GENETIC ANALYSIS OF COWPEA (*Vigna unguiculata* L. WALP) AGRONOMIC VARIABLES IN PHOSPHORUS LIMITING SOIL.

By

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DECLARATION

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

Signature: ....

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APPROVAL

This dissertation of Emmanuel Chikalipa has been approved by the University of Zambia as partial fulfilment of the requirements for the award of the degree of Master of Agricultural Science in Plant Breeding and Seed Systems.

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DEDICATION

I dedicate this dissertation to my dearest and loving late mum Priscah Mutale, Emmanuella Priscah, Emmanuel Elijah and those who love education and

To all who endeavors to dream and see visions. To those who have progressive ideals but cannot influence decisions.

ABSTRACT

Cowpea is an important cultivated grain legume pulse, vegetable and fodder crop of African origin. However, yields have been characteristically low as a result of both abiotic and biotic stresses. Among the abiotic stresses, yield losses due to phosphorus (P) deficiency is an important factor. Identification and selection of cowpea genotypes possessing suitable agronomic traits is key for cultivar design and development to increase production. Accordingly, genetic improvement in cowpea aims to develop cultivars with improved grain yields and tolerance to abiotic and biotic stress factors. The objectives of the study were to (i) identify high yielding cowpea genotypes when exposed to P-limiting soil, (ii) determine the cowpea heritability response of yield and its associated variables when exposed to P-limiting soil and (iii) identify the variables with high discriminating capability among cowpea genotypes in P-limiting soil. Twenty cowpea genotypes comprising of twelve crosses and eight progenitors were evaluated for agronomic variables performance in P-limited soil at the University of Zambia Greenhouse in Lusaka. The experiment was arranged as a two set experiment (i.e. applied rate of i) 0kg P_2O_5 /ha and ii) 60kg P_2O_5/ha [a control]) and laid as a completely randomized design (CRD) with 3 replications. Comparisons using a paired t- test revealed a significant increase in performance in a control (60kg P_2O_5 /ha) for all measured variables across genotypes when compared to a 0 Kg P_2O_5 / ha experimental set. In this study, genotype [LT 11-3-3-12] and a cross [LT 3-8-4-1 X LT 11-5-1-1] were identified as the highest performers exhibiting a mean yield of 1994.90 kg/ha and 1984 kg/ha respectively in a P-limiting soil. The narrow sense heritability for measured variables ranged from 11- 35 % and 12 -72% for 0 Kg P_2O_5 and 60 Kg P_2O_5 experimental set respectively. The highest narrow sense heritability scores in a limiting P experimental set were for pod length (h^2= 0.35) and root biomass (h^2= 0.28) while in the control set the variable yield (h^2= 0.72) had the highest score. Variables plant biomass, root biomass, number of pods and pod length were identified as ideal at discriminating genotypic performance responses in P- limiting soil. These findings imply that high-yielding cowpea genotypes with acceptable P deficiency can be developed.

Keywords: Cowpea, yield, phosphorus, discriminating, principal component analysis, Heritability

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ADP	Adenosine Diphosphate
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
CRD	Completely Randomized Design
DAP	Di Ammonium Phosphates
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organisation
FAOSTAT	Food Agriculture Organisation Statistics
ha	Hectares
IITA	International Institute of Tropical Agriculture
K	Potassium
Kg	Kilogram
LSD	Least Significant Difference
LT	Lutembwe
MAP	Mono Ammonium Phosphates
MT	Metric tons
N	Nitrogen
P	Phosphorus
SSA	sub-Saharan Africa
SSP	Single Superphosphates
STP	Single Triple phosphates
UNZA	University of Zambia
PR	Phosphate Rock
$\mathbf{P_2O_5}$	Phosphate

CHAPTER ONE

1.0 INTRODUCTION

Cowpea [*Vigna unguiculata (L.) Walp*] is an annual grain legume that belongs to the family Fabaceae (Ehlers and Hall, 1997; Singh *et al.,* 2002). It is a diploid (2n = 2x = 22) species with a genome size of 620 million base pairs and a self-pollinated species (Boukar *et al*., 2018). Cowpea is one of the important edible grain legumes in Africa contributing to food security among small-scale farmers in sub-Saharan Africa (SSA) (Tarawali *et al*., 2002). It is also an important seed-pod crop in the dry savanna areas of SSA and it is a source of dietary protein, fodder, and income to farmers. Cowpea is mainly cultivated in areas with annual rainfall ranging from 500mm to 1200mm. However extra-early and early varieties can be grown in zones having less than 500 mm (Dugje *et al*., 2009).

Cowpea is cultivated for its fresh leaves, green pods, and grain which are rich sources of protein, vitamins, and minerals (Bai *et al.,* 2020; ElMasry *et al.*, 2021; Silva *et al.,* 2021). This renders cowpea a key crop for food, nutrition, and health security. The grain protein content of cowpea is about 250 mg/g (Boukar *et al*., 2019), which is comparable to that of soybeans (Kumar *et al.*, 2017). Cowpea grain contains essential nutrients such as iron, zinc, calcium and magnesium (Boukar *et al*., 2019). In addition, the high amount of antioxidants in its grain contributes in lowering the risk of cancer (Mahamane *et al*., 2008). Young succulent leaves, and pods of cowpea are used as cooked vegetable, while the grains are ground and processed into powder for making thick porridge, gravy, or sometimes consumed as a boiled delicacy (Silva, 2018). In some areas, cowpea is mostly grown as a key component of agricultural systems in association with cereals especially maize, finger millet and sorghum as cover crop for prevention of wind and water erosion. The presence of cowpea in cropping systems is a cheap alternative to improve soil fertility and subsequently yield. The crop is also used as a key companion crop in mixed cropping systems useful for suppressing weed infestation, enhancing soil fertility and reducing water evaporation (Meena *et al*., 2015). Importantly, cowpea forms symbiosis with the root nodule bacterium, *Rhizobium*, and fixes 70 to 350 kg/ha of atmospheric nitrogen and some 40 to 80 kg of this is deposited into soils as a natural source of mineral nitrogen, which contribute to soil health (Meena *etal*.,2015).

Global production of cowpea is estimated to be 7.4 million tons per annum on 12.5 million hectares (ha) land (FAOSTAT, 2020). Cowpea is widely cultivated by small-scale farmers in southern African countries (Horn *et al.,* 2015, Molosiwa *et al*., 2016). It is one of food security crops in Zambia widely cultivated in the eastern, southern and western regions. The mean grain yields of cowpea in SSA is between 100 to 599 kg/ha which is far less than the potential yield of 3000 kg/ha (Horn *et al*., 2015, Gerrano *et al.*, 2017).

Cowpea production is, however, hampered by both biotic and abiotic factors and these constraints severely affect cowpea productivity. Biotic stresses including weeds (e.g., *Striga gesnerioides* (Willd.), etc.) (Tchiagam *et al.,* 2010; Horn *et al.,* 2015), insects (e.g., aphids, flower thrips, etc.) (Karungi *et al*., 2000; Agele *et al*., 2006; Abudulai et al., 2017), bacterial diseases (e.g., bacterial blight, bacterial pustule, etc.) (Viswanatha *et al*., 2011), fungi (e.g., leaf smut, anthracnose, etc.) (Adejumo *et al*., 2001; Fery and Dukes, 2011) and viruses (e.g., yellow mosaic, aphid borne mosaic, southern bean mosaic, etc.) (Mbeyagala *et al*., 2014). Among the abiotic stresses, yield losses due to phosphorus (P) deficiency is an important factor. Phosphorus is one of the most important plant nutrients and is required in large quantities and comprises of 0.2% of the plant dry weight (Lambers and Shane, 2007; Schachtman, Reid, and Ayling, 1998), ranging from 0.05% to 0.5% (Vance, Uhde-Stone, and Allan, 2003). However, P is one of the least available plant nutrients and is deficient in many soils of the world (White, 2010; Bieleski, 1973). Fundamentally, plants require P for growth throughout their life cycle, particularly during the early stages of growth and development. In cowpea, the demand for P is greatest during pod and seed development where more than 60% of P ends up in the pods and seeds (Usherwood, 1998). As one of three essential mineral macronutrients, P plays critical roles in plant growth and development as a key component of fundamental biomolecules participating in multiple cellular activities (Zeng *et al*., 2016). Phosphorous uptake by cowpea is essential for ensuring proper nodule formation and improving yield and quality of the crop (Anon, 2004). A previous study indicated that high soil phosphate depressed seed protein and oil content, while yield would be low if available phosphorus was less than 30kg P/ha (DAFF, 2010). However, due to low mobility and high rate of fixation in soils, P is often a limiting factor of cowpea productivity, especially in acid soils, where Phosphate ($PO4^{3-}$) is easily fixed by Ferrous ion (Fe^{2+}), Aluminium (Al^{3+}) and Manganese (Mn^{2+}). In order to realize and maintain high yields, large amounts of P synthetic fertilizers, often in excess of plant requirements, are regularly applied in agricultural systems (Dobre *et al*. 2014).

1.2 Statement of Research Problem

Cowpea like most other leguminous crops requires higher amounts of P (Singinga *et al.,* 2000). Presently, the use of P-formulated fertilizers appears to be a quick and easy fix for P deficiency soils, but that option has not been largely adopted by most smallholder cowpea growers because P-fertilisers are costly. Furthermore, most of the P applied in the form of inorganic fertilizer and organic fertilizer is easily bound and becomes unavailable in the soil by reacting with complexes of Fe and Al oxides in acidic soils, and Calcium (Ca) in alkaline soils, making the amount of available P for plant use extremely low (Horn *et al*., 2015; Lynch, 2011; Mullins and Thomason, 2009). Amending such P deficiency through increased application of inorganic fertilizer is neither sustainable, economically feasible nor environmentally safe. Therefore, the most sustainable solution is to identify and select cowpea genotypes that have high yield in P-limiting soil.

1.3 Justification of Study

Continuous cropping without commensurate nutrient replenishment is reported to contribute to low P content in many soils. This scenario is typical of the cowpea growing soils of Zambia where the smallholder farmers are resource-poor. Fertilizer use by farmers as a strategy for replenishing soils with limiting nutrients is a critical component in improving the production of cowpea (FAO, 2004). However, phosphorus fertilizers are costly, nonrenewable, and potentially ineffective because of P immobilization in the soil. Therefore, breeding genotype with high yield will help resolve the problem associated with low P in soil. Employment of a breeding strategy for P-limiting soil requires an understanding of the heritability of the agronomic variables. This enables breeders to design appropriate breeding strategies. Genetic analysis of cowpea agronomic variables in P-limiting soil provides information for devising viable breeding strategies for developing cowpea genotype that are high yielding in P-deficient soil. Multivariate analysis such as principal component analysis (PCA) has been used where factor assessment involves utilization of several associated measured variables. In this study, variables such as, plant height, shoot biomass, root biomass, plant biomass, 100 seed weight, pod length, root length, number of pods per plant, number of seeds per pod, yield/hectare and chlorophyll concentration index were used to evaluate genetic analysis of cowpea yield in P-limited soil.

However, studies to determine the relevance of these variables in discriminating genotypes in P limited soils are limited. This research serves as a foundation for breeding low P efficient cowpea varieties for farmer use in Zambia and similar agro-ecological regions.

1.4 General objective

To evaluate agronomic performance of cowpea genotypes in P-limited soil.

1.4.1 Specific objectives

The specific objectives were to:

1. Identify high yielding cowpea genotypes when exposed to P-limiting soil.
2. Determine the cowpea heritability response of yield and its associated variables when exposed to P-limiting soil.
3. Identify the variables with high discriminating capability among cowpea genotypes in P-limiting soil.

1.5 Research Hypotheses

The hypotheses tested in the study.

1. High yielding cowpea genotypes which perform well in phosphorus-limiting soil exist.
2. Heritability of agronomic variables vary in P- limiting soil.
3. Variations in discriminating capability among agronomic variables exists in cowpea.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Origin and domestication of cowpea

Cowpea is one of the most important pulse crops and it belongs to the family Fabaceae. Cultivated cowpea, which is in subspecies *unguiculata,* is divided into five cultivar groups namely: *Unguiculata, Sesquipedalis, Textilis, Biflora* and *Melanophthalmus* (Boukar *et al.*, 2018). The origin of cowpea has been controversial as different authors contradicts with their suggestions (Pasquet and Padulosi, 2012, Feleke *et al*., 2006). Ehlers and Hall (1997) stated that West Africa was the first center of cowpea domestication. Their claim corroborates with the findings of Ng (1995) who asserted that the greatest diversity of cultivated cowpea is found in the Northwest of Cameroon, part of Burkina Faso, Savanna of Nigeria, Northern Benin, Togo and Southern Niger. Other authors claim that cowpea was first discovered in central Ghana (Flight, 1976; CluttonBrock, 1989).

The molecular study have supported two gene pools attributed to Western and Southern Africa regions (Huynh *et al*., 2013). The investigations were done following a single-nucleotide polymorphism (SNP) marker analysis carried out on 1,200 cowpea lines from West Africa and East Africa (Huynh *et al.,* 2013). The authors concluded that cowpea has dual domestication. Another molecular marker diversity study also confirmed West and Central Africa as the region of cowpea domestication (Xiong *et al.,* 2016).

2.2 Botany and characterization of cowpea.

Cowpea is a diploid and self-pollinating crop with 22 chromosomes and a genome size of about 620 million base pairs. Cowpea is an herbaceous short term, annual leguminous plant that is grown in many tropical and subtropical countries (Singh and Sharma, 1996). The crop is characterised by a very strong taproot and more lateral branching roots spreading on top soil layer as compared to other legume crops. Furthermore, the categorization of the crop includes the number of pods per plant, number of days to reach flowering and pod formation (Cobbinah *et al*., 2011). Cowpea is known by different names throughout the world such as Southern pea, black eye pea, Crowder pea, lubia, niebie, cowpea or frijole (Davis *et al*., 1991). Cowpea follows an epigeal emergence pattern which makes it prone to seedling injury especially when the seed bed is not well prepared (Shiringani, 2007).

Cowpea growth habit varies among different genotypes; it may be erect, semi-erect, trailing, climbing or bushy. There is a high variability within species, in terms of growth habits and range from indeterminate to moderately determinate and non-vining types are more determinate. The crop is more tolerant to low soil fertility due to its high rates of N fixation (Sanginga, 2003). In addition, it can grow better and tolerate soils over a wide range of pH when compared with other grain legumes (Fery, 1990). For optimum production, well-drained sandy loam or sandy soil with pH ranges from 5.5 to 6.5 is required (Davis *et al*., 1991).

Like most other legumes, cowpea is self-pollinated and is a typical day neutral plant; it may flower within 30 days after sowing when temperatures are around 30°C. Whereas, other photosensitive varieties would flower at approximately 100 days after planting as influenced by time of what and location. Flowers are borne in alternate pairs at the tip of the branches, and two or more flowers can be found per inflorescence (Gomez, 2004). Flowers are borne on short pedicels with corollas that are either white, dirty yellow, pink, pale blue or purple and are displayed above the foliage such that they can attract insects for pollination.

Cowpea seeds differ in size and, seed shape is used as the major characteristic associated with seed development in the pod. Initially, a single pod can contain about 6- 13 seeds. The seed develops into a kidney shape and it maintains that shape until maturity when the pod is not obstructive during growth (Gomez, 2004). However, the pod tends to restrict seed shape to a more globular shape and the seed coat can either be smooth or wrinkled. The plant produces smooth, cylindrical and curved pods. As the seeds dry up, pod colour of the green and yellow types becomes tan or brown (Aeling, 1999). Seed colour varies from white, cream, brown, red, black, speckled, mottled, and blotchy or eyed (black eye, pink eye, purple eye) (Aeling, 1999).

2.3 Importance of cowpea

Cowpea is an important source of protein especially for low income household in SSA. It also plays a role in soil fertility or nutrient replenishment especially if it's rotated or intercropped with cereals. In addition, its importance as a source of income for farmers, small and medium-size entrepreneurs cannot be underestimated (Timko and Singh, 2008). Farmers often sell the grain and leaves in the local markets (Alemu *et al*., 2016). Cowpea is among the dominating grain legumes traded almost in all local markets. Trading of fresh cowpea leaves, fresh produce and processed food provide both rural and urban communities opportunities for earning some money, particularly women (Ngalamu *et al.*, 2015). Trading of cowpea haulms as fodder for large and small ruminants can also be economically rewarding. The majority of farm households selling at least part of their cowpea produce in the local market from their previous harvests and grain marketing was more important in all cowpea producing areas (Beshir *et al.*, 2019). Fresh cowpea leaves marketing is also the most important particularly in an area where it is widely grown. Besides, good number of the farmers appreciated the market prices of cowpea as the price is comparable to that of common bean price which is part of the commodity exchange market (Ngalamu *et al.*, 2015).

2.3.1 Nutritional Status and Composition

Cowpea leaves provide a good source of amino acids, vitamins, minerals and proteins with a higher nitrogen content in the younger leaves (Ahenkora *et al.*, 1998). Protein content in cowpea leaf ranges from 29% to 43%, however, these amounts decrease with increasing leaf age (Nielson *et al.*, 1993). Seed protein content ranges from 22 to 32% of seed weight (Hall *et al.*, 2003, Nielson *et al.*, 1993; Tembo *et al.*, 2016).

Cowpea grain is also a rich source of minerals and vitamins and it has one of the highest levels of any food of folic acid, a crucial vitamin B that helps prevent spinal tube defects in unborn children. It is rich in micronutrients, nutraceuticals, and antioxidants (Hall *et al.*, 2003). Among the antioxidants found in the leaves include alpha tocopherols, flavonoids, lycopene, and anticancer agents (Shetty *et al.*, 2013). Cowpea vegetables also contain important nutrients including vitamins and minerals that can improve the nutritional status of individuals and households (Okonya and Maass, 2014). The rich nutritional property of cowpea seeds, hay and leaves makes them ideal for efforts aimed at reducing food and nutrition insecurity (Tables 1 and 2).

Table 1*:* Chemical composition of cowpea (%)

Nutrition content	Seeds	Hay	Leaves
Proteins	56-66	-	8
Protein	22-24	-	4.7
Water	11	18	85
Crude Fibre	5.9-7.3	9.6	2
Ash	3.4-3.9	23.3	-
Fat	1.3-1.5	11.3	0.3
Phosphorus	0.146	2.6	0.063
Calcium	0.104-0.076	-	0.256
Iron	0.005	-	0.005

Sources: FAO (2004).

Table 2: Cowpea leaves nutritional content

Nutrient content	Fresh boiled	Fresh with groundnuts	Dried with groundnuts
Protein(g)	27.48	26.82	45.03
Gross Energy(Kcal/g)	3.96	5.65	6.15
Calcium(mg)	2.53	1.71	1.80
Vitamin C(mg)	209.82	1.45	-
Iron (mg)	71.08	23.95	7.6
Zinc(mg)	0.54	0.43	1.93

Source: Dakora and Belane (2019)

2.4 Cowpea distribution and production

As earlier stated cowpea is a versatile legume crop that tolerates to a large extent harsh climatic conditions. Most of the production in Africa is by smallholder farmers in marginal conditions, often as an intercrop with maize, sorghum, or millet. Globally, Africa is by far, the leading cowpea producing continent (Table 3).

Table 3: Top 20 world cowpea producing countries in 2017

Rank	Country	Production (tons)	Area (ha)	Yield (Kg/ha)
1	Nigeria	2,137,900	3,701,500	578
2	Niger	1,593,166	5, 325,168	299
3	Burkina Faso	573,048	1,205,162	475
4	Tanzania	190,500	197,323	965
5	Cameroon	174,251	209,019	834
6	Mali	149,248	353,382	422
7	Kenya	138,673	281,877	492
8	Myanmar	115,200	132,000	873
9	Mozambique	103,837	377,900	275
10	Sudan	80,000	260,000	308
11	DRC	70,042	159,945	438
12	Senegal	64,088	153,142	418
13	Malawi	35,903	81,753	439
14	Haiti	29,895	41,525	720
15	USA	21,591	12,060	1.790
16	Peru	17,588	12,779	1.376
17	Serbia	16,189	4,777	3.389
18	Sri Lanka	15,281	11,519	1.327
19	China, mainland	13,500	13,000	1.038
20	Uganda	10,100	25,000	404

Source: FAOSTAT, 2019.

From Table 3 above, 77% of the world production is from Africa, the major producing countries are Nigeria, Niger and Burkina with 2,137,900, 1,593,166 and 573,048 tons respectively.

In Zambia, the crop is grown in all the provinces on either ridges or flat land as a monocrop or mixed intercropping system with cereal crops such as maize, sorghum and finger millet. Southern Province is ranked first and accounts for 53% of the national annual production followed by Central Province in the hierarchy with 28%, and Lusaka with 7% (Ministry of Agriculture and Central Statistics Office, 2018).

Table 4: Zambia Cowpea production 2017/ 2018 farming season

Province	Area planted (ha)	Area Harvested (ha)	Expected production (MT)	Yield (MT/ha)
Central	3,263	2,988	1,949	0.6
Copperbelt	13	13	12	0.9
Eastern	292	267	162	0.6
Luapula	19	19	6	0.3
Lusaka	359	234	527	1.5
Muchinga	18	18	13	0.7
Northern	2	2	2	0.8
North-western	211	204	132	0.6
Southern	8,771	6,967	3,632	0.4
Western	1,074	979	390	0.4
Total	14,022	11,691	6,824	0.5

Source: Central Statistics Agency (CSA), compiled by Conservation Farming Unit (CFU) – 2018.

2.5 Constraints to Cowpea production.

Cowpea production is subject to a complex of biotic and abiotic limiting factors that severely reduce its yield. The average yield in the SSA in 2014 was 444 kg/ha (FAOSTAT, 2017), a very low compared to the potential yield which was reported to be more than 3000 kg/ha (Singh *et al.*, 1997; FAOSTAT, 2017). Among the biotic factors drought, heat stress, low soil fertility and meagre use of improved varieties are among the critical factors which highly dwindle cowpea production. Phosphorous (P) deficient has been found to be a key nutrient affecting productivity and cowpea production. In this dissertation a review of literature on P as a factor in crop productivity is undertaken in the following sub-sections.

2.6 Natural sources and availability of phosphorus

Phosphate rock is the naturally occurring materials containing one or more phosphate minerals as well as possessing chemical characteristics that make it acceptable for commercial use as a source of phosphorus (Weil and Brady. 2017).

Phosphate rock is insoluble and cannot be taken up by cowpea plant. Acidulation using sulphuric acid or phosphoric acid and even hydrochloric acid transforms the material into soluble form for plant uptake. While the resultant products are in general effective for crop production, the production of Single/Triple Superphosphates (SSP/TSP) as well as Mono/Di Ammonium Phosphates (MAP/DAP) require high capital investments (Van Straaten, 2007). In much of SSA industries, acidulation or even partial acidulation to break down the phosphate minerals and making phosphorus more available is constrained by lack of local Sulphur or inadequate infrastructure to allow for economical transportation of Sulphur or sulphuric acid and lack of capital.

Several innovative techniques to enhance Phosphate Rock (PR) solubility have been investigated, including modification techniques like partial acidulation, heap leaching, thermal treatment, mechanical activation, as well as modification through biological processes (Van Straaten, 2002). Although there are several options open to transform PR into a form that is more available to plant, the options for small-scale farmers are limited.

The use of organic fertilizer like compost plays an important role in the dissolution of phosphate rocks. However, making compost is generally laborious to farmers and the decomposition of crop residues takes longer time, usually not less than two months.

2.6.1 Utilization of Phosphorus

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

The above stated P forms exist in complex equilibria with each other. Empirical evidence shows that P availability can be depressed by P fixation (Al, Fe, Ca, various clays and organic matter) leading to the formation of precipitates (Al-Fe-Ca Phosphates) of low solubility (Weil and Brady, 2017).

However, primary P minerals including apatites, strengite and variscite are very stable (Weil and Brady, 2017), and the release of available P from these minerals by weathering is generally too slow to meet the crop demand though direct application of phosphate rocks (i.e. apatites) has proved relatively efficient for crop growth in acidic soils. In contrast, secondary P minerals including Ca, Fe, and Al phosphates vary in their dissolution rates, depending on size of mineral particles and soil pH (Oelkers and Valsami-Jones, 2008).

2.7 Evaluation of Cowpea genotypes in P limited soil

Phosphorus is an important ingredients for Rhizobium to convert atmospheric N to ammonium (NH4) which can be used by plants. Phosphorus influences nodule development through its basic functions in plants as an energy source when Adenosine Triphosphate (ATP) are converted to Adenosine Diphosphate (ADP) as each molecule of N is reduced to NH_3 (Berg, 2009). The translocation of photosynthate from leaves to root and the movement of N-containing compounds from nodules to other plant parts are vital to an efficient symbiotic system (Zahran, 1999; Meena *et al.*, 2017). It's therefore understandable why lack or insufficient phosphorous causes low productivity in cowpea and legume at large. Previous studies have reported increased N-fixation in legumes by adding phosphate to the P-deficient soil (Ahlawat and Ali, 1993; Bekere and Hailemariam, 2012; Hayat *et al*., 2000). However, the cost on P fertiliser is a challenge to resource poor famers. Identifying cowpea genotypes which can produce reasonable yield is a feasible option. The genetic control of response to P use efficiency in P-limited soils was found to be primarily additive although non-additive was also found to be important (Weil and Brady, 2017; Chaubey *et al*., 1994). Previous work identified and selected genotypes with high genotypic responses in P- limiting soils (Rao *et al*., 1997). However, the type of gene action and genotypic responses are limited to the germplasm and environment under study. Although primary traits such as grain yield are an important criterion in selecting genotypes for stress tolerance, there is wide agreement that selection under stress is less efficient than under optimal conditions, mainly because heritability of grain yield declines under suboptimal conditions (Banzinger and Cooper, 2001).

2.7.1 Heritability

The term "heritability" describes how much of the variance in a trait results from genetic variation. Often, this term is used in reference to the resemblance between progenitors and their offspring. In this context, high heritability implies a strong resemblance between parents and offspring with regard to a specific trait, while low heritability implies a low level of resemblance. The additive genetic value of genotype, called the breeding value, is the sum of the average effects of all the alleles the genotype carries (Falconer and Mackay, 1996). Broad sense heritability, defined as $H^2 = V_G/V_P$, captures the proportion of phenotypic variation due to genetic values that may include effects due to dominance and epistasis. On the other hand, narrow sense heritability, $h^2 = V_A/V_P$, captures only that proportion of genetic variation that is due to additive genetic values (V_A). Note that often, no distinction is made between broad sense heritability and narrow sense heritability; however, narrow sense is most important in plant selection programs, because of response to selection depends on additive genetic variance. Moreover, resemblance between progenitor and offspring is mostly driven by additive genetic variance (Hill *et al*., 2008). On the other hand, selecting under optimal conditions especially high inputs increases genetic variance relative to environmental variance and thus increases heritability of yield and its components. Heritability estimates in crops are classified as high (>0.50), medium (0.30-0.50), and low (<0.30) (Bhateria *et al.*, 2006).

2.7.2 Principal Component Analysis

Principal component analysis (PCA) is the oldest and most admired method developed by Karl Pearson way back in 1901, but still rules the data analytics because of its recent advancements in visualization and ability to reduce the multiple variables to fewer uncorrelated variables (Chiquet *et al*., 2018). It helps in decomposing large number variables into fewer variables without losing much information (Abdi and Williams, 2010). Moreso, it is an adaptive data analysis technique which is effectively used to visualize the similarity and difference between the genotypes and helps in identifying the variables contributing towards genetic divergence (Jindal *et al*., 2018, Ringnér and Markus, 2008). The dataset is reduced to fewer variables based on the eigen values by creating new uncorrelated variables called principal components (PC). These principal components helps in minimizing the data lose by maximizing the variance (Jolliffe and Cadima, 2016). Biplot of the principal component analysis are constructed to identify genotypes with suitable agronomic traits for effective selection and breeding. Application of PCA is one of the strategies breeders adopt to identify influential traits for effective selection in cultivar development (Mofokeng *et al.,* 2020). It is useful to identify influential traits for effective selection and use in cultivar development. Grain yield is a complex trait and influenced by many component traits with varying magnitude.

Multivariate analytical approaches such as PCA has been used by breeders to compound the effect of all measured variables with the aim of evaluating the genotypic performance or identifying genotypes variables which are influential in differentiating genotypes (Simasiku *et al.,* 2021; Chiseche *et al* .,2020) in both positive and negative directions. Direct selection for grain yield is not sufficiently effective and it is desirable to select indirectly for improved yield. In plant breeding program direct selection for yield as such can be misleading, successful selection may depend on genetic variability and agronomic variables related to yield.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Germplam used

Twelve F1 crosses and their respective eight derived cowpea mutant progenitors were used in the study. The crossing block was laid at the University of Zambia (UNZA), screen house in Lusaka (15°23'S and 28°25'E) at 1250m above sea level.

3.1.1 Crossing Procedure

Hand emasculation procedure was used to generate the F1s. The progenitors used for crossing were based on the hydroponic system preliminary conducted for phosphorus resistance. Ultimately, twelve F1 crosses were successful (Table 5). These were harvested at harvest maturity and were manually sorted to remove extremely small grains, seeds with defective seed coats and extraneous materials such as unhealthy seeds and insect-infested seeds.

Table 5: Genotype used in the study

Genotypes Codes	Genotypes	Type
AXH	LT 3-8-4-6 X LT 11-3-3-13	Cross
BXG	LT 11-5-1-1 X LT 10-7-1-12	Cross
AXD	LT 3-8-4-6 X LT 4-2-4-1	Cross
CXE	LT 11-5-2-2 X LT 3-8-4-1	Cross
DXA	LT 4-2-4-1 X LT 3-8-4-6	Cross
EXB	LT 3-8-4-1 X LT 11-5-1-1	Cross
FXE	LT 16-7-2-5 X LT 3-8-4-1	Cross
AXE	LT 3-8-4-6 X LT 3-8-4-1	Cross
GXC	LT 10-7-1-12 X LT 11-5-2-2	Cross
CXG	LT 11-5-2-2 X LT 10-7-1-12	Cross
EXD	LT 3-8-4-1 X LT 4-2-4-1	Cross
HXA	LT 11-3-3-13 X LT 3-8-4-6	Cross
A	LT 3-8-4-6	Parent
B	LT 11-5-1-1	Parent
C	LT 11-5-2-2	Parent
D	LT 4-2-4-1	Parent
E	LT 3-8-4-1	Parent
F	LT 16-7-2-5	Parent
G	LT 10-7-1-12	Parent
H	LT 11-3-3-13	Parent

3.2 Conduct and location of experiment

The study was laid as a two-set potted experiments. The soil with limited phosphorous was collected from a poor phosphorous site in UNZA Liempe farm ($15^0$22'S and $28^0$26'E, at 1171m above sea level) in Chongwe District. The soil was collected from the top layer (0–30 cm) and was analyzed for soil P content (Table 6), using the Bray II method.

Table 6: Soil characteristics of UNZA Liempe farm soil used in the study

Name	Threshold	Measured quantity
Available Phosphorus	20 mg/kg soil	7.30 mg/kg soil
pH		4.7

A total of 12 F1 genotypes and 8 progenitors were evaluated in a screen house using a completely randomized design (CRD), with three replications in two independent experimental sets (1 set applied phosphate level of 0 Kg P_2O_5 /ha and the other one control with applied level of 60 kg P_2O_5 /ha). The experimental plot was 15 cm diameter polythene plastic bags filled with 3kg of soil and Single Super Phosphate (SSP) was applied three weeks after planting. The quantity of SSP added was calculated to achieve the soil P levels at two levels 60kg P_2O_5/ha (optimal) and 0kg P_2O_5/ha (suboptimal). However, the recommended quantities of potassium (K) (30 kg K/ha) and nitrogen (N) (20 kg N/ha) was applied in form of potassium sulphate and urea (46% N) fertilisers at the rates of 64 kg/ha and 25 kg/ha respectively as basal dressing in experimental. In each bag, three genotypes were planted which were later thinned 15 days after planting to two plants per bag. Other standard cowpea production agronomic practices were followed.

3.4 Data collection

The following variables were measured:

1) **Plant height**- was measured using a measuring tape in centimeters after plant have reached physiological maturity after 100 days. Measurement was done from the main stem; ground level to the tip of the plant.

2) **Shoot biomass**- the weight of the above ground total dry matter which includes stalk and leaves in grams was measured using digital weighing scale. This was done after harvesting.

3) **Root biomass**- the weight of the below ground total dry matter in grams this was done after harvesting using digital weighing scale.

4) **Plant biomass**- derived variable of the sum of shoot biomass and root biomass.

5) **Root length** – the root length was measured using a measuring tape in centimeters and was done after harvesting.

6) **Chlorophyll concentration index** – was recorded during the vegetative growth stage from the adaxial surface of healthy leaf using a chlorophyll meter (model SPAD-502, Japan). The chlorophyll meter was calibrated to zero every time before taking on a new measurement. The measurement was done before and after applying SSP.

7) **100 Seed weight** –was calculated in grams from 100 sampled seeds and weighed using digital weighing scale in grams. This was done after harvesting.

8) **Pod Length** –This was taken as the length of the pod from base to the tip of pod using 30cm meter ruler in centimeters.

9) **Number of seeds per pod** – mature seeds per pod were counted after harvest.

10) **Yield Kg/ha-** this was done by converting measured yield per plot to yield per hectare.

3.5 Data Analyses

Analysis of Variance (ANOVA) was performed assuming a fixed model to determine significant differences among measured genotypic variables in specific treatment levels. Means of measured variables was separated using fisher protected Least Significant Difference (LSD) method, at a significant level of $\alpha = 0.05$. The variations of the expected mean squares were estimated using the methodology explained by Singh and Chaudhary (1985).

$$\mathbf{Y}_{ik} = \mu + \alpha_i + \gamma_k + €_{ik}$$

Where: $\mathbf{Y}_{ik}$ was the response variable., i was the genotype, k was the replication; μ is the mean; α_i was the fixed variable effect of the genotype i; Υ_k was the random variable effect of a k replication; $€_{ik}$ was the residual association with replication k in genotype i.

A paired t-test was used to compare the performance of genotypic mean performance in two experimental sets at added value of 0kg P_2O_5/ha on one hand and a control experiment with added value of 60kg P_2O_5/ha. Heritability in the narrow sense was estimated by regressing mid-parent means on the offspring means. Further, evaluation was computed by performing a multivariate approach tool, principal component analysis (PCA). PCA was undertaken using genotypic means of measured variable across replications and discriminating capability of measured variables computed using factor analysis and associated angle created by a variable and each principal component on a two dimensional scatter plot. All data was performed using GenStat statistical software and XLSTAT in excel.

CHAPTER FOUR

4.0 RESULTS

4.1 Comparison of variable genotypic mean performance in 0 Kg P_2O_5 and 60 Kg P_2O_5 experiments

The paired t-test showed that the mean performance of measured variables across genotypes was higher in a control experiment at an applied rate of 60kg P_2O_5/ha than at applied rate of 0Kg P_2O_5/ha (Table 7).

Table 7: Comparisons of mean performance of measured variables at 0kg P_2O_5 and 60kg P_2O_5 using a paired t- test

Variables	Mean-0^{X}	Mean 60^{Y}	Difference	P-value
Yield	2569.02	2644.54	75.52	<0.001
100 seed weight	18.99	16.81	2.18	<0.001
Number of seed per pod	16.12	17.45	1.33	<0.001
Number of pods	15.75	16.68	0.93	<0.01
Pod length	16.95	17.86	0.91	<0.001
Root Biomass	27.90	29.64	1.74	<0.01
Plant height	52.68	59.07	6.38	<0.01

X-mean value of measured variables at fertilizer application rate of 0 Kg P_2O_5 across genotypes,
Y-mean value of measured variables at fertilizer application rate of 60 Kg P_2O_5 across genotypes.

4.2. Analysis of variance of measured variable in each experimental set

The analysis of variance (ANOVA) in both levels of phosphorous treatment showed significant ($P< 0.01$) genotypic mean differences on all measured variables except chlorophyll concentration index which was non-significant ($P >0.05$) in the 0 Kg P_2O_5 (Table 8 and 9).

Table 8: Mean squares for genotypic agronomic variable response in 60 Kg Phosphate per hectare control set

		Mean squares agronomic variables									
SOV	**DF**	**100SW**	**CCI**	**NDP**	**NSPD**	**PBM**	**PDL**	**PH**	**RBM**	**SBM**	**Yield**
Genotype	19	450.64***	384.3***	30.26***	8.906***	104.74***	2.20***	162.79**	29.27***	60.82***	423772.00***
Error	40	12.22	118.30	5.80	1.15	1.37	0.34	58.77	1.14	0.27	277.30

***highly significant at P< 0.001, **Significant at P < 0.01. **DF**= Degree of freedom, **100SW**=Hundred Seed Weight, **CCI**= Chlorophyll Concentration Index, **NDP**= Number of Pods per Plant, **NSPD**= Number of Seed per Pod, **PBM**= Plant Biomass, **PDL**= Pod Length, **PH**=Plant Height, **RBM** =Root Biomass, **SBM**=shoot biomass, **Yield**= Yield/ha, **SOV**=Source of variation

Table 9: Mean Squares for genotypic agronomic variable response in a 0 kg Phosphate per hectare experimental set

		Mean Squares for agronomic variables									
SOV	**DF**	**100SW**	**CCI**	**NDP**	**NSPD**	**PBM**	**PDL**	**PH**	**RBM**	**SBM**	**Yield**
Genotype	19	486.14***	80.40NS	31.19***	9.90***	113.78***	4.33***	47.39**	21.44***	51.91***	451635.40***
Error	40	8.88	2.6	5.42	2.12	1.44	0.21	30.92	0.94	0.44	954.00

***highly significant at P< 0.001, **Significant at P < 0.01, NS=non-significant at P=0.05, **DF**=degree of freedom, **100SW**=Hundred Seed Weight, **NDP**= Number of Pods per Plant, **NSPD**= Number of Seed per Pod, **PBM**= Plant Biomass, **PDL**= Pod Length, **PH**=Plant Height, **RBM** =Root Biomass, **SBM**=shoot biomass, **Yield**= Yield/ha, **RTL**=Root Length, **CCI**= Chlorophyll Concentration Index, **SOV**=Source of variation

4.2.1 Mean performance of genotypes for measured variables

Tables 10 and 11 further represents a summary of means for cowpea genotypes across replications for all evaluated agronomic variables with significant different genotypic responses in applied 0kg and 60 Kg P_2O_5/ha sets respectively.

Table 10: Means of genotypes for agronomic variable responses evaluated in 60Kg Phosphate per hectare control set

Genotype	PH	CCI	NDP	PDL(cm)	NSPD	100SW(g)	RTL(cm)	RBM(g)	SBM(g)	PBM(g)	Yield(Kg/ha)
AXH	50.67	32.00	11.00	16.82	14.56	17.50	36.60	23.23	42.37	65.60	2524.70
BXG	65.00	45.20	13.33	18.30	16.44	19.00	34.70	22.50	38.47	60.97	2698.20
AXD	67.00	54.40	13.67	17.80	15.89	19.00	31.40	24.93	29.40	54.33	2826.40
CXE	63.00	62.50	10.33	18.22	17.78	14.75	27.40	25.37	32.70	58.07	2754.70
DXA	49.00	34.10	15.67	17.89	16.67	21.00	23.90	20.57	36.47	57.03	2752.90
EXB	54.67	40.60	18.00	18.26	19.44	17.50	27.60	15.17	35.53	50.70	2909.40
FXE	65.33	48.50	14.67	16.42	15.22	15.25	24.50	19.53	36.23	55.77	2615.20
AXE	59.33	27.70	16.33	17.08	16.44	17.25	24.30	26.53	40.43	66.97	2349.40
GXC	73.67	35.40	21.00	18.24	16.00	14.67	22.60	26.77	43.07	69.83	2385.20
CXG	59.00	30.10	21.33	18.39	16.33	15.58	33.20	22.17	34.10	56.27	2751.00
EXD	68.33	33.10	22.33	18.42	19.00	22.25	32.70	25.07	39.93	65.00	3305.30
HXA	69.00	22.90	19.33	16.24	17.00	16.75	22.90	23.40	37.27	60.67	3194.10
A	59.67	61.80	16.67	19.30	22.33	22.00	39.60	16.50	34.93	51.43	2142.00
B	48.00	38.80	17.33	19.00	18.33	14.25	35.90	20.53	38.60	59.13	2334.30
C	56.67	32.00	15.67	18.06	18.33	17.00	33.80	23.67	32.20	55.87	2977.20
D	61.00	47.50	18.00	18.11	18.11	12.50	23.80	26.33	38.27	64.60	2177.80
E	51.67	31.30	17.33	17.27	18.22	17.33	25.50	20.63	35.47	56.10	2057.10
F	51.33	25.70	17.00	16.53	18.00	12.08	23.70	23.50	39.20	62.70	2724.60
G	55.00	36.50	19.33	18.28	16.67	19.00	33.50	22.60	23.63	46.23	3252.50
H	54.00	32.40	15.33	18.63	18.22	11.50	34.50	24.07	34.33	58.40	2158.90
Grand Mean	59.07	38.60	16.68	17.86	17.45	16.81	29.61	22.65	36.13	58.78	2644.50
$LSD_{0.05}$	12.65	17.95	3.97	0.97	1.77	5.77	2.76	1.76	0.87	1.93	27.48

A= LT 3-8-4-6, **B**= LT 11-5-1-1, **C**= LT 11-5-2-2, **D**= LT 4-2-4-1, **E**= LT 3-8-4-1, **F**= LT 16-7-2-5, **G**= LT 10-7-1-12, **H**= LT 11-3-3-13, **PH**=Plant Height, **CCI**= Chlorophyll Concentration Index, **NDP**= Number of Pods per Plant, **PDL**= Pod Length, **NSPD**= Number of Seed per Pod, **100SW**=Hundred Seed Weight, **RTL**=Root length, **RBM** =Root Biomass, **SBM**=shoot biomass, **PBM**= Plant Biomass, **Yield**= Yield Kg/ha.

Table 11: Means of genotypes for agronomic variable responses evaluated in 0Kg Phosphate per hectare experimental set

Genotype	**PH**	**CCI**	**NDP**	**PDL(cm)**	**NSPD**	**100SW(g)**	**RTL(cm)**	**RBM(g)**	**SBM(g)**	**PBM(g)**	**Yield(Kg/ha)**
AXH	59.00	29.60	12.00	15.41	12.56	15.50	32.63	21.60	35.20	56.80	1396.50
BXG	48.33	37.50	18.00	18.17	15.89	17.67	35.40	19.33	34.13	53.47	1607.70
AXD	52.00	38.50	13.00	14.90	15.22	22.75	34.23	28.00	34.87	62.87	1683.10
CXE	51.33	46.40	13.00	16.39	16.89	16.00	23.83	14.67	21.70	36.37	1620.90
DXA	49.67	32.90	14.67	16.72	16.33	24.75	22.87	21.53	34.40	55.93	1636.00
EXB[Y]	52.33	37.70	11.67	15.67	15.22	15.00	24.50	18.10	29.37	47.47	1984.80
FXE	55.67	27.30	15.00	14.83	11.89	23.00	23.47	20.60	35.30	55.90	1671.80
AXE	53.67	38.90	10.67	18.19	17.22	20.50	24.93	21.33	38.23	59.57	1355.00
GXC	56.67	35.10	16.33	17.82	14.89	16.75	23.67	17.70	30.13	47.83	1330.50
CXG	54.67	34.40	18.00	17.41	15.22	22.75	35.90	18.70	32.80	51.50	1530.40
EXD	58.00	50.10	24.00	16.90	17.89	19.25	39.47	21.00	38.10	59.10	1175.20
HXA	56.67	31.80	19.33	17.93	16.33	23.00	33.60	19.10	30.03	49.13	1737.80
A	46.00	50.40	16.00	18.51	16.44	16.75	23.63	18.57	30.07	48.63	1251.30
B	50.67	29.10	18.00	17.90	15.44	23.00	23.80	18.20	38.07	56.27	1370.10
C	55.33	39.50	18.33	18.13	19.22	17.75	26.60	16.63	28.70	45.33	1047.00
D	48.33	29.20	13.67	15.62	14.89	19.75	25.63	18.97	30.27	49.23	1055.20
E	55.33	41.50	13.33	17.79	18.22	15.50	25.57	19.67	29.77	49.43	1859.10
F	48.67	30.70	17.67	17.56	18.22	15.75	25.87	21.50	37.30	58.80	1703.80
G	55.67	22.40	14.33	15.71	17.00	17.67	27.47	20.60	29.20	49.80	1369.40
H[Y]	45.67	28.00	18.00	17.47	17.44	16.75	24.93	17.73	31.23	48.97	1994.90
Grand Mean	52.68	35.60	15.75	16.95	16.12	18.99	27.90	19.68	32.44	52.12	1551.79
$LSD_{0.05}$	9.18		3.84	0.76	2.40	4.89	2.59	1.57	1.09	1.98	50.97

A= LT 3-8-4-6, **B**= LT 11-5-1-1, **C**= LT 11-5-2-2, **D**= LT 4-2-4-1, **E**= LT 3-8-4-1, **F**= LT 16-7-2-5, **G**= LT 10-7-1-12, **H**= LT 11-3-3-13. **PH**=Plant Height, **CCI**= Chlorophyll Concentration Index, **NDP**= Number of Pods per Plant, **PDL**= Pod Length, **NSPD**= Number of Seed per Pod, **100SW**=Hundred Seed Weight, **RTL**=Root length, **RBM** =Root Biomass, **SBM**=shoot biomass, **PBM**= Plant Biomass **Yield**= Yield Kg/ha. Y=genotype selected as high yielding in P- limiting soil.

4.3 Heritability of the measured variables

Genotypic performance of measured variables showed varying narrow sense heritability (h^2). Narrow sense heritability ranged from 11- 35 % and 12-72 % for 0Kg P_2O_5 experimental trial and 60 Kg P_2O_5 control trial respectively across measured variables (Table 12).

Table 12: Narrow sense heritability of measured variables

Measured variables	h^2 0Kg P_2O_5	h^2 60Kg P_2O_5
PH	0.19	0.12
NPD	0.13	0.22
PDL	0.35	0.16
NSPD	0.11	0.46
100SW	0.12	0.24
RBM	0.28	0.16
PBM	0.12	0.38
SBM	0.13	0.37
Yield	0.11	0.72

h^2=Heritability, **PH**=Plant Height, **NDP**= Number of Pods per Plant, **PDL**= Pod Length, **NSPD**= Number of Seed per Pod, **RBM** =Root Biomass, **SBM**=shoot biomass, **PBM**= Plant Biomass, Yield= Yield Kg/ha.

4.4 Multivariate evaluation of measured agronomic variables

Principal component analysis revealed three PCs contributing 63.36% and 60.56% total phenotypic variation explained for treatment application of 0Kg P_2O_5/ha and control (60 kg P_2O_5/ha) respectively (Table 13). In the applied rate of 0kg P_2O_5/ha, variables, plant biomass (PBM), shoot biomass (SBM) and root biomass (RBM) were identified as important at differentiating genotypes with regards to principal component (PC1), attaining factor loading values 0.9, 0.8 and 0.8 respectively. On the other hand, in the control experiment PBM and yield were identified as important with regards to PC1 and PC2 respectively, each attaining factor loading value of 0.8 and 0.9 respectively (Table 13).

Further analysis in the applied rate 0kg P_2O_5/ha indicated RBM and NDP as the leading associated linked variables to PC1 and PC2 as evident by the smallest acute angles on the respective axis respectively (Figure 1). In the control experiment PBM and yield were identified as the leading associated linked variables to PC1 and PC2 as evident by the smallest acute angles on the respective axis respectively (Figure 2).

Table 13: Factor loadings of measured variables

	$0Kg\ P_2O_5$			$60Kg\ P_2O_5$		
Variables	**1(30.40%)**	**2(20.39%)**	**3(12.57%)**	**1(30.58%)**	**2(16.22%)**	**3(13.76%)**
PH	0.3	0.1	0.7	0.3	0.4	0.4
CCI	-0.3	0.5	0.1	-0.5	-0.3	-0.1
NDP	0.1	0.8	0.1	0.1	0.4	0.7
PDL(cm)	-0.4	0.7	-0.3	-0.6	-0.3	0.5
NSPD	-0.5	0.6	-0.3	-0.6	-0.3	0.5
100SW(g)	0.6	0.1	-0.1	-0.5	0.4	0.2
RTL(cm)	0.4	0.5	0.5	-0.7	-0.2	0.1
RBM(g)	0.8	0.1	-0.1	0.7	0.1	0.1
SBM(g)	0.8	0.3	-0.3	0.6	-0.4	0.4
PBM(g)	0.9	0.2	-0.3	0.8	-0.3	0.3
Yield(Kg/ha)	0.1	-0.4	-0.4	-0.1	0.9	0.1

PH=Plant Height, **CCI**= Chlorophyll Concentration Index, **NDP**= Number of Pods per Plant, **PDL**= Pod Length**,** **NSPD**= Number of Seed per Pod, **100SW**=Hundred Seed Weight**, RTL**=Root length, **RBM** =Root Biomass, **SBM**=shoot biomass, **PBM**= Plant Biomass**, Yield**= Yield Kg/ha.

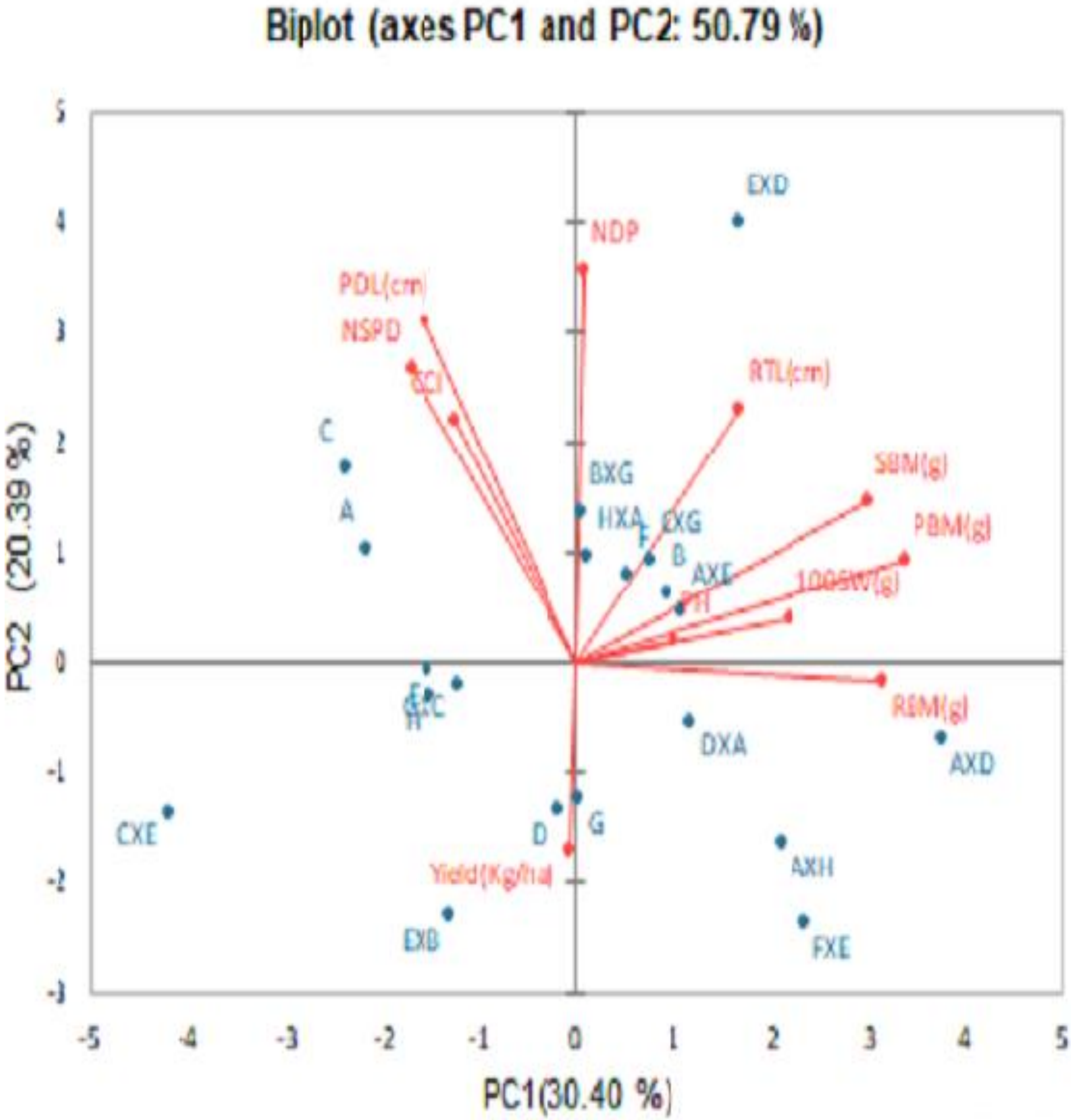

Figure 1: *Principal component score plot of PC1 and PC2 describing the variation among cowpea genotype estimated using the data set of measured traits for 0kg P_2O_5. The first two PCs explained 50.79% of the total variation. Blue dots=genotype; red dots= measured variables.*

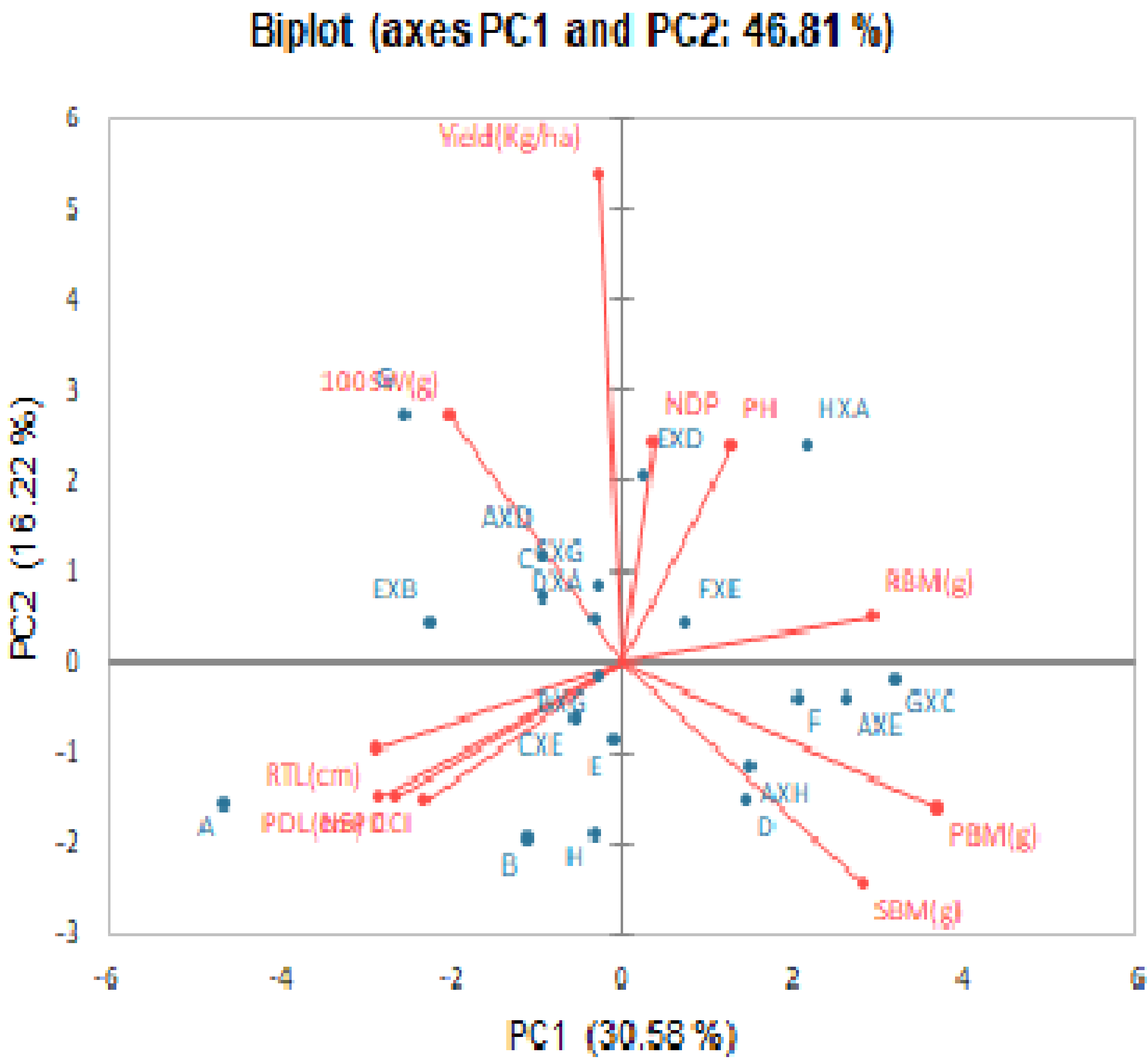

Figure 2: *Principal component score plot of PC1 and PC2 describing the variation among cowpea genotype estimated using the data set of measured traits for 60kg P_2O_5. The first two PCs explained 46.81% of the total variation. Blue dots=genotype; red dots= measured variables.*

CHAPTER FIVE

5.0 DISCUSSION

Cowpea production in Zambia is usually constrained by both biotic and abiotic factors. Among the biotic stresses, yield losses due to phosphorus deficiency is an important factor. In this study, mean performance of genotypic response of measured variables in P-liming medium compared to medium with optimum P attest to that fact (Table 7).

The study was undertaken with an endeavor to; (i) identify high yielding cowpea genotypes when exposed to P-limiting soil, (ii) determine the cowpea heritability response of yield and its associated variables when exposed to P-limiting soil and (iii) identify the variables with high discriminating influence among cowpea genotypes in P-limiting soil.

5.1 Response of genotypes in phosphorus-limiting soil

The genotypes evaluated in P-limited soil showed highly significant differences among genotype with regards to all measured variables except for chlorophyll concentration index (CCI) which was non-significant. Genetic response variation in P-limited soils exists in cowpea genotypes (Menssen *et al.,* 2017**)** and successful selection depends upon the information on the genetic variability and morpho-agronomic traits related to grain yield (Hassan *et al.*, 2005).

In this study, genotype [LT 11-3-3-12] and a cross [LT 3-8-4-1 X LT 11-5-1-1] had the highest mean yield of 1994.90 kg/ha and 1984 kg/ha respectively in a P-limiting experimental set (Table 11). Previous studies agree that higher cowpea yields in P- limited soils can be obtained within the same range, 1900- 2000 kg/ ha (Iseki *et al.,* 2021; Owusu *et al.,* 2021). On the other hand, genotypic cowpea yields as high as 3305.3 kg/ ha were obtained in optimum P medium (60 Kg P_2O_5/ ha) (Table 10). This implies that the identified cowpea genotypes efficient at utilizing P in P- limiting soils can be supplemented by addition of sub optimal fertilizer levels.

5.2 Heritability of evaluated agronomic variables

The narrow sense heritability (h^2) for yield and associate variables ranged from 11- 35 % and 12 -72% for 0Kg P_2O_5 and 60 Kg P_2O_5 experimental trial respectively (Table 12). Heritability estimates in crops are classified as high (>0.50), medium (0.30-0.50), and low (<0.30) (Bhateria *et al.*, 2006). Estimates for narrow sense heritabilities obtained are similar to reported narrow sense heritability ranging from 20 - 85% in cowpea (Jacinto-Hernandez *etal.*, 2003; Mashi, 2006). In the experimental set (0Kg P_2O_5/ ha) variables, pod length (h^2= 0.35) and root biomass (h^2= 0.28) had the highest heritability values while in the control set, the variable yield (h^2= 0.72) had the highest value. Responses to pod length (PDL) and root biomass (RBM) could be used as an indirect selection criteria for desirable advanced cowpea crosses [e.g. LT 3-8-4-1 X LT 11-5-1-1] in P-limiting soil especially as early generation selection criteria. However for late generation selection (F_5 onwards) screening based on yield response should be employed as heritability for yield was found to be low (h^2= 0.1). Previous studies have shown that PDL and RBM can be used as an indirect selection criteria for yield in common beans (Cichy *et al*., 2019). The higher yield heritability value (h^2= 0.7) in control set (Table 12) could be due to sufficient P which permitted full expression of genes associated with yield. In the case of the identified desirable genotype LT 11-3-3-12 efficient at utilizing P in P- limiting soil, it can either be evaluated for further release as a variety or used as parent in further breeding.

5.3 Multivariate evaluation of measured agronomic variables

In the study, plant biomass (PBM),root biomass (RBM),number of pods (NDP) and pod length (PDL) variables were identified as ideal at discriminating genotypic performance responses, exhibiting a factor loading score benchmark of 0.8 and above in P limiting soils. Generally genotypic screening is costly and identification of important traits/variables with ideal discriminating capability helps to narrow down to only few essential variables to utilize and this ultimately saves cost (Simasiku *et al*., 2021). In this study variables with smallest acute angles were identified. Interestingly, yield response in P- limiting medium didn't positively correlate with any other measured variables in the experimental set as observed by the created obtuse angles (Figure 1). This implies that under cowpea genotypic selection in P-limiting soils variable responses PBM, RBM, NDP and PDL cannot solely be used as an indirect selection criteria for yield response but can be used as supplement to yield response as earlier alluded. However, in the control set yield was positively corrected with 100 seed weight, number of pods, plant height and root biomass exhibiting an acute angle with yield.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

In this study, genotype [LT 11-3-3-12] and a cross [LT 3-8-4-1 X LT 11-5-1-1] were identified as the highest performers exhibiting a mean yield of 1994.90 kg/ ha and 1984 kg/ ha respectively in a P-limiting soil. The narrow sense heritability for measured variables ranged from 11- 35 % and 12-72% for 0Kg P_2O_5 and 60 Kg P_2O_5 experimental set respectively. The highest narrow sense heritability scores in a limiting P experimental was for pod length (h^2= 0.35) and root biomass (h^2= 0.28) while in the control set the variable yield (h^2= 0.72) had the highest score. Variables PBM, RBM, NDP and PDL were identified as ideal at discriminating genotypic performance responses in P- limiting soil.

6.2 Recommendation

Further research should focus on molecular marker analysis to identify genes associated with performance of cowpea genotypes in phosphorus limiting soil.

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