RESILIENCE TO LOW PHOSPHORUS AND WATER STRESS TOLERANCES IN NEW COWPEA (*Vigna unguiculata* [L] Walp.) GENOTYPES AND ASSOCIATED MORPHOLOGICAL ADJUSTMENTS

BY

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A Thesis Submitted to the University of Zambia in Partial Fulfilment of The Requirements for the Degree of the Master of Science in Agronomy

SCHOOL OF AGRICULTURAL SCIENCES

DEPARTMENT OF PLANT SCIENCES

UNIVERSITY OF ZAMBIA

LUSAKA

2022

DECLARATION

I, Japhet Mutunga hereby declare that the work presented in this dissertation was my own and has never been submitted for a degree at this or any other university

Signature……………………………………………………….

Date…………………………………………………………...

APPROVAL

This dissertation of Mr Japhet Mutunga was approved as fulfilling part of the requirements of the award of Master of Science Agronomy by the University of Zambia

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ABSTRACT

Cowpea (*Vigna unguiculata* L. Walp) is an important legume crop, but drought and soil infertility, especially Phosphorus (P) deficiency are primary constraints for its production. Three studies, a field, greenhouse and hydroponics experiments were carried out at the University of Zambia to determine the effect of low soil phosphorus and water stress on the performance of new selected mutation derived cowpea genotypes. In the field experiment, 23 genotypes were planted in a Randomized Completely Block Design (RCBD) to identify cowpea genotypes with dual tolerance to low phosphorus and water stress condition. The field that was used had suboptimal soil phosphorus, and the cowpea was subjected to terminal water stress during the growing period. The effect of genotype on the number of pods per plant, 100 seed grain weight, yield per hectare, number of seeds per pods and pod length were highly significant ($p<0.001$). The genotypes LT 11-3-3-12, BB 3-9-7-5-2, BB 8-1-5-2 and MS 1-8-1-4 were identified to be better performing, hence were selected for the greenhouse experiment. The greenhouse study separated the effect of P and water stress in order to determine the effect of low P and water stress on the morpho agronomic traits of cowpea. The greenhouse study was conducted using Completely Randomized Design (CRD) and the treatments replicated three times. It was a factorial experiment with three factors; P application at 2 levels, water application at 3 levels and 7 cowpea genotypes. Phosphorus (P_2O_5) was applied at planting at the rate of 23 and 80 kg P_2O_5 ha $^{-1}$ as low and high P levels, respectively. The water regime was imposed by irrigation at 40% and 60% (water stress) and 100% (no stress) based on Plant Available Water (PAW). Water stress was used as proxy for drought condition. Moisture stress significantly reduced shoot biomass, the number of branches, plant height, root biomass, pod and grain yield. P had a significant effect on shoot biomass, root biomass, grain yield and grain yield partitioning. The studies established that some mutation derived cowpea genotypes were resilient to both low soil phosphorus and water stress conditions, and can be directly released as varieties or used as parents in breeding for improved tolerance to low phosphorus and water stress. Therefore, they can be grown in drought-prone areas, and soils that are deficient in Phosphorus.

Key terms: Genotypes, phosphorus, mutation derived lines, water stress.

DEDICATION

I dedicate this piece of work to my family for their inspiration and support.

ACKNOWLEDGEMENT

I wish to acknowledge the following;

Dr Kalaluka. Munyinda and Dr K Kamfwa for the supervisory and inspirational support

Mr. Musukwa for providing technical assistance and guidance.

Mr. Stanley Phiri and Mr. Alex Bwalya for their technical assistance in the study.

The International Institute for Tropical Agriculture (IITA) for providing cowpea accessions for testing.

The Plant Science Department for providing the materials for research and supervision.

Agriculture Productivity Programme for Southern Africa (APPSA) for providing funds for the study.

International Atomic Energy Agency (IAEA) and National Institute for Industrial and Scientific Research (NISIR) for radiating the seeds in the development of mutation derived lines.

The Ministry of Agriculture for granting me study leave.

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CHAPTER ONE

1.0 Introduction

Cowpea ((*Vigna unguiculata* L. Walp.) is an important legume crop in sub- Saharan Africa, with parts of Asia and the Americas representing other regions of production (Food and Agriculture Organization [FAO], 2021). Cowpea is a versatile crop because it provides nutrition to humans and livestock and improves soil fertility (Carneiro da Silva et al., 2018). It contributes to the improvement of soil fertility by the fixation of Nitrogen (N). According Simunji et al. (2019) cowpea can fix up to about 60 - 86 kg N ha $^{-1}$ in the soil. Therefore, it is an important component of traditional cropping systems for soil fertility improvement particularly in smallholder farming systems where little or no fertilizer is used (Kyei-Boahen et al., 2017).

The total world production of cowpeas in 2019 was 8.9 million metric tons (FAO, 2021), though Africa being the highest producer, the average yields of 250 -300 kg ha $^{-1}$ are low especially in some regions as compared with yields of up to 5000 kg ha^{-1} reported from the best performing countries (FAOSTAT, 2016). According to the Ministry of Agriculture and Central Statistics Office Crop forecast (2018), the average yield of traditional cultivars of cowpea in Zambia has remained low, ranging from 300 to 500 kg ha^{-1}. This is caused by attacks from insects, diseases, low soil fertility, drought, inadequate planting systems, inappropriate cultivars and lack of inputs (Ojiewo et al. (2018)

Amongst various abiotic stresses, soil infertility especially inadequate soil phosphorus and insufficient soil moisture due to drought is noted to cause a significant yield loss in many crops, including cowpea (Osakabe et al., 2014). This is because cowpea's growth and development are influenced by the interactions between soil water levels and morpho-physiological factors, including mineral uptake, cell division, photosynthesis, respiration, and protein synthesis (Taiz et al., 2015). Increased environmental fluctuations resulting from climate change have been forecasted in many agricultural regions to increase the frequency, intensity, and duration of drought episodes (Cramer et al., 2011). According to Hamududu et al. (2018) the spatial distribution of rainfall in Zambia has changed from the historical reference period in 1960s – 2000s and the present, and it is projected to change even further by mid – to end of the century in 2100.

Rainfall is projected to decrease by about 3% by mid - century and only marginally by about 0.6% toward the end of the century across the country.

Zambian soils are inherently low in P (figure 1) and farmers rarely apply P in cowpea production because inorganic P fertilizers are expensive (Adetunji, 1995). Low soil P is caused by continuous cultivation and nutrient depletion (Magani and Kuchinda, 2009).

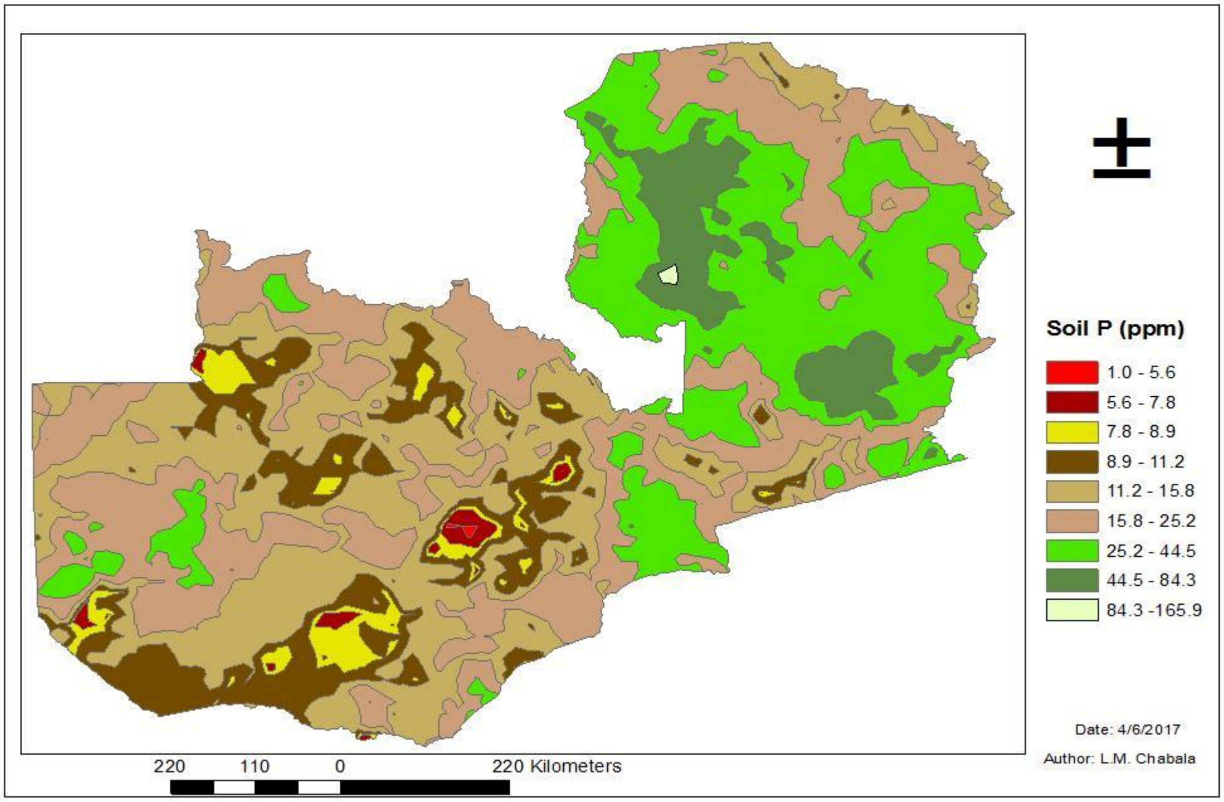

Figure 1: Map of Zambia showing soil Phosphorus levels

Source: The University of Zambia, School of Agricultural Sciences, Department of Soil Science

Phosphorus (P) is an essential nutrient for the general health and vigour of all plants based on its contribution to the biomass as a macronutrient and maintenance of the energy metabolic processes (Jin et al., 2014).

With the simultaneous occurrence of both low P and soil water deficit scenarios on plant development and yield production, this study was initiated to identify new cowpea genotypes that are resilient to low phosphorus and water stress conditions. The new mutation derived cowpea lines which were used addressed the stresses under consideration.

Main objective

- To determine the effect of low soil phosphorus and water stress on the performance of new selected mutation derived cowpea genotypes.

1.2. Specific Objectives

1. To identify cowpea genotype with dual tolerance to low Phosphorus and water stress conditions.
2. To determine the effect of Phosphorus and water stress on the morpho-agronomic traits of selected cowpea

1.3. Hypothesis

1. Some cowpea genotypes have dual tolerance to low Phosphorus and water stress conditions.

2. Phosphorus and water stress affect the morpho- agronomic traits of selected cowpea genotype

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Botanic characteristic

Cowpea (Vigna unguiculata (L.) Walp) is a versatile, vascular, annual, and warm season legume. It belongs to the class Dicotyledonae, order Fabales, family Fabaceae, subfamily Papilionoideae, tribe Phaseoleae, subtribe Phaseolinae, and genus Vigna (OECD 2016; Padulosi and Ng, 1997). Linnaeus described cowpea as *Dolicho sunguiculatus* L. (later renamed *Vigna unguiculata* (L.) Walp.) in 1753. Between 1753 and 1845, more than 20 binomials were described from cultivated *Vigna unguiculata* species. However, annual cowpea has two botanical varieties, the cultivated *Vigna unguiculata unguiculata var. unguiculata* and the wild form *Vigna unguiculata unguiculata var. spontanea*, both of which are inbreeding. All cultivated cowpeas are grouped under *Vigna unguiculata* subspecies *unguiculata*, which is the largest cultivar group (Kabede et al., 2020)

2.2 Origin and distribution

Cowpea originated in Africa, which, according to the Food and Agriculture Organization Corporate Statistical Database (FAOSTAT, 2021), accounts for the majority of the world's production. The precise location of its centre of domestication is still controversial. A study reported that southern Africa is where most primitive wild varieties occur (Singh, 1997) specifically, wild cowpea has been found to occur with a relatively higher frequency within the territory encompassing Botswana, Eswatini, Mozambique, Namibia, South Africa, Zambia, and Zimbabwe (Padulosi et al, 1997)

However, based on the oldest archaeological evidence in Ghana and the existence of weedy intermediates between wild and domesticated cowpeas, eastern and western Africa have been proposed as the centre of domestication (D'Andera et al, 2007; Herniter et al, 2020; Vaillancourt et al,1992). Benin, Burkina Faso, north-western Cameroon, Niger, Nigeria, and Togo in West Africa have been considered the centres of maximum diversity for cultivated cowpea (Singh, 1997; Fatokun et al, 2018; Munoz-Amatrian et al, 2021). While cowpea was introduced into Europe around 300 BC and Asia (India) around 200 BC, its introduction into South America dates back to the 17th century. In the United States (US), cowpea was first introduced into the south in the

18th century. Like in the Caribbean, cowpea's introduction into the Americas was possibly through the "Columbian Exchange" (Herniter et al, 2020; Singh, 1997)

2.3 Importance

2.3.1 World and National production

The total world production of cowpeas in 2019 was 8.9 million metric tons (Food and Agriculture Organization [FAO], 2021), representing 2.7-folds increase since 2000. According to Kamara et al. (2018), over 12.61 million ha are grown to cowpea worldwide, with an annual grain production of about 5.59 million tons. Africa accounts for 84% of grain production; Nigeria being the largest cowpea producer in the world and accounts for over 2.5 million tons of grain production from an estimated 4.9 million ha. Niger, Burkina Faso and Tanzania are the leading cowpea producer both in terms of area coverage (ha) and production (tons) following Nigeria. Other important production areas include lower elevation areas of eastern and southern Africa and in South America (particularly in northeastern Brazil and in Peru), parts of India, and the southeastern and southwestern regions of North America. Uganda and Kenya are also the largest cowpea-producing countries in eastern Africa (Ojiewo et al., 2018).

In Zambia cowpea is mainly grown on ridges in the northern region of the country as a mixed intercropping system with cereal crops such as millet, sorghum and maize. Cowpea is grown in almost all the Provinces of Zambia (table 1). 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 1: 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 Province	1,074	979	390	0.4
Total	14,022	11,691	6,824	0.5

Source: Central Statistics Office (CSO), compiled by Conservation Farming Unit (CFU) - 2018

2.3.2 Human Nutritional /Composition

As indicated by Carneiro da Silva et al. (2018), cowpea plays a critical role in the lives of millions of people in the developing world, which were estimated as 38 million households (194 million people) in Sub – Saharan Africa (SSA), providing them a major source of dietary protein that nutritionally complements low-protein cereal and tuber crop staples. According to the United States Department of Agriculture [USDA] (2021), cowpea has the following nutrient content; protein (~24%), dietary fibre (~11%), and potassium (1112 mg/100 g) while low in lipids (<2%) and sodium (16 mg/100 g). Cowpea protein has appreciable amounts of essential amino acids except cysteine and methionine. This makes cowpea to be a multifunctional crop, providing food for humans and feed for livestock (Alemu et al., 2016).

2.3.3 Soil improvement (N fixation)

According to Kyei-Boahen et al. (2017), cowpeas play a critical role in the management of soil fertility in cereal-based intercropped and rotational cropping systems where they are often grown in sub-Saharan Africa, in terms of nutrient improvement and resistance to certain pests. It is an important component of the traditional cropping systems because it fixes atmospheric nitrogen and contributes to soil fertility improvement particularly in smallholder farming systems where little or no fertilizer is used. Cowpea has the ability to fix atmospheric nitrogen with its nodules in association with soil dwelling bacteria, known as rhizobia. Cowpea can fix about 240 kg ha $^{-1}$ of atmospheric nitrogen and make available about 60–70 kg ha^{-1} nitrogen for succeeding crops grown in rotation with it (Crops Research Institute, 2006; Simunji et al 2019). In addition to that, it provides ground cover and suppresses weeds (Beshir et al., 2019).

2.4 Major cowpea production constraints

The production and productivity of legumes are low especially in Africa, with average yields of 250–300 kg ha $^{-1}$ in some region as compared with yields of up to 5000 kg ha $^{-1}$ reported from the best performing countries (FAOSTAT, 2016). This is caused by severe attacks of insects, diseases, low soil fertility, drought, inadequate planting systems, inappropriate cultivars and lack of inputs. Ojiewo et al. (2018) also indicated that the low productivity of cowpea is attributed to various production and socio-economic constraints including persistent drought episodes, deteriorating soil fertility, market failures and limited access to improved varieties on account of challenges in the seed systems.

2.5 Agronomic Requirements

Cowpea is adapted to wide range of soil types, from sandy to clay loam with moderate amounts soil water and salinity (DAFF, 2011). It has been observed to grow well in sandy soils where root growth is not restricted (DAFF, 2011). It can survive under infertile acid soils but it is reported to be less tolerant to cold soils (DAFF, 2011). The crop requires well drained soils with a pH of 5.6 - 6.0 (Smith, 2006). Germination occurs best when the soil is at least 19 ℃ (DAFF, 2011), and the required optimum temperature range is 20℃-30℃ (Sys et al., 1993). The optimum rainfall conditions for cowpea ranges from 400 to 700 mm per annum (Smith, 2006). It is important that the rainfall is well–distributed for normal growth and development.

2.6 Plant nutrition and development

2.6.1 Phosphorus

Phosphorus is among the most needed elements for cowpea production in many tropical soils. It is critical to cowpea yield because of its multiple effects on nutrition. P is a vital component of adenosine diphosphate (ADP) and adenosine triphosphate (ATP) (Nesme et al. 2014). These are high-energy phosphate compounds that control most processes in legume crops including respiration, photosynthesis, nucleic acid synthesis, and protein and plant cell formation through nutrient transport (Nesme et al. 2014; Meena et al. 2014). According to Smyth and Cravo (1990) cowpea optimum levels for soil P was 60 kg P ha^{-1}, but due to P retention as adsorbed P on the surface of soil particles and the association with amorphous Aluminium (Al) and Iron (Fe) oxides (Mitran and Mani, 2017), a much higher recommendation of up to 80 kg P ha^{-1} may be required for optimum production.

2.6.2 Phosphorus Use Efficiency in Legumes

The Phosphorus Use Efficiency (PUE) is the amount of total biomass produced per unit of P uptake (Hammond et al., 2009; Meena et al., 2016). The PUE is low in agricultural soils. When P is applied to the soil through a source of fertilizer or organic manure, it undergoes several biochemical reactions which remove phosphate ions from the soil solution (Kruse *et al*.2015). It is measured that only 15–30% of applied fertilizer P is taken up by crops in the year of its application (Swarup 2002; Syers et al. 2008). However, the remaining 70–90% becomes part of the soil P pool, which is fixed but subsequently released to the crop over the following months and years (Roberts and Johnston, 2015). Improving the PUE for growth in legume crops requires enhanced P acquisition from the soil, and enhanced use of P in the processes that lead to faster growth and a greater allocation of biomass to the harvestable parts (Kruse et al. 2015)

2.6.3 Importance of Phosphorus in Nitrogen fixation in cowpea

P is one of the important ingredients for *Rhizobium* to convert atmospheric N to ammonium (NH_4) which can be used by plants (Berg, 2009). P influences nodule development through its basic functions in plants as an energy source. 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). Researchers across the world have reported increased N-fixation in legumes by adding phosphate to the P-deficient soil (Ahlawat and Ali, 1993; Bekere and Hailemariam, 2012). The significant role of P in the symbiotic N-fixation process includes; increased top and root growth of cowpea plant, enhanced early formation of active nodules for benefitting from hosting legumes, increased size and number of nodules, improving the amount of N assimilated nodules per unit weight, increased total amount of N in the harvested portion of the host legume plants and helps in improving the root of the density of crop plants.

2.6.4 Nitrogen

According to Iruhvwu (2011), cowpea plants do not require too much nitrogen fertilizer because they fix their own nitrogen from the air using the nodules in the roots. However, in areas where soils are poor in nitrogen, a small quantity of about 15 kg ha $^{-1}$of nitrogen in the form of NPK 15:15:15 is needed as a starter dose for a good crop. If too much nitrogen fertilizer is used, the plant will grow luxuriantly with poor grain yield (Iruhvwu, 2011)

2.6.5 Root and Canopy development

The recent studies indicated that P enhanced root system which provides greater root-soil contact and eventually higher uptake of P and other important and low mobility nutrients and absorption of higher concentration of mineral nutrients (Zafar et al. 2011). Almost all the legumes required P in relatively large amounts for growth and have been reported to promote leaf area, biomass, yield, nodule number, and nodule mass (Kasturikrishna and Ahlawat, 1999). P supplement in legumes has great potential for promoting growth and higher yield, increases nodule number, as well as enhances symbiotic establishment for increased N-fixation (Ndakidemi et al. 2006).

2.7 Stress and plant development

Changes in rooting depth and rooting density due to water stress and inadequate soil phosphorus exist among many crop species (Smit et al., 1994). Extraction of P from the soil by plant depends on the root physiological and morphological properties such as root length, root exudates, high-affinity for inorganic P transporter and arbuscular mycorrhizal colonization (Quan et al., 2010). Cowpea roots can be adapted to low soil P conditions by increasing root growth such as basal and adventitious roots, modified root architecture (Lynch, 1995) and the ability to extract P from the soil (Niu et al., 2012). These have been reported to be root traits that are necessary for the adaptation of cowpea to P limiting environments. Miller et al. (2003) reported that an increase in production of adventitious roots in common beans helps in P acquisition by improving plant foraging in the most P rich soil environment and shallower root systems were more competitive than deep root systems for top soil P.

Water stress causes a decrease in the number of leaves per plant and reduced leaf size of the crop Mitra (2001). Agbicobo et al. (2009) also reported that plants develop strategies for maintaining turgor by increasing root depth or developing an efficient root system to maximize water uptake, and by reducing water loss through reduced epidermal (stomatal and lenticular) conductance, reduced absorption of radiation by leaf rolling or folding and reduced evapotranspiration surface (leaf area).

Water stress cause reduction in N fixation (Sinclair et al., 1987), and also cause significant crop yield losses. Nitrogen fixation in legumes is highly sensitive to water stress conditions (Wery et al., 1994). Water stress affects nodule formation and function (Serraj, 2003) as well as their longevity.

2.8 Mechanism of Drought Resistance

Many authors (Jones et al., 1989; Passioura et al., 1993; Kramer and Boyer, 1995; Sinclair and Purcell, 2005) pointed out that drought tolerance is an emergent property involving a wide range of component processes such as drought escape, drought avoidance, and biochemical tolerance of tissue water deficits and efficiency of water use. Three primary types of drought resistance mechanisms in crop plants have been identified and these are:

2.8.1 Drought escape

This describes the situation where an otherwise drought susceptible variety performs well in drought environment simply by avoiding the period of drought. For example, early maturing varieties tend to escape drought if it comes late in the season. Early maturing varieties escape terminal drought (Singh, 1987), but if exposed to intermittent moisture stress during the vegetative growth stage, they perform very poorly (Mai-Kodomi et al., 1999). Moreover, the early maturing cowpea cultivars tend to be very sensitive to drought that occurs during the early stages of reproductive development phase (Thiaw et al., 1993)

2.8.2 Dehydration avoidance

This is the ability of a plant to retain a relatively higher level of hydration under conditions of soil or atmospheric water stress. The common measure of dehydration avoidance is the tissue water status as expressed by water or turgor potential under conditions of water stress. Dehydration avoidance can be as a result of reduced transpiration, osmotic adjustment, cuticular wax and other leaf characteristic such as leaf pubescence. Plants can endure or withstand a dry period by maintaining a favourable water balance under drought conditions. Osmotic adjustment, in which the plant increases the concentration of organic molecules in the cell water solution to bind water, is another example. A thicker layer of waxy materials at the plant surface and a more extensive and deeper rooting are others.

2.9 Water use efficiency

Water-use efficiency (WUE) is an important physiological trait in legumes. It is a complex and single trait that is important for the improvement of drought tolerance in crops (Zhou *et al.,* 2011). It is defined as the efficiency of the crop to use water in producing dry matter and harvest index (Siddique *et al.*, 2001), which varies among crop species. It is affected by various soil factors such as soil structure and depth as well as root distribution (Yada, 2011). Drought stress decreases WUE, leaf production and root proliferation; and consequently, crop productivity (Farooq *et al.*, 2009). It is, therefore, important to identify or develop new genotypes of cowpea that are sufficiently drought tolerant and possess better WUE to provide solutions to the multiple challenges of global and household food insecurity. When crops experience drought stress, physiological mechanisms such as stomata closure, reduced transpiration and reduced photosynthetic rate take place as responses to insufficient water availability (Costa and Lobato, 2011).

2.10 Compensatory growth

According to the study conducted by Subramanian et al., (1992) to identify compensatory growth responses in cowpea after relief of water stress. It was established that assimilate supply seemed to have limiting early pod growth upon relief of water stress due to low photosynthesis rate, reduced leaf area per plant and increased partitioning to leaf expansion. However, later pod growth was not limited by assimilate supply and final dry matter per pod was similar in both non-stressed and stress-affected plant. It was observed in the study that cowpea exhibited an increase in leaf area, shift in dry matter partitioning in favour of leaf expansion, extended green leaf duration, and increase in pod number as growth responses during pod-fill stage upon relief of water stress. Therefore, Subramanian et al (1992) concluded that the partially compensating physiological responses probably ensured reasonable productivity of cowpea during rainy season when drought occurred.

Morphological traits such as delayed leaf senescence (DLS) may contribute to drought adaptation (Gwathmey et al., 1992). DLS trait helps to improve plant survival after mid-season drought damage. A typical example is the first flush of pods that enable a substantial second flush to be produced, which allows the crop to stay alive through mid-season drought and recover when rainfall resumes (Agbicobo et al., 2009).

2.11 Crop improvement

While the primary objectives of most existing cowpea breeding programs are to improve grain yield and quality, efforts have also been made to develop varieties with resistance to insects, diseases and other abiotic factors which are major constraints to cowpea production (Timko et al., 2008; Ndeve et al., 2019). Despite the progress in research, cowpea has been lacking essential genetic and genomic resources and investment until recently (Timko et al, 2008).

Several studies have been conducted in cowpea and a conclusion has been made that genetic improvement of cowpea genotypes for drought tolerance depicts the best and low-cost strategy for mitigating the impacts of drought stress and ensuring sustainable crop yield (Agbicodo et al., 2009; Ravelombola et al.,2020). Lonardi et al. (2019) documented the genome of improved cowpea genotypes. This could provide a significant resource for understanding the morpho-physiological response of cowpeas to drought stress.

Mutation breeding is one of the conventional breeding methods in plant breeding. It has become increasingly popular in recent times as an effective tool for crop improvement (Acharya et al.,2007) and an efficient means supplementing existing germplasm for cultivar improvement in breeding programs (Dubinin, 1961)

The International Atomic Energy Agency (IAEA) has been supporting member states in genetic improvement of various crops including cowpea through the use of artificial mutagenesis such as gamma rays, X-rays, and ethyl methanesulphonate (EMS) (Horn and Shimelis, 2013; Jain, 2005; Maluszynski et al., 2000). This has led to the development and release of improved cowpea cultivars in Africa, Asia, and Latin America with different agronomic traits of interest as well as resistance to insect and diseases. The application of induced mutation breeding techniques in cowpea has increased in most countries across Africa as a faster way to enhance genetic variation (Goyal and Khan, 2010; Singh et al., 2013). Induced mutation fit well in cowpea genetic enhancement as most cowpea breeding initiatives aim at broadening the genetic bases of the crop to adapt to various cropping systems and agro-ecologies, and also in the development of consumer-preferred varieties (Lima et al., 2011; Singh et al., 2003).

The University of Zambia has produced some cowpea mutation derived genotypes from the parent varieties with mutation breeding using gamma radiation. This is in order to address the problem of low yields caused by low yielding varieties which are susceptible to biotic and abiotic stresses among other things.

CHAPTER THREE

3.0 MATERIALS AND METHODS

The study involved three experiments, the field, green house and hydroponics studies. To evaluate moisture stress in the field study, the cowpea genotypes were subjected to terminal water stress by planting late in the season. Similarly, to evaluate the effect of low P, the Cowpea genotypes were planted in a soil with a P concentration below critical level of less than 10 ppm. The follow up greenhouse study separated the effect of the moisture and P stress by planting the experiment in a factorial design with P at 2 levels and moisture stress at 3 levels.

3.1 Identification of new cowpea genotypes which are low phosphorus and water stress tolerant

3.1.1 Site

The field experiment was carried out at the University of Zambia School of Agricultural Sciences, Lusaka, field station located at $28^{o}33'E$ & $15^{o}39'S$. The site falls in Agro- ecological region IIa.

3.1.2 Plant Materials

Twenty-four genotypes which included six from IITA, three parents, and 14 mutant derived lines were used in the field study (table 2). The mutation bred materials were a result of plant improvement work of the Plant Science Department, School of Agricultural Sciences, the University of Zambia while the parental lines are released varieties from Zambia Agriculture Research Institute (ZARI)

Table 2: Cowpea genotypes used in the field study

Genotype	Source	Genotype	Source	Genotype	Source
Bubebe (BB PRT)	ZARI	Lutembwe (LT PRT)	ZARI	Musandile (MS PRT)	ZARI
BB 10-4-2-3	UNZA	LT 10-7-1-12	UNZA	MS 10-11-1-1	UNZA
BB 14 -16-2-2	UNZA	LT 11-3-3-13	UNZA	MS 1-8-1-4	UNZA
BB 3-9-7-5	UNZA	LT 16-7-2-5	UNZA	MS 1-8-4-1	UNZA
BB 7-9-7-5	UNZA	LT 3-8-4-6	UNZA	Namuseba	ZARI
BB 8 -1-5-2	UNZA	LT 4-2-4-1	UNZA	ITOOK-126-3	IITA
IT 97K-390-2	IITA	LT 11-3-3-12	UNZA	IT 98K-131-2	IITA
ITOOK-126-3	IITA	IT 18	IITA		

3.1.3 Experimental Design

The experiment was set in a Randomized Completely Block Design (RCBD) with three replications.

3.1.4 Agronomic Management of Field experiment

A 12m × 25m site with low Phosphorus (<10 ppm) soil was used. Soil testing was done before planting. The pH of the soil was slightly acidic 5.7 with available Phosphorus which was at 7.13 ppm which was at suboptimal for cowpea production. Planting was done in 22nd February 2018. Which was toward the end of the rain season in Zambia weather pattern. This was to allow the plants to be subjected to terminal water stress at flowering. The weather pattern experienced during the growing period is shown in table 3. About 2 to 3 seeds were planted per hole at a spacing of 0.15 m ×0.5 m and later thinned down to one after 2 weeks of germination. The experimental plots were manually weeded twice, and plants were sprayed with cypermethrin at the rate of 20 g active ingredient ha-^{1}initially at 3 weeks after germination and subsequently for every fortnightly until pod maturity to prevent insect pest infestation.

Table 3: Weather experienced during the growing season at UNZA field station (2018)

Parameter	February	March	April	May	June
Total rainfall mm	360.6	143.6	8	32.2	0
Rain days	7	18	4	3	0
Max air temp ℃	26.43	27.14	26.09	25.49	23.45
Min air temp ℃	18.34	18.24	16.3	15.09	11.52
Average soil temp ℃	25.02	25.01	23.4	21.71	19.44
Relative humidity %	89.85	86.42	78.3	73	61.26
Average solar irradiance W/m^2	189.06	218.07	221.72	209.04	211.61
Total solar irradiance MJ/m^2	454.85	588.81	574.71	559.87	548.5

Source: Metrological Weather Station, Soil Science Department, School of Agricultural Sciences UNZA (2018

3.1.5 Data collection

Data collected were grain yield, number of pods per plant, pod length, 100 seed weight and maturity score.

3.1.5.1 Number of pods per plant

This was taken after maturity by counting the number of pods for four representative plants per plot and the average of the four was taken.

3.1.5.2 Number of seeds per pod

This was taken at harvesting by counting the number of seeds from four pods from four representative plants and the average was taken.

3.1.5.3 100 seed weight

This was taken after harvest as the weight of 100 shelled grains per plot in grams

3.1.5.4 Pod length

This was taken at maturity by measuring the length using 30cm ruler of four pods from four representative plants per plot and the average of four was taken.

3.1.5.5 Yield

This was calculated by converting the grain weight to kilograms per hector.

3.1.6 Statistical Analysis

The Analysis of Variance was analysed using GenStat 18th Edition statistical software. The mean separation was carried out with standard error of mean at 5% probability Level (S.E 0.05).

3.2 Effect of low soil phosphorus and water stress on the morpho-agronomic characteristics of selected cowpea

3.2.1 Site

The pot experiment was conducted in the greenhouse at the University of Zambia, Lusaka, School of Agricultural Sciences. Located at 28^{o}33'E & 15^{o}23'S

3.2.2 Plant Materials

Better performing mutation derived genotypes alongside their parental lines from the field experiment were selected and planted in the greenhouse for further screening. The selected cowpea genotypes used are listed in table 4.

Table 4: Cowpea genotypes used in the pot experiment

Genotype	Source	Genotype	Source	Genotype	Source
Bubebe (BB PRT)	ZARI	Lutembwe (LT PRT)	ZARI	Musandile (MS PRT)	ZARI
BB 3-9-7-5	UNZA	LT 11-3-3-12	UNZA	MS 1-8-1-4	UNZA
BB 8 -1-5-2	UNZA				

3.2.3 Soil testing

Soil samples were randomly collected from the field that was previously used in the field experiment using an auger. The soil samples were sent to the soil science laboratory, UNZA for analysis. The subsoil used for the study was slightly acidic and low in organic matter (table 5). The macro elements were below the critical levels required for the proper growth and development of cowpea. This indicates that the soil was depleted. The textural classification of the soil was sandy clay loam (table 5).

Table 5: Soil chemical and physical properties of the soil used in the field and pot experiment

Parameter	Values	Remarks
Sand % - Hydrometer	51	
Silt% - Hydrometer	23	
Clay% - Hydrometer	26	
Texture class (USDA)		Sandy clay loam
pH (0.01 $CaCl_2$)	5.71	Moderately acidic
Organic Carbon % - Walkley & Black	1.84	Very low
Total Nitrogen N % - Kjeldahl	0.12	Low
Available P (mg/kg) - Bray P1	11.07	Low
Sulphur S (mg/kg) - Na – Acetate	10.68	
Copper Cu (mg/kg) – DTPA	< 0.025	
Manganese Mn (mg/kg) – DTPA	12.91	
Zinc Zn (mg/kg) – DTPA	0.43	
Iron Fe (mg/kg) – DTPA	2.67	
Exchangeable bases (cmol Kg^{-1}) - Ammonium Acetate		
Sodium Na	0.03	Low
Potassium K	0.31	Very low
Calcium Ca	3.25	Low
Magnesium Mg	0.8	Low
Field Capacity (1 bar) - Pressure Plate Apparatus		
Volumetric moisture content %	21.77	
Permanent Wilting Point (15 bar)		
Volumetric moisture content %	7.46	
Plant Available Water (PAW) %	9.53	

Source: Soil Science Department, School of Agricultural Sciences UNZA (2019)

3.2.4 Pot establishment and Crop management

Planting was done on 22nd October, 2019. The plants were grown in 10-litre plastic pots perforated at the base and filled with 12 kg top soil. The soil was comprehensively tested (Table 6) before planting and was found to be low in P. At planting, two levels of Single Super Phosphate (P_2O_5) chemical fertilizer were added to the pots at the equivalent rate of 23 and 80kg P_2O_5 ha^{-1} representing low and high phosphorus levels respectively. The three soil moisture levels were 40% and 60% (water stress) and 100% (no stress) of Plant Available Water (PAW) determined volumetrically (Kramer, 1983). Water stress was used as a proxy for drought condition. The soil moisture levels were maintained throughout the experiment by periodic application of water as required. Four seeds of each genotype were planted in a pot and later thinned to two seedlings per pot at two weeks after emergence. Weed control was achieved by hand pulling at weekly intervals. Weekly application of insecticide, Karate 2.5 EC (25 g/l lambda-cyhalothrin), at a rate of 5 ml per litre of water was made from the onset of flowering until pod maturity using a hand sprayer to control flower and pod boring insects.

During the first three (3) weeks of cowpea development, the mean air temperature and relative humidity inside the greenhouse were 28 ± 1 °C and 66 ± 3%, respectively, but during the late vegetative and early reproductive phase, mean higher temperatures of 37 ± 1 ℃ and mean low Relative Humidity (RH) of about 37 ± 3 % were recorded.

3.2.5 Experimental Design

The study was conducted using Completely Randomized Design (CRD) and the treatments were replicated three times. It was a factorial experiment with three factors, Phosphorus application at 2 levels, water application at 3 levels and 7 cowpea genotypes.

3.2.6 Data Collection

Parameters measured were; plant height, plant biomass, stem diameter at 5 cm above ground, root biomass, root length, root diameter at 5cm below ground, grain and pod yield.

3.2.6.1 Plant height

This was taken as the average height, from the ground to the top most part of the plants, per pots in centimeter using a meter ruler. This was taken after maturity.

3.2.6.2 Plant Biomass

Four representative plants were selected, cut at the base and packed in the envelops and oven dried, weights were taken and averaged.

3.2.6.3 Stem diameter

This was measured at 5 cm above ground using a Vernier caliper. This was taken at physiological maturity.

3.2.6.4 Root Biomass

This is the mass of dried roots. Four representative plants were selected, cut at the base and lifted from wetted soil. The roots were then washed on a sieve, packed in envelops and oven dried. Weights were taken when the roots attained a constant dry weight. Then the weights were averaged.

3.2.6.5 Root diameter at 5 cm below ground

Representative plants were pulled out of the pots and the roots were cleaned and root diameter was measured at 5 cm below ground using a Vernier caliper.

3.2.6.6 Grain yield

Grains were obtained from four representative plants and the weights were averaged to represent yield in g per plant.

3.2.7 Statistical Analysis

The experiment was arranged in a completely randomized design, the Analysis of Variance was analysed using R statistical software. The mean separation was carried out with standard error of mean at 5% probability Level (S.E 0.05).

3.3 Effects of phosphorus on Morpho agronomic characteristics of selected cowpea genotypes- Hydroponics Experiment

The study was conducted in the green house at the University of Zambia, School of Agricultural Sciences, Lusaka. Located at 28^{o}33'E & 15^{o}33'S. Four (4) cowpea genotypes which included 2 mutants (BB 8-1-5-2 and MS 1-8-1-4) and their parental lines (Bubebe and Musandile) were selected for further screening to confirm the results that were obtained from the greenhouse experiment on their morpho agronomic characteristic. The seeds were first sterilized with 1% Sodium hypochlorite for a minute, rinsed in sterilized water, and then germinated on a white filter paper that was placed on separate Petri dishes in an incubator at 25℃ for 72 hours (Sauer and Burroughs, 1986). Once the seeds germinated, they were transferred. The nutrient solutions were prepared according to the description proposed by Kerridge et al. (1971). The concentration of P nutrient in mg L^{-1} was prepared at 0 mg L^{-1}, 3.1 mg $L^{-1}$and 9.6 mg L^{-1}. The 0 mg L^{-1} and 3.1mg L^{-1} represents low P level whereas 9.6 gm L^{-1}represents high P level. The seedlings were selected and transferred into the nutrient in 1.25 ml plastic containers covered with black polythene to prevent the growth of green algae. The seedlings were allowed to grow for 10 days. Aeration of the solution was done using an electric aeration pump twice a day.

3.3.1 Experimental Design

The experiment was conducted in a Completely Randomized Design replicated 3 times.

3.3.2 Data collection

After ten days, shoot length and root length were taken using a thirty cm ruler. Number of root hairs were counted with the aid of magnifying glass. The plants were cut into two; the root and shoot parts, parked in small envelops and taken to the oven to dry to a constant weight. The shoot and root biomass were then measured.

3.3.3 Statistical Analysis

All data collected were analyzed using Genstat 18th software. Mean separation was carried out with least significant difference at 5% probability Level (LSD 0.05).

CHAPTER FOUR

4.0 RESULTS

4.1 Identification of cowpea genotype tolerant to low P and water stress conditions - Field Experiment

The effect of genotype on yield per hectare, number of pods per plant, 100 seed weight, number of seeds per pod and pod length was highly significant at $p < 0.001$ (table 6).

Table 6: Summary ANOVA mean squares for measured traits parameters

Source of Variation	D.f	Yield/ ha	Pods/ plant	100 Seed Weight	Seed/pods	Pod Length
Replication	2	5339^{NS}	4.7^{NS}	4.3^{NS}	1.2^{NS}	4.8^{NS}
Genotype	23	493363***	25.1***	11.7***	17.1***	5.3***
Error	42	25848	1.2	1.8	3.2	1
CV %		19.9	14.8	10.1	15.2	5.6
S. E		71.7	1.0	1.1	1.5	0.8

Key: NS = Not-significant, *** = highly significant at $p<0.001$, S.E = standard error of mean, CV% = coefficient of variation.

4.1.1 Grain Yield

There was significant difference in yield between parents and their respective mutation derived genotypes (figure 2). BB 7-9-7-5 had a higher grain yield of 1734 kg ha $^{-1}$ as compared to the parent (BB PRT) which had 927 kg ha $^{-1}$. The genotype LT 11-3-3-13 and LT 4-2-4-1 had a higher grain yield of 1491 kg ha^{-1} and 1019 kg ha^{-1}, respectively as compared to the LT PRT, which had the yield of 470 kg ha^{-1}. MS PRT was out performed by all its mutation-derived genotypes.

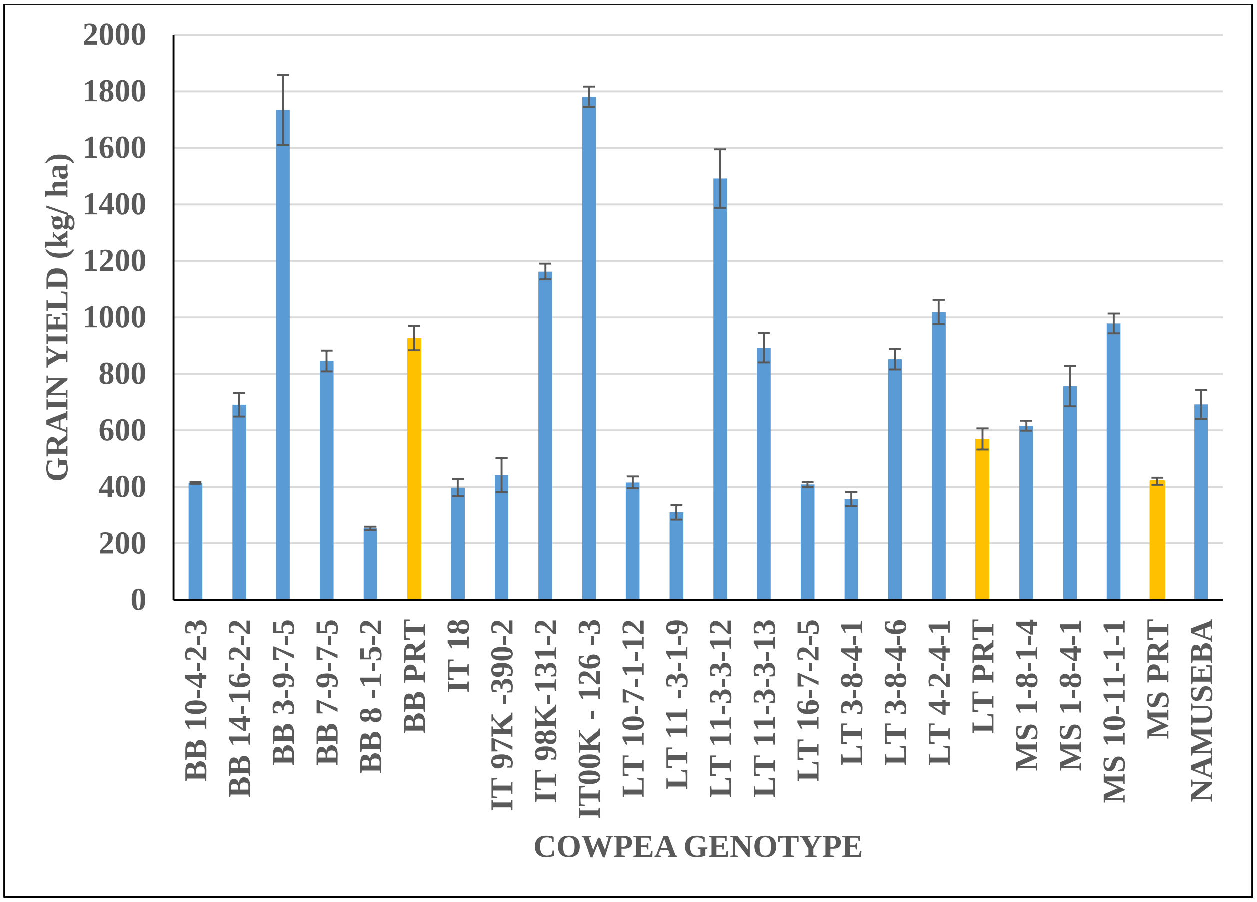

Figure 2: Variation of Grain Yield of Cowpea Genotypes

Error bars are representing standard error of the means (S.E)

4.1.2 Number of pods per plant

The genotype LT 13-3-3-12 had the highest number of pods per plant (16) while LT PRT had (4). There was no significant difference in the number of pods per plant between the LT 3-8-4-6 and LT 4-2-4-1 which had both 6. LT PRT had the lowest number of pods per plant (4). There was no significant difference in the number of pods per plant between MS PRT (8) and MS 10-11-1-1 (8), and that of MS 1-8-4-1 and MS 1-8-1-4 (9) (figure 3)

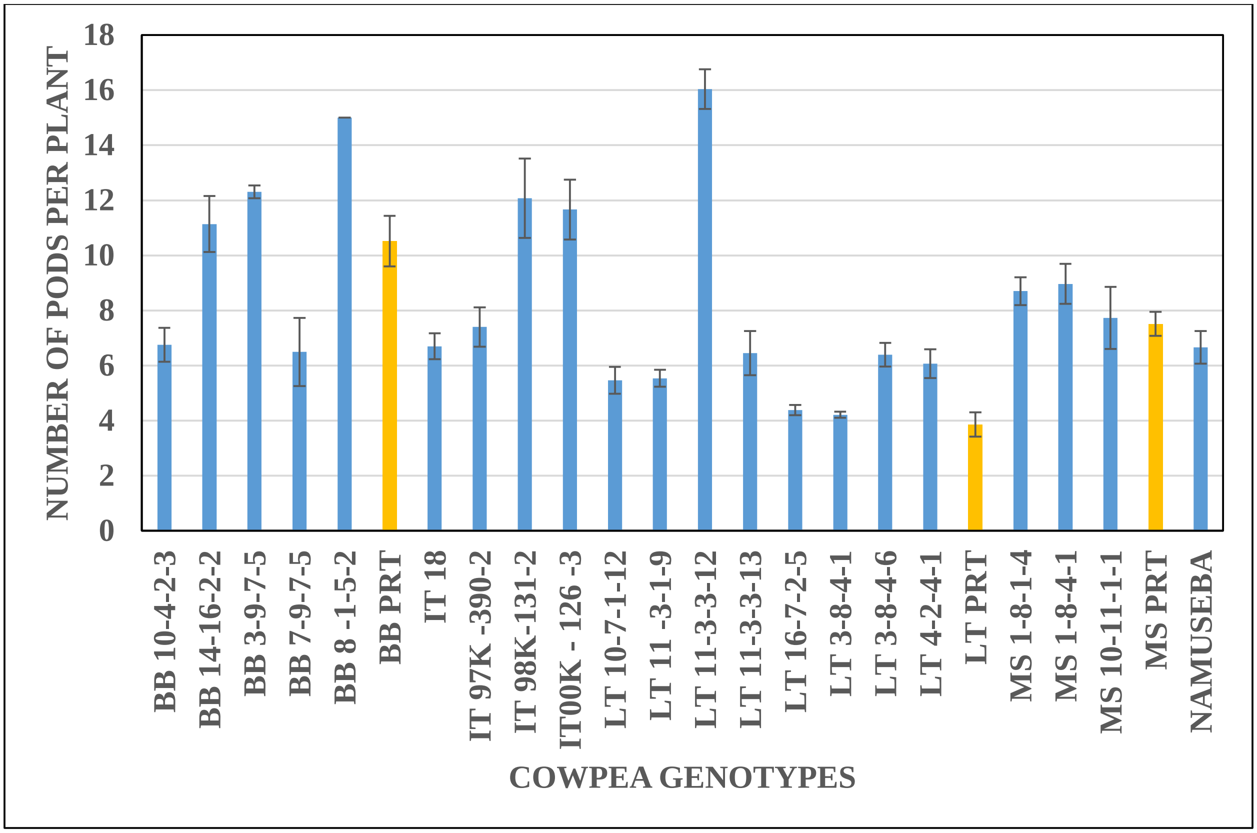

Figure 3: Effect of cowpea genotype on number of pods per plant

Error bars are representing standard error of the mean (S.E)

4.1.3 Hundred Seed Weight

The hundred seed weight ranged from 10 g to 19 g. MS PRT had the highest 100 seed weight of 17.8 g as compared to its mutation derived genotypes. There was no significant difference in the 100 seed weight of the genotypes BB 10-4-2-3, BB 3-9-7-5, BB 3-9-7-5 and BB PRT which had 12 g. The genotype BB 8-1-5-2 had the lowest 100 seed weight of 9.5 g (figure 4)

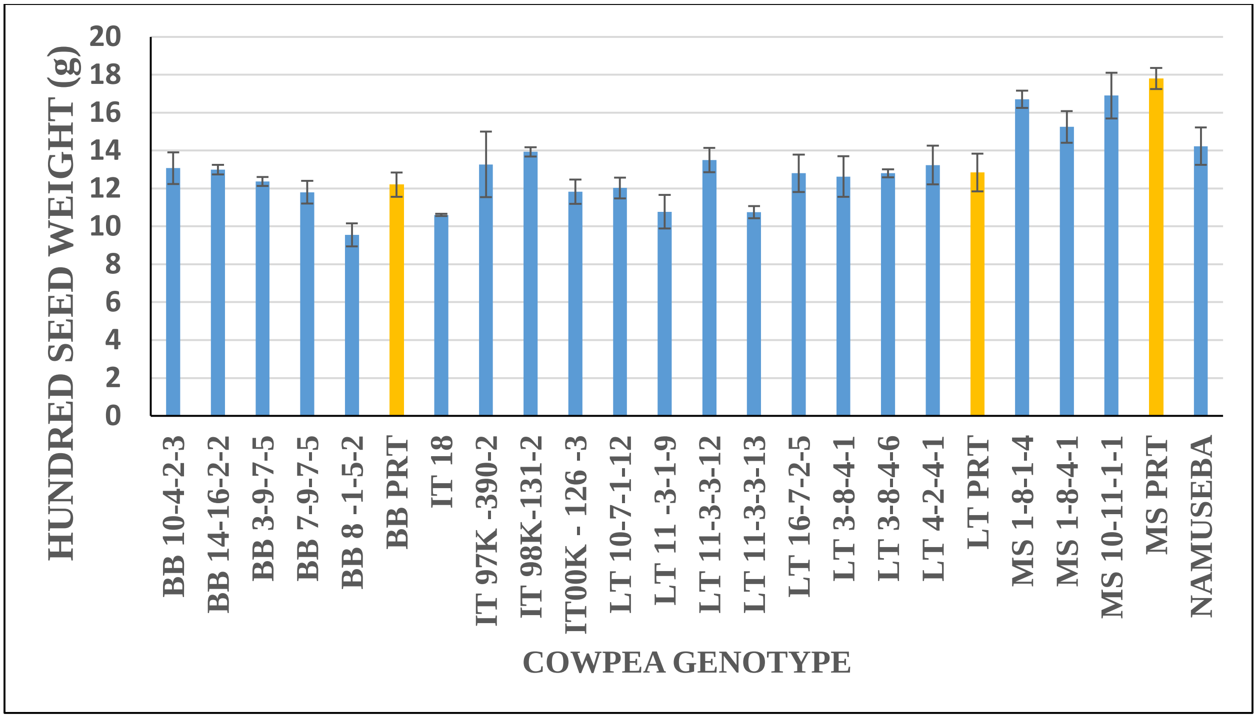

Figure 4: Variation of Hundred Seed Weight with cowpea genotype

Error bars are representing standard error of the mean (S.E)

4.1.4 Pod length

The highest mean pod length (21 cm) was recorded LT 11-3-3-12 and the lowest was in MS 1-8-4-1 and BB 8-1-5-2 (15 cm). There was no significant difference in pod length between BB PRT and BB 14-16-2-2 (17 cm), and between BB 3-9-7-5 and BB 7-9-7-5 (19 cm) (figure 5).

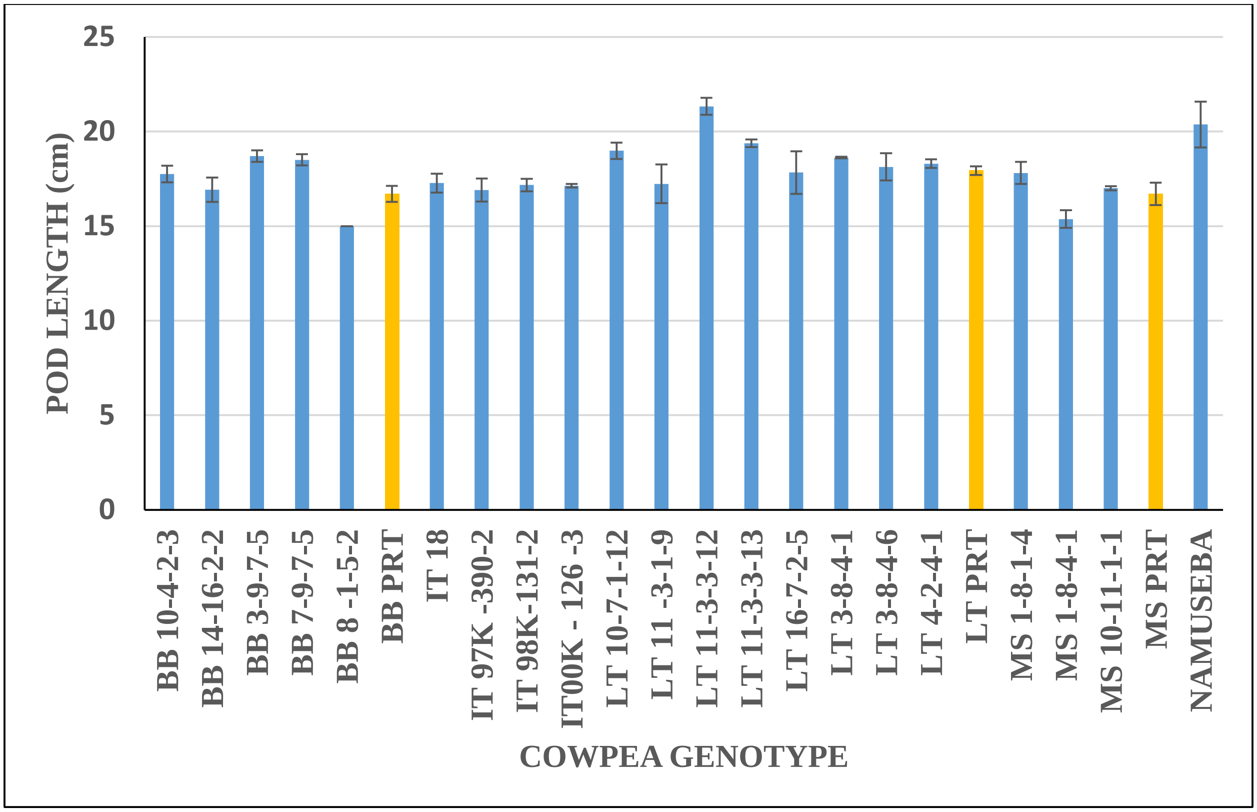

Figure 5: Variation of Pod Length with Cowpea genotype

Error bars are representing standard error of mean (S.E)

4.1.5 Number of seeds per pod

The mean number of seeds per pod ranged from 8 to 17. BB PRT was outperformed by BB 8-1-5-2, BB 7 -9-7-5 and BB 10- 4-2-3, but there was no significant difference between BB PRT and BB 3-9-7-5. There was no significant difference in the number of seeds per pod between LT 11- 3-3-12 and LT 11-3-3-13. MS PRT had the lowest mean number of seeds per pod (8), and was outperformed by all of its mutation-derived genotypes (figure 6)

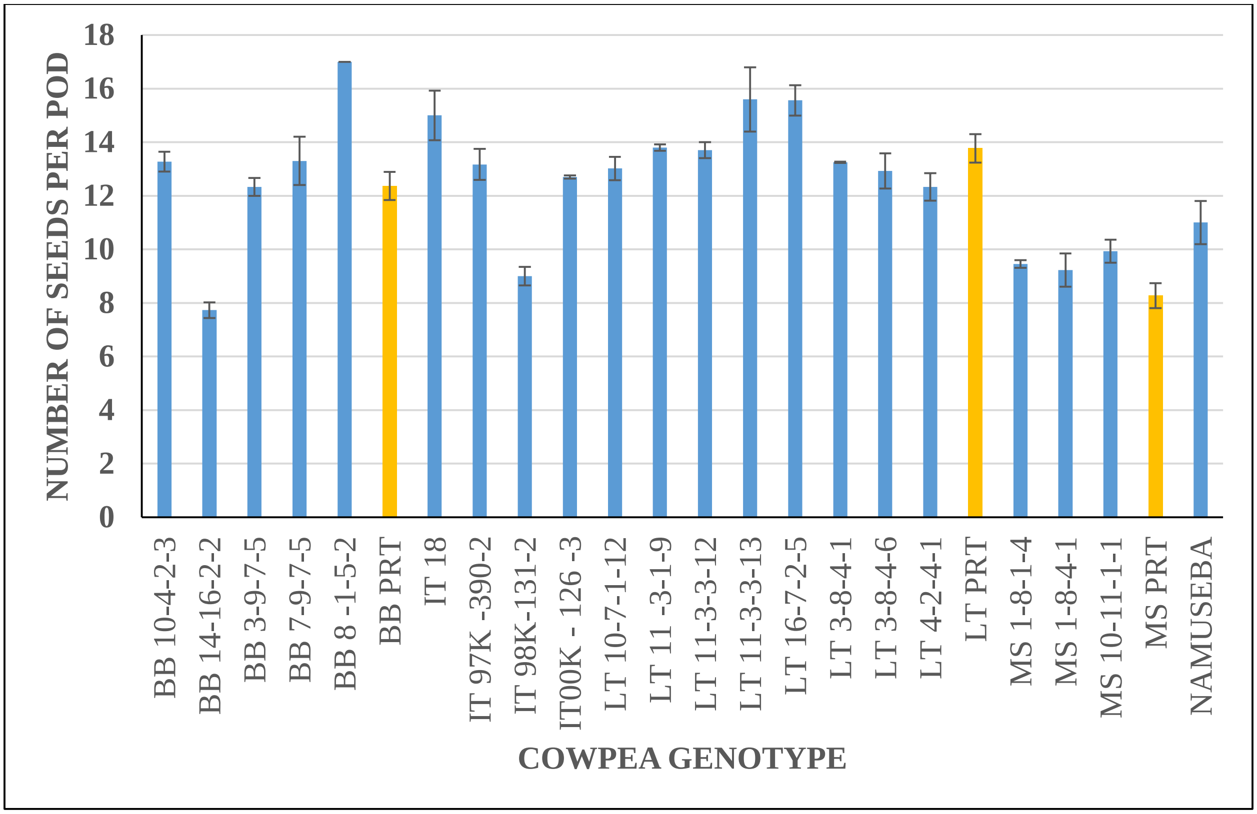

Figure 6: Effect of genotype on number of seeds per pod

Error bars are representing standard error of the mean (S.E)

4.2. Effect of Phosphorus and water stress on morpho agronomic characteristic of cowpea genotypes - Greenhouse Experiment:

4.2.1 Effect of phosphorus on vegetative parameters

Phosphorus had no effect on number of brunches. The effect of phosphorus on plant height was non- significant. The effect of phosphorus on shoot biomass was highly significant ($P < 0.01$) and plant height, but had significant ($P<0.001$) effect on shoot biomass. Higher shoot biomass was recorded under low P (table 7)

4.2.2 Effect of moisture on vegetative parameters

The effect of water stress on shoot biomass was highly significant ($P<0.01$). The highest shoot biomass was recorded at 100% moisture content with 6.5 g plant $^{-1}$ and the lowest was at 60%

moisture level with 52.7 g $plant^{-1}$ (table 7). The effect of water stress on number of branches was highly significant (P<0.01). The highest was recorded at 100% moisture level with 5.6 number of branches and the lowest was at 40% moisture level with 3.2 number of branches. The effect of water stress on plant height was highly significant (P<0.01). The highest plant height was recorded at 100% moisture level with 99.7 cm and the lowest was at 40% moisture level with 42.8 cm plant height. The interaction effect of genotype and moisture was significant (P< 0.05) (figure 7).

4.2.3 Effect of cowpea genotype on vegetative parameters

The effect of genotype on shoot biomass was highly significant (P <0.01). The highest shoot biomass was recorded in LT 11-3-3-12 which had 69.9 g and then followed BB 3-9-7-5 which had 59.4 g, MSPRT (60.0 g) and MS 1-8-1-4 (58.8 g). The lowest were BB 8-1-5-2 and LTPRT. The genotype LT 11-3-3-12 had 29% higher shoot biomass as compared to the parent. The effect of genotype on number of branches was highly significant (P <0.01). BB 8-1-5-2 had the highest number of branches of 5.1 and then followed by BB 3-9-7-5 and MS 1-8-1-4 which had both 4.9 number of branches. Genotypes MS PRT and BBPRT were out performed by their respective mutation lines (table 7)

Table 7: Effect of genotype, phosphorus and moisture level on vegetative parameter of cowpea

Treatment	Shoot biomass (g/plant)	Branches (number)	Plant height (cm)	Stem diameter (cm)
Phosphorus level (P)				
23	62.6a	4.5a	78.3a	2.51a
80	51.5b	4.56a	80.76a	2.58a
Moisture status (M)				
40	57.3ab	3.2c	42.8b	2.3c
60	52.7b	4.9b	95.2a	2.6b
100	61.5a	5.6a	99.7a	2.8a
Cowpea Genotype (G)				
BB 3-9-7-5	59.4b	4.9ab	68.2c	2.6a
BB 8-1-5-2	48.2c	5.1a	81.5abc	2.5a
BB PRT	54.4bc	4.0c	75.7bc	2.6a
LT 11-3-3-12	69.9a	4.7abc	74.9bc	2.6a
LT PRT	49.5c	4.3bc	84.1ab	2.5a
MS 1 -8 -1-4	58.8b	4.9ab	82.5ab	2.4a
MSPRRT	60.0b	4.0c	89.8a	2.6a
P values				
G	***	**	*	***
M	***	***	***	NS
P	***	NS	NS	**
G × M	***	*	NS	NS
G× P	***	NS	NS	NS
M × P	***	*	NS	NS
G × M × P	***	NS	NS	NS

Mean followed by the same letter in a column are not significantly different at $P \leq 0.05$; *** = Significant at $P \leq 0.001$, **= Significant at ≤ 0.01, * = Significant at ≤ 0.05, NS = Non-Significant,

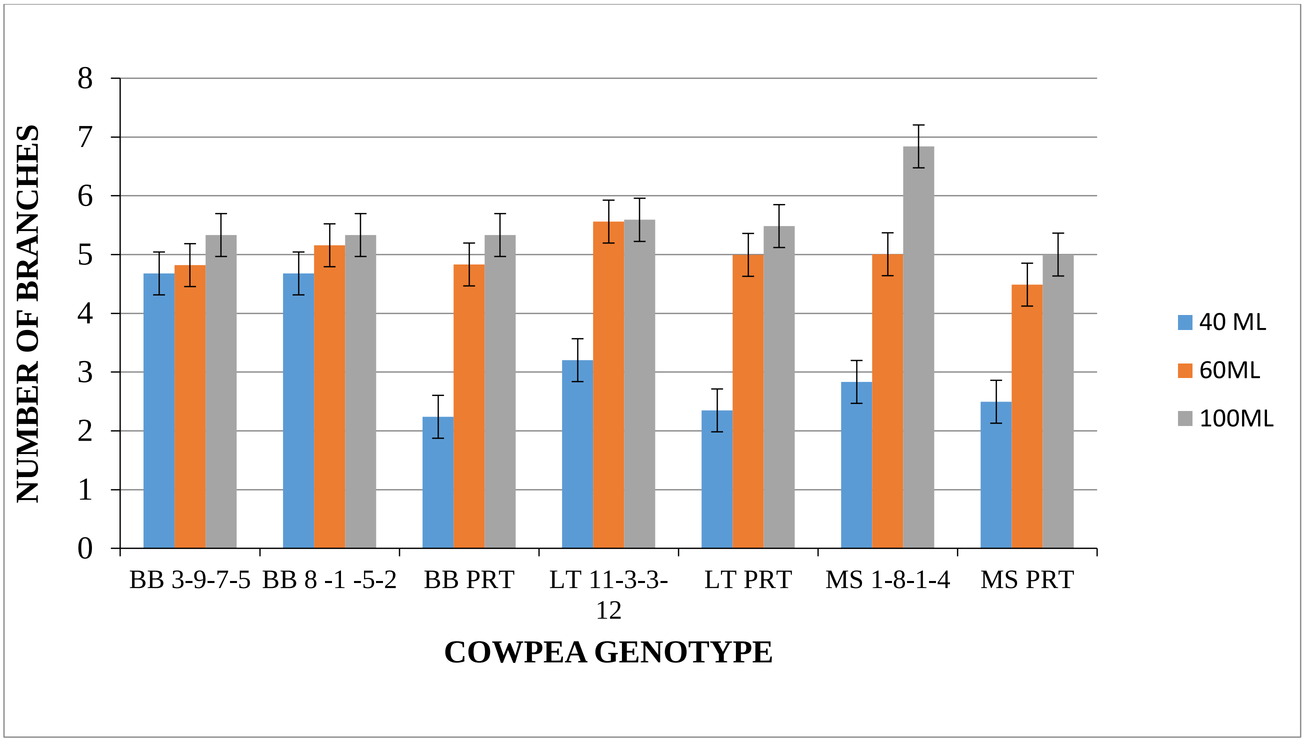

Figure 7: The interaction effect of genotype and moisture level (ML) on number of branches.

Error bars are representing standard error of mean (S.E)

4.2.4 Effect of phosphorus on root characteristics

Phosphorus had significant ($P<0.01$) effect on root biomass. The highest mean root biomass of 2.1 g plant^{-1} was recorded at high phosphorus level. At low P level, the mean root biomass was 2.0 g plant^{-1}. The interaction effect of P level and genotype on root biomass was highly significant ($P< 0.001$) (table 8)

4.2.5 Effect of water stress on root characteristics

The effect of moisture on root biomass was highly significant ($P <0.001$). At 100% and 60% moisture level, there was no significant difference in root biomass, a mean 2.2 g plant^{-1} of root biomass was recorded at both moisture levels. The effect of moisture on root diameter at 5cm below ground was highly significant ($P< 0.001$). The highest was recorded at 100% and 60% moisture levels, with 0.4 cm root diameter at both moisture level. The lowest was at 40% moisture with 0.3 cm root diameter. The effect of moisture on root length was significant ($P< 0.05$). The highest mean root length was recorded at 40% moisture level with 34.4 cm mean root length (table 8)

4.2.6 Effect of cowpea genotype on root characteristics

The effect of Genotype on root biomass (P< 0.001) was highly significant (Table 9). There was significant difference in root biomass between MS PRT and MS 1-8-1-4, which had 2.4 and 1.3 g plant^{-1} of root biomass, respectively. There was no significant difference in root biomass between LT PRT (2.2 g plant^{-1}) and LT 11-3-3-12 (2.3 g plant^{-1}). BB PRT had 2.2 g plant^{-1} root biomass which was 10% higher as compared to BB 8-1-5-2. (table 8)

Table 8: Effect of genotype, phosphorus and moisture level on root characteristics

Treatment	Root biomass (g/plant)	Root diameter (cm)	Root length (cm)
Phosphorus level (P)			
23	2.0b	0.4a	33.8a
80	2.1a	0.4a	33.1a
Moisture status (M)			
40	1.8b	0.3b	34.4a
60	2.2a	0.40a	31.9b
100	2.2a	0.40a	34.1a
Cowpea Genotype (G)			
BB 3-9-7-5	2.3b	0.46b	26.7d
BB 8-1-5-2	2.0c	0.36b	36.9b
BB PRT	2.2b	0.36b	43.4a
LT 11-3-3-12	2.3b	0.43a	39.3b
LT PRT	2.2b	0.29c	32.6c
MS 1 -8 -1-4	1.3d	0.38ab	31.4c
MSPRRT	2.4a	0.39ab	22.6e
P values			
G	***	**	***
M	***	***	*
P	**	NS	NS
G × M	***	NS	***
G× P	***	NS	***
M × P	NS	NS	NS
G × M × P	***	NS	***

Means followed by the same letter in a column are not significantly different at P ≤ 0.05; *** = Significant at P≤ 0.001, **= Significant at ≤ 0.01, * = Significant at ≤ 0.05, NS = Non-Significant.

4.2.7 Effect of phosphorus on grain yield

The effect of phosphorus on cowpea grain yield was highly significantly (P<0.001). Higher mean grain yield of 3.1g plant-1 was recorded at low P level and the lowest mean grain yield of 2.8 g plant^{-1} was recorded higher P level. (table 9)

4.2.8 Effect of water stress on grain yield

The effect of water stress on grain yield was highly significant (P<0.001). The highest mean grain yield of 3.7 g plant^{-1} was recorded at 100% water level, 2.8 g plant^{-1} mean grain yield was recorded at 60% water level and the lowest grain yield of 2.3 g plant^{-1} was recorded at 40% water level. Similar results on grain yield partitioning was obtained, on which the highest grain partitioning was also recorded at l00% water level and the lowest was at 40 % water level. (table 9)

4.2.9 Effect of water stress on pod yield

The effect of water stress on pod yield was highly significant (P< 0.001). The highest (10.8 g plant^{-1}) and lowest (7.3 g plant^{-1}) mean pod yield was recorded at 100% and 40% moisture levels, respectively. (table 9)

4.2.10 Effect of Cowpea Genotype on Yield Components

The effect of cowpea genotype on grain yield was highly significant (P<0.001). There was significant difference in yield between the parent genotype and their mutants, and also among all the genotypes. MS PRT was the highest with 5.32 g plant^{-1} of grain yield, and it performed better than MS 1 -8 -1-4 by 28%. Mutant LT 11-3-3-12 had a higher grain yield of 2.8 g plant^{-1} as compared to LT PRT with 2.7 g plant $^{-1}$. BB 8-1-5-2 had a higher grain yield of 2.3 g plant^{-1} as compared to BB PRT (2.0 g plant^{-1}) and BB 3-9-7-5 (1.8 g plant $^{-1}$). The effect of genotype on pod yield was highly significant (P< 0.001). Genotypes with higher pod yield had a higher grain yield. There was also variation among genotypes on grain yield partitioning. (table 9)

4.2.11 Interaction effect of cowpea genotypes, phosphorus and water stress on yield component

The interaction effect of cowpea genotype, P and moisture levels on grain yield was highly significant (P< 0.001). The highest mean grain yield of 48.4 g plant^{-1} and 48.3 g plant^{-1} was recorded in MS PRT and MS 1-8-1-4, respectively at high P level and 100% moisture level. At low P and water stress conditions, the genotypes MS 1- 8-1-4 had a higher mean grain yield of 19 g plant^{-1} as compared with MS PRT with 14.9 g plant^{-1}, similar results were obtained in LT 11-3-3-12 and BB 3-9-7-5 which had higher grain yields as compared to their respective parents. (Figure 8)

Table 9: Effect of genotype, phosphorus and moisture on yield parameter of cowpea

Treatment	Grain yield (g/plant)	Pod yield (g/plant)	Grain yield partitioning	Pod yield partitioning
Phosphorus level (P)				
23	3.13a	62.62a	11.98a	9.23b
80	2.77b	51.54b	11.83a	10.85a
Moisture status (M)				
40	2.32c	7.30b	8.89c	8.98b
60	2.79b	10.55a	10.99b	12.04a
100	3.76a	10.80a	15.84a	9.06b
Cowpea Genotype (G)				
BB 3-9-7-5	1.72g	5.78cd	4.81e	6.94de
BB 8-1-5-2	2.38e	4.43d	9.41c	7.08cde
BB PRT	1.98f	3.67d	6.61d	4.70e
LT 11-3-3-12	2.80c	6.86c	9.38c	8.08cd
LT PRT	2.65d	7.69c	10.94c	9.46c
MS 1 -8 -1-4	3.83b	23.53a	17.25b	20.66a
MS PRT	5.32a	14.90b	24.91a	13.26b
P Values				
G	***	***	***	***
M	***	***	***	***
P	***	NS	NS	***
G × M	***	***	***	***
G× P	***	***	***	***
M × P	***	***	***	*
G × M × P	***	***	***	***

Means followed by the same letter in a column are not significantly different at P ≤ 0.05; *** = Significant at P≤ 0.001, **= Significant at ≤ 0.01, * = Significant at ≤ 0.05, NS = Non-Significant.

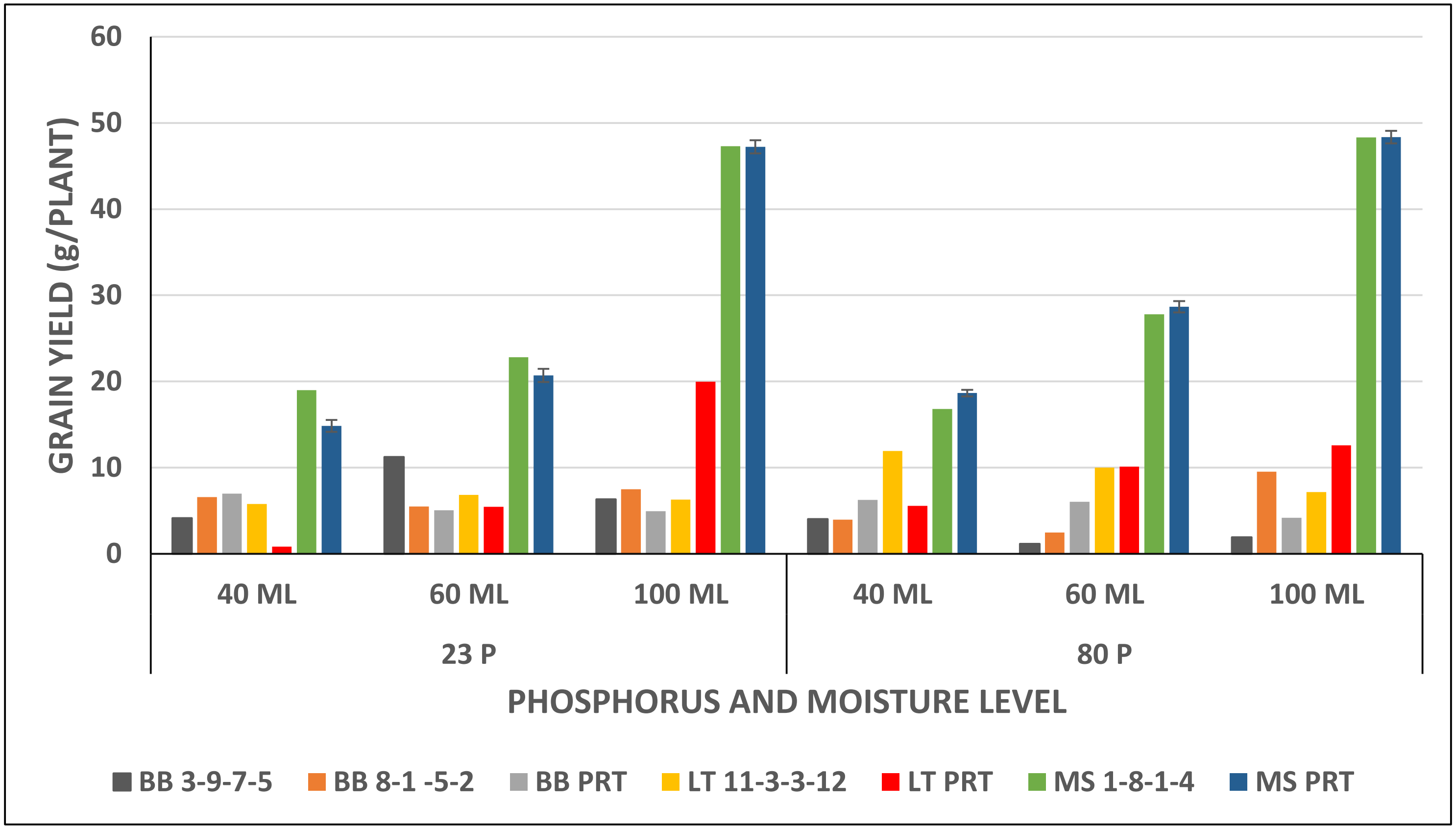

Figure 8: Interaction effect of genotype, phosphorus and moisture level on grain yield

Error bars are representing the standard error of mean (SE)

4.3. Effect of phosphorus on morpho agronomic characteristics of selected cowpea genotypes - Hydroponics Experiment

In the hydroponics experiment, the summary of the analysis of variance on all measured parameters was significant (table 10). The interaction effect of genotype and phosphorus on all measured parameters was also significant with the exception on root length which was non – significant (table 10)

Table 10: Analysis of Variance for the parameters measured in the hydroponics.

Source of Variation	df	Shoot length	Root length	No of root hairs	Shoot biomass	Root biomass
Genotype (G)	3	9.6*	37.7***	1278.2***	0.02***	0.002***
Phosphorus (P)	2	34.4***	55.0 **	1369.3***	0.01**	0.002***
G* P	6	4.4**	6.8^{NS}	353.5***	0.01***	0.001***
Error	24	0.9	3.6	14.4	0.001	0.000
Cv %		10.1	10.6	5.3	19.9	21.7
Lsd		0.28	0.53	0.27	0.33	0.25

CV= Coefficient of Variance *** = Significant at P≤ 0.001, **= Significant at ≤ 0.01, * = Significant at ≤ 0.05, NS = Non-Significant, Lsd = Least significant difference

4.3.1 Effect of phosphorus level on shoot length

The effect of P on shoot length was highly significant (P<0.001). The highest mean shoot length of 11.4 cm was recorded at 9.3 mg L^{-1} followed by mean shoot length of 9.1 cm at 3.1 mg L^{-}1 and the lowest mean shoot length was 8 cm at 0 mg L^{-1} (table 11)

4.3.2 Effect of phosphorus on Root Length

The effect of phosphorus on root length was highly significant (P< 0.001). The highest mean root length of 19.7 cm was recorded at 9.3 mg L^{-1} P level followed by mean root length of 18.5 at 3.1

mg L^{-1} and the lowest was recorded at 0 mg L^{-1} P level with mean root length of 15.5 cm. (table 11)

4.3.2.1 Effect of Phosphorus on Number of Root Hairs

The effect of P on the number of root hairs was highly significant (P<0.001). The highest mean number of root hairs was 81. 5 at 9.1 mg L^{-1} followed by 73 number of root hairs at 3.1 mg L^{-1} and the lowest mean number of root hairs was 60 at 0 mg L^{-1} (table 11)

4.3.2.2 The interaction effect of Genotype and Phosphorus on Root Biomass

The interaction effect of genotype and P on root biomass was highly significant (P<0.001). At 0 and 3.1 mg L^{-1} P levels, the mutation derived lines had a higher root biomass as compared to their respective parents, except under high P level (9.1 mg L^{-1}) on which MS PRT had a higher mean root biomass of 53 mg plant $^{-1}$ as compared to MS 1-8-1-4 (35 mg plant-^{1}) (figure 9).

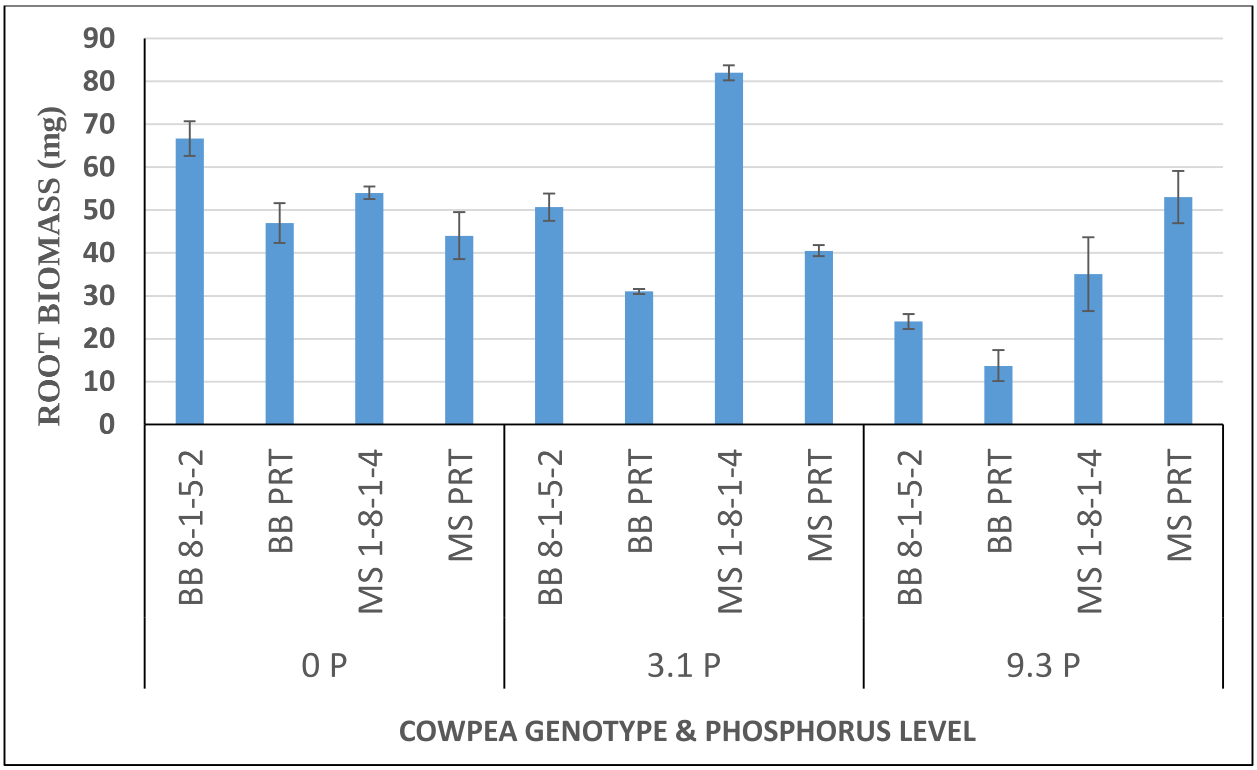

Figure 9: Effect of genotype and level of P on Root biomass in hydroponics

Error bar are representing standard error of the mean (SE)

Table 11: Means of parameters measured of cowpea genotypes in hydroponics experiment

Genotype		Shoot length (cm)			Root length (cm)			Root hairs (n)			Shoot biomass (g)		
	Phosphorus level (mg/L)	**0**	**3.1**	**9.3**	**0**	**3.1**	**9.3**	**0**	**3.1**	**9.3**	**0**	**3.1**	**9.3**
BB PRT		8.3	8.1	8.6	15.3	15.3	15.5	43.5	45.8	74.7	0.1	0.2	0.1
BB 8-1-5-2		7.2	9.3	13.3	14	17.8	19.8	61	92.3	74	0.2	0.2	0.2
MS PRT		7.7	8.4	11.3	17.2	21.5	21.5	65.7	67	88.3	0.2	0.2	0.3
MS 1-8-1-4		9.1	10.6	12.5	15.7	19.2	22	71	87	89	0.2	0.3	0.3
Means		**8**	**9.4**	**12.4**	**15.6**	**19.5**	**21.1**	**65.9**	**82.1**	**83.8**	**0.2**	**0.2**	**0.3**
LSD			**0.28**			**0.53**			**0.27**			**0.33**	

LSD = least significant difference at 5% probability Level (LSD 0.05).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Identification of cowpea genotypes tolerant to low soil p and water stress condition- Field Experiment

The experimental field used had a suboptimal soil phosphorus, and during the growing season, the cowpea genotypes were subjected to terminal water stress. Under terminal water stress condition, there could be enough water for early establishment and growth of crop, but later phenological stages are exposed to soil water deficit (Wortmann et al., 1998).

According to Hassan et al., (2005), yield is a complex trait or super character and is influenced by many component traits. Therefore, successful selection depends upon the information on the genetic variability and morpho-agronomic traits related to grain yield. Despite the aforementioned, grain yield among other factors was used as a criterion in selecting mutant derived genotypes for the pot experiment. Grain yield in cowpea is determined by the product of three components: the number of pods per plant that reach maturity, the average number of seeds in each pod and mean dry weight of individual seed (Akyeampong, 1985). Of these yield components, it has been shown that the most important is the number of pods that reach maturity (Doku, 1970). This agreed with the results that was obtained in which the highest grain yield was obtained with the highest number of pods per plant. The results were further supported by Thosago (2015) who found a significant correlation between the number of pods per plant and grain yield.

5.2. Effect of Phosphorus and water stress on morpho agronomic characteristic of cowpea genotypes - Greenhouse Experiment:

5.2.1 Effect of phosphorus level on vegetative parameters

Phosphorus is important in cowpea production because of its multiple effects on nutrition, and ultimately on growth and yield (Nesme et al., 2014). Higher shoot biomass was recorded under low P. The result disagreed with Thosago (2015) who found that an increase in P levels increased the number of branches, and also that of Nkaa et al. (2014) who indicated that application of Single Super Phosphate increased the number of branches, plant height and dry weight of cowpea and soybean plant. At higher P level, the genotypes did not show any P toxicity associated symptom such as reduced shoot biomass. Therefore, the applied P did not reach the toxicity level.

5.2.2 Effect of P level on root characteristics

Phosphorus is important for root development, but despite its importance in the soil, it only affected root biomass. Similar result was obtained by Nkaa *et al.* (2014) who found that P fertilizer application enhanced the weight of cowpea root biomass. In the experiment, genotypes that performed well under low soil phosphorus level had the highest root biomass and the lowest root length. According to Mataa et al. (2019), genotypes with shallow and adventitious root systems can forage for P more efficiently under low P soils. Therefore, identification and use of genotypes with improved root traits that can unlock and absorb P from soil P resources may be important for increasing the efficiency or utilization of applied P fertilizers (Abelson, 1999).

5.2.3 Effect of Phosphorus on Yield Components

Phosphorus is critical in cowpea production (Nesme et tal., 2014). However, higher grain yields were recorded under low phosphorus levels. The genotypes that exhibited higher yields at low P was possibly due to change in critical limits that triggered certain responses. The critical limit to P uptake might have become much lower. Another possibility was that the genotypes had a higher P foraging and were not able to utilize the high application of P to increase grain yield (Mataa *et tal*.,2019) The result may imply that some cowpea genotypes have a threshold at which they can utilize P fertilizer. Based on the findings of the study, it was economical to apply lower rates of P fertilizers to obtain higher grain yield.

5.2.4 Effect of water stress on vegetative parameters

Moisture stress significantly reduced shoot biomass, the number of branches, and plant height. The reduction in the vegetative parameters and plant growth was attributed to decrease in cellular expansion resulting from decrease in plant water content (Farooq et al., 2009). The effect of moisture stress on branching was in contradiction with Abayomi and Abidoye (2009) who showed that there was no significant effect of moisture stress on branching.

5.2.5 Effect of water stress on root characteristics

Cowpea is a hardy crop, but still suffer considerable damage from abiotic and biotic conditions. Abiotic stress such as soil water deficit affects root and nodule development, physiological and biochemical activity (Farooq, et al., 2009). Moisture affected root biomass, root diameter and root length. Some genotypes with longer roots were recorded. These genotypes showed resilience to water stress condition. Similar results were found by Scholz et al. (2002) who reported that under water limiting conditions plant roots grow longer than those in rain-fed. The result was also in support of the annual report of the Science Daily (2008) that plants growing under water stress conditions have roots that grow longer to ascent for nutrients and moisture around the growth environment. Thus, being adaptive to water stress condition.

5.2.6 Effect of water stress on yield and yield parameters

Although cowpea is regarded as drought tolerant, water stress causes a serious drop in crop grain yield and reduces the quality of the crop (Abayomi and Abidoye, 2009). The decreased grain yield due to water stress was due to reduced sink rather than the source (Abayomi and Abidoye, 2009). There was reduction in pod density as water stress exacerbates the loss of immature pods (Shonse et al., 1981). The result agreed with Abayomi and Aderoru (2000) who reported that water stress conditions significantly reduced grain yield and growth at any stage in cowpea.

5.2.7 Effect of Genotype on Vegetative Parameter

Genotypes showed a variation in shoot biomass and the number of branches. A similar result was obtained by Ali et al. (2009) who showed that significant differences in several branches per plant were a result of varietal differences in cowpea. The higher the number of branches of a plant, the higher the chances to obtain a higher grain yield. Boutraa (2009) and Liao et al. (2004) suggested that improved PUE is related to the capacity of the plant to accumulate dry matter despite the inadequacy of soil P. The genotypes with higher shoot biomass are good for the duo purpose of fodder for livestock and soil improvement.

5.2.8 Effect of Cowpea Genotype on Yield Components

According to de Ronde and Spreeth, (2007) much variation occur within the cowpea genotypes. There was variation in genotypes on pod and grain yield as well as on their respective partitioning. Genotypes with higher grain yield had a higher grain partitioning. The results agreed with Nkaa *et al*. (2014) who found a significant effect on yield between three cowpea varieties tested but contradicted with Singh *et al*. (2011) who reported that cowpea variety had no significant effect on the grain yield.

During the vegetative and reproductive growth phases of the cowpea, mean higher temperatures of 41℃ and a mean low Relative Humidity (RH) of 37% was recorded in the greenhouse. This affected the grain yield performance of some genotypes by causing flower abortion and non-pod settings. Thus, reducing on the major yield components of cowpea (Doku, 1970). The results also provided some indication of tolerance of some cowpea genotypes to heat. The genotype MS 1-8-1-4 exhibited some compensatory growth characteristic, a morphological trait of delayed leaf senescence (Gwathmey *et al.,* 1992. This was observed when the genotype MS 1-8-1-4 rejuvenated the reproductive growth phase when conditions became favourable after almost drying. Therefore, screening for heat tolerance should also be considered, and Lobell et al. (2012) provided evidence that developing crops to adapt to high temperatures should be a top priority for plant physiologists and crop breeders.

5.2.9 Interaction Effect of Genotype, Phosphorus and Moisture level on Yield Components

The simultaneous occurrence of low soil phosphorus and water stress condition, and their interactions thereof had a profound effect on the yield components. The results also agreed with the study by Magani and Kuchinda (2009), who found a significant interaction between cowpea varieties and P level on cowpea grain yield.

5.3. Effect of phosphorus on morpho agronomic characteristics - Hydroponics Experiment

The hydroponics results confirmed the variation in root biomass between the selected mutation derived lines and their respective parents. It also helped to explain relationship phosphorus and root hairs which was very difficult to observe in the field and pot experiments. Hence helped to consolidate with the findings from the green house experiment. Phosphorus had an increasing effect on root hairs and that confirmed with the findings by Nkaa al tal. (2014) who reported that P application increases the number of root hairs and nodulation in cowpea.

CHAPTER SIX

6.0 CONCLUSION

Cowpea varieties that are resilient to low phosphorus and water stress were identified in the study. The genotypes MS 1-8-1-4, BB 3-9-7-5 and LT 11-3-3-12 performed better under low soil phosphorus and water stress condition. The new genotypes will be less costly to produce especially by small -scale farmers who are in drought-prone areas, and soils that are depleted of phosphorus.

There was sufficient variation in the morpho agronomic characteristic of the cowpea genotypes. The genotypes exhibited different morpho agronomic characteristics in order to be adaptive to low soil phosphorus and water stress conditions. Water stress had a more depressing effect on most measured parameters as compared to phosphorus.

The study also contributed to the release of BB 8-1-5-2 as a mutation derived cowpea variety called Lunkwakwa.

6.1 RECOMMENDATION

Given the importance of cowpea, and in ensuring food and nutritional security, the identified cowpea genotypes can be directly released as new varieties or used as parents in breeding programmes for improved cowpea production. There is need to further develop and select available germplasm for improved yields and nutrition. Therefore, researchers and plant breeders should make efforts to identify cowpea varieties with improved nutritional content, enhanced levels of drought and heat tolerance and high biological nitrogen fixation, among other important traits.

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APPENDICES

Appendix 1: Analysis of Variance table for Grain Yield for field Experiment

Source of Variation	D.F.	S.S.	M.S.	V.R.	F pr.
Rep	2	8381	4191	0.54	
Genotype	23	1280112	556570	72.15	<.001
Error	42	323976	7714		
Total	67	1254659			

Appendix 2: Analysis of Variance table for Number of pods/Plant for field Experiment

Source of variation	D.F.	S.S.	M.S.	V.R.	F pr.
Rep	2	6.53	3.27	2.16	
Genotype	23	1617.20	70.31	3.61	<.001
Error	42	63.56	1.51		
Total	67	1034.02			

Appendix 3: Analysis of Variance table for Hundred Seed Weight for field Experiment

Source of Variation	D.F.	S.S.	M.S.	V.R.	F pr.
Rep	2	6.62	3.31	1.76	
Genotype	23	313.02	13.61	7.24	<.001
Error	42	79.00	1.89		
Total	67	334.68			

Appendix 4: Analysis of Variance table for Number of Seed per Pod for field Experiment

Source of Variation	D.F.	S.S.	M.S.	V.R.	F pr.
Rep	2	3.78	1.89	0.59	
Genotype	23	372.58	16.20	5.02	<.001
Error	42	135.61	3.03		
Total	67	453.05			

Appendix 5: Analysis of Variance table for Pod Length for field Experiment

Source of Variation	D.F.	S.S.	M.S.	V.R.	F pr.
Rep	2	6.69	3.34	3.71	
Genotype	23	132. 86	5.78	6.40	<.001
Error	42	37.88	0.9888		
Total	67	161.85			

Appendix 6: Analysis of Variance table for Pod Yield for Green House Experiment

Source	Df	SS	MS	F value	Pr (>F)
Genotype					
G	6	5578.7	929.78	946.2861	< 2.2e-16 ***
Water W	2	320.3	160.17	163.0131	< 2.2e-16 ***
Phosphorus					
P	1	0.3	0.29	0.2928	0.5899
G* W	12	2067.5	172.29	175.3471	< 2.2e-16 ***
G* P	6	279.9	46.65	47.4787	< 2.2e-16 ***
W: P	2	26	13	13.2292	1.012e-05 ***
G* W*P	12	695.6	57.96	58.9918	< 2.2e-16 ***
Residuals	84	82.5	0.98		

Appendix 7: Analysis of Variance table for Plant Biomass for Green House Experiment

Source	Df	SS	MS	F value	Pr (>F)
Genotype	6	5880.5	980.1	104.561	< 2.2e-16 ***
Water	2	1615.4	807.7	86.172	< 2.2e-16 ***
Phosphorus	1	3510.4	3510.4	374.509	< 2.2e-16 ***
G* W	12	8154.1	679.5	72.493	< 2.2e-16 ***
G* P	6	4253.7	708.9	75.634	< 2.2e-16 ***
W* P	2	1321.9	661	70.515	< 2.2e-16 ***
G*W*P	12	9193.2	766.1	81.732	< 2.2e-16 ***
Residuals	84	787.4	9.4		

Appendix 8: Analysis of Variance table for Stem Diameter for Green House Experiment

Source	Df	SS	MS	F value	Pr (>F)
Genotype	6	0.3775	0.06291	0.6623	0.680205
Water	2	4.5892	2.2946	24.1562	5.158e-09 ***
Phosphorus	1	0.2074	0.20741	2.1835	0.143234
G* W	12	2.9087	0.24239	2.5518	0.006323 **
G* P	6	0.2938	0.04897	0.5156	0.79501
W* P	2	0.4254	0.2127	2.2392	0.112869
G*W*P	12	0.6735	0.05612	0.5908	0.84387
Residuals	84	7.9792	0.09499		

Appendix 9: Analysis of Variance table for Root Diameter for Green House Experiment

Source	Df	SS	MS	F value	Pr(>F)
Genotype	6	0.2132	0.035534	3.7348	0.002425 **
Water	2	0.21878	0.109391	11.4976	3.859e-05 ***
Phosphorus	1	0	0	0	0.997114
G* W	12	0.17776	0.014814	1.557	0.120489
G* P	6	0.08922	0.01487	1.5629	0.168129
W* P	2	0.03422	0.017109	1.7982	0.171913
G* W*P	12	0.15724	0.013104	1.3773	0.193071
Residuals	84	0.7992	0.009514		

Appendix 10: Analysis of Variance table for Plant Height for Green House Experiment

Source	Df	SS	MS	F value	Pr(>F)
Genotype	6	5456	909	2.2203	0.0490 *
Water	2	82654	41327	100.9009	<2e-16 ***
Phosphorus	1	388	388	0.9469	0.3333
G* W	12	7606	634	1.5475	0.1239
G*P	6	1774	296	0.7218	0.6331
W*P	2	1082	541	1.3205	0.2726
G*W*P	12	7406	617	1.5068	0.1382
Residuals	84	33995	410		

Appendix 11: Analysis of Variance table for Number of Branches for Green House Experiment

Source	Df	SS	MS	F value	Pr (>F)
Genotype	6	21.937	3.656	3.0558	0.009395 **
Water	2	121.333	60.667	50.7065	3.613e-15 ***
Phosphorus	1	0.149	0.149	0.1248	0.724723
G * W	12	34.089	2.841	2.3744	0.010947 *
G * P	6	10.199	1.7	1.4208	0.216283
W * P	2	7.584	3.792	3.1695	0.047093 *
G * W *P	12	21.422	1.785	1.4921	0.143356
Residuals	84	100.5	1.196		

Appendix 12: Analysis of Variance table for Grain Yield for Green House Experiment

Source	Df	SS	MS	F value	Pr (>F)
Genotype	6	10992.8	1832.14	2188.215	< 2.2e-16 ***
Water	2	3190.6	1595.31	1905.353	< 2.2e-16 ***
Phosphorus	1	50.6	50.58	60.405	1.731e-11 ***
G * W	12	6300.7	525.06	627.1	< 2.2e-16 ***
G * P	6	1095.1	182.51	217.981	< 2.2e-16 ***
W* P	2	33.2	16.59	19.816	8.915e-08 ***
G * W *P	12	742.1	61.84	73.862	< 2.2e-16 ***
Residuals	84	70.3	0.84		

Appendix 13: Analysis of Variance table for Root Length for Green House Experiment

Source	Df	SS	MS	F value	Pr(>F)
Genotype	6	5406.5	901.08	51.4721	< 2.2e-16 ***
Water	2	128.9	64.43	3.6806	0.029461 *
Phosphorus	1	22.7	22.73	1.2982	0.257854
G * W	12	2153.9	179.5	10.2533	5.549e-12 ***
G * P	6	478.7	79.78	4.5574	0.000489 ***
W * P	2	63.3	31.65	1.808	0.170463
G * W* P	12	1093.3	91.11	5.2042	2.017e-06 ***
Residuals	84	1435.5	17.51		

Appendix 14: Analysis of Variance table for Grain Yield Partitioning for Green House Experiment

Source	Df	SS	MS	F value	Pr(>F)
Genotype	6	5209	868.17		< 2.2e-16 ***
Water	2	1068.6	534.32	554.9111	< 2.2e-16 ***
Phosphorus	1	0.3	0.29	0.2988	0.5861
G *W	12	3106.6	258.88	268.858	< 2.2e-16 ***
G* P	6	1445.4	240.9	250.1788	< 2.2e-16 ***
W *P	2	29.2	14.61	15.175	2.365e-06 ***
G * W *P	12	1039.9	86.65	89.9939	< 2.2e-16 ***
Residuals	84	80.9	0.96		

Appendix 15: Analysis of Variance table for Root Biomass for Green House Experiment

Source	Df	SS	MS	F Value	Pr(>F)
Genotype (G)	6	683.82	113.971	63.572	< 2.2e-16 ***
Water (W)	2	424.25	212.126	118.3224	< 2.2e-16 ***
Phosphorus (P)	1	0.08	0.077	0.0428	0.8366
G* W	12	609.22	50.768	28.3182	< 2.2e-16 ***
G* P	6	580.88	96.814	54.0019	< 2.2e-16 ***
G*W*P	12	196.22	16.351	9.1206	5.927e-11 ***
Residuals	84	150.59	1.793		

Appendix 16: Analysis of Variance table for Number of Root Hairs for Hydroponics Experiment

Source of variation	D.F.	S.S.	M.S.	V.R.	F pr.
Genotype	3	3834.53	1278.18	88.92	<.001
Phosphorus	2	2738.51	1369.26	95.25	<.001
Genotype*. Phosphorus	6	2120.76	353.46	24.59	<.001
Residual	24	345.00	14.38		
Total	35	9038.81			

Appendix 17: Analysis of Variance table for Root Biomass for Hydroponics Experiment

Source of variation	D.F.	S.S.	M.S.	V.R.	F pr.
Genotype	3	0.00321958	0.00107319	11.19	<.001
Phosphorus	2	0.00340362	0.00170181	17.74	<.001
Genotype * phosphorus	6	0.00465499	0.00077583	8.09	<.001
Residual	24	0.00230250	0.00009594		
Total	35	0.01358069			

Appendix 18: Analysis of Variance table for Root Length for Hydroponics Experiment

Source of variation	D.F.	S.S.	M.S.	V.R.	F pr.
Genotype	3	113.024	37.675	10.46	<.001
Phosphorus	2	110.195	55.097	15.29	<.001
Genotype * phosphorus	6	40.541	6.757	1.88	0.127
Residual	24	86.460	3.602		
Total	35	350.220			

Appendix 19: Analysis of Variance table for Shoot Biomass for Hydroponics Experiment

Source of variation	D.F.	S.S.	M.S.	V.R.	F pr.
Genotype	3	0.057318	0.019106	19.02	<.001
Phosphorus	2	0.014968	0.007484	7.45	0.003
Genotype * Phosphorus	6	0.047939	0.007990	7.95	<.001
Residual	24	0.024106	0.001004		
Total	35	0.144330			

Appendix 20: Analysis of Variance table for Shoot Length for Hydroponics Experiment

Source of variation	D.F.	S.S.	M.S.	V.R.	F pr.
Genotype	3	28.9076	9.6359	10.36	<.001
Phosphorus	2	68.7587	34.3794	36.97	<.001
Genotype*. Phosphorus	6	26.5403	4.4234	4.76	0.003
Residual	24	22.3173	0.9299		
Total	35	146.5239			