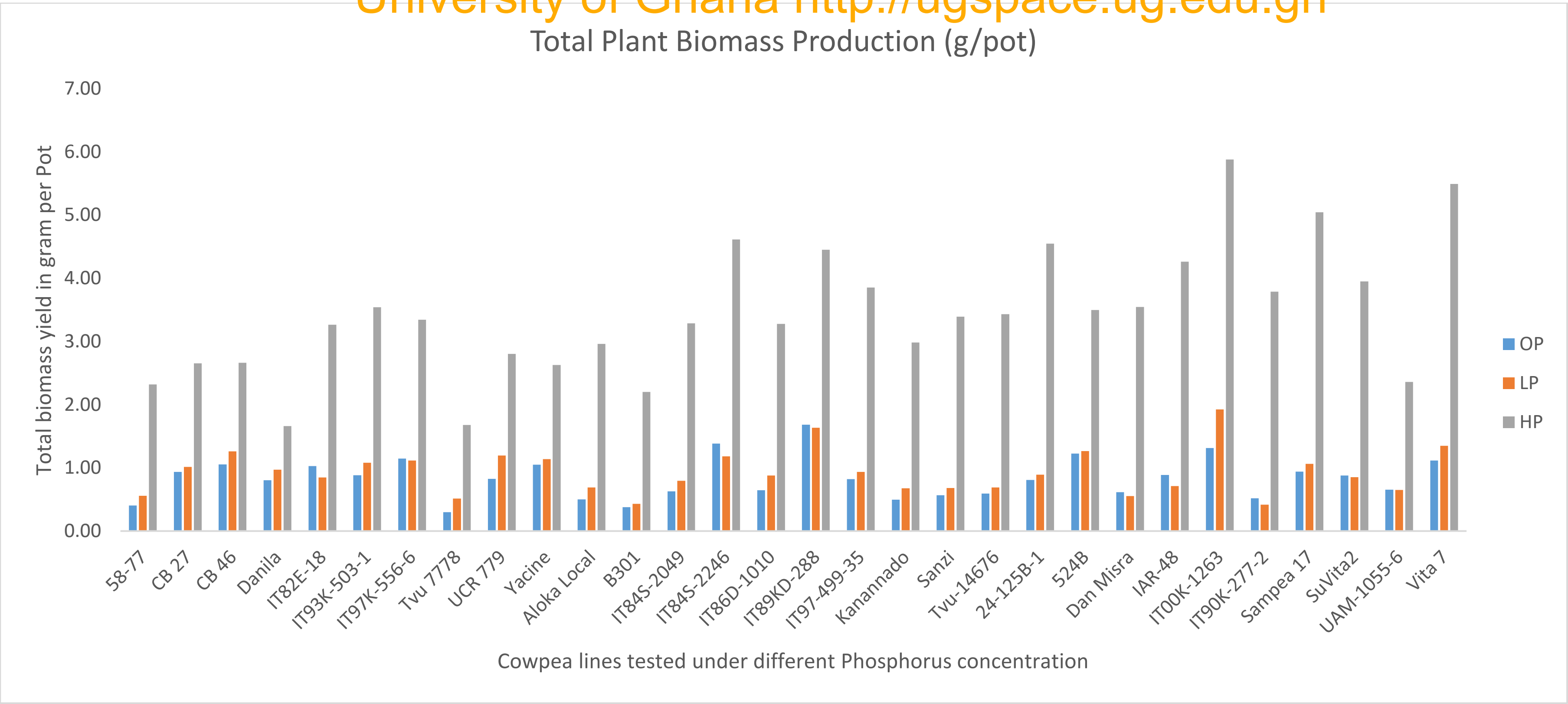


Figure 4.6: Differential response of cowpea lines to varied phosphorus concentration evaluated in Screenhouse using Hoagland Nutrient solution

(OP = No application of P, LP = low P and HP = high P)

4.3.2 Assessing the relationship between growth parameters and tissue P concentration

Phosphorus element added in the nutrient media had significant effects on P content of cowpea lines and led to a significant correlation between several pairs of parameters (Fig. 4.7). There were high positive correlations between shoot dry weight and shoot P content at OP (r = 0.8) and HP (r = 0.9). Shoot dry weight at OP and HP were moderately correlated (r = 0.6), likewise the root dry weight at OP and HP (r = 0.6). Shoot: root ratio at HP and OP were negatively correlated (r = -0.2, r = -0.3) with root dry weight at OP. As expected, shoot and root dry weights at all P conditions had a positive significant association with total plant biomass, likewise shoot and root P concentraion were associated with the shoot and root dry weights.

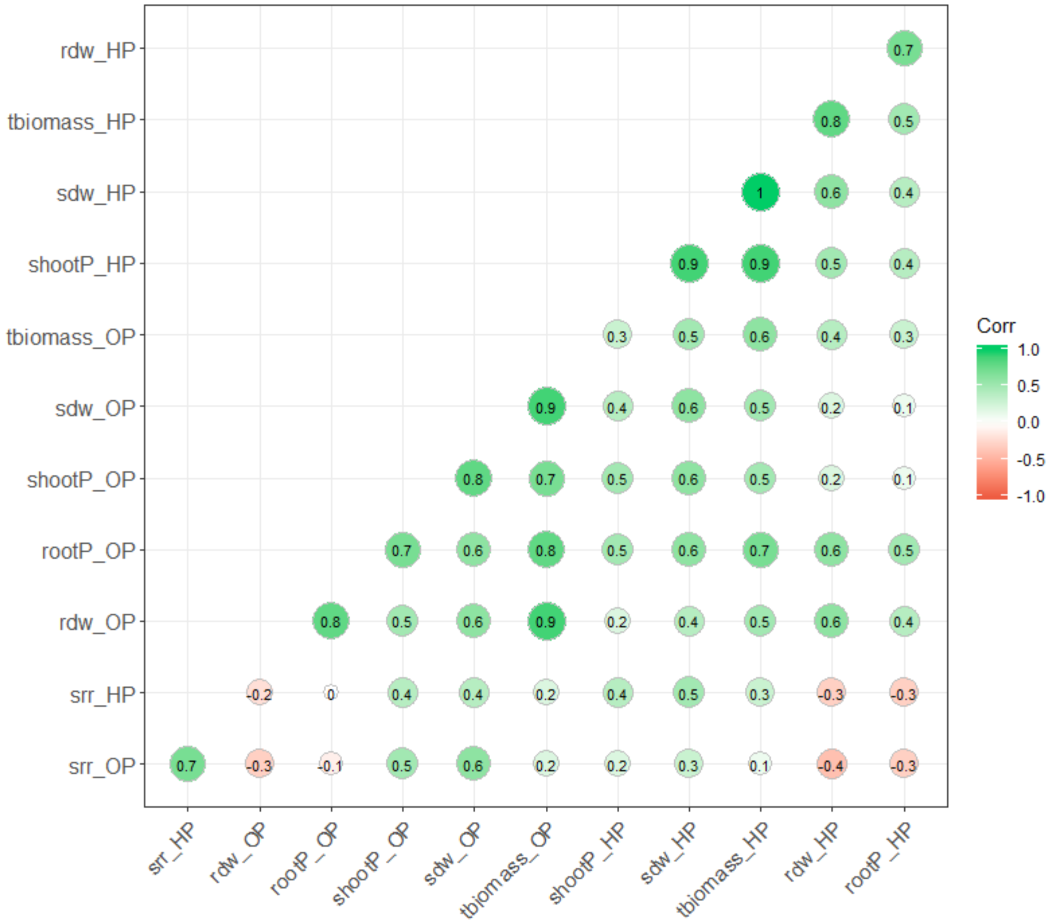


Figure 4.7: Pattern of the relationship between plant parameters and phosphorus contents in shoot and root organs. **Note:** positive correlation increases with the intensity of greenness while negative increases with increasing red colour

4.3.3 Grouping of cowpea lines based on performance in Low P & Response to P addition

The plant materials used in this work were grouped into four groups based on their shoot dry weight, as the most reliable criteria for accessing P use for this study. The following cowpea lines with shoot yield above mean OP (0.41 g) and HP (2.22 g); IT89KD-288, IT84S-2246-4, IT00K-1263, SAMPEA-17, Vita7, IT97K-499-35, 524B, IT93K-503-1, IT97K-556-6, IAR-48 and SuVita2 were grouped as efficient responsive (ER) lines. Efficient non-responsive (ENR) lines included CB27, CB46, Yacine, and Danila that had shoot yield of above average in OP (0.41 g) and below (2.22 g) in HP. Inefficient responsive (IER) consisted of IT90K-277-2, Sanzi, IT84S-2049, Tvu-14676, Aloka-local, Dan-Misra, and IT86D-10-10 with shoot yield below mean yield in OP (0.41 g) and above average in HP (2.22 g) while the inefficient non-responsive (IENR) lines; 58-77, Tvu-7778, B301, UCR779, Kanannado, IT82E-18 and UAM-1055-6 were those with shoot yield below average in OP (0.41 g) and HP (2.22 g) media (Figure 4.8).

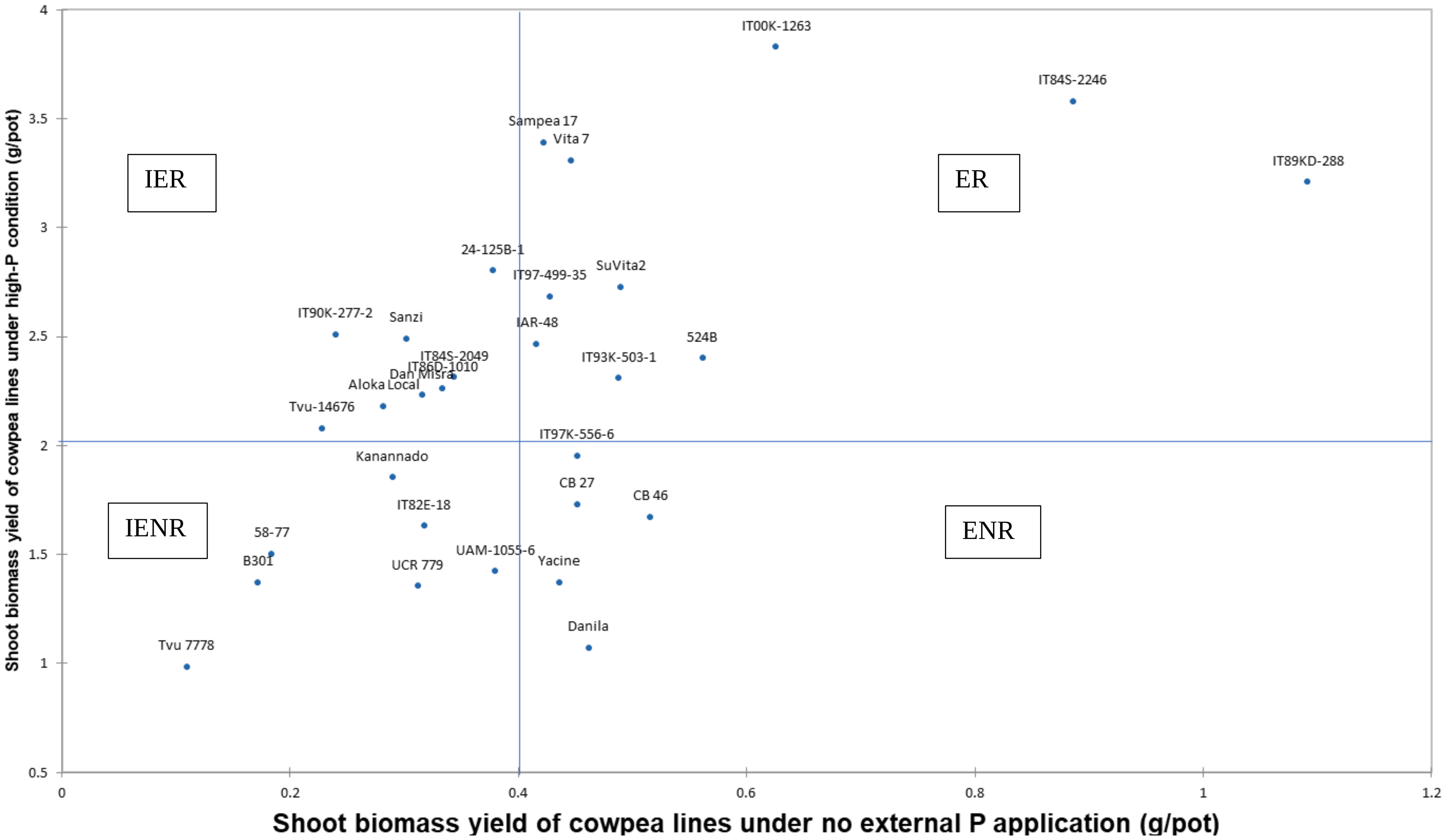


Figure 4.8: Biplot of cowpea shoot dry weight (g/plot) at low P and high P of Screenhouse experiment
ER= efficient responsive, ENR = efficient non-responsive, IER = inefficient responsive, IENR =inefficient non-responsive

4.3.4 Results of the field experiment

The field soil had low to very low physical and chemical properties such as pH in H_2O (1:1 water: soil mixture) and pH in 0.01M $CaCl_2$ was acidic, mean available P content was 3.70 mg kg^{-1} (Bray 1 method), total N and organic carbon were very low and other physicochemical properties (Table 4.7). The trend from the field experiment was similar to performance of plants from the pots experiment.

There was significant variation among the lines in response to P in the growth environment for all measured parameters; plant height, days to first flowering, days to maturity, shoot dry weight, pod yield, total plant biomass, and P concentrations in shoot and root tissue (Table 4.8).

Generally, the performance increased with increasing P concentration. Phosphorus treatments lead to a reduction in the number of days to first flowering and maturity of the lines evaluated under medium and high P treatments. Delayed flowering and maturity were observed for lines under no-external P application (Table 4.9). Like the screenhouse results, the pattern in shoot biomass and pod yields were smaller in the OP and LP treatments of all the lines compared to HP outputs for the same lines (Table 4.10).

Table 4.7: Physical and chemical properties of the low soil P of field experimental site

Analysis	**Results (Field soil)**				**Unit**	**Rating**
	Composite Sample 1	**Composite Sample 2**	**Composite Sample 3**	**Mean**		
pH (H2O)	6.37	6.4	6.27	6.34	NA	Acidic
pH (0.01M CaCl2)	5.67	5.7	5.40	5.59	NA	Acidic
ECE (dsm)	0.35	1.0	1.05	0.80	dsm	
Organic Carbon	0.70	0.7	0.93	0.79	g kg soil^{-1}	Very low
Total N	0.11	0.1	0.13	0.11	g kg soil^{-1}	Very low
Available P	4.65	2.8	5.03	4.15	mg kg^{-1}	Very low
Calcium^{++}	4.02	2.6	3.38	3.32	Cmol/kg	Very low
Magnesium^{++}	1.37	0.6	0.91	0.97	Cmol/kg	Very low
Potassium	1.20	0.6	0.28	0.68	Cmol/kg	Very low
Sodium	1.73	1.6	1.58	1.63	Cmol/kg	Very low
H^{+} Al	0.40	0.5	0.60	0.49	Cmol/kg	Very low
CEC	8.72	5.8	6.75	7.08	Cmol/kg	Very low
Clay	23.33	17.3	16.00	18.89	%	
Silt	30.67	30.0	32.67	31.11	%	
Sand	46.00	52.7	51.33	50.00	%	
Texture	Clay Loam	Sandy Loam	Sandy loam	Sandy Loam		

Table 4.8: Probabilities ($p < 0.05$) of F-test for plant height, phenological traits, shoot dry weight, pod yield, total biomass, and P concentrations of cowpea lines in the field

SOURCE	**PHT**	**DFF**	**MAT**	**SDW**	**PodHa**	**Tbiomass**	**ShootPC**	**RootPC**
Lines	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0087	0.0002	< 0.0001	< 0.0001
Phosphorus	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.3348	0.1514
Line x P	0.2698	0.0042	0.4137	0.0138	0.2938	0.1091	0.2064	0.0177
Mean	23.18	53.96	82.41	107.93	1582.80	237.60	1159.1	1171.46
CV	33.16	8.27	5.53	43.73	53.23	46.02	16.21	20.95

PHT = plant height, DFF = days to first flowering, MAT = days to maturity, SDW = shoot dry weight, PodHa = pod yield per ha, Tbiomass = total plant biomass, ShootPC = shoot P concentration in mg kg^{-1}, RootPC = root P concentration in mg kg^{-1}

Table 4.9: Performance of cowpea lines under different P treatments plant height, and days to first flowering and maturity evaluated in the field environment

	Plant Height (cm)				**Days to first flowering**				**Days to maturity**			
Lines	**OP**	**LP**	**HP**	**Mean**	**OP**	**LP**	**HP**	**Mean**	**OP**	**LP**	**HP**	**Mean**
24-125B-1	17.9	19.4	23.7	20.3	67	53	48	56	87	96	80	87
524B	15.4	26.2	49.9	30.5	48	43	43	44	90	77	75	81
58-77	16.4	26.0	35.5	26.0	47	41	45	44	85	76	73	78
Aloka_Local	15.2	20.9	27.7	21.3	70	52	52	58	92	89	84	88
B301	11.0	16.7	18.9	15.5	57	49	45	50	82	77	74	78
CB27	24.4	26.4	39.2	30.0	48	49	44	47	81	81	82	81
CB46	17.5	34.6	63.8	38.6	48	45	44	46	83	81	80	81
Danila	11.1	17.9	25.4	18.1	85	53	49	62	93	92	87	91
DanMisra	17.8	23.0	25.5	22.1	75	75	66	72	90	89	88	89
IAR-48	15.1	24.4	36.2	25.2	64	61	53	59	91	89	86	88
IT00K-1263	16.0	18.0	28.4	20.8	58	51	49	52	88	77	74	80
IT82E-18	15.9	17.4	21.5	18.3	54	49	47	50	81	79	77	79
IT84S-2049	16.4	23.8	34.4	24.9	52	45	45	47	83	76	75	78
IT84S-2246-4	15.9	20.5	24.0	20.1	61	50	46	52	84	81	77	81
IT86D-1010	30.3	36.5	78.2	48.3	48	48	45	47	78	76	72	75
IT89KD-288	9.8	16.7	21.2	15.9	70	69	64	67	93	89	84	89
IT90K-277-2	14.9	20.0	28.9	21.2	78	68	68	71	94	91	91	92
IT93K-503-1	13.7	18.0	38.9	23.5	57	59	52	56	89	84	76	83
IT97K-499-35	18.8	22.8	25.3	22.3	51	46	48	48	87	81	79	82
IT97K-556-6	15.9	18.9	29.4	21.4	59	53	48	53	86	82	82	83
Kanannado	15.2	22.2	31.7	23.0	73	73	70	72	104	95	87	95
SAMPEA-17	13.7	25.5	29.9	23.0	67	54	49	56	89	86	80	85
Sanzi	15.9	33.4	29.2	26.1	50	45	41	45	76	69	64	70
SuVita2	13.0	21.3	22.7	19.0	55	49	44	49	88	76	60	75
Tvu-14676	11.7	18.2	26.8	18.9	60	50	50	53	86	86	81	84
TVU-7778	18.3	23.1	36.7	26.0	51	47	46	48	81	76	76	77
UAM-1055-6	13.8	14.8	32.3	20.3	56	48	48	51	89	87	75	83
UCR 779	9.7	14.4	20.5	14.8	56	53	52	53	84	85	73	81
Vita7	15.8	23.0	33.4	24.0	77	53	49	59	90	85	79	85
Yacine	12.7	16.2	20.0	16.3	61	47	47	52	82	76	74	77
Min	9.7	14.4	18.9	14.8	47	41	41	44	76	69	60	70
Max	30.3	36.5	78.2	48.3	85	75	70	72	104	96	91	95
Mean	15.64	22.0	32.0	23.2	60	53	50	54	87	83	78	83

OP = no-P application, LP = 10 kg P kg^{-1} soil, HP = 60 kg P kg^{-1}

Table 4.10: Shoot biomass and pod yield of cowpea lines under contrasting soil P in the field

	Shoot Dry Weight (g/plot)				**Pod yield (kg)/ha**			
Lines	**OP**	**LP**	**HP**	**Mean**	**OP**	**LP**	**HP**	**Mean**
24-125B-1	43.8	55.8	221.8	107.1	495.4	625.4	3328.1	1483.0
524B	15.8	39.5	70.5	41.9	215.5	643.2	1112.5	657.1
58-77	40.1	48.3	152.5	80.3	523.2	351.0	2202.8	1025.7
Aloka-local	20.8	57.0	96.2	58.0	479.9	964.8	1571.8	1005.5
B301	52.9	103.8	102.2	86.3	792.6	1891.8	1927.9	1537.4
CB27	65.6	40.9	68.9	58.5	731.8	646.1	1009.9	795.9
CB46	26.9	79.3	105.5	70.5	328.8	990.6	1477.7	932.4
Danila	37.6	75.8	188.7	100.7	35	775.8	2242.9	1007.4
DanMisra	82.2	83.6	382.8	182.8	1022	1179.7	5263.7	2488.5
IAR-48	85.7	81.9	222.6	130.0	971.8	1127.4	2578.5	1559.2
IT00K-1263	21.8	91.0	277.7	130.2	449.3	703.7	3451.4	1534.8
IT82E-18	74.2	89.6	169.5	111.1	1531.8	1204.2	2409.4	1715.1
IT84S-2049	18.5	80.9	148.8	82.7	340.5	1141.4	2601.6	1361.1
IT84S-2246-4	37.0	85.4	195.9	106.1	967.5	811.5	3240.9	1673.3
IT86D-1010	46.8	123.8	213.4	128.0	727.9	2345.0	3929.0	2334.0
IT89KD-288	14.0	68.0	194.1	92.0	311.6	1238.0	2831.5	1460.4
IT90K-277-2	106.1	134.8	345.9	195.6	1120.8	1702.9	3246.4	2023.4
IT93K-503-1	23.2	132.6	248.6	134.8	548.4	1491.4	3707.2	1915.7
IT97K-499-35	71.0	120.5	190.0	127.2	1306.3	2312.8	3323.6	2314.2
IT97K-556-6	139.9	48.9	225.1	138.0	1988.2	700.8	3469.6	2052.9
Kanannado	100.6	178.5	147.8	142.3	764.3	3495.2	2761.5	2340.3
SAMPEA-17	31.4	122.0	255.4	136.3	443.2	1068.1	3631.3	1714.2
Sanzi	33.7	41.1	147.1	74.0	564.9	925.6	3910.7	1800.4
SuVita2	21.4	61.3	145.9	76.2	312.7	1055.3	2333.9	1234.0
Tvu-14676	22.1	69.0	125.9	72.3	426.8	854.0	2128.4	1136.4
TVU-7778	30.7	88.6	254.0	124.4	772.8	1413.4	3454.7	1880.3
UAM-1055-6	51.5	49.3	200.2	100.3	837.3	606.7	4222.5	1888.8
UCR-779	7.0	51.3	155.0	71.1	126.6	961.4	1995.1	1027.7
Vita7	95.1	152.3	362.5	203.3	944.8	1417.4	3944.0	2102.1
Yacine	46.1	78.0	103.8	76.0	478.2	1429.1	2541.6	1483.0
Min	7.0	39.5	68.9	41.9	35.0	351.0	1009.9	657.1
Max	139.9	178.5	382.8	203.3	1988.2	3495.2	5263.7	2488.5
Mean	48.8	84.4	190.6	107.9	685.3	1202.5	2861.7	1582.8

OP = no-P application, LP = 10 kg P kg^{-1} soil, HP = 60 kg P kg^{-1}

4.3.5 Grouping of cowpea lines based on Performance in Low P and Response to P under Field Conditions

Based on their performance in OP and HP treatments, lines were categorized as efficient or inefficient in low P soil and as responsive or non-responsive when P is applied using pod yield, giving four classes as earlier stated in the results of the pots experiment.

The efficient responsive lines had an above average pod yield (684 kg/ha) in OP and HP (2,862 kg/ha), these include IT97K-556-6, IT84S-2246-4, IT97K-499-35, IT86D-1010, Vita7, IAR-48, IT90K-277-2, *DanMisra*, and UAM-1055-6. The efficient non-responsive had an above-average yield in the OP but below average in HP: these lines are CB27, IT82E-18, B301 and *Kanannado*. The Inefficient responsive were low yielding in OP and higher yielding in HP; they are IT93K-503-1, IT89KD-288, *Sanzi*, IT00K-1263, 24-125B-1 and SAMPEA-17 while Inefficient non-responsive had lower yield in OP and HP; UCR 779, *Yacine*, 58-77, CB 46, *Danila*, IT84S-2049, *Aloka_local*, Tvu-14676, Suvita2, and 524B (Figure 4.9).

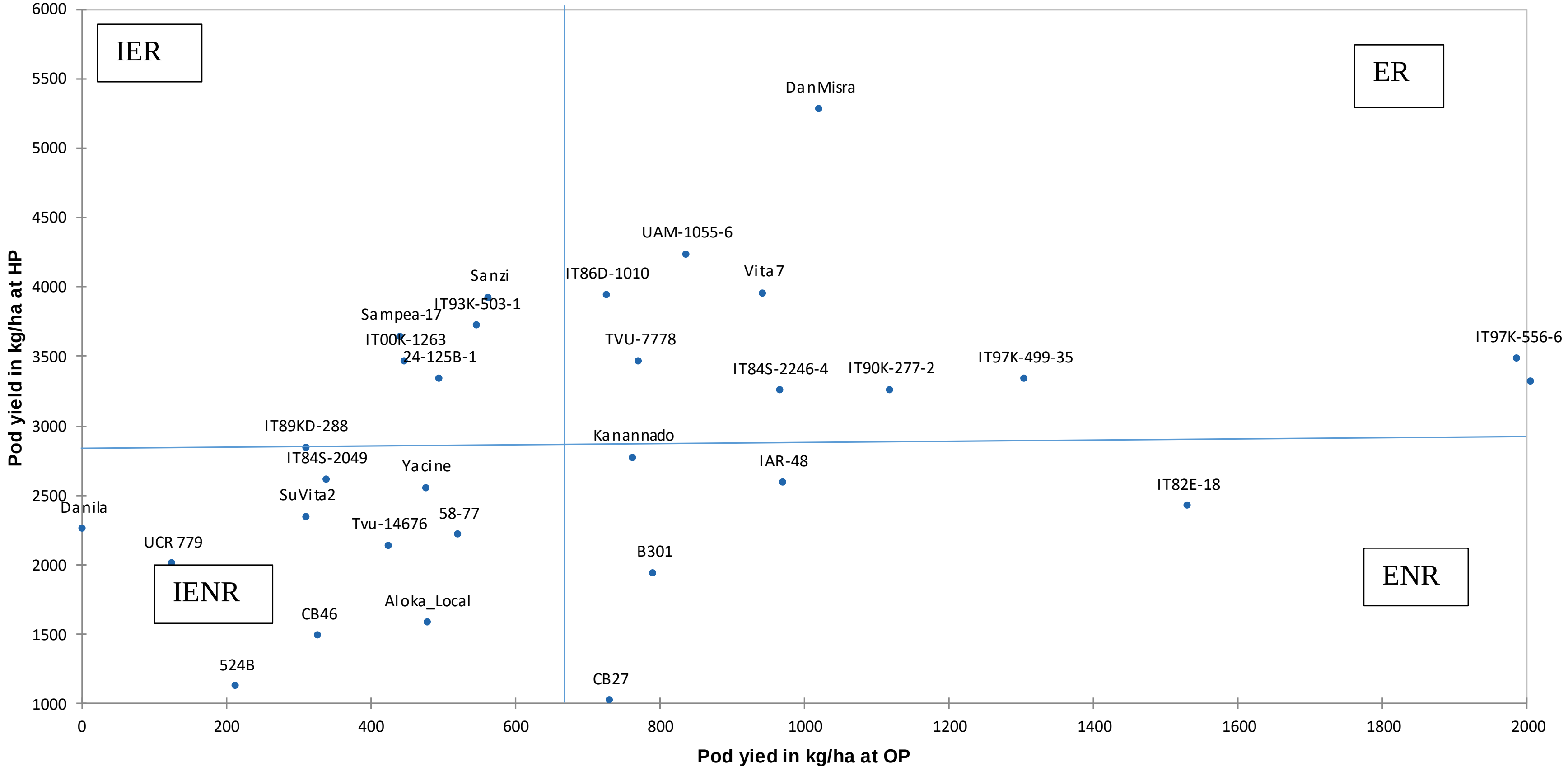


Figure 4.9: Biplot of cowpea lines for pod yield at low and high P treatments under field conditions

ER = efficient responsive, ENR = efficient non-responsive, IER = inefficient responsive, IENR = inefficient non-responsive

4.3.6 Root hairs and seedling root architecture

For all the traits measured, the mean, standard deviation, range, CV and probability of F-test are summarized in Table 4.11. The result of the analysis of variance indicated significant differences ($p < 0.05$) among the lines for all the traits assessed. There were wide ranges for primary root length (PRL) from 18 - 43.5 cm, basal root number (BRN) from 7 - 18, taproot branching density I (TBD5) from 17 - 37, taproot branching density II (TBD10) from 13 - 33, the weight of 100 seeds from 9.2 - 29.1 g, basal root hair density (BRHD) from 32.6 - 135.3, lateral root hair density (LRHD) from 53.7 - 155.9 and tap root hair density (TRHD) ranged from 19.9 - 76.9. The CV was higher for the root hair traits 33.7 – 41.1% for lateral root hair density (LRHD) and taproot hair density (TRHD) respectively (Table 4.11). Root hairs of the lines varied from 0.2 to 1.3 mm in length and density from 20 to 156 root hairs/mm^2. In addition, correlation analysis revealed a strong association between root hairs on tap and basal roots ($r = 0.44$) (Figure 4.10).

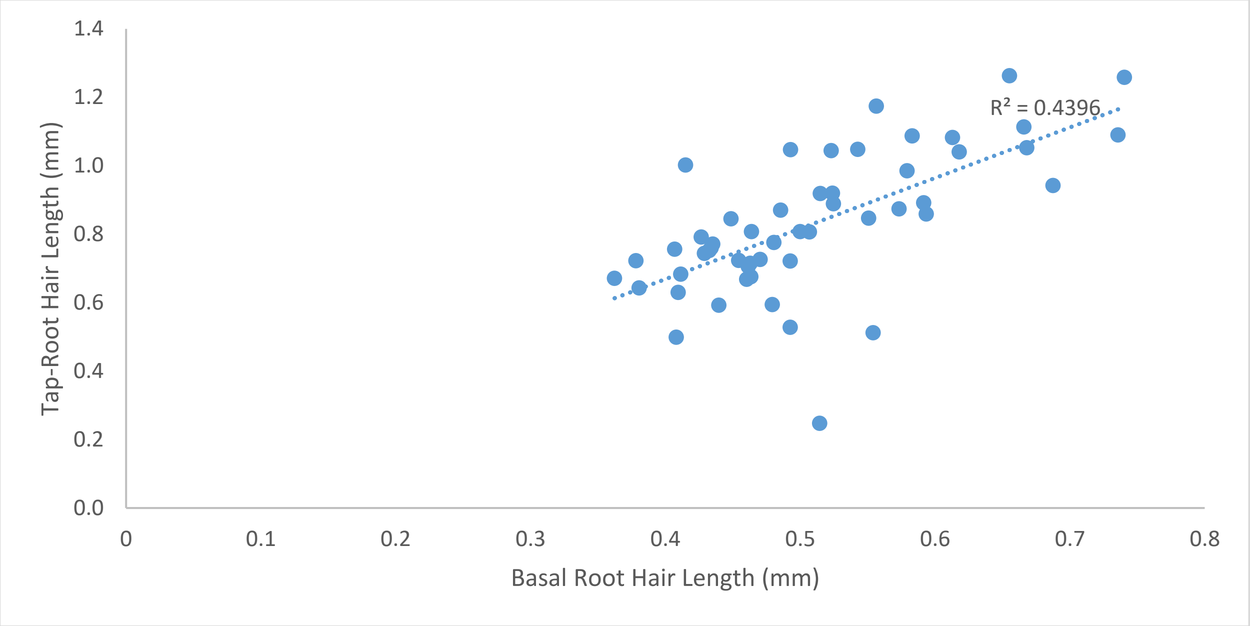


Figure 4.10: Strong association of taproot hair and basal root hair length

Table 4.11: Means, SD, ranges, F-test prob (0.05) and coefficient of variation (CV %) for cowpea seed and seedling root traits measured.

Variables	**Mean**	**Std Dev**	**Min**	**Max**	**Range**	**CV (%)**	**Prob (0.05)**
Primary Root Length (cm)	28.4	5.0	18.0	43.5	25.5	20.3	<.0001
Basal root number	10.9	2.0	7.0	18.0	11.0	24.0	<.0001
Tap root branching density	26.8	4.5	17.0	37.0	20.0	25.0	<.0001
Tap root branching density	23.7	4.4	13.0	33.0	20.0	26.0	<.0001
Weight of 100 seeds (gram)	18.1	4.4	9.2	29.1	19.9	0.6	<.0001
Basal root hair length (mm)	0.5	0.1	0.4	0.7	0.4	38.4	<.0001
Basal root hair density	82.3	19.7	32.6	135.3	102.7	39.3	<.0001
Tap root lateral root hair length (mm)	0.5	0.1	0.4	0.8	0.4	33.7	<.0001
Tap root lateral root hair density	89.8	20.0	53.7	155.9	102.2	38.5	0.0062
Tap root hair density	51.9	12.6	19.9	76.9	57.0	41.1	<.0001
Tap root hair length (mm)	0.8	0.2	0.2	1.3	1.1	33.9	<.0001

4.4 Discussion

Use of sand and nutrient solution to screen cowpea plants' response to P nutrient has been reported (Saidou, 2005). The pattern of variation observed in this study is comparable to reports of earlier works on adaptation and response of cowpea to different P rates (Adusei *et al.*, 2016; Karikari *et al.*, 2015; Kolawole *et al.*, 2008; Mawo *et al.*, 2016; Saidou *et al.*, 2007; Sanginga *et al.*, 2000). These authors have shown that different concentrations of P on cowpea lead to a significant increase in shoot and root biomass production, number and weight of nodules, and P content. The differential performance among tested lines in this study is an indication that selection for superior performing lines with the potential to yield well under low and high P is possible and varieties can be developed to fit different edaphic conditions. Variation in shoot and pod yield among lines treated with P fertilizers appeared to be genetically controlled and needs to be transferred to adapted cowpea lines. Similar observations have been made by previous workers (Krasilnikoff *et al.*, 2003*;* Singh *et al.*, 2002).

Cowpea lines were grouped based on their potential to produce above-average yield on low and high P soil into four categories; as efficient and inefficient under low P condition and responsive and non-responsive under high P condition. Such grouping will permit recommendation of lines that fit into specific agro-ecologies and breeding programmes target nutrient efficiency. This grouping method was earlier suggested by Gerloff (1987) and has been used by several workers to group crop plants (Hammond *et al.*, 2009; Mahamane, 2008; Zapata & Roy, 2004). Lines with higher response to P application and higher performance under minimal or low available P are most desirable. Some of the lines identified from this current work as P efficient and good responders to applied P-fertilizer have been reported. These include IT90K-277-2 (Kolawole *et al.*, 2008) and IT84S-2246-4 (Krasilnikoff *et al.*, 2003) while lines like Danila, a landrace from Nigeria was found to be P efficient in the screenhouse but inefficient from field results. Similar contradictory reports have been made about this landrace.

Krasilnikoff *et al.* (2003) reported Danila to be good at P uptake and attributed that to its long root hairs, while Rothe (2014) reported the same line to be poor P efficient. These contradictions may be due to many variants of Danila seeds in existence, as there were several landraces that farmers called Danila due to the similarity in their seed coat.

There was a high positive significant correlation between low and high P conditions, as most lines that produced high shoot yield in OP were also higher yielder when P was supplied. This finding contradicts the earlier report that lines with tolerance to limited P conditions were not good at responding to added P condition (Caradus & Snaydon, 1986). Impact of P nutrition was visible in other parameters measured such as phenology (flowering and maturity time) and plant height. Root dry weight was not measured under field condition due to difficulty in recovering whole root architecture from the soil in the field. There were 20% and 30% reduction in root growth as a result of plant's efforts to maintain shoot growth under limited P condition, this further attests to the critical role played by P in cowpea growth and development.

Even though several traits could be used as indices for measuring P performance, use of shoot dry weight and total plant biomass were more discriminating for adjudging P adaptation and response. Shoot dry weights have been used in several studies to measure adaptation to low P and response to P fertilization (Caradus *et al.*, 1991; Hammond *et al.*, 2009; Korkmaz *et al.*, 2009; Leiser *et al.*, 2015).

4.5 Conclusions

Extensive genetic differences in cowpea lines for the uptake of P from deficient soils and efficient use of P applied through fertilizers or P-containing nutrient solutions were observed. Results from this study enabled classification of lines into efficient, inefficient, responsive and non-responsive groups based on their performance in the low and high P growth media. This classification will help in identifying lines suitable for cultivation under different agro-ecologies and for farmers with different levels of access to fertilizer inputs.

Since most of the lines used in this study were parents of biparental and multi-parent advanced generation inter-cross RIL populations. RILs with contrasting adaptation for P use and response to P fertilization were identified for further studies. Strong associations were found between root hairs on tap and basal root hairs. This finding will help direct breeding programmes objectives for P use efficiency.

There were high positive significant correlations between performance of cowpea lines evaluated under low and high P conditions. Tissue P concentrations in shoot biomass were positively correlated with biomass and grain yield. In addition, seedling root architecture traits such as primary root length, basal root number, lateral root branching density and root hair traits (length and density) were varied significantly between the lines tested.

P acquisition and use efficiency should be taken as complementary strategies to reduce the use of chemical or synthetic fertilizers instead of complete replacement of chemical fertilization.

CHAPTER FIVE

5.0 Phenotypic evaluation and QTL mapping for phosphorus use efficiency and yield in RIL population under two phosphorus rates

5.1 Introduction

Several screening studies have revealed genetic variation for adaptation to low soil P and response to applied P, indicating the possibility of developing varieties for different soil conditions (Abdou, 2018; Gyan-Ansah *et al.*, 2016; Timko & Singh, 2008). The quantity of P available in soil solution for plant uptake is conditioned by several factors such as soil pH, soil type, association with arbuscular mycorrhizal fungi (AMF), root architecture, and genotype of the crop type (Niu *et al.*, 2013; Richardson *et al.*, 2011; Vandamme *et al.*, 2013). Legumes like cowpea can fix a considerable amount of N through biological N fixation in association with *Bradyrhizobium* spp when there is adequate soil P, this is because the *rhizobium* found in root nodules are able to reduce atmospheric N gas to ammonium for plant use (Diaz *et al.*, 2017; Kyei-Boahen *et al.*, 2017; Zahran, 1999).

Indicators for P use and acquisition efficiency reported in cowpea include shoot dry biomass, root dry biomass, shoot to root ratio, P concentration in shoot and root, grain yield and phenological attributes (Rothe, 2013; Ravelombola *et al.*, 2017; Saidou *et al.*, 2012) and computed P efficiency traits such as agronomic P use efficiency, physiological P use efficiency, P efficiency ratio and P utilization efficiency (Fonji, 2015; Solomon Gyan-Ansah, 2012; Hammond *et al.*, 2009). There are few reports on QTLs and markers for P efficiency traits in cowpea especially using high-density SNP markers. This has limited the capacity of breeders to deploy markers in selection to increase precision and reduce the time taken to achieve genetic gains in developing P efficient varieties. Few SSR markers and QTLs for P use efficiency using shoot dry biomass and tissue P concentration have been reported (Rothe, 2014).

A cowpea biparental recombinant inbred lines population (TVu-14676 x IT84S-2246-4) was evaluated under high and low soil P conditions representing common conditions of most farmers' field. The RIL population used in this work have been investigated and used by different workers to map QTLs for yield components, resistance to *Striga*, and nematode and yield components (Huynh *et al.*, 2013; Lucas *et al.*, 2013; Muchero *et al.*, 2009). Initial screenings (unpublished yet) showed cowpea line; IT84S-2246-4 to be efficient in low P soil and respond positively to applied P under high P environments, while TVu-14676 has contrasting performance under low and high P conditions. In the present study, P efficiency, phenology, and yield traits were evaluated to investigate performance in contrasting P conditions, identify the pattern of relationship between traits and identify QTLs associated with P efficiency traits in cowpea for future breeding work on marker-assisted selection targeting varieties with potential to yield well in low P soils and respond to applied P.

5.2 Materials and Methods

5.2.1 Plant Materials

The biparental recombinant inbred lines (RIL) set of TVu-14676 x IT84S-2246-4 (TV x IT) consisting of 130 RILs (Lucas *et al.*, 2011; Muchero *et al.*, 2009; Muñoz-Amatriaín *et al.*, 2017) were evaluated with their two parents. The RILs were F_9 lines advanced by single seed descent (SSD) (Huynh *et al.*, 2013) and seeds were kindly provided by the Cowpea team of the University of California Riverside (UCR), USA. The IT84S-2246-4 and Tvu-14676 were from IITA's worldwide collection and segregated for nematode and *Striga* resistance (Muchero *et al.*, 2009). In the initial screening experiments, these lines segregated for biomass and grain yield under low and high P growth media (nutrient-sand solution and natural field conditions).

5.2.2 Phenotyping sites and experimental design:

The field evaluation of RIL lines was undertaken at Zaria (N11^{0}09'49.6'' E007^{0}37'13.8'' at 668m elevation), located in the northern guinea savannah of Nigeria in 2017 and 2018 cropping seasons, each with three replications in an alpha lattice design of 12 x 11. Prior to planting, seeds were treated with a commercial fungicide (*AllStar*) at 10 g 4 kg^{-1} of seeds according to the manufacturer's recommendation. Phosphorus (P) was applied to high P (HP) plots using the commercial single super phosphate (SSP) fertilizer at the rate of 60 kg ha^{-1} seven days after sowing (DAS). The SSP used contained 20% total P_2O_5, 15% water soluble P_2O_5, 16.5% water and citrate-soluble P_2O_5, 11% Sulphur, 18% Ca and 4% moisture (*TAK-AGRO SSP 20%*). Urea (46% N) and muriate of potash (MOP) fertilizers were applied at 30 kg ha^{-1} for both low and high treatments to avoid confounding effects of N and K. Plots were one row of 2 m length at a 0.20 x 0.75 m intra and inter-row spacing consisting of 10 plants per row.

The Low P (LP) treatments did not receive an application of SSP fertilizer and plants in the LP plots were maintained on the inherent soil P (6 mg kg^{-1}), contrary to the high P (HP) treatments that received 60 kg P ha^{-}1. Soil available P was earlier measured at 0 - 0.2 m soil depth (mean of 6 mg kg^{-1} of soil, Bray I method) before planting and other physical and chemical properties of the field were determined.

5.2.3 Phenotypic data collection and analysis:

Plant parameters assessed were phenological traits (days to first flowering & days to maturity), yield components and P efficiency traits as physiological P use efficiency (PPUE), P utilization efficiency (PUtE), agronomic P use efficiency (APE), and P uptake efficiency (PUpE). Phosphorus concentration was determined using Vanadate-molybdate (Yellow) method (Kitson & Mellon, 1944) at the Phosphorus Lab of the Department of Soil Science, Ahmadu Bello University Nigeria. Dried plant shoot and grain samples were ground to a fine powder using grinding mill and 1g of each sample was weighed out using a digital balance and packed into small zip lock bags. Total P content of lines was calculated as a product of P concentration (in dry shoot or grains) and dry weight of shoot and grain per line per replication. Yield and yield components were measured as dry weight of pods, grains per plot, and shoot biomass. Data were recorded with the aid of Field-Book App on an android tablet (Rife & Poland, 2014).

When all measurements were taken and recorded, harvesting was done on a plot basis, into individually labelled bags. For each plot, pods were threshed, and seeds retained in a secured and labelled bag to preserve the identity of the seed. Phenotypic data were analysed to generate means of RIL lines over replications using the linear mixed model (R software). Phenotypic traits correlation using *ggcorrplot* R package on the pooled means of RIL lines (Kassambara, 2017) was undertaken to identify the important pattern of relationship in the data set.

5.2.4 Genotypic data acquisition and genetic linkage map construction

The genotypic data for the RILs were sourced from the Cowpea team at the UCR, USA. The procedure used for the genotyping is briefly described. Genomic DNA from each RIL line and parents were extracted from dried young seedling leaves using Plant DNeasy procedure, and DNA was quantified with Qunat-IT dsDNA kit. The detailed explanation for DNA extraction, quantification and purity check have been described (Lo *et al.*, 2018; Muñoz-Amatriaín *et al.*, 2017). The RILs were genotyped at the University of Southern California (USA) using the Cowpea Iselect Consortium Array with 51, 128 SNPs. The linkage map of IT x TV population used in this study has been published (Muñoz-Amatriaín *et al.*, 2017). It has 14,660 SNP markers across 11 the linkage groups of cowpea. The map was constructed using MSTmap50 (http://www.mstmap.org/) with the below criteria; "grouping LOD criteria = 10; population type = DH (doubled haploid); no mapping size threshold = 2; no mapping distance threshold: 10 cM; try to detect genotyping errors = no; and genetic mapping function = kosambi." after SNPs were called with Illumina GenomeStudio software, and curated by removing SNPs with

> 20% missing data, heterozygous calls, and as well as removing individual lines with duplicate or non-parental alleles, leaving SNPs that are polymorphic between parents and RILs with minor allele frequencies of > 25%. The Centimorgan distances were later divided by two for the RIL lines to correct for potential inflation of cM in the MSTmap50 that used double haploids as population. The linkage groups in the current map of this population have been oriented and numbered according to synteny with common bean (Muñoz-Amatriaín *et al.,* 2017).

5.2.5 QTL analysis and marker-trait association

The QTL identification was conducted with a linear mixed effect model that controls polygenetic background effects using marker-inferred kinship matrix between the lines. The detailed description of the model has been given (Xu, 2013) and implemented in R using an in-house code (Personal communication, Sassoum Lo, UCR, USA) as used by Lo *et al.* (2018) in mapping QTLs for domesticated related traits in cowpea.

In this model, the effect of the marker is fitted as a fixed factor and tested using the Wald test statistic (squared effect divided by the variance of estimated effect). The Wald test follows a chi-square distribution under the null model with one degree of freedom, from which a p-value was calculated for each marker. SNP markers of the entire genome were scanned and a test statistic, $-\log_{10}P$ (*P*-value) profile was computed (Lo *et al.*, 2018) and a window of ±2 cM around the testing interval (marker) was excluded from kinship matrix when scanning for putative QTL region. The thresholds for declaring QTLs as significant were determined with adjusted Bonferroni correction using a trait specific “effective number of SNPs” as the denominator (Wang *et al.*, 2016). The percentage of phenotypic variance explained by each SNP was calculated (Lo *et al.*, 2018). QTL peaks were displayed using TIBCO Spotfire student license (TIBCO Software Inc., Palo Alto, CA, USA).

5.3 Results

5.3.1 Phenotypic analysis of recombinant inbred lines population in contrasting P soils

The phenology, yield components and phosphorus efficiency traits were investigated in low and high phosphorus (P) soils in a TV x IT biparental RIL population. The low and high P conditions applied (0 and 60 kg ha^{-1}) using SSP, reflects the typical P fertility in farmers' fields and experimental conditions, respectively. The phenotypic data revealed that performance for all traits were generally superior under high P compared to the low P conditions with some lines having higher output in low over high P treatment. The two parents had contrasting performance across all P conditions. IT84S-2246-4 was overall superior to Tvu-14676 for all the traits measured under both P conditions. The parental lines maintained near mean values for phenological traits (DFF, D50F and DTM) under OP condition (Table 5.1).

In the TV x IT population, application of SSP (HP) lead to increased shoot biomass yield (34 - 85 g plot^{-1}), pod yield (287 - 664 kg ha^{-1}), grain yield (172 - 454 kg ha^{-1}) and P accumulation in grain and shoot (Table 5.1). The performance was reduced by more than half in OP relative to HP performance. Adequate soil P resulted in earlier flowering and maturity of parents and RILs while P stress delayed flowering and maturity from 1 - 4 days among parents (Table 5.1).

5.3.2 Phenotypic correlations of the RIL lines in contrasting soil P conditions

Grain yield at both LP and HP were positively correlated with biomass and P use efficient traits (grain APUE, grain PPUE and grain P content) in both P conditions. Shoot P content and biomass at both P conditions were positively correlated while significant positive correlations were observed between P efficiency traits (grain APE vs PPUE and grain P content vs PPUE) at both P conditions (Figure 5.1).

Table 5.1: Descriptive statistics of parents and TVu-14676 x IT84S-2246-4 RIL lines evaluated under contrasting soil P

		Parents			**RIL Lines**				
Trait	**P-Level**	**TVu-14676**	**IT84S-2246-4**	**SD**	**Min**	**Max**	**Range**	**SD**	**Mean**
Days to flowering	OP	48	47	1	43	58	16	4	50
	HP	48	44	2	40	58	18	4	49
Days to 50% flowering	OP	53	52	0	46	66	20	4	55
	HP	54	50	3	44	59	15	4	54
Days to maturity	OP	65	64	1	60	72	12	2	66
	HP	67	61	4	57	73	16	2	65
Shoot Biomass yield	OP	24	43	14	6	104	98	17	34
	HP	138	74	45	20	217	197	36	85
Pod yield(kgha-1)	OP	145	439	208	41	908	867	164	287
	HP	647	966	226	53	2024	1970	362	664
Grain yield(kgha-1)	OP	70	300	162	11	609	598	114	172
	HP	685	698	9	28	1257	1229	255	454
Grain P uptake (gkg-1)	OP	124.8	466.5	242	9.8	1078.8	1069	183.4	269.5
	HP	1044	1189.7	103	57.3	2558.9	2501.6	452.7	802.5
Shoot P uptake (gkg-1)	OP	29.63	57.77	20	7.1	156.7	149.6	27.0	39.0
	HP	163.53	67.9	68	18.17	252.2	234.0	45.0	102.8

OP = 0 kg SSPha^{-1}, HP = 60kgha^{-1}, P uptake = dry weight x P concentration of tissue, SD = standard deviation

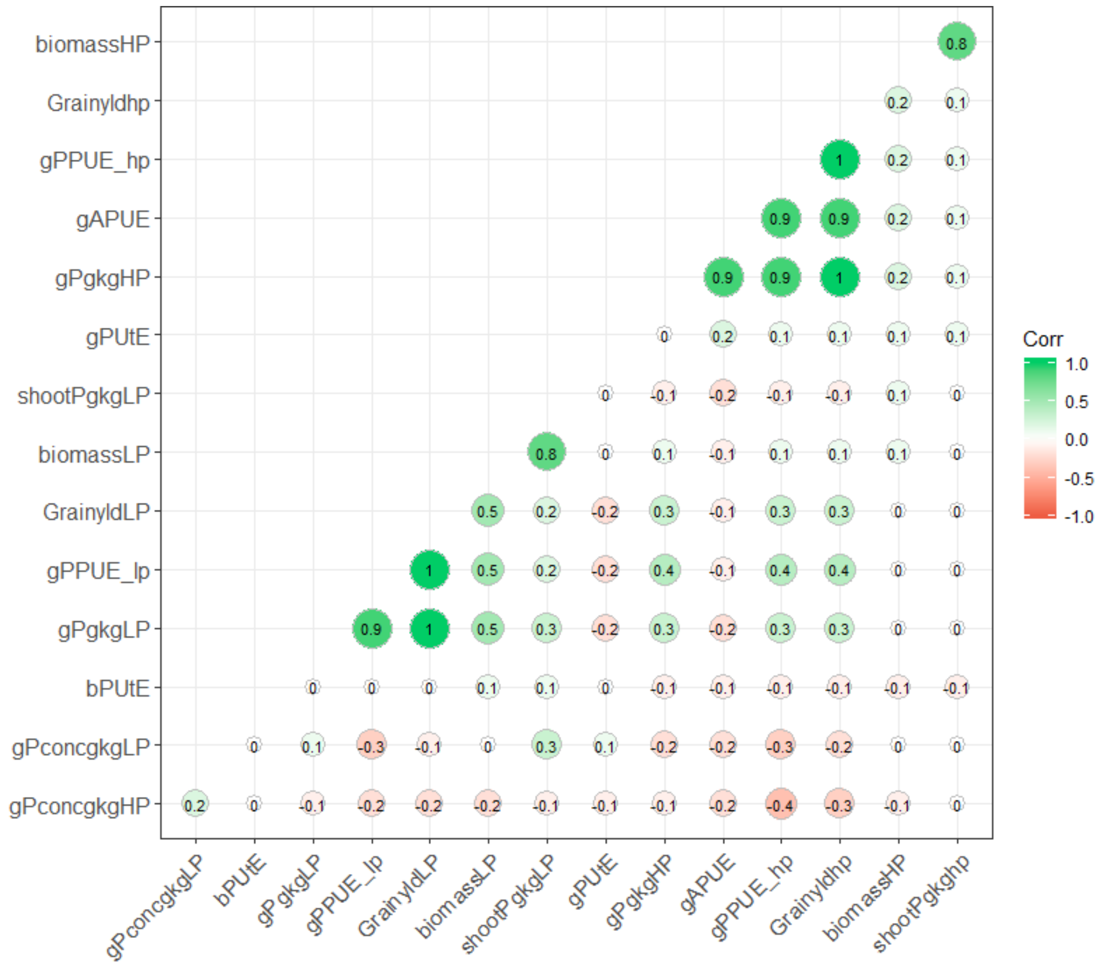


Figure 5.1: Phenotypic correlations of TV x IT RIL population under no-P and high P conditions. **Note:** Positive correlation increases with the intensity of greenness while negative increases with increasing red colour.

5.3.3 Linkage map and marker-trait association analysis

The linkage map of TV x IT used in this study had 14, 660 SNP markers over a distance of 812.90 cM mapped into 1216 bins on 11 linkage groups with an average of 12 SNPs per bin. The linkage grouping in this map was based on reference genome sequence (cowpea pseudomolecules) available on (www.phytozome.net), making the current numbering different from the previously published map of this population.

The mixed model QTL mapping used, identified a total of 27 QTLs for 17 traits on seven out of the 11 cowpea linkage groups (Table 5.2 – 5.3) with $-\log_{10}$ (p-value) ranging from 2.41 for shoot dry weight at LP to 6.10 for days to maturity at LP (Table 5.2). The percentage of phenotypic variance explained (PVE) for the mapped QTLs ranged from 6.11 for shoot dry weight at LP to 28.38 for days to flowering at LP. A region (65.07 cM) on chromosome Vu01 was identified showing a cluster of QTLs for days to flowering (LP), pod and grain yield at HP and three grain P use efficiency traits (gPUpE, gPPUE, and gAPE).

5.3.4.1 QTLs for phenological traits

One major QTL for days to flowering was identified on chromosome Vu08 under HP explaining 27.80% of the PVE with the allele conferred by IT84S-2246-4 and two QTLs were mapped for the same trait under LP on Vu08 and Vu01 explaining 28.38% and 13.5% of PVE respectively, with alleles for the trait contributed by IT84S-2246-4 on Vu08 and TVu-14676 on Vu01 (Figures 5.2, Tables 5.2). Two QTLs were detected on Vu06 and Vu08 for days to maturity explaining 15.2% and 13.7% PVE under HP while one major QTL was detected on Vu01 for the trait under LP explaining 17.34% PVE. Negative alleles for the trait for all the QTLs were conferred by the female parent (TVu-14676) (Table 5.2, Fig. 5.2).

5.3.4.2 QTLs for yield components

A total of 8 QTLs were mapped for yield and its component traits at HP and LP conditions. QTL analysis revealed the presence of a main QTL on Vu09 for shoot dry biomass at HP with PVE of 21.0%, spanning within 39.89 - 40.27 cM region, the allele for the effect was contributed by IT84S-2246-4. For shoot dry weight under LP, a minor QTL was located on Vu07 explaining 6.1% of the PVE with the allele effect contributed by TVu-14676 at LP condition (Table 5.2, Fig. 5.3).

Pod yield QTLs were identified on Vu01 and Vu05 for HP and LP conditions, the PVE for this QTLs ranged from 11.8 – 14.6% with both parents contributing positive alleles. The region ranges from 52.2 - 65.07 cM on Vu01 and 12.4 - 14.6 cM on Vu01. A QTL governing grain yield with 13.1% PVE was identified on Vu01 under both P conditions with the allelic effects contributed by IT84S-2246-4. One additional region was mapped on Vu05 for grain yield under LP condition explaining 12.0% of the PVE with the alleles conferred by TVu-14676. These QTLs were found on the same region with QTLs identified for pod yield on Vu01. The peak SNPs for pod and grain yield at HP were the same (2_07925 and 2_33992) while pod and grain yields at LP had similar peak SNPs (2_26276 and 1_1013) (Table 5.2, Fig. 5.3).

5.3.4.3 QTLs for P use efficiency traits

QTLs for PUE traits were mapped on chromosomes; Vu01, Vu02, Vu05, Vu08 and Vu09 of cowpea. One QTL for gAPE was mapped on Vu09 with PVE of 13.26% and spanning 1.12 cM distance, with positive alleles conferred by the female parent of the RIL population. The P uptake (content) of grains at HP and LP QTLs were identified on Vu01, Vu02 and Vu05 jointly explaining 23% and 19% under HP and LP conditions, respectively. Both grain P content traits whose QTLs were mapped on Vu01 had their alleles contributed by the two parents (Table 5.3).

One main QTL was mapped for gPPUE on HP environment on Vu01 with 14.3% PVE, gPPUE on LP had two QTLs on Vu08 and Vu01 with 15% and 12.6% PVE and allele effects derived from the female parent. Two QTLs on Vu01 and Vu05 were detected for gPUpE and explained 23.1% of PVE with both parents contributing positive alleles for the trait while gPUpE under LP had two QTLs on Vu01 and Vu02 jointly explaining 20.3% PVE. Taken together, P use efficiency traits had QTLs in five regions from both parents, with those on Vu02 only observed in LP and VuG05 and Vu08 observed in HP conditions (Table 5.3, Fig 5.4).

Table 5.2. Quantitative trait loci for cowpea phenological traits and yield components mapped using linear mixed model analysis

Env	Trait	QTL	Peak SNP (s)	ChrNo	Position(cM)	$-\text{Log}_{10}\text{P}$	QTL region (cM)	PVE (%)	Effect
HP	Days to flowering_HP	Cfthp8	2_00706, 2_00707, 2_03649	8	31.5	5.89	31.75-34.01	27.8	-2.00
LP	Days to flowering_LP	Cftlp8	2_06417, 2_53317, 2_03632	8	31.75	4.36	31.38-32.89	28.4	-1.86
		Cftlp5	2_07925, 2_27671, 2_13572	5	65.07	3.85	64.70-65.07	13.3	1.28
HP	Days to maturity _HP	Cdtmhp6	2_06829, 2_53681, 2_13759	6	4.90	4.22	3.40-4.90	15.2	-0.96
		Cdtmhp8	2_12561, 2_32956, 2_41633	8	26.13	3.33	25.38-26.13	13.7	-0.83
LP	Days to maturity _LP	Cdtmlp1	2_03564, 2_09007, 1_0082	1	28.97	6.16	28.97-29.35	17.34	-0.83
HP	Shoot Dry Weight_Hp	Csdwhp9	2_46682, 2_10636, 2_01022	9	39.89	5.81	39.52-40.27	21.02	-16.71
LP	Shoot Dry Weight_LP	Csdwhl7	2_00706, 2_07522	7	21.59	2.41	21.59-33.19	6.11	4.28
HP	Grain yield_HP	CgrainHP1	2_07925, 2_33992	1	**65.07**	4.10	64.7-65.07	13.12	-13.83
LP	Grain yield_LP	CgrainLP1	1_1013, 2_26276	1	53.35	3.19	52.22-54.10	13.12	-6.19
		Cgrainlp5	2_03774	5	14.61	3.00	13.11-14.61	12.39	6.09
Hp	Pod yield_hp	Cpodyldhp1	2_07925, 2_33992	1	**65.07**	4.59	64.7-63.95	14.61	-138.34
		Cpodyldhp5	2_00867	5	12.36	3.00	11.99-12.36	11.77	124.81
LP	Pod yield_lp	Cpodyldlp1	1_1013, 2_26679	1	53.35	3.55	52.22-54.10	14.13	-61.35
		Cpodyldlp5	2_03774	5	14.61	3.31	13.11-14.61	13.62	60.91

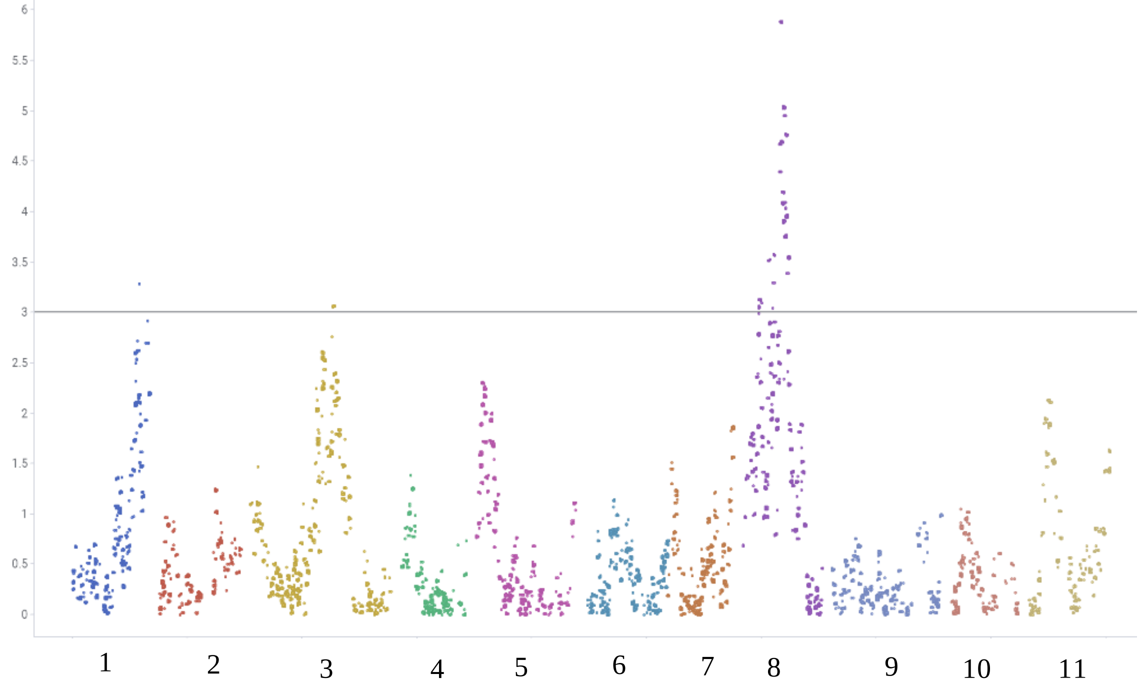
Env. = Environment (P condition), HP =high phosphorus, LP = low phosphorus, PVE = percent of variance explained. QTL are designated as follow: “C” to indicate cowpea, followed by the trait code, then followed by the chromosome number. Positive or negative effect alleles, for which a positive value indicates allele of the TVu-14676 is present and a negative value indicates the allele of the IT84S-2246-4 is present.

Table 5.3. Quantitative trait loci for cowpea phosphorus use efficiency traits mapped using linear mixed model analysis

Env	**Trait**	**QTL**	**Peak SNP (s)**	**ChrNo**	**Position(cM)**	**-Log$_{1}$0P**	**QTL region (cM)**	**PVE (%)**	**Effect**
HP&LP	bAPE	Capeb9	2_46682, 2_03151, 2_07790	9	39.89	4.43	38.77-39.89	13.26	-0.32
HP	gPAE	CgrainPhp5	2_00867	5	12.36	3.30	11.99-12.36	13.07	24.68
		CgrainPhp1	2_07925	1	**65.07**	3.24	64.7-65.07	10.19	-21.68
LP	gPAE	CgrainPLp1	1_1013	1	53.35	2.72	52.22-53.35	10.96	-9.08
		CgrainPLp2	2_27234	2	46.82	2.63	46.45-46.82	8.22	7.92
Hp	gPPUE	Cppue_ghp1	2_07925, 2_33992	1	**65.07**	4.47	64.7-65.83	14.28	-8.57
LP	gPPUE	Cppue_glp8	2_09958	8	33.26	3.49	31.75-33.26	14.98	4.63
		Cppue_glp1	2_05224	1	55.22	3.28	52.22-53.35	12.61	-4.24
HP	gPUpE	CpUpe_ghp1	2_07925	1	**65.07**	3.30	64.7-65.83	10.45	-21.73
		CpUpe_ghp5	2_00867	5	12.36	3.23	11.99-16.49	12.65	24.03
LP	gPUpE	CpUpe_glp1	1_1013	1	53.35	2.87	52.22-54.1	12.00	-9.18
		CpUpe_glp2	2_27234	2	46.82	2.59	46.07-46.82	8.26	7.67

Env. = Environment (P condition), HP =high phosphorus, LP = low phosphorus, PVE = percent of variance explained, bAPE: agronomic P use efficiency for shoot biomass, gPPUE= physiological P use efficiency for grain, gPUpE: P uptake efficiency for grain, gPAE: P accumulation efficiency for grain. QTL are designated as follow: "C" to indicate cowpea, followed by the trait code, then followed by the chromosome number. Positive or negative effect alleles, for which a positive value indicates allele of the TVu-14676 is present and a negative value indicates the allele of the IT84S-2246-4 is present.

High P



Low P

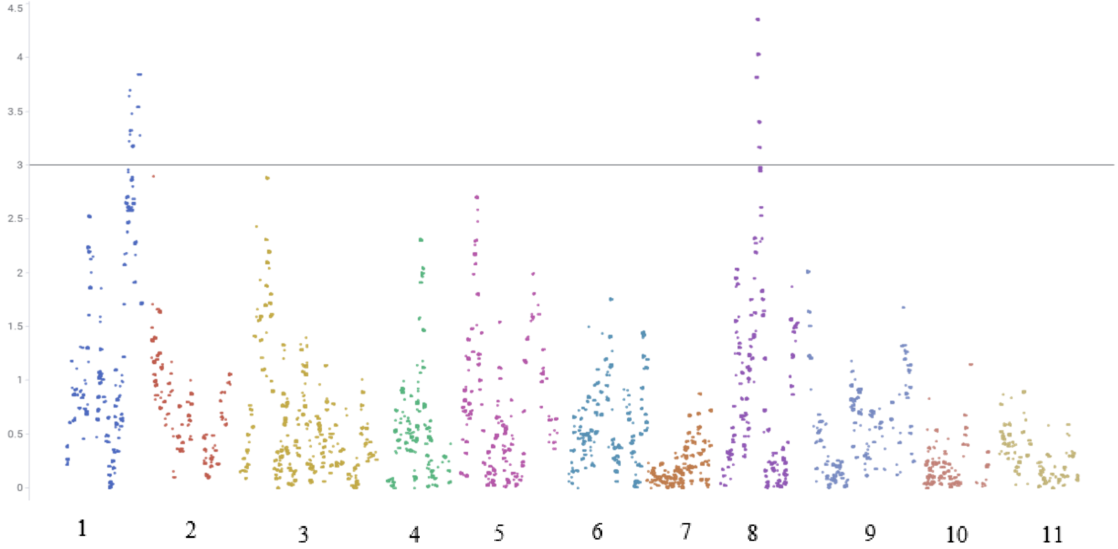
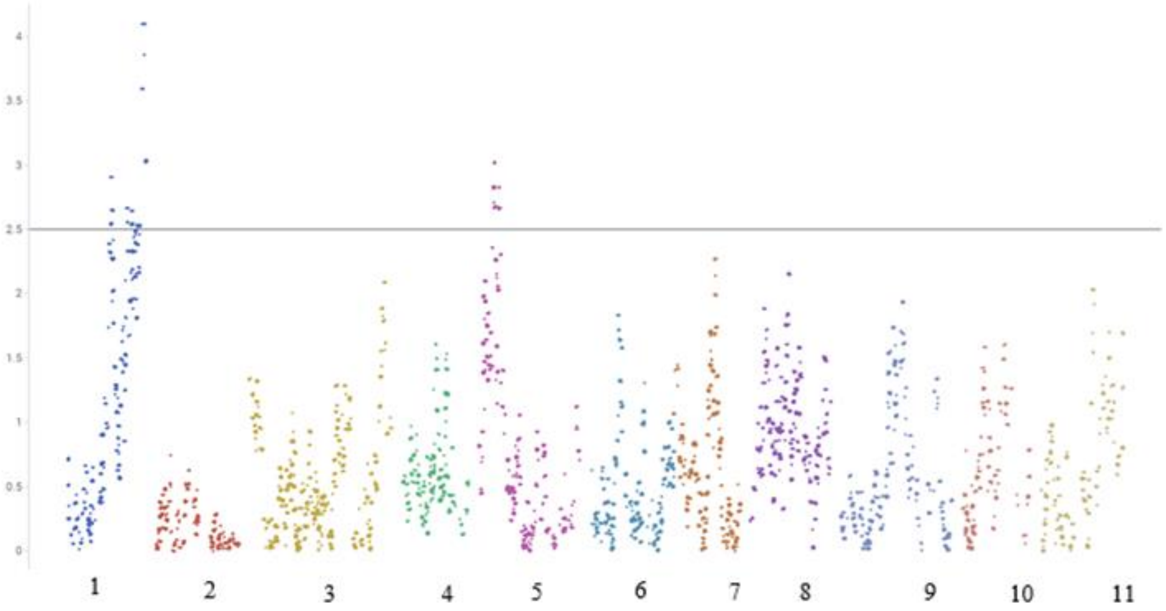


Figure 5.2: QTL plots for days to flowering time. The X-axis indicates the chromosomes, the Y-axis indicates the $-\log_{10}P$ of the probability (p-values). The horizontal line indicates the significance threshold at 0.05.

High P



Low P

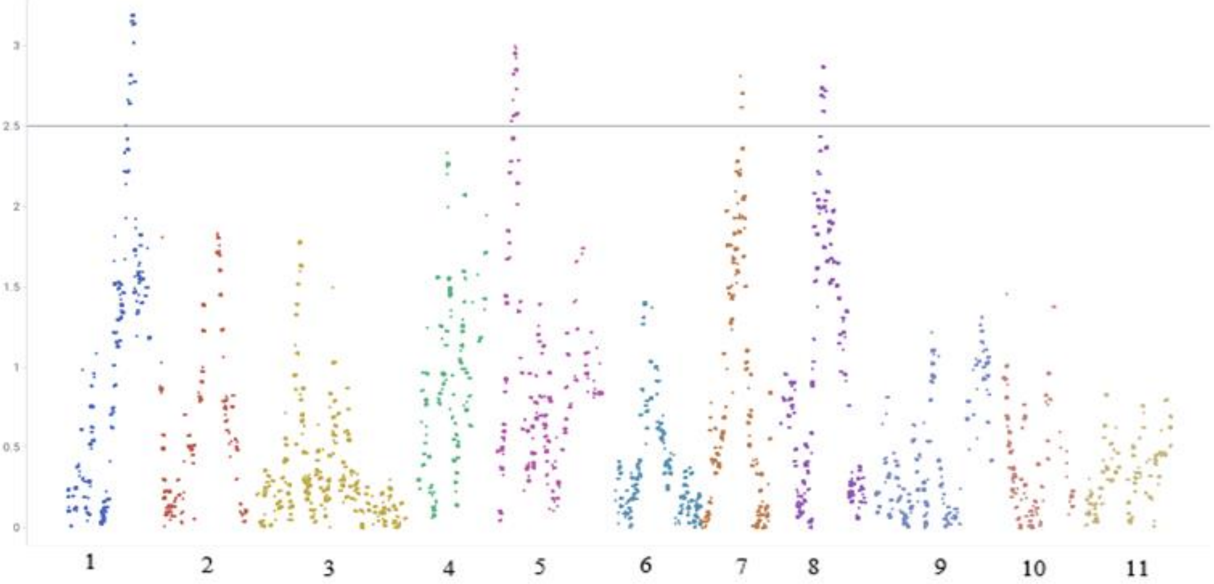
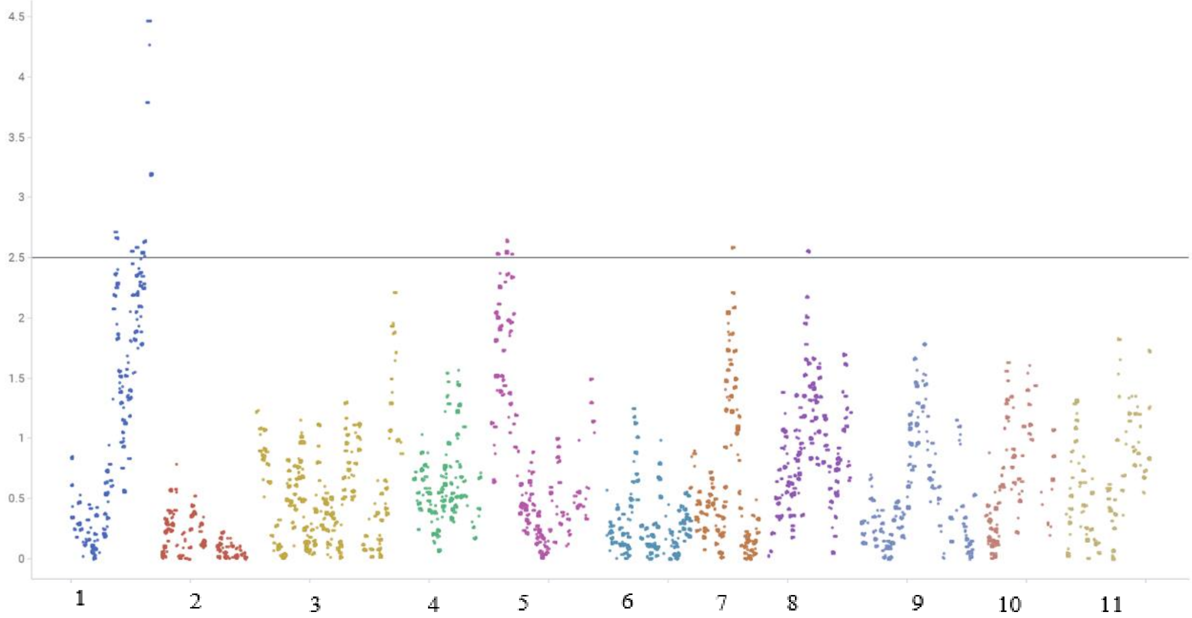


Figure 5.3: QTL plots for the grain yield. The X-axis indicates the chromosomes, the Y-axis indicates the $-\log_{10}P$ of the probability (p-values). The horizontal line indicates the significance threshold at 0.05.

High P



Low P

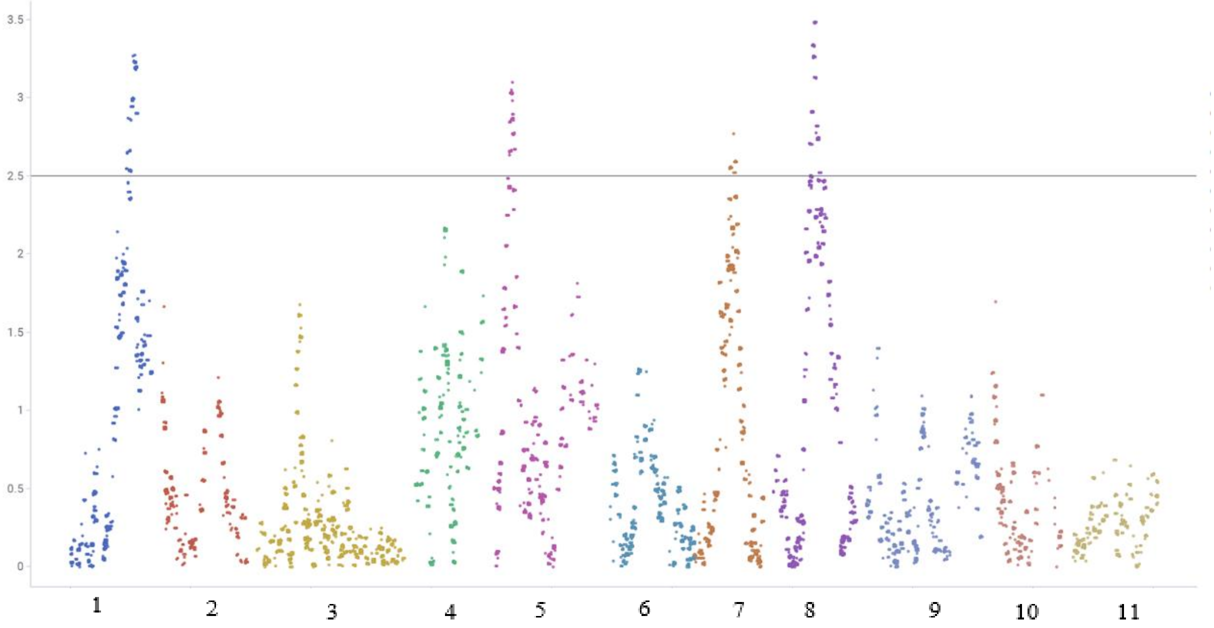


Figure 5.4. QTL plots for the physiological P use efficiency in cowpea. The X-axis indicates the chromosomes, the Y-axis indicates the $-\log_{10}P$ of the probability (p-values). The horizontal line indicates the significance threshold at 0.05.

5.4 Discussion

This research investigated the phenological, yield components and P use efficiency traits of a RIL population contrasting for P use and acquisition under low and high P soils. The levels of P used as low and high was drastic but reflects what is found in farmers' fields, where cowpeas are grown under little to no P fertilization. Growing of cowpeas under limited P condition has been documented (Belko *et al.*, 2016; Kugblenu *et al.*, 2014; Saidou *et al.*, 2012). The HP conditions provided plants with sufficient P, while LP conditions had sub-optimal P such that yield was negatively impacted, similar reports have been made (Rothe *et al.*, 2013; Saidou *et al.*, 2012). To avoid confounding effects of major nutrient deficiencies in the experiment, N, K, Mg, and S were applied through the application of Urea and muriate of potash (MOP) fertilizers. The use of 60 kg ha^{-1} SSP as high P was based on a previous recommendation in the literature (Adusei et *al.*, 2016; Boukar *et al.*, 2018; Sanginga *et al.*, 2000).

The increased performance was observed for IT84S-2246-4 parent under both LP and HP over the TVu-14676 that would be expected by chance for most of the traits measured. This is probably due to increased selection pressure for yield, an important component of PUE. Numerous studies have established within species variation of PUE in cowpea and these point to the fact that these traits are governed by quantitative trait loci. Variation in yield and PUE traits found in cowpea is in line with previous studies on PUE in rice, *Brassica* and common bean (Diaz *et al.*, 2017; Hammond *et al.*, 2009; Wang *et al.*, 2014). The pattern of relationship between traits showed high biomass and P efficiency traits were important performance indicators for cowpea grown in low P soil. Results further revealed that cowpea lines under LP had lower P concentration in biomass and grains relative to lines grown under high soil P.

Fodders were sampled at a similar growth stage; 8 weeks after sowing to observe the response to contrasting P of the lines in both P conditions. Results revealed significant reduction in biomass and grain yield of LP while the performance of the same lines was generally superior under HP relative to LP, though few RILs had a superior performance by producing higher yields in LP than HP. To have a better understanding of the role of P nutrition in this study, three categories of traits; phenology, yield components and P use efficiency were investigated. Earlier studies have focused on estimating genetic variation and identifying lines with adaptation to low P soils and those with good response to P fertilization.

A total of 27 QTLs over seven linkage groups were identified for TV x IT RIL population under low and high P environments. For most traits, one or two major QTLs were detected. QTLs were identified for days to flowering and maturity, important phenological traits for adaptation of varieties to different agro-ecologies. In the present study, four QTLs were associated with flowering and maturity on cowpea chromosomes Vu01, Vu05, Vu06 and Vu08. Significant QTLs associated with yield and PUE traits were mapped on chromosomes Vu01, 2, 5, 8 and 9 and this provided information to advance breeding for improved PUE in cowpea.

Studies on cowpea P traits are scarce and comparisons are mostly made with related species. This is the first major report of QTL mapping in cowpea with high-density SNP markers. The earlier known report identified QTLs for shoot dry matter, a component of P use efficiency with three SSR markers (Rothe, 2014) while another study targeted the effects of high and low soil P on biological N fixation and yield using nodule number, nodule dry weight, shoot dry weight, number of pods per plant, and hundred seed dry weight as indicators and identified QTLs for N fixation traits (Fonji, 2015). However, there are reports of QTLs associated with P use traits under varying P conditions in common bean (Blair *et al.*, 2009; Cichy *et al.*, 2009; Diaz *et al.*, 2017; Hong *et al.*, 2004), a close relative of cowpea and other crops especially rice where P uptake efficiency has been mapped (Wang *et al.*, 2014; Wissuwa *et al.*, 1998), soybean (Zhang *et al.*, 2017) and *Brassica* spp (Hammond *et al.*, 2009).

P use efficiency attributes used in this study were grain agronomic P use efficiency measured as increased yield per unit of applied fertilizer (g DM g^{-1} P), grain P uptake efficiency (PUpE) as increased amount of plant P content per unit of added P fertilizer measured in g P g^{-1} and P utilization efficiency (PUtE) measured as increased yield resulting from increased in plant content g DM g^{-1} P. Several measures of PUE have been used in the literature to study P use and acquisition of crop plants (Leiser *et al.*, 2015; Wang *et al.*, 2014; Wissuwa & Ae, 2001).

Yield QTLs were discovered for grain yield under both low and high P conditions on Vu01, Vu05, Vu07 and Vu09 chromosomes. These QTLs and SNP markers mapped in this study would lay the foundation for marker-assisted selection for developing P efficient cowpea varieties for cowpea breeding programmes. Mapping QTLs for same traits under different stress conditions as undertaken in this study has been previously conducted. For instance, days to flowering under long and short day for cowpea (Huynh *et al.*, 2018), nitrogen fixation traits and hundred seed weight of cowpea RILs grown under low and high P in the field soil and pot (Fonji, 2015) and symbiotic N fixation and yield traits in common bean under low and moderate soil P *(*Diaz *et al.*, 2017).

5.5 Conclusions

There was significant phenotypic variation between RILs of TV x IT cross that would be expected by chance. The wide range of variation as a result of differences in P contained in the growth media indicates the traits conferring P use efficiency are quantitative in nature. QTLs for phenology, yield and P use efficiency traits were mapped on seven out of the 11 chromosomes of cowpea. Several genomic regions of cowpea were associated with PUE traits and many of them co-localized on Vu08 chromosome including QTLs for flowering, maturity, and yield with phenotypic variance explained by these QTLs ranging from 6 – 28% with desirable alleles mainly contributed by parent 2 (IT84S-2246-4).

Further fine mapping of these genomic regions is required for higher resolution and identification of candidate genes underlying the QTLs. Such information will allow utilization of QTLs for P use efficiency in cowpea. Varieties with higher PUE will require less P-based fertilizers, thereby reducing the cost of production on fertilizer inputs for cowpea growers and will aid in having a cleaner environment especially in areas where P is heavily used.

CHAPTER SIX

6.0 Genome-wide association mapping of cowpea for adaptation to low phosphorus soils and response to phosphate fertilizer

Breeding efforts to develop cowpea varieties for adaption to low P soils and cowpea's response to applied P fertilizers have already begun and several reports have identified lines with tolerance to low P soils and response to applied P (Gyan-Ansah, 2012; Belko *et al.*, 2016). These lines used a different type of mechanism such as P use efficiency, a situation where cowpea can use and recycle internal P with less uptake from soil and there is also P uptake efficiency in which cowpea is able to efficiently remove P from soil. However, most of these efforts were achieved using conventional screening tools. With the advent of molecular markers and next-generation sequencing technologies, several genomic resources including SNP array genotyping platforms, consensus genetic, and publicly accessible reference genome sequences are now available and are promising to be more efficient tools that will allow rapid progress when combined with conventional breeding techniques (Bohra & Abhishek, 2013; Boukar *et al.*, 2016).

Several QTLs and SNP markers for abiotic and biotic factors from biparental mapping populations have been identified in cowpea (Agbicodo *et al.*, 2010; Huynh *et al.*, 2015; Lo *et al.*, 2018; Lucas *et al.*, 2013; Muchero *et al.*, 2009; Pottorff *et al.*, 2012; Santos *et al.*, 2018) and including QTLs for low P tolerance using SSR markers (Rothe, 2014) and adaptation to low P tolerance and response to rock phosphate using diversity panel (Ravelombola *et al.*, 2017). These QTLs were identified across the 11 linkage groups of cowpea with some of them transferred into several elite parents to improve their tolerance level through marker-assisted backcrossing and recurrent selection process (Boukar *et al.*, 2016; Batieno *et al.*, 2016).

However, it is a known fact that the resolution of identified QTLs from biparental mapping is limited due to few recombination events of alleles from the two parents used for the population development. QTL resolution can now be improved with available multiple parent population like genome-wide association panel and multi-parent advanced generation inter-cross population and next-generation sequencing platforms like diversity array technologies (Huynh *et al.,* 2018; Ravelombola *et al.,* 2017). The genome-wide association approach takes advantage of historical recombination of loci, making identified linked regions to be more reliable and with high resolution as against rare recombination events exploited in bi-parental mapping. The linkage disequilibrium decay is low in such a population. The availability of multiple and relatively cheaper platforms like diversity array technologies (DArT) and other NGS makes genome-wide association approach appealing especially as it does not require the expensive, laborious and time-consuming procedure of developing a mapping population (Korte & Farlow, 2013).

The genome-wide association studies (GWAS) has been extensively used in maize, rice, sorghum, cassava, pearl millet and animal breeding programmes leading to simultaneous discovery of useful diversity and identification of SNP markers associated with traits of breeding importance and genomic prediction of quantitative traits (Akbari *et al.*, 2006; Begum *et al.*, 2015; Kilian *et al.*, 2012; Sánchez-Sevilla *et al.*, 2015). There are few reports on the use of GWAS to map QTLs and identify markers associated with traits of interests in cowpea breeding (Burridge *et al.*, 2017). With the availability of a link to cowpea reference genome, it is now possible to conduct genotyping-by-sequencing (GBS) (Elshire *et al.*, 2011) and GWAS to identify regions and SNPs associated with biotic and abiotic traits with high resolution. The objectives of the present work was to conduct a GWAS mapping on 400 cowpea lines for adaptation to low P soils and response to applied phosphate fertilizer based on GBS generated markers on DArTseq platform.

6.2 Materials and Methods

6.2.1 Plant materials

The study used a collection of 400 cowpea lines consisting of entries from the worldwide collection of IITA, 14 commercial varieties and several breeding lines from the of IAR/ABU, Nigeria.

6.2.2 Experimental design and phenotyping:

The field trials were laid in 20 x 20 alpha lattice design with two replications under low and high P conditions at Zaria (11o 11’N, 07o 38’E) in Nigeria for two years. The lines were sown in mid-September and were later supplemented with irrigation when the rain ceased in October 2017 and replanted in 2018 between 5th June – 30th September. Plots comprised of one row of 1 m length with 1.5 m space between blocks. Seeds were sown at the rate of 10 stands at 0.2 m intervals within rows and 0.75 m between rows and later thinned to 5 stands per plot. Prior to sowing, seeds were treated with a broad-spectrum commercial fungicide *Apron star* at a rate of 4 kg seeds to a sachet of 10 g to reduce the incidence of fungal diseases. Plants were protected against insect pests at all stages by spraying insecticides at a rate of 1.0 l ha^{-1}.

The trial was kept weed-free by hand-hoe weeding. There were two factors, namely cowpea lines and phosphorus (P) application rates. The P rates were low and high P, where SSP fertilizer was applied to high P treatments at a rate of 60 kg ha^{-1} while no SSP was applied to low P treatments. All treatments received Urea and MOP at the rate of 30 kg ha^{-1} applied between plant stands and buried in the soil a week after sowing.

6.2.3 Data collection and phenotypic analysis

Evaluation of cowpea lines for tolerance to low P conditions and response to applied P fertilizer was assessed using shoot dry biomass, P uptake and P use efficiency. Fodder samples were taken at the mid-vegetative stage by sampling two plants from each plot. Sampled shoot biomass from the field was air-dried in the screenhouse till constant weight was maintained and weighed with a digital scale (Kerro BL20001). The P concentration of shoot samples were determined using Vanadate-molybdate method (Kitson & Mellon, 1944).

Cowpea's tolerance to low P and response to P fertilization were ranked on a scale of 1 – 5 using dry shoot biomass, P uptake and P use efficiency data, where a score of 1 = most efficient, 2 = efficient, 3 = moderately efficient, 4 = inefficient, and 5 = most inefficient for adaptation to low P condition, whereas response to applied P fertilizer was scored as 1 = highly responsive, 2 = responsive, 3 = moderately responsive, 4 = poorly responsive, and 5 = least responsive (GRIN, 2008; Ravelombola *et al.,* 2017). The scoring was based on grouping system Gerloff (1987) that group plants under nutrient stress as efficient or inefficient and responsive or non-responsive for plants under high nutrient condition, as adopted previously by earlier workers (Hammond *et al.*, 2009; Mahamane *et al.*, 2006; Saidou, 2005).

6.2.4 DNA isolation and genotyping

Young leaves from 2-3 weeks old cowpea seedlings were collected into LGC genomics sample collection kits and shipped to the Integrated Genotyping Support Service (IGSS) facility at BeCA-ILRI Kenya for DNA extraction using an in-house protocol available on (https://ordering.igss-africa.org/files/DArT_DNA_isolation.pdf). The quality and quantity of the extracted DNA was checked on 0.8% agarose gel prior to sending genomic DNA to the GBS service provider, ensuring the quality was at least 50 ng/ul but not exceeding 100 ng/ul and the quantity is at least 30 ul but not exceeding 50 ul. DNA of at least 30 ul of each line was sent to the Diversity Arrays Technology (DArT) facility based at Canberra, Australia (https://www.diversityarrays.com/) for GBS as described by Elshire *et al.* (2011) using DArTseq GBS (1.0) high-density SNP. Generated sequences were aligned to the cowpea reference genome on *Vunguiculata_469_v1.0* publicly accessible on Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Vunguiculata_er).

6.2.5 SNP calling and curation

SNP markers derived from DArTseq sequencing were filtered to remove SNPs with more than 20% missing data or no calls (80% and above call rates retained) and greater than 30% heterozygous calls. In addition, SNPs with < 5% minor allele frequencies (MAF) and those missing in more than 20% of lines were pruned using TASSEL 5.1 (Bradbury *et al.*, 2007) leaving only 5, 621 informative SNPs from a total of 18, 056 called SNPs and 386 lines, out of a total of 400 evaluated, which were used for further downstream analysis.

6.2.6 Statistical analyses

6.2.6.1 Phenotypic data

The phenotypic data were analyzed using a linear mixed effects (lme4) model that assumed lines as a fixed factor while replications, years, and blocks within replications as random factors to get the best linear unbiased prediction (BLUP) means of the lines using the R *lme4* package. A second model that assumed intercept as fixed while lines, reps, years and blocks with reps as random was used to estimate the variance components (Bates *et al.*, 2015). An estimate of broad-sense heritability estimate was obtained as the proportion of the total variance explained by the genetic variance.

6.2.6.2 Population structure analysis and linkage disequilibrium

The level of genetic structure and relatedness among the cowpea lines used was investigated using DArTseq derived SNP markers that passed the filtering test as described in 6.2.5 above. The population structure, referred to as "Q" matrix as described (Pritchard *et al.*, 2000) was checked by conducting a principal component analysis (Zhao *et al.*, 2007) with the R-*GAPIT* package (Lipka *et al.*, 2012). The DArT derived SNP markers were scored as 0, 1, and 2 representing major, minor, and heterozygous alleles while missing data was scored as "-". The compressed mixed linear model (CMLM) used assumes effects of individuals as random and used this to determine the level of relatedness between individuals conveyed through the Kinship "K" matrix as a variance-covariance matrix between individuals (Zhang *et al.*, 2010). Highly related individuals were assigned to the same group using a clustering algorithm that measures the level of similarity in the cluster analysis using the average method.

Linkage disequilibrium (LD) analysis was performed with R-*GAPIT* package as a plot of R-squared values between pairs of markers against their distance. LDs were calculated on a sliding window with 100 adjacent genetic markers with a moving average of 10 markers for adjacent markers (Lipka *et al.*, 2012).

6.2.6.3 Genome-wide association analysis

The phenotypic data for shoot dry biomass, P uptake and use efficiency and curated DArT SNP markers were used to conduct the analysis with *GAPIT* package implemented in R *(*Lipka *et al.*, 2012) using the basic scenario of the compressed mixed linear model (MLM). The MLM method used compression approach to assign individual markers into groups for the analysis, in other words, markers were not treated as an individual entity but considered as a group. The MLM model used relatedness between individuals using Marker-inferred similarity between lines (Kinship) generated via VanRanden method (VanRaden, 2008) and considers the existence of potential population structure as described by Zhang *et al.* (2010). The marker inferred the relationship between individuals and the population structure helped to improve the statistical power of the MLM model to detect a true association between traits and markers. The MLM equation of the model used is represented as below;

$$Y = X\beta + Zu + e$$

Where Y is a vector of phenotype measured, β is unknown vector with fixed effects (including SNP markers, population structure (Q) and the intercept); u is an unknown vector of random additive genetic effects such as effects of multiple QTL for individuals; X and Z are the known design matrices; and e is the unobserved vector of residuals. The u and e vectors are assumed to be normally distributed with a mean of zero and homogenous variance (Zhang *et al.*, 2010).

The default critical threshold in *GAPIT* for declaring SNP significantly associated with traits based on *p*-values of ≤ 0.0001 based on Bonferroni correction test for false positives at 5% in the MLM model was too stringent for the traits in this study. Considering the MLM used has accounted for population structure that may cause false positive associations between SNPs and traits, a less stringent threshold was set at $-\log_{10}P$ (*p*-values) > 3 by considering the bottom 0.1 percentile distribution of *p*-values as significant, as proposed by Chan *et al.* (2010) and adapted by Pasam *et al.* (2012).

6.3 Results

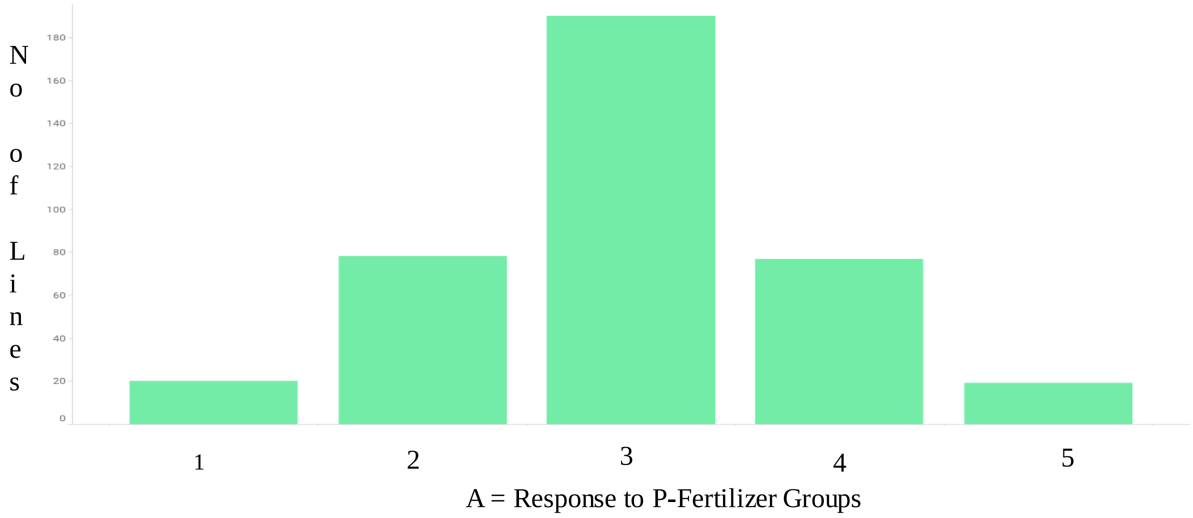
6.3.1 Descriptive data of the phenotypes measured

Summary statistics from the linear mixed effects analysis and estimated broad-sense heritability (h^2) are presented in Table 6.1. The range revealed significant variation between the cowpea lines used for the study. A plot of phenotypic data further showed that this variation was normally distributed (Figure 6.1). The heritability for the traits was low to moderate for shoot dry biomass and P use under the low and high P conditions.

Table 6.1: Summary statistics of cowpea lines under two phosphorus conditions

Traits	Min	Max	Range	Mean	SD	h^2
SDW-HP	4.7	89.6	84.9	33.0	10.2	0.11
SDW-LP	2.0	35.0	33.0	9.3	4.4	0.12
PuPE-HP	2.8	210.0	207.2	73.4	32.6	0.29
PuPE-LP	2.6	81.6	79.0	17.0	9.4	0.19
PUE-HP	1.8	123.7	121.9	17.4	10.3	0.45
PUE-LP	0.7	29.0	28.3	5.5	3.3	0.28

SDW-HP = shoot dry weight at HP, SDW-LP = shoot dry weight at LP, PuPE-HP = phosphorus uptake efficiency at HP, PuPE-LP = phosphorus uptake efficiency at LP, PUE-HP = phosphorus use efficiency at HP, PUE-LP = phosphorus use efficiency at LP



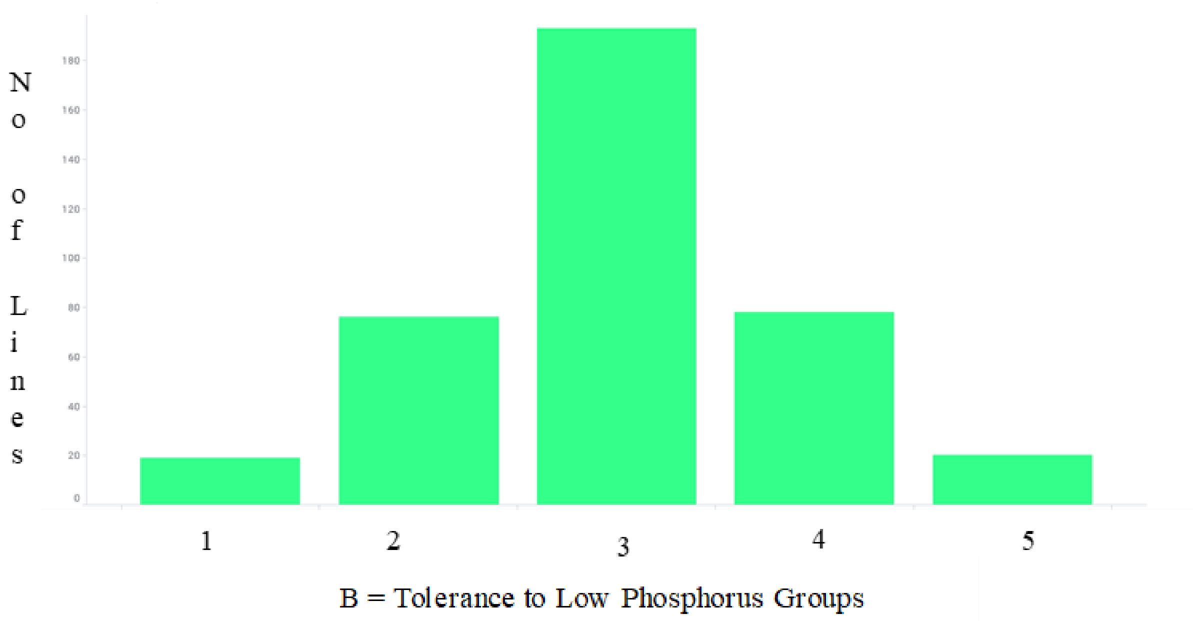


Figure 6.1 Bar charts showing the distribution of A) Response to P Fertilization, and B) Tolerance to low phosphorus condition scores.

Where 1 = highly responsive, 2 = responsive, 3 = moderately responsive, 4 = poorly responsive, and 5 = least responsive in (A) and a score of 1 = most efficient, 2 = efficient, 3 = moderately efficient, 4 = inefficient, and 5 = most inefficient for adaptation to low P condition in (B)

6.3.2 Maker distribution, population structure and linkage disequilibrium

A total of 5,621 cleaned SNPs that passed filtering were distributed across the 11 linkage groups of cowpea with an average density of 511 SNPs per cowpea chromosome (Vu)/linkage group. A total of 382 SNPs were placed on Vu01, 399 Vu02, 721 on Vu03, 564 on Vu04, 433 on Vu05, 528 on Vu06, 634 on Vu07, 459 on Vu08, 441 on Vu09, 540 on Vu10, and 510 on Vu11. SNPs ranged from 382 being the lowest on Vu01 to 721 SNPs on Vu03. The 3D PCA plots showed no obvious population structure between the lines. The average clustering algorithm used grouped the lines into four sub-populations based on their inferred kinship with the mean method (Figure 6.2). Linkage disequilibrium decay plot showed that LD ranged between 0 and 0.81 and started to decay below 0.2 at about a distance of 1.0 – 2kb.

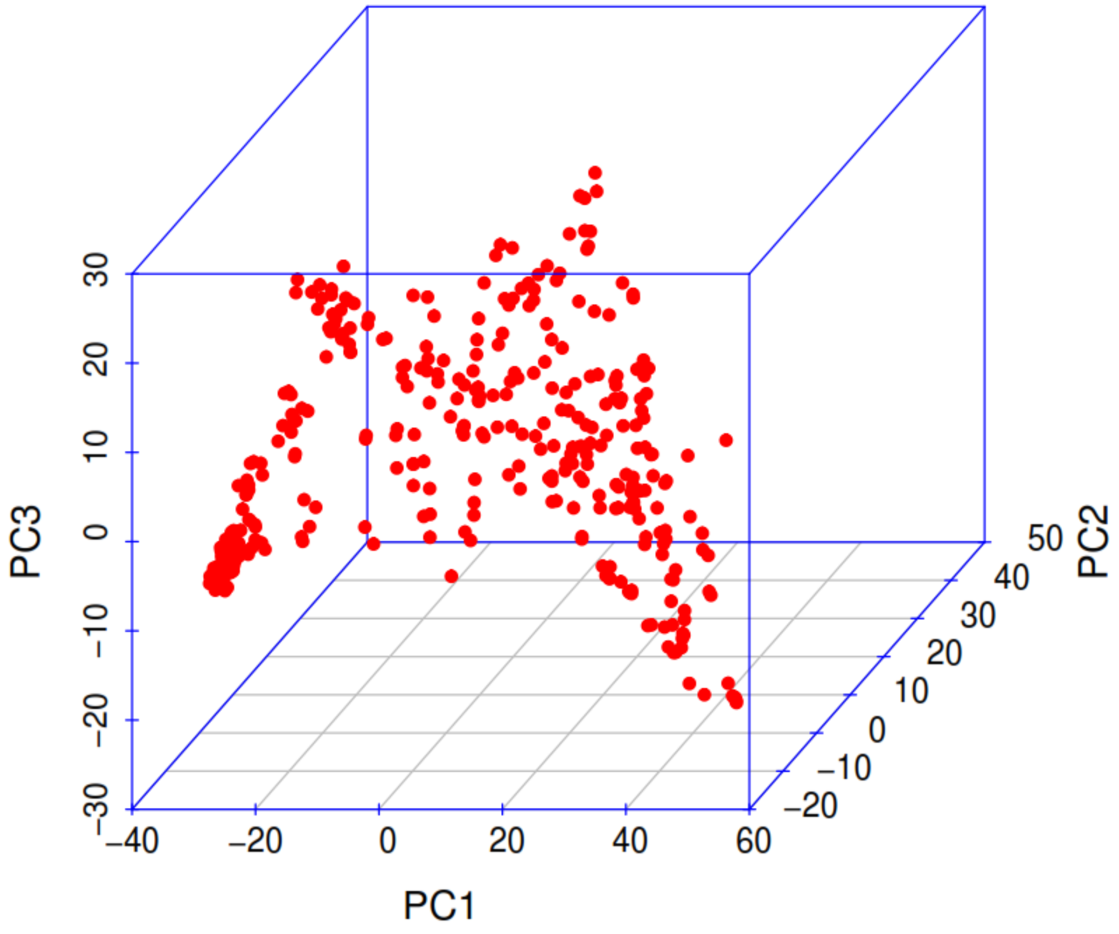


Figure 6.2: A three-dimensional PC view of the grouping of 400 cowpea lines

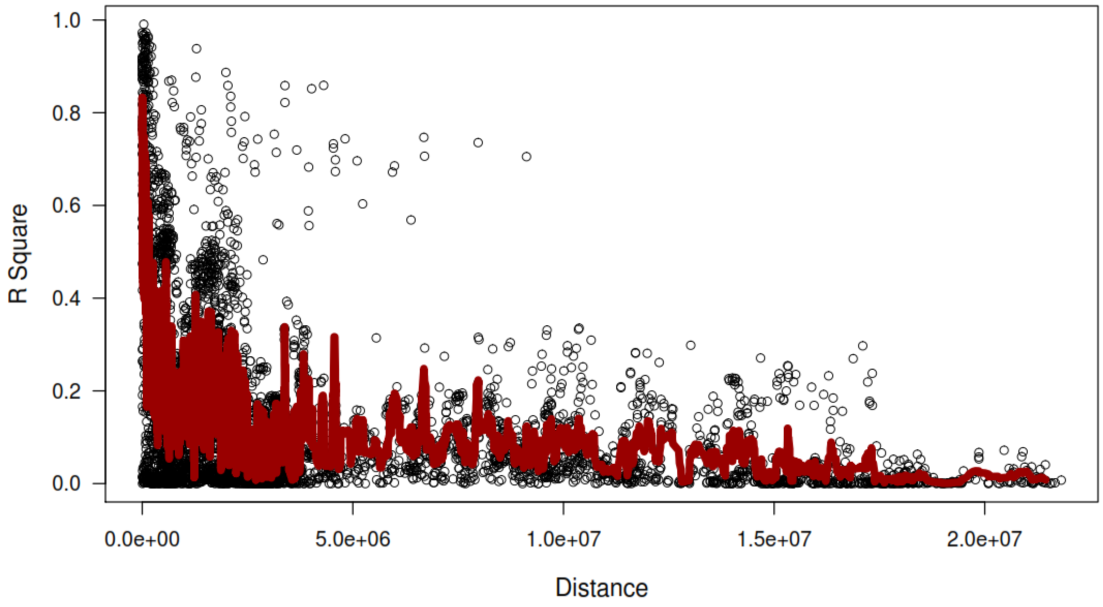


Figure 6.3: A Linkage disequilibrium decay plot of cowpea lines over distance

6.3.3 Genome-wide association mapping for Low P tolerance and response to P fertilization

The genome-wide association analysis conducted with 386 lines and 5, 621 DArT derived SNPs using the compressed MLM in *GAPIT* resulted in the identification of significant SNPs associated with adaptation to low P tolerance and response to applied mineral P fertilizer. The threshold for declaring significance for SNPs was set at 10^{-3} for the traits assessed. The quantile-quantile (QQ) plots showed that observed distribution was close to normal and the model fit well between the observed and expected *p*-values with some outliers indicating significant SNPs for shoot dry weight, P uptake and use efficiency (Figures 6.4 – 6.5)

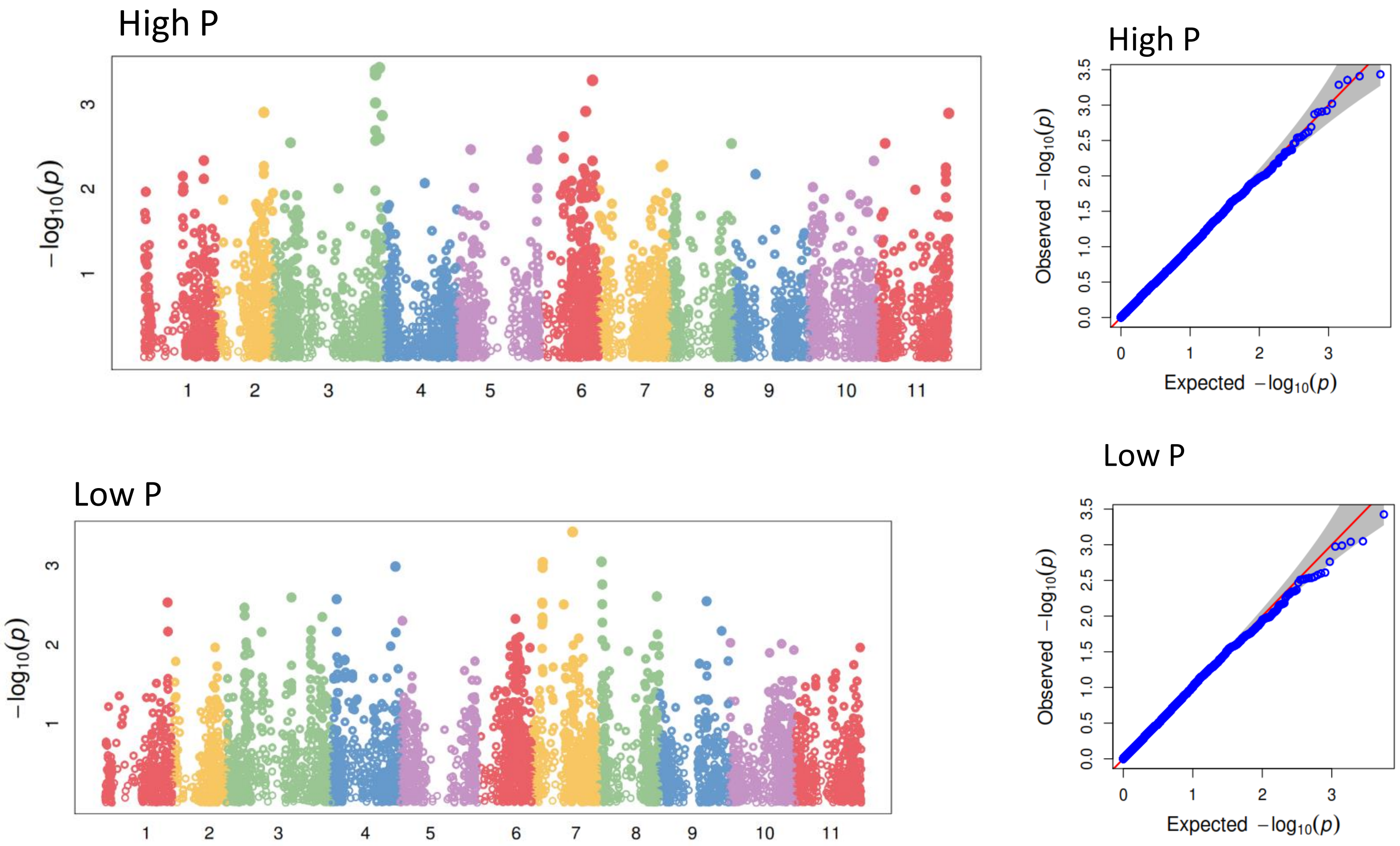


Figure 6.4: Manhattan plots and quantile-quantile plots for shoot dry weight at high and low P conditions

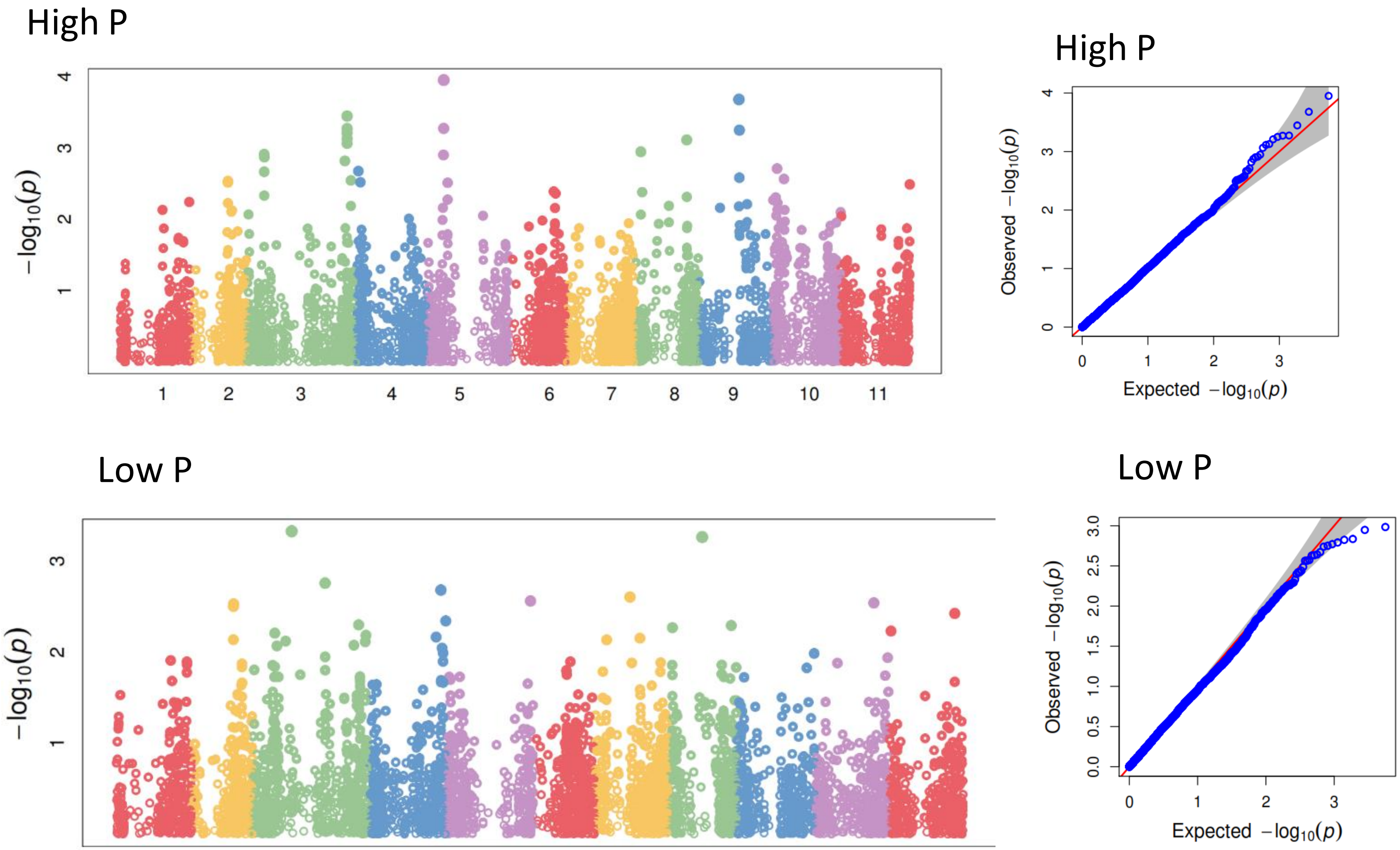


Figure 6.5: Manhattan plots and quantile-quantile plots for phosphorus uptake efficiency at high and low P conditions

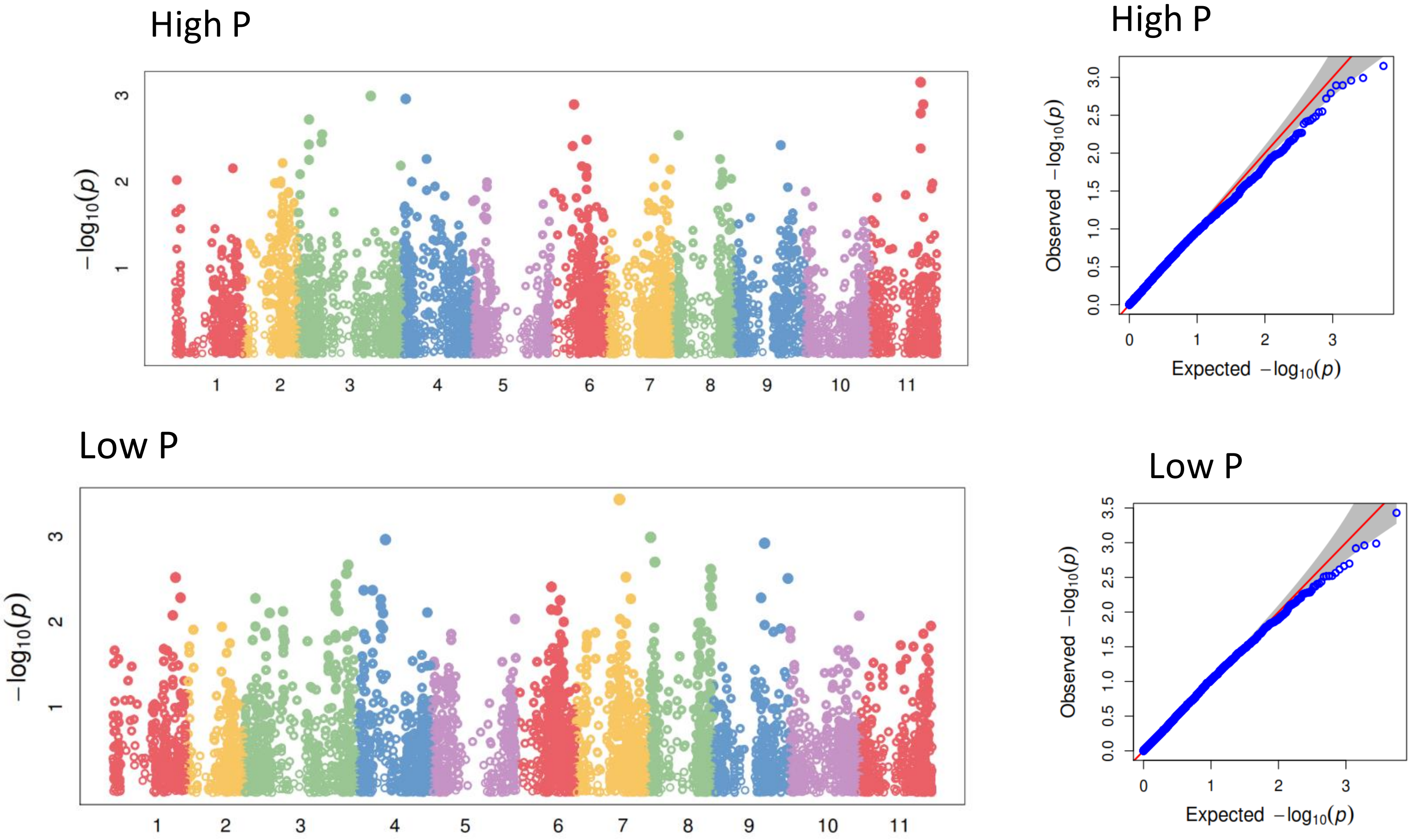


Figure 6.6: Manhattan plots and quantile-quantile plots for phosphorus use efficiency at high and low P conditions

A total of 65 markers with percentage of phenotypic variance explained (PVE) of greater or equal to 3% were associated with tolerance to low P and response to applied P fertilizer conditions measured via shoot dry weight, P uptake and use efficiency (Table 6.2 - 6.4), the phenotypic variance explained (PVE) (R-squared) ranged from 3 – 5%. Most of the markers detected for response to applied P fertilizer measured as shoot dry weight were placed on chromosome Vu03 while markers for tolerance to low P condition measured using the same trait were detected on Vu07 (Table 6.2).

Seven SNPs found on chromosome 3, 5 and 9 were significantly associated with response to P-fertilization measured via P uptake efficiency with PVE ranging from 3 – 3.9% while tolerance to low P condition measured as P uptake efficiency had 10 significant SNPs with PVE that ranged from 3.1 - 4.2% on chromosomes 2, 3, 4, 5, 7, 8, 10 and 11 (Table 6.3). The MLM revealed ten SNPs significantly linked to response to P and tolerance to low P as measured from P use efficiency, these SNPs were found on chromosomes 3, 4, 6, 7, 8, 9, 11 explaining on 3.0 - 3.7% and 4.1 – 5.1% respectively. SNPs with the highest peak had both positive and negative allelic effects that increased and decreased the value of the trait under both P conditions (Table 6.4).

Table 6.2: SNPs associated with shoot dry weight at high and low P conditions

	SNP	Chrom	Position	P.value	MAF	R.square	Allelic effect
HP	Vu_100355825_7007	3	62253733	0.00037	0.07	0.05	-0.11
	Vu_100155267_2554	3	60000957	0.00039	0.46	0.05	0.01
	Vu_14077134_3423	3	60007556	0.00044	0.46	0.05	-0.01
	Vu_100425302_10397	6	30604767	0.00052	0.11	0.05	0.06
	Vu_100472254_13698	3	59985754	0.00096	0.38	0.05	0.00
	Vu_14074546_15962	6	26647588	0.00120	0.35	0.04	0.01
	Vu_14075813_6801	2	28324743	0.00124	0.39	0.04	0.01
	Vu_14074035_12086	11	41268412	0.00127	0.08	0.04	-0.13
	Vu_100158904_3719	3	63878878	0.00135	0.18	0.04	0.02
	Vu_14082997_7772	3	59983984	0.00204	0.44	0.04	-0.12
	Vu_14086785_17186	6	13767246	0.00239	0.23	0.04	0.05
	Vu_14076927_7576	3	62279455	0.00250	0.07	0.04	-0.06
	Vu_14082997_7771	3	59983984	0.00267	0.45	0.04	-0.13
	Vu_14077501_7958	3	10167577	0.00283	0.11	0.04	-0.09
LP	Vu_100149271_602	7	25039544	0.00038	0.14	0.05	0.06
	Vu_100358211_7923	8	2169987	0.00090	0.16	0.05	0.09
	Vu_14080179_16625	7	6377515	0.00091	0.32	0.05	-0.01
	Vu_14075625_12451	4	40215362	0.00103	0.18	0.05	-0.17
	Vu_14084227_14493	7	6284711	0.00106	0.32	0.04	-0.09
	Vu_14074876_1600	8	2654522	0.00174	0.13	0.04	-0.02
	Vu_100455158_12154	8	36719407	0.00246	0.45	0.04	-0.04
	Vu_100354086_6297	3	40520235	0.00253	0.42	0.04	0.06
	Vu_100483211_14785	4	3525842	0.00266	0.23	0.04	0.00
	Vu_14074942_119	9	29574865	0.00282	0.37	0.04	-0.03
	Vu_100454153_11739	1	39106476	0.00292	0.32	0.04	0.00
	Vu_14083246_16909	7	6034243	0.00293	0.41	0.04	-0.11
	Vu_14086622_17295	7	6031166	0.00298	0.40	0.04	-0.05
	Vu_14073620_42	7	6137759	0.00305	0.32	0.04	0.00

HP = high P (representing response to applied P fertilizer), LP = low P (indicating tolerance to low P)

Table 6.3: SNPs associated with P uptake efficiency at high and low P conditions

	SNP	Chrom	Position	P.value	MAF	R.square	Allelic effect
HP	Vu_100479597_14367	5	9820268	0.000112	0.188	0.039	-0.066
	Vu_100454775_12003	9	23986375	0.00021	0.088	0.036	-0.145
	Vu_100472254_13698	3	59985754	0.000358	0.381	0.033	0.000
	Vu_14054709_5364	5	9784841	0.000533	0.141	0.031	0.078
	Vu_100155267_2554	3	60000957	0.000536	0.460	0.031	0.004
	Vu_14075593_8065	9	24261588	0.000563	0.098	0.031	-0.074
	Vu_14082997_7772	3	59983984	0.000618	0.443	0.030	-0.007
LP	Vu_100152691_1695	3	21914473	0.00047	0.127	0.042	-0.484
	Vu_100423375_9585	8	18887233	0.000545	0.214	0.041	-0.324
	Vu_100354086_6297	3	40520235	0.001747	0.416	0.035	-0.098
	Vu_14087299_17924	4	39860454	0.002081	0.183	0.034	-0.137
	Vu_14078738_11153	7	19499315	0.002479	0.259	0.034	-1.645
	Vu_100352419_5612	5	47108004	0.002742	0.181	0.033	-0.135
	Vu_14056133_14827	10	32654849	0.002879	0.168	0.033	-0.858
	Vu_14085209_14149	2	23373744	0.002951	0.339	0.033	-0.061
	Vu_100352225_5524	2	23273789	0.00314	0.222	0.032	0.067
	Vu_14057854_12585	11	36681904	0.003758	0.155	0.031	-0.264

HP = high P (representing response to applied P fertilizer), LP = low P (indicating tolerance to low P)

Table 6.4: SNPs associated with P use efficiency at the high and low P conditions

	SNP	Chrom	Position	P.value	MAF	R.square	Allelic effect
HP	Vu_100154399_2285	11	30941527	0.000708	0.196	0.037	-0.088
	Vu_100455625_12350	3	45363364	0.001018	0.172	0.035	-0.017
	Vu_14082376_8140	4	1775753	0.001102	0.465	0.035	-0.076
	Vu_14083701_14366	11	32477000	0.001275	0.282	0.034	0.035
	Vu_14087168_14382	6	14581072	0.001276	0.439	0.034	0.013
	Vu_100423814_9773	11	30951943	0.001616	0.214	0.033	0.124
	Vu_14079476_13189	3	7149479	0.001906	0.184	0.032	-0.070
	Vu_14082750_7332	3	15271540	0.002825	0.247	0.030	-0.098
	Vu_14084028_8631	8	3995911	0.002904	0.201	0.030	-0.020
	Vu_14086413_11353	6	22351425	0.00327	0.166	0.030	0.077
LP	Vu_100149271_602	7	25039544	0.000372	0.136	0.051	0.110
	Vu_100358211_7923	8	2169987	0.001029	0.162	0.046	0.012
	Vu_14083552_14308	4	16058911	0.001095	0.183	0.046	-0.117
	Vu_14074942_119	9	29574865	0.001207	0.370	0.045	-0.026
	Vu_14086780_18030	8	4631171	0.002004	0.078	0.043	-0.087
	Vu_100160047_3946	3	59782784	0.002171	0.127	0.043	0.020
	Vu_100455158_12154	8	36719407	0.002431	0.446	0.042	0.010
	Vu_14076603_16728	3	58772456	0.002728	0.372	0.041	0.144
	Vu_14075749_6769	7	28778480	0.003009	0.078	0.041	0.075
	Vu_14081851_4855	8	37387039	0.003036	0.150	0.041	0.023

HP = high P (representing response to applied P fertilizer), LP = low P (indicating tolerance to low P)

6.4 Discussion

In this study, differences in performance of cowpea under low P stress and response to synthetic P-fertilizer were found among the 400-cowpea lines tested. Several authors have reported significant differences in cowpea for adaptation to low P conditions and response to rock phosphate application or synthetic P-fertilizers (Abdou, 2018; Karikari & Arkorful, 2015; Mawo *et al.*, 2016). Diversity panels serve as important sources of genes desired for improving cowpea for tolerance to biotic and abiotic stresses such as poor soil fertility of P. The frequency distribution from scores of shoot dry weight used to define tolerance to low P and response to applied P showed continuous distribution and further indicated that the traits are quantitative, which agrees with previous findings (Mahamane *et al.*, 2006; Ravelombola *et al.*, 2017).

Existence of potential population structure that could lead to the false marker-traits association was investigated among the collection of lines used. There were small four sub-groups present among the lines used, revealed with the aid of 3D principal component clustering. Absence of major structure in the population used was good for accurate genome-wide mapping, as association mapping tends to be skewed with the presence of obvious population structure (Lucas *et al.*, 2013; Xiong *et al.*, 2016). Existence of two major gene pools have been identified in the worldwide collection of cowpea in a study that used SNP markers to inferred relatedness and diversity between lines of cowpea, with one gene pool representing materials from western Africa and the second for materials from eastern and southern Africa (Huynh *et al.*, 2013; Muñoz-Amatriaín *et al.*, 2017).

Several DArT derived SNPs were associated with adaptation to low P condition and response to mineral P fertilization. A total of 65 SNP markers were detected to be associated with cowpea adaptation to low P tolerance and response to applied mineral P fertilizer across all the 11 chromosomes of cowpea except chromosome 1. The number of SNPs used for association mapping in this study was 5,621 and resulted in detection of 65 markers, this is over 5-fold more than 1,018 SNPs that lead to the detection of 18 markers by a similar study on cowpea (Ravelombola *et al.*, 2017). A low percentage of phenotypic variance explained (PVE) was observed for these SNPs (3 - 5%) compared to PVE for SNPs mapped from a biparental QTL mapping studies (Boukar *et al.*, 2016; Lo *et al.*, 2018), where PVE of 20 - 85% have been reported for some traits like flowering, seed size and pod shattering. The low PVE reported in this study are comparable with 2 - 7% PVE from recent studies at Texas A & M University, USA on some 375 USDA cowpea accessions for low P tolerance and response to rock phosphate (Ravelombola *et al.*, 2017). The work by these authors is the first known report on SNP association with P traits in cowpea. The present work is a complement and further improvement over the reports of Ravelombola *et al.* (2017) for a few reasons; response to P fertilization in that study was investigated using rock phosphate, a relatively less soluble form of P compared to synthetic and readily soluble P source (SSP) used in the present study. Secondly, their work was conducted in the greenhouse, an artificial growth environment where cowpea plant does not express full potential compared to field-grown cowpea plants used in the present study.

Studies on the association of cowpea with P use efficiency traits are generally scarce. The first attempt of using molecular markers to detect an association between cowpea and P use was made by Rothe (2013), where four SSR markers using a biparental population were reported to be associated with P use efficiency measured as shoot dry weight and the PVE explained by those SSR markers ranged from 6 – 15%. In addition, similar work demonstrating effects of low and high P soil conditions on N-fixation traits identified QTLs for number of nodules, nodules dry weight, shoot dry weight, number of pods and hundred seed dry weight in cowpea (Fonji, 2015).

However, there are more reports in common bean and soybean on P traits than in cowpea and such reports can help explain the genetic architecture underlying P use and uptake in cowpea due to close synteny between these crops (Lucas *et al.*, 2011; Lucas *et al.*, 2013). The close relationship between cowpea and common bean was further strengthened by the recent consensus of cowpea community to align the numbering of cowpea linkage groups with those of common bean (Lo *et al.*, 2018). QTLs for

P uptake have been identified to be associated with common bean's performance in the low P field (Yan *et al.*, 2004) explaining about 14% of PVE. In another study by Beebe *et al*. (2006), 26 QTLs were detected to be associated with P acquisition (same as uptake) in common bean using 86 biparental recombinant inbred lines.

Results from the present study and from close relatives of cowpea will serve as an important source of knowledge in mapping loci for P association in cowpea. It will also provide a basis for implementing marker-assisted selection for cowpea improvement for adaptation to low P soils and response to external P application. Marker-assisted selection is promising as an effective approach to screening and selection with higher precision for quantitatively inherited traits and especially those difficult to measure like P use and uptake efficiency (Asoro *et al.*, 2013; Batieno *et al.*, 2016; Lucas *et al.*, 2013). The DArT GBS technology used in the current work has been used successfully in other crops for genetic linkage construction, QTL mapping in biparental and genome-wide association mapping, studying genetic diversity, and genomic prediction (Sánchez-Sevilla *et al.*, 2015).

6.5 Conclusions

An association panel consisting of 400 lines of cowpea was evaluated under two contrasting soil phosphorus regimes for adaptation to low P tolerance and response to applied synthetic P fertilizers. There were significant differences in the performance of the lines under the different P regimes. A total of 65 DArT SNPs were detected to be associated with adaptation to low P tolerance and response to P fertilizer in cowpea. The diversity in performance and SNPs association will provide a basis for selecting superior lines for P improvement in cowpea. This is the first report on cowpea SNPs association with P traits on cowpea grown under field conditions and using materials relevant to African breeding programmes.

CHAPTER SEVEN

General Summary, Conclusions and Recommendations

Cowpea is the major grain legume with widespread cultivation across the study areas. This research investigated the level of phosphate-based fertilizer use among cowpea farmers and found that farmers are aware that fertilizers were important for sustaining crop growth and increased yield, but few were aware that cowpeas require P-based fertilizers to achieve increased yield. An investigation into prevailing cowpea cropping systems revealed that the traditional approach of planting the crop in mixed intercrop with cereals is still in place despite the availability of higher yielding planting patterns such as 2 - 4 cereal - cowpea intercropping system. The study found that farmers predominately cultivated landraces and the use of improved varieties of cowpea was very low among the farming communities studied. Higher grain yield was the most important trait cowpea farmers desired to have in a variety and insect pests were identified as the top constraints impeding cowpea productivity across the study areas.

Thirty diverse cowpea lines were screened at varying levels of P nutrient under both nutrient-media and field conditions. Results revealed significant differences in the performance of elite cowpea lines under sub-optimal and high P conditions. Four different groups were identified among cowpea lines regarding soil P conditions; these were efficient responsive, inefficient responsive, efficient non-responsive and inefficient non-responsive lines. Classification based on ability to use inherent low soil P and response to applied P is critical to identify lines suitable for cultivation in different agro-ecologies. As per expectation, performance under high P was generally superior over low P condition.

A biparental RIL population differing in performance under low and high P was evaluated for identification of QTLs and SNP markers associated with cowpea performance under varying soil P conditions. The results revealed genomic regions and SNPs underlying P use and uptake in cowpea.

Furthermore, a genome-wide association mapping study was employed in addition to biparental QTL mapping to identify historic recombination events between important genomic loci and SNPs associated with tolerance to low P and response to applied P fertilization.

There is a need for advocacy and extension outreach to educate farmers on the need to use P based fertilizers on cowpea, implement higher yielding sole or intercropping systems and use available improved varieties. Breeding programmes should take into account grain yield in addition to any tolerance to biotic and abiotic tolerance that a new variety will possess to ensure adoption by farmers. Cowpea varieties grouped as P efficient responsive should be used by resource-poor farmers. Further studies are necessary to validate SNPs and QTLs identified in this study in different genetic backgrounds and more environments before they could be used in marker-assisted selection for P use and acquisition efficiency.

Findings would be relevant for breeding cowpea varieties with potential to produce good yield in soils with sub-optimal P content for the benefits of smallholder cowpea farmers and consumers using marker-assisted selection, this will facilitate the development of more efficient cowpea varieties with adaptation to low soil P and desired yield qualities using a combination of conventional and molecular breeding approaches.

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APPENDICES

Appendix 1: Villages/Sites Visited and their Waypoints

S/No	Village	Local Govt. Area	State	Lat.	Long.	Elev.(m)
1	Mando	Birnin Gwari	Kaduna	10.712086	6.566043	447.57
2	Ungwar Shitu	Birnin Gwari	Kaduna	10.661994	6.547136	438.274
3	Ungwar Shehu Rimi	Birnin Gwari	Kaduna	10.663703	6.426499	447.138
4	Giwa Town	Giwa	Kaduna	10.663753	6.426494	623.329
5	Gangara	Giwa	Kaduna	11.33893	7.384577	642.998
6	Ungwar Sarki	Giwa	Kaduna	11.240454	7.299287	665.741
7	Kallah	Kajuru	Kaduna	10.41908	7.802887	604.615
8	Rimau	Kajuru	Kaduna	10.411118	7.76336	642.366
9	Kasuwa Magani	Kajuru	Kaduna	10.401078	7.713589	692.842
10	Dan Guzuri	Makarfi	Kaduna	11.367094	7.830234	652.654
11	Tudun Wada	Makarfi	Kaduna	11.380765	7.885615	683.793
12	Angwar Bazai	Makarfi	Kaduna	11.231088	8.056419	666.302
13	Bataiya	Albasu	Kano	11.683822	9.031088	400.887
14	Panda	Albasu	Kano	11.605747	9.045033	453.9
15	Tsangaya	Albasu	Kano	11.612426	9.214769	476.157
16	Bunkure	Bunkure	Kano	11.698308	8.542983	439.993
17	Fallungu	Bunkure	Kano	11.735604	8.531023	467.997
18	Gurjiya	Bunkure	Kano	11.752846	8.560069	437.772
19	Yarganda	Tsanyawa	Kano	12.329073	8.075072	555.08
20	Harbau	Tsanyawa	Kano	12.326418	8.065062	565.122
21	Kabagiwa	Tsanyawa	Kano	12.310815	8.022003	573.567
22	Wasai	Minjibir	Kano	12.153197	8.67926	414.1
23	Zura Malam Lade	Minjibir	Kano	12.220525	8.574501	465.845
24	Dingin	Minjibir	Kano	12.171027	8.647709	450.027
25	Karaduwa	Matazu	Katsina	12.310354	7.685409	496.798
26	Sayaya	Matazu	Katsina	12.272257	7.644313	518.656
27	Matazu Town	Matazu	Katsina	12.242213	7.676314	533.293
28	Nasarawa Shadakotoma	Kaita	Katsina	13.087478	7.69949	445.824
29	Kafin Mashi	Kaita	Katsina	13.054806	7.700289	465.031
30	Yan Hoho	Kaita	Katsina	13.060463	7.734234	464.5
31	Tandama	Danja	Katsina	11.441281	7.439383	692.248
32	Kahutu	Danja	Katsina	11.37923	7.693465	678.526
33	Danja Town	Danja	Katsina	11.375105	7.565422	684.628
34	Mahuta A	Dandume	Katsina	11.444371	7.26894	716.186
35	Dantankari	Dandume	Katsina	11.444875	7.15717	719.654
36	Tumburkai	Dandume	Katsina	11.305977	7.231209	674.851