UNIVERSITY OF CAPE COAST

GENETIC VARIATION IN RHIZOSHEATH, ROOT HAIR, ROOT SYSTEM ARCHITECTURE AND PHOSPHORUS USE EFFICIENCY AMONG COWPEA GENOTYPES (*Vigna unguiculata* (L) Walp)

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BY

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A thesis submitted to the Department of Crop Science of the School of Agriculture, College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Master of Philosophy degree in Crop Science.

SEPTEMBER 2020

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature: ..... Date: .....

Name: ……

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised per the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature: ..... Date: .....

Name: …….

Co-Supervisor's Signature: ..... Date: .....

Name: ……

ABSTRACT

Cowpea (*Vigna unguiculata* (L) Walp) is an economically important legume crop of vital importance to the livelihood of several millions of people. Even so, cowpea yields on African farmers' fields are still below the potential yield of the crop. This is, largely, as a result of the use of unimproved genotypes and farming on phosphorus (P)-poor soils, which are pervasive in the tropics. Plants have evolved several strategies to obtain adequate P for their growth under P limiting conditions. These strategies include modification of root system architecture (RSA) and longer root hairs. Root hairs can be measured using rhizosheath, sheet of soil retained on roots after excavation and shaking. Breeding for cowpea genotypes with superior RSA, rhizosheath and root hair traits will help in achieving food security. The variation in rhizosheath, RSA and root hair were quantified among sixty (60) cowpea genotypes grown up to 21 d under greenhouse conditions. Twenty (20) genotypes were selected for further screening on three (3) external P concentrations $[P]_{ext}$ (i.e.: 0, 250 and 500 mg P/kg soil). Analyses were conducted on rhizosheath, root hair, RSA and biomass traits. The cowpea genotypes were also analysed for variation in P use efficiency (PUE) parameters including agronomic P use efficiency (APE), P uptake efficiency (PUpE), and P efficiency ratio (PER). There were genetic variations among cowpea genotypes in almost all the traits examined. Genotypes with longer root hairs produced larger rhizosheath mass compared to genotypes with larger root hair density. Increasing $[P]_{ext}$ resulted in a significant increase in rhizosheath mass, root hair density, biomass, shoot P concentration and content except for root hair length, which was reduced with increasing $[P]_{ext}$. Substantial variation was observed for shoot-P, root-P and various measures of PUE among the cowpea genotypes. Some genotypes, including MU9, IT91, Sunshine and WC10*WC36 developed longer root hairs under low P conditions and these were categorised as P-efficient genotypes. Root system and root hair traits including root hair density, root hair length and total root length correlated with PUE in cowpea. The results could be used to select for cowpea -genotypes with improved PUE for use on P-poor soils and provide potential materials and targets for breeding new cowpea cultivars better adapted to P-poor soils in Ghana.

KEYWORDS

Cowpea

Rhizosheath

Root hairs

Root system architecture

Phosphorus use efficiency

Genotypic variation

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DEDICATION

This work is dedicated to my uncle Mr Ben Ankomah and his wife Mrs Mary Ankomah, who in their limited financial capacity has assisted me throughout my education since I lost my father. God bless anybody who has once made an orphan or a needy smile and realized their aspirations.

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LIST OF ACRONYMS

AL	Aluminum
APE	Agronomic Phosphorus Use Efficiency
ARW	Absolute Rhizosheath Weight
CEC	Cation Exchange Capacity
KH_2PO_4	Potassium dihydrogen phosphate
LMA	Leaf Mass per Area
MT	Metric Tons
N	Nitrogen
P	Phosphorus
$[P]_{ext}$	External P source
PAE	Phosphorus Acquisition Efficiency
PCA	Principal Component Analysis
PER	Phosphorus Efficiency Ratio
PUE	Phosphorus Use/Utilization Efficiency
PUpE	Phosphorus Uptake Efficiency
PUtE	Phosphorus Utilization Efficiency
PPUE	Physiological Phosphorus Use Efficiency
RFW	Root Fresh Weight
RDW	Root Dry Weight

RHD	Root Hair Density
RHL	Root Hair Length
RRW	Relative Rhizosheath Weight
RFW	Root Fresh Weight
RSA	Root System Architecture
SDW	Shoot Dry Weight
SFW	Shoot Fresh Weight
SLA	Specific Leaf Area
SRW	Specific Rhizosheath Weight
TLA	Total Leaf Area
TRL	Total Root Length

CHAPTER ONE

INTRODUCTION

Background to the study

Cowpea (*Vigna unguiculata* (L) Walp) is essential food security and income generation crop grown in the tropics and sub-tropics (Carlos, 2000; Tharanathan & Mahadevamma, 2003). Cowpea is cultivated mostly for leaves, green pods, grain, and haulm used as animal feed. The leaves have dietary importance and can be served as a vegetable crop at all stages of development (Ahenkora *et al.*, 1998). Also, the capacity to fix atmospheric nitrogen is highly useful when cowpea is grown in a rotation sequence with other crops (Timko *et al.*, 2007). Despite the role cowpea plays in achieving global food security and poverty reduction (Coulibaly & Lowenberg-deboer, 2014), the production of the crop is challenged by increasing marginal soils, climate instability and low soil fertility (Lynch, 2007b; Wortmann, 1998) especially low P levels of most tropical soils (Sanginga *et al.*, 2000). A gap between the actual and potential yield of cowpea has been reported (Adu *et al*., 2019). Farm level yields of cowpea on area basis have remained low (600 - 800 kg ha^{-1}) compared to research fields (1600 - 2500 kg ha^{-1}) (Yirzagla et al., 2016). Hence, improving the yield of crops such as cowpea would be a significant tool in achieving food security goals (López-Arredondo *et al.*, 2015).

Phosphorus (P) is an important macro element needed for plant growth and development (Brown *et al*., 2012). It forms an essential part of nucleic acids, phospholipids, and ATP molecules (Schachtman *et al.*, 1998) and, therefore, performs a critical function in the morphology, physiology of plants (Theodorou & Plaxton, 1993). Adequate quantity of P is required for cell division in young

shoot and root tissues of plants. It promotes the initiation of flower and enhances seed and fruit development (Ndakidemi & Dakora, 2007). A suitable quantity of P enhances the growth of root and shoot, early maturity, water use efficiency, and grain yield (USDA, 1994).

Despite the relevance of P in crop productivity, 40 per cent (approximately 2 billion hectares) of the world's agricultural land are P deficient (Vance *et al.*, 2003). Cochran *et al.* (1986) suggested that 86% of tropical soils have low P concentrations (< 7 parts per million) in the soil horizon.

Phosphorus is absorbed predominantly in the form of orthophosphates (H_2PO^{4-} and HPO_4^{2-}) by plant roots (Schachtman *et al*., 1998). However, the concentration of these ions in the soil solution is low and seldom exceeds 10 µM (Schachtman *et al*., 1998). Also, diffusion of P ions to plant roots in the soil solution is generally hindered because diffusion is a significant controlling factor that moves the process (Syers *et al.*, 2008; Marschner, 2011). Consequently, inadequate supply of P poses considerable constraints on plant growth and productivity (Busman *et al.*, 2006). Low P level accounts for a 50% reduction in crop yield in both natural and agricultural ecosystems (Vitousek *et al*., 2009). External P application has been used as an option to overcome P deficiency, however, up to 15 - 30 per cent of applied fertilizer P is absorbed by plants in the year of its application (Syers *et al*., 2008). This marginal effect is due to the fixation of P by Iron (Fe) and Aluminum (Al) oxides in most soils (Sample *et al.*, 1980).

Many plant species have evolved numerous mechanisms that improve their ability to absorb soil P (Vance *et al*., 2003; White *et al.*, 2005). According to White *et al*. (2013), such mechanisms include root hair development,

developing suitable root system architecture, symbiotic associations and improving rhizosphere conditions. Among these adaptive mechanisms, root hair production and rhizosheath formation are of unique importance in accessing resources (Bailey & Scholes, 1997; McCully, 1999; White *et al*., 2013; George *et al*., 2014). Root hairs are tubular extensions on plant roots that enhance plant-soil contact considerably and thus increase the acquisition of soil nutrients (James *et al*., 2016). It provides a physical structure for rhizosheath expansion (Brown *et al*., 2012; George *et al*., 2014; Haling *et al*., 2014).

Rhizosheath is defined as a unique case consisting of soil particles intertwined with roots resulting from the intertwining of prolific epidermal hair formed by the roots (Bailey & Scholes, 1997). Thus, the mass of persistent soil coating encasing the roots on which they occur (Bailey & Scholes, 1997; George *et al*., 2014). Several factors influence rhizosheath formation. These include root system traits such as root hair length, density and morphology (Haling *et al*., 2010), root and microbial mucilage (Barré & Hallett, 2009), soil water content (Watt *et al*., 1994), soil texture (Haling *et al*., 2014), mycorrhizal fungi (Moreno-Espíndola *et al*., 2007) and free-living bacteria (Unno *et al*., 2005). It has also been shown that both the root hair size and the formation of rhizosheath affect soil – water relation (Young, 1995), tolerance to phosphorus and zinc deficiency (Brown *et al*., 2012; Haling *et al*., 2013). Rhizosheath is reported to enhance tolerance to hard soils, water deficit and soil acidity due to aluminium-oxides (Brown *et al*., 2012).

Breeding more efficient roots is increasingly recognized as a high-priority target to achieve yield improvements (Araus & Cairns, 2014) since roots are important plant element for nutrient acquisition and water uptake

(Beebe *et al*., 2006). Despite several observational studies on rhizosheath, there has been a lack of work in legumes (Delhaize *et al.*, 2012). This study is designed therefore to screen for variability in the rhizosheath and root system architecture among cowpea populations as well as the impact of phosphorus on these features.

Statement of the problem

Cowpea is an important grain legume grown in tropical and subtropical regions (Tan *et al*., 2012). The crop serves as a key source of dietary protein, which nutritionally complements staple cereals and tubers with low protein content. Cowpea is a reliable revenue and income-generating crop for both farmers and traders (Langyintuo *et al*., 2003). The crop is of paramount importance in farming systems worldwide because of its capacity to restore soil fertility for later cereal plants grown in a rotational scheme (Sanginga *et al*., 2003). Despite the significance, the yield potential of cultivated cowpea is not achieved owing to low soil fertility especially P (Osodeke, 2005). Low availability of soil P significantly affects plant growth (Vitousek *et al*., 2009) since P boost development, initiate nodule formation and improves rhizobium-legume symbiosis (Haruna *et al.*, 2011).

Currently, cowpea production in many regions relies heavily on P fertilizers. However, an average of about 70 – 90 per cent of applied P is fixed in multiple low-solubility soil P complexes (Holford, 1997; McBeath *et al*., 2012). The concentration of soil P could be high, but it's uptake and utilization by crops cause an important nutritional constraint to the growth of plants (Bates, Terence & Lynch, 2000). In addition to the above, global P reserves are

predicted to decline in the next 50 to 100 years (Smil, 2000) due to the present mining and consumption rate of P reserves.

Plants have developed characteristics that contribute to better utilization of accessible soil P and mobilize P from less accessible soil P fractions. These mechanisms involve changes to the root hair growth and structure, the production of root exudates and soil microbe interactions (White & Hammond, 2008). Root hair is described by Gahoonia *et al*., (2001) as one of the important traits of plants essential for P acquisition in the soil. A significant correlation between the length of root hair and specific weight of rhizosheath in cereals has been reported (Delhaize *et al*., 2012).

In cereals, rhizosheath and root hairs have been shown to present a prospect characteristic for long term sustainability in nutrient deficiency and drought conditions (Brown *et al.*, 2012; Adu *et al*., 2017). However, limited literature covers the presence of such a trait in legumes. Significant genetic diversity has been identified in cowpea for architectural features of the root system responsible for growth in nutrient-poor and dry environments (Singh & Matsui, 2002; Krasilnikoff *et al.*, 2003; Matsui & Singh, 2003). Hence, exploring genetic variation in root hair, rhizosheath and RSA traits among cowpea will serve as a prominent tool for breeding for improved resource acquisition and use efficiency.

Justification

The world population is expected to reach nine billion by 2050 (George *et al*., 2014) however, resources to support this population are finite (White *et al*., 2013). Improving soil fertility will play a vital role in meeting the food

demand by this escalating world population (Byrnes & Bumb, 1998). Besides, adequate P supply leads to increase grain production, high-quality crop, greater stalk strength, more root growth and early crop maturity (Douglas & Philip, 2002), thus it is essential for achieving and maintaining food security.

Most tropical soils are deficient in P despite its importance in crop production (Osodeke, 2005). Low soil P availability accounts for poor food production situation in most African soils (Krasilnikoff *et al*., 2003). Approximately, one-third of the total arable land has insufficient P for sustainable crop production (Vance *et al*., 2003). As a result, external P is used to compensate for a limited P level. However, numerous concerns are related to global P fertilizer use such as; finite nature of P reserves (White *et al*., 2013), reaction with Fe- and Al-oxides to form insoluble complexes (Sample *et al*., 1980), contamination by cadmium and other heavy metals (van de Wiel *et al*., 2016) and eutrophication (Gaxiola *et al*., 2011).

Plant roots have evolved different adaptations to enhance abiotic stress tolerance and the acquisition of soil resources (White *et al*., 2013). Such mechanisms include; growth of root hairs and alteration of root system architecture (White *et al*., 2013) and beneficial symbiotic association (Ho, 2004). Root hairs are essential root traits for the acquisition of resources (water and nutrients) and tolerance to abiotic stress (George *et al*., 2014). The morphology of root hair is important in rhizosheath formation (Haling *et al*., 2010). Both the root hairs and rhizosheath influence soil – water relation (Young, 1995), prevents P and zinc (Zn) deficiencies (Brown *et al*., 2012; Haling *et al*., 2013) and enhances tolerance to hard soils, water deficit and Al-induced acidic soil condition (Brown *et al*., 2012).

Reports on genetic variation in cowpea genotypes for RSA traits related to growth in poor resource environments (Krasilnikoff *et al*., 2003; Matsui & Singh, 2003) in other crops present an opportunity for exploiting such variation to develop cowpea genotypes with superior adaptation to low P soils (Lynch, 1998). Also, root characteristics accountable for yield variability has been noted to induce the greatest capacity for evergreen revolution (Tester & Langridge, 2010; Gregory & George, 2011). The choice of plant cultivars with improved root development would, therefore, be a strategy to increase P uptake and grain output, particularly in tropical and subtropical settings where P deficiency is a major challenge to crop production.

Objective(s)

General objective

The research aims to contribute to the achievement of food security by generating knowledge that will provide the fundamental basis for breeding P efficient cowpea cultivars.

Specific objectives

Specifically, the research seeks to:

1. determine genotypic variation in rhizosheath, root hair and RSA traits among cowpea genotypes.
2. evaluate the effect of external P on rhizosheath, root hair and RSA traits among cowpea genotypes.
3. evaluate the effect of external P on biomass production among cowpea genotypes.
4. determine P use efficiency among cowpea genotypes.

Research hypothesis

The hypothesis tested by the study were:

1. Rhizosheaths are pronounced in grain legumes such as cowpea.
2. Variations exist among cowpea lines in rhizosheath, root hair and RSA traits.
3. External P application influences rhizosheath, root hair and RSA traits.
4. External P application influences the yield of biomass among cowpea genotypes.

CHAPTER TWO

LITERATURE REVIEW

Origin and distribution of cowpea

Cowpea (*Vigna unguiculata*) is cultivated throughout the tropics and subtropics around the world. The domestication of cowpea is dated in the Neolithic times (Summerfield *et al.*, 1980). Cowpea is thought to have originated from Africa, however inadequate archaeological evidence makes this hypothesis debatable (Johnson, 1970; Tindall, 1983; Coetzee, 1995). Cowpea is believed to have been introduced to India and other neighbouring countries from Africa between 2000 to 3500 years ago (Allen, 1983) from where it reached Europe before 300 BC. Padulosi and Ng (1997) reported that slave trade during the early 18th century resulted in the introduction of cowpea from West Africa to the southern part of the USA. Another opinion was that the Republic of South Africa's Transvaal area was the *V. unguiculata* centre of origin due to the existence of the most primitive wild species (Padulosi & Ng, 1997).

According to Ng (1995), there was a modification in the growth habit of *V. unguiculata* from the perennial growth habit to annual reproduction and predominantly inbreeding during evolution. However, cultivated cowpea (subsp. unguiculata) developed through annual wild cowpea (var. dekindtiana) domestication and selection. Despite these speculations, the variety of geographic distribution of dekindtiana in sub-Saharan Africa, indicates that the species could have been cultivated in any portion of the region. Cultivation centre of maximum diversity is situated in West Africa, notably Nigeria's savannah region, southern Nigeria, part of Burkina Faso, northern Benin, Togo, and north-eastern Cameroon (Padulosi & Ng, 1997). The carbon dating of wild

cowpea remains in Ghana within the rock protection in the central region reported that the oldest archaeological evidence of cowpea is found in Africa (Flight *et al.*, 1976).

Botanical description of cowpea

Cowpea is an annual herbaceous hot season grain legume with a large morphological diversity. Cowpea is reported to be autogamous, however, 5 per cent of out-crossing was recorded in the cultivated varieties due to insect activities (Fery, 1985; Badiane *et al.*, 2014). Cowpea has different growth habit, ranging from the prostate (trailing), semi-prostate, semi-erect, erect, and climbing. The growth habit of cowpea is influenced by genotype, photoperiods and conditions of development (Timko *et al.*, 2007). Depending on cultivars, cowpea can grow up to 2 m in height.

Germination of cowpea is epigeal with the first couple of true leaves being simple. At the seedling stage, the first leaves above the seed leaves are simple and opposite. Succeeding leaves are alternate and trifoliate with the terminal leaflet often longer than the two asymmetrical laterals (Steele & Mehra, 1980). Leaflets are linear, lanceolate, or broadly or narrowly elliptic, entire or obscurely toothed, broadly cuneate or rounded at the foot and gradually tapering to a pointed crest (Owusu, 2015a). The leaves are mostly oval-shaped (6-15 cm long and 4-11 cm wide) (Steele & Mehra, 1980). The petiole is strong, grooved, and 5-25 cm long. The stem is striated, smooth or mildly hairy and laced with purple at times (Summerfield *et al*., 1980).

Most cultivated cowpeas have indeterminate growth habit and the ability to generate numerous flowers (Gwathmey *et al.*, 1992). Flowers appear at the

lateral part of lengthy peduncles in alternate pairs on racemes, generally with two flowers per inflorescence. Flowers generally dwindle and collapse within a short period after blossoming (Ige *et al.*, 2011). Corollas may consist of purple, mauve-pink, yellowish, or white colours. Flowers are mostly 2-3 cm in diameter, with a straight cap, diadelphy stamens. The ovary is a sessile ovary with many ovules and an oblique stigma (James & Robert, 2002).

The peduncle usually grows two or three pods with variation in the size, shape, colour and texture of the pods (Timko *et al*., 2007). Nevertheless, the peduncles are cylindrical but may be smooth, mildly distorted, bent or coiled, and when matured, the colour may differ from yellow to brown or dark purple (Danso, 2017). The pod length is normally 10 - 25 cm. However, sub-species called sesquipedalis (found in Asia), which is frequently used as green beans can grow 40 - 100 cm long (Timko *et al*., 2007).

The colour of cowpea seeds varies from white, cream, green, yellowish-brown, red, brown, or black and maybe kidney, ovoid, crowder, globose or rhomboid-shaped, distinguished by distinct pigmentations around the hilum (Danso, 2017). 100 seed weight of cowpea varies from 1 g in some wild species to 34 g in cultivars (Steele & Mehra, 1980).

Importance of cowpea

Cowpea is a reliable crop for food security in the semi-arid and forest border regions of West and Central Africa (Skerman, 1988). It is a very significant and reliable crop that provides farmers and traders with revenue (Langyintuo *et al*., 2003). Cowpea is mostly cultivated by subsistence farmers

for grain and leaves which are important sources of food because of their rich protein, mineral and vitamin content.

Cowpea plays a central function in the nutrition of many people in developing countries (Diouf & Hilu, 2005; Nielsen *et al.*, 1993). The grain of cowpea is referred to as a poor man's meat due to rich protein (~30%) and carbohydrate (50 - 60%) content (Diouf & Hilu, 2005). Cowpea grain is an excellent source of minerals, vitamins and amino acids (lysine and tryptophan) (Timko & Singh, 2008). Dry leaves of cowpea contain 3.0 - 6.7 mg/g phosphorus, 0.3 - 1.5 mg/g ascorbic acid and 27 - 35 per cent protein (Ahenkora *et al*., 1998).

Cowpea plays a substantial role in farming systems when cultivated in rotation cropping systems due to its ability to fix atmospheric nitrogen for successive crops (Sanginga *et al*., 2003). This increases the flow of nitrogen in the cropping scheme. Cowpea is a suitable green manure crop due to its early establishment and fast ground cover to reduce soil erosion (Davis *et al*., 1991). Some genotypes of cowpea can minimize the germination of parasitic weeds such as *Striga hermonthica*, which is a major pest of most cereal crops (Singh & Matsui, 2002). Some cowpea genotypes can decrease the replication of parasitic nematodes including *Scutellonema cavenssi* that can attack pearl millet, sorghum and peanut (Hall *et al*., 2003).

Cowpea is used as livestock feed particularly in preparing high-quality legume hay during the drought periods when animal feed is scarce. The crop is therefore important in both farming and animal production systems particularly in Africa (Ortiz & Crouch, 2001). Cowpea haulms contain 13 to 17 per cent of crude protein content with high digestibility and low fibre. The nutritional

content makes cowpea fodder a good protein supplement to cereal stalks for sustainable livestock production (Tarawali *et al.*, 1997).

World production of cowpea

An estimated area of 12 million hectares was under cultivation worldwide in 2013 (Singh & Matsui, 2002; FAOSTAT, 2015). Cowpeas are cultivated annually in tropical and subtropical areas as a warm-season crop (Hall, 2003) in most sub-Saharan African nations as well as in Asia, South America, Central America, Caribbean, United States and the Mediterranean. Among various developed countries, the United States remains the leading producer and exporter of cowpea (Tettey, 2017), exporting approximately 2 000 tons per year of very high-quality cowpea (Lowenberg-DeBoer, 2000). In Northeast Brazil, cowpea production covers approximately 1.5 million hectares providing food to around 25 million individuals. According to Singh *et al.*, (1997), in Brazil, cowpea consumption per capita is approximately 20 kilograms. By comparison, about 40, 000 hectares of cowpea is produced in the southern United States with an estimated 45, 000 tonnes of dry cowpea seed production per year. Asia accounts for less than 3% of worldwide output on average during the period 1993 - 2013, the majority of which is grown in Myanmar (FAOSTAT, 2015)

Most of the cowpea cultivation occurs in sub-Saharan, West and Central Africa. These regions account for over 95.4 per cent of drier savannah cowpea production (FAOSTAT, 2016). Major cowpea producing countries in Africa are Nigeria, Niger, Senegal, Ghana, Mali and Burkina Faso (Langyintuo *et al.*, 2003). Nigeria is responsible for 61 per cent of cowpea production in Africa and

58 per cent of production worldwide with about 5 million ha and over 2 million tons annual production (FAOSTAT, 2012) Niger is the second greatest producer, followed by Brazil, Burkina Faso, Myanmar, Cameroon, and Mali (Guazzelli, 1988). Most African farmers grow cowpea and most of these farmers are women who engage in subsistence cropping (Langyintuo *et al*., 2003). An estimate of 38 million families (194 million people) has been suggested to grow cowpea in sub-Saharan Africa.

Cowpea production in Ghana

Ghana is noted as one of the principal producers of cowpea in the world with an estimated annual production of about 14,3000 MT on about 156,000 ha of land making Ghana the fifth-highest producer of cowpea in Africa (Ibrahima, 2012). However, Ghana imports about 10,000 MT annually, about 30 per cent of which are from Burkina Faso and Niger (Seferiadis, 2009). The importation of cowpea in Ghana is because the domestic production of cowpea cannot meet the national requirements (Quaye *et al*., 2011). Also, production gap is as a result of pest and diseases, drought, low soil fertility, the unavailability of farming inputs, improved seeds, poor cultural practices and lack of tools and equipment for large-scale production (Ibrahima, 2012). Despite these problems, there is a projected 11 % increase in production between 2010 and 2020. This huge production and consumption gap could be cut back by breeding improved cultivars desired by farmers (Azam *et al*., 2013).

Constrains of cowpea production

The yield of cowpea is influenced by several factors such as pests and diseases, deteriorating land and climate conditions, lack of credit to farmers, inadequate storage facilities and inadequate transportation networks among others (Quaye *et al*., 2011). Yields of cowpea are generally low in Sub-Saharan Africa as a result of various biotic and abiotic constraints. Constraints of cowpea production can be categorized broadly into biotic and abiotic constraints.

Biotic factors

Insect pests and diseases

Numerous pests and diseases affect cowpea, which affects crop yield and general productivity (Rusoke & Rubaihayo, 1994). *Striga gesnerioides* and *Alectra* vaguely have been keyed out as common parasitic weeds that reduce the yield of cowpea noticeably in Africa (Parker & Riches, 1993; Rugare *et al.*, 2013). Up to 75% of the cowpea damage is done by these weeds before the crop emerges from the soil (Singh & Ram, 2005; Dugje *et al.*, 2009).

Common insect pests of cowpea include cowpea weevil (*Callosobruchus maculatus*), cowpea calculus (*Chalcodermus sermus*), and the southern cowpea weevil (*Mylabris quadrimaculatus*). If not controlled, these pests can cause up to a hundred per cent loss to cowpea grain (Ezueh, 1981). In Sub-Saharan Africa, thrips aphids and maruca are the major field insect pests of economic importance to the cowpea (Singh & Ram, 2005).

Cowpea is prone to a broad variety of diseases at all phases of its development cycle (Allen, 1983). A typical example includes cowpea wilt caused by *Fusarium oscysporium*, cowpea root rust caused by a nematode

(*Meloidogyne sp*), aphid-borne mosaic virus, cowpea bacterial blight caused by *Xanthomonas vignicola* and stem rot caused by *Phytophthora vignae*. Losses due to diseases can be as high as 100% (Owusu, 2015b).

Abiotic factors

A broad range of abiotic limitations significantly restricts cowpea development and yield. Poor soil fertility, drought, heat, soil acidity and competition stress due to intercropping with other crops, especially cereals are common abiotic factors that affect cowpea production (Singh & Ajeigbe, 2002). According to Ludlow and Muchow (1990), drought remains the most prominent abiotic stress that limits crop performance more, especially in drier savanna and Sahelian regions. This substantially affects plant efficiency and survival, resulting in plant functioning limitations, including several morphological, physiological, and metabolic modifications.

Although cowpea is characteristically more resistant to drought compared to other food plants, it still suffers significant harm owing to frequent drought in areas with low and irregular rainfall (Singh & Ram, 2005). In West Africa's savanna area, the vast bulk of cowpea production is concentrated at 10º and 20º N latitude, where droughts frequently occur thereby affecting the potential yield of the crop (Wien, 1979). Irregular rainfall adversely impacts plant population and flowering capacity, leading to a dramatic decrease in grain yield and overall total biomass (Timko & Singh, 2008).

Soil fertility status in Africa

Low soil fertility status is a major problem of 13% of arable land found in Africa (FAO, 2015). The average decline rate of soil fertility in Africa over the past 30 years was estimated at 660, 75 and 450 kg ha^{-1} for nitrogen, phosphorus and potassium, respectively (Smaling *et al.*, 1997) Compared to most other areas of the globe, the vast majority of African soils are poor (Bationo, 2009). Poor farming practices by most peasant farmers result in an annual depletion of 22, 2.5, and 15 kg ha^{-1} of nitrogen, phosphorus and potassium respectively (Bationo, 2009). Africa loses $4 billion a year because of the mining of soil nutrients (Smaling *et al*., 1997).

Most African soils are highly weathered resulting in an increased amount of Ca, Mg and K in the soil (Brady & Weil, 2008). As a result, soil solution becomes predominated by Fe and Al which limit the phytoavailability of soil nutrient (Dhillon *et al.*, 2017). High acidic soils such as cambisols, ferralsols and vertisols dominate in Africa (Zake, 1992). These soils are described mostly to be P deficient (Baligar *et al.*, 2001), due to high Ca deposits which interact with P to form insoluble complexes, which in turn affects P availability for plant acquisition.

Soil fertility status in Ghana

A total of 57.1% (13,628,179 ha) Ghanaian land is suitable for agriculture but most of the soils are of low inherent fertility (Jayne *et al*., 2015). Ghana has a relatively vast cultivated land per capita; however, most of these lands are characterized by poor fertility due to degradation. Annual soil nutrient depletion rate is estimated to be around 35 kg N, 4 kg P and 20 kg K ha^{-1} (Jayne

et al., 2015). Hence, Ghana is graded among countries with the highest rates of soil nutrient depletion in Africa (Jayne *et al*., 2015). The extent of nutrient depletion is widespread in all the agro-ecological zones with N and P being the most prominent. Most of Ghana's soils are formed through weathering of parental rocks, hence they are old and leached over decades especially in the humid (high rain forest and semi-deciduous) (Jayne *et al*., 2015).

High mineralization due to warm and humid climate and continuous cropping especially in the Sudan savanna zone result in low organic matter content of the soil. Generally, the soils have low buffering capacity due to the low mineral reserves, low organic matter content and coarse-textured topsoil (Jayne *et al*., 2015). Most Ghanaian soils are predominated by kaolinite which affects nutrient retention of the soil due to low cation exchange capacity (less than 10 cmol $_{(+)}$ kg $^{-1}$ clay). Factors such as leaching, soil erosion, poor fertilizer management and indiscriminate burning have been linked to major causes of low soil fertility in Ghana (Jayne *et al*., 2015).

Soil P availability

Soil P may occur in solution or bound to soil particles. However, soil particles bounded P are less available for uptake by plant roots (Tirado & Allsopp, 2012). According to Syers *et al*. (2008), soil P exists in four (4) different pools depending on their rate of availability for uptake. The first pool of P is mostly in soil solutions and are readily available for root absorption. The pool of P held on specific sites on surfaces of soil particles makeup the second pool. These pools are readily transported into soil solution for uptake by plants if the concentration of P in the soil solution is lowered by plants uptake. The

third pool includes, soil P strongly fixed to the surface of soil particle and less readily absorbed by plant roots but becomes available for extraction over time. Lastly, soil P which is available for plant uptake over time but mostly at a slower rate constitutes the fourth pool.

In the soil, P exist chemically in the inorganic and organic forms. These P forms differ in their behaviour and fate in soils (Hansen *et al.*, 2004; Turner *et al*., 2008). About 35 – 70 per cent of total soil P is in the inorganic P form. Plant roots absorb P as phosphate ions or orthophosphate of $H_2PO^-_4$ or HPO_4^{2-} (Marschner, 1995; Turner *et al.*, 2002). The concentration of P ions in the soil solution ranges from 10^{-4} M (very high) to 10^{-6} M (deficient) and 10^{-8} M (low) in most marginal tropical soils (Syers *et al*., 2008). The amount of P in the soil solution and crop uptake of P are the key factors that affect the concentrations of soil P.

According to Marschner (1995), the diffusion coefficient controls the movement of P ions in the rhizosphere. The diffusion coefficients of H_2PO_4 mostly common in soil solution is 0.9×10^{-9} m^2 s^{-1}, but in soil, estimated values range from 10-12 to 10-15 m^2 s^{-1}. The slow diffusion rate of P ions necessitates the need for an adequate supply of readily available P to meet plant P demand (Syers *et al*., 2008).

Effect of soil P on cowpea production

Phosphorus is a crucial plant nutrient for growth, development and yield of the crops (Karikari & Arkorful, 2015). Several key functions are associated with P availability such as energy metabolism, N-fixation, nucleic acids and membranes synthesis, photosynthesis, respiration and enzyme regulation

(Karikari & Arkorful, 2015). Additionally, physiological functions such as; early root growth and the primordial for reproductive components of crops are enhanced by P during the early phases of plant growth (Raghothama, 1999).

Phosphorus inputs obtained from soil reserves or manufactured fertilizers are essential for successful grain legume production (Ndakidemi *et al.*, 2006). According to Tomar and Jajoo (2014), shoot development, plant leaf area and dry matter of the legume plants increased with P availability and application of external P. Cowpea requires P in large amounts because it also helps in root growth and energy transfer during photosynthesis (Raemaekers, 2001). Concerning the above, P encourages flower initiation, delayed physiological maturity, plant growth, enhanced N fixation by enhanced nodulation and N use (Raemaekers, 2001).

Meena *et al.*, (2005) using chickpea plants reported that dry matter production increased significantly with each increase in phosphorus levels. Singh and Ahuja (1985) reported that applied P increased leaf area and accumulation of more dry matter in groundnut. Fageria *et al*., (2006) reported that partitioning of photosynthate and their effects on dry matter distribution was influenced by several environmental factors such as low temperature, drought, and mineral nutrient deficiency. Dry matter yield of cowpea per plant increased significantly with levels of phosphorus fertilizer for all the sampling periods (Magani & Kuchinda, 2009).

Despite the relevance of P in crop production, P deficiency due to either environmental or cultivation factors limits plant growth and production (Ndakidemi & Dakora, 2007) on over 5.7 billion hectares of land (Hinsinger, 2001). Widespread low P availability is a major soil fertility problem that affects

cowpea production (Sanginga *et al*., 2000). Phosphorus deficiency is usually the most determining factor for the poor yield of legume crops on most of the tropical soils because apart from playing an essential role in root development, phosphorus is needed for the growth of rhizobium bacteria responsible for nitrogen fixation (Adusei *et al.*, 2016).

Constrains associated with mineral P fertilizer use

Phosphorus deficiency spreads over 67 per cent of global arable soil (Batjes, 1997). This problem accounts for low crop productivity over approximately 5.7 billion hectares of land (Hinsinger, 2001) resulting in global food insecurity. According to Lynch and Brown (2008), the issue of P soil deficiency can be mitigated by applying external fertilizers that provide the plant with soluble inorganic P. However, the usage of P to increase agriculture production s associated with several problems (Lynch & Brown, 2008).

Approximately 50-80 per cent of total P in fertilizer is retained by the soil during the year of application (McBeath *et al*., 2012). Although the use of P fertilizer improves sustainable agricultural systems, however, excessive build-up of P results in eutrophication of freshwater habitat (Tiessen, 2008). Phosphorus fertilizers are excellent sources of heavy metals such as cadmium that can build up in arable soils when applied (Adu *et al*., 2014). Additionally, deposits of rock phosphate have been revealed to be exhausted due to the current consumption patterns in the next 50 years due to current consumption patterns (Gilbert, 2009). Heffer *et al.*, (2006) concluded that P reserves are adequate for roughly 100 years about present usage. Likewise, Smil (2000) projected the depletion of P reserves over the next 50 to 100 years. Phosphorus fertilizers are mainly obtained from finite rock phosphate deposit which is becoming

increasingly costive due to the present demand and declining rate of this resource (Dawson & Hilton, 2011).

Mechanisms for plant adaptation to low P condition of the soil

Plants have evolved numerous morphological, physiological and biochemical processes, in response to the insufficiency of P (Suriyagoda *et al.*, 2010). Morphological mechanisms for plants to cope with inadequate soil P accessibility include prolific root growth, such as greater root: shoot ratio, finer roots and longer root hairs to promote the exploration of the soil (Raghothama, 1999). Plants enhance general soil exploration through increased root length, increased root branching and changed branching angle (Gahoonia & Nielsen, 2004; Lynch, 2007a; Lynch & Brown, 2001). According to Gahoonia *et al.* (2001), root hair under marginal resource conditions is a particularly important feature for P acquisition. Root hair is a single-cell expansion on the root surface that increases the root surface area and thus increases contact between the root and ensures about 80 per cent P absorption (Jungk, 2001). Plant genotype with longer, denser root hairs was found to increase P absorption when grown in P-deficient soils (Brown *et al*., 2012; Gahoonia *et al.*, 2004). Mechanisms for adapting to low P in the low resource environment are described by Ramaekers *et al.* (2010) to include: development of extra and longer adventitious roots, growth of more basal roots horizontally oriented, development of more taproot laterals, growth and distribution of laterals roots and increased density and length of root hair (in combination with enhanced exudation of organic acid).

Roots of grain legumes adapt to poor soil P conditions by enhancing root development, such as basal and adventitious roots, root architecture alteration

(Lynch, 1995). Miller *et al.*, (2003) concluded the development of adventitious bean roots is an adaptive mechanism that helps to acquire P by enhancing the foraging of plants in marginal soil. The acquisition of soil P by plant relies on the physiological and morphological characteristics of root systems such as root size, root exudates (citrate and malate), high affinity for inorganic P transporters and arbuscular mycorrhizal colonization (Liang *et al.*, 2010).

Phosphorus acquisition efficiency

Phosphorus acquisition efficiency (PAE) describes the ability of plants to absorb sparsely soluble soil P (Aziz *et al*., 2014). It defines the plant's capacity to mobilize P ions from poorly soluble sources or acquire soil P (Narang *et al.*, 2000). Adu *et al*. (2014) described phosphorus acquisition as the increase in the P content in a plant tissue per unit of added P fertilizer (g P g^{-1} P_{fert}). Phosphorus acquisition efficiency (PAE) is based on the capacity of the root to obtain P from the soil and often expressed as the relative difference in P acquired in low and high availability conditions of P (Gahoonia & Nielsen, 1996; Narang *et al*., 2000; Zhang *et al*., 2009; Ramaekers *et al*., 2010).

Several factors such as root architecture, root morphology, mycorrhizal associations, strong affinity transporters and rhizosphere modification have been correlated with PAE (Lambers *et al.*, 2006). Three procedures for taking P from the soil were outlined: root interception, diffusion, and mass flow (Syers *et al*., 2008). Despite the association between these processes, the prevailing conditions of the soil determine which is utilized at any moment (Syers *et al*., 2008). When roots only occupy a limited soil volume, soil acquisition of P ions is regularly insufficient to balance plant P requirement (Barber, 1995). In the

soil solution, soluble P flows into the root as water and is taken up by the roots during the mass flow cycle. Nutrient uptake capacity of roots depend on root metabolism and concentration of P in plant tissues (Hinsinger, 2001; Syers *et al*., 2008). Breeding for enhanced P uptake, by changing the root architecture, is often proposed as a significant tool to increase crop PAE (Liao *et al*., 2004; Zhu & Lynch, 2004). According to Lynch (2007a), variations in root traits (length, branching, hair formation and topsoil foraging) are significant architectural modifications that increase nutrient absorption as it improves root absorption.

Phosphorus use efficiency

Numerous researchers have described the concept of PUE. Phosphorus use efficiency denotes the capacity of plants to produce higher biomass per unit of absorbed nutrient (P) (Aziz *et al*., 2014). Phosphorus use efficiency is the total production or yield of biomass per unit of P in biomass (Hammond *et al*., 2009). Phosphorus use efficiency (PUE) designates the increase in crop yield as a result of a unit increase in the crop's P content (g DM g^{-1} P) (Adu *et al*., 2014). Agronomic PUE is mostly used to represent PUE (White *et al*., 2005; Hammond *et al*., 2009). This denotes the capacity of plants to yield higher output per unit of P applied as fertilizer or available soil P. This corresponds to the product of phosphorus utilization efficiency and phosphorus uptake efficiency (Hammond *et al*., 2009).

Rhizosheath

Plants in poor resource environments use numerous processes to improve resource access and adaptation to abiotic stress, particularly nutrient and water deficiencies (White *et al*., 2013). These mechanisms include the growth of root hair, development of suitable root system architecture, promoting useful symbiotic connections and enhancement of physical and biological rhizosphere conditions (White *et al*., 2013). Improvement in soil conditions generally results from rhizosheath formation, particularly among cereals and other grasses (Vermeer & McCully, 1982; Duell & Peacock, 1985; Goodchild & Myers, 1987). This feature was first seen in North African desert grasses gathered over a hundred years ago (Volkens, 1887), and is believed to be limited to the Poaceae (Duell & Peacock, 1985).

Rhizosheath refers to a unique sheath consisting of agglutinated sand particles (Volkens, 1887). Rhizosheath relates to the soil mass that heavily adheres to plant roots on excavation (George *et al*., 2014). Thus, the soil weight adhering to the roots when removed from the pot or field. There have been substantial studies of rhizosheath in desert grasses (Goodchild & Myers, 1987; Othman, Amer, Fayez, & Hegazi, 2004), as well as in cereal species including wheat, barley and maize (Watt *et al*., 1994; Young, 1995; Delhaize *et al*., 2012; George *et al*., 2014; Haling *et al*., 2014). Although McCully has reported the presence of rhizosheath in the fine root of legumes and eudicotyledonous crops, these have not been investigated further.

Root hair development

Root hairs are protrusions from single epidermal cells on a root surface that extend a plant's influence into the surrounding soil (Ma *et al.*, 2001). They originate from epidermal cells called trichoblasts (Dolan, 2001). According to Bibikova and Gilroy (2002), trichoblasts initiate localized growth processes that result in the development of a hair-like projection from the epidermal cell wall. Bibikova and Gilroy (2002) described the development of root hairs using three specific schemes. In these schemes, type I plants are commonly ferns, monocots and most dicots in which root hairs are produced by epidermal cells. Type II plants are mostly smaller cells resulting from asymmetric cell division in meristem and produces root hairs. These plants include *Lycopodium*, *Selaginella* and *Equisetum*, some monocots, and dicot family Nymphaeaceae (Cutter & Feldman, 1970; Cutter & Hung, 1972). In Type III plants, root hairs emerge from root epidermal cells in files made of either atrichoblasts or trichoblasts. It is common with Brassicaceae.

The growth of root hairs occurs from the differentiation region of the root after cessation of diffuse elongation growth of the cell. Root hairs are particularly key for the acquisition of ions of low availability due to low soil diffusion. Root hairs enhance nutrient and water uptake by increasing root surface area and the volume of soil explored by roots. Additionally, they support anchoring the root system more closely to the soil (Bibikova & Gilroy, 2002).

Relationship between root hair and rhizosheath formation

Root hair is an essential element for rhizosheath formation, such that longer root hairs are associated with larger rhizosheaths (Pang & Ryan, 2017). Several pieces of research have demonstrated the significance of developing root hair for the production of rhizosheath (Moreno-Espíndola *et al*., 2007). Root hairs enmesh soil particles around the root (Bristow *et al.*, 1985) hence, crucial for rhizosheath formation (Haling *et al*., 2010; Brown *et al*., 2012). Root hairs ensure root-soil contact (Gregory, 2008) and absorption of soil water and nutrients. Rhizosheath size has proven to be a credible root hair surrogate in several studies (Pang & Ryan, 2017).

A strong correlation has been established between the length of root hair and the weight of rhizosheath (Delhaize *et al.*, 2015), however, this varies greatly in intensity depending on the species of the plant (Brown *et al.*, 2017). Genotypes with longer and denser root hairs produced greater rhizosheath mass (Haling *et al*., 2010). Studies in young wheat by Delhaize *et al*. (2012) revealed that, under acidic growth conditions, rhizosheath of seedlings strongly correlated with the length of root hairs. Similar results were obtained with Barley where mutants lacking root hair either have no rhizosheath or the rhizosheath is significantly lower than the wild type (Haling *et al*., 2010; George *et al*., 2014).

Nevertheless, the connection between rhizosheath size and root hair length has conflicting results (Pang & Ryan, 2017). In barley, rhizosheath size poorly correlated with root hair length, indicating factors other than root hair length substantially influences rhizosheath formation (George *et al*., 2014). A similar investigation was carried out by Haling *et al*. (2010) using barley and

wheat lines by regressing rhizosheath size against the volume of the root hair cylinder. The variation in the quantity of root hair cylinder only described 52 per cent of the variation in the size of rhizosheath in wheat and 66 per cent in barley. Using a fitted linear model, Brown *et al*., (2017) reported that specific rhizosheath weight (dry soil grams per gram of fresh root weight) and root hair length across a broad spectrum of species revealed weak association. These suggest that root hair length and rhizosheath size association vary considerably between plant species (Brown *et al*., 2017).

Role of rhizosheath and root hairs in tolerance to edaphic stress

Root hairs are among the various mechanisms used by plants to improve their access to resources and abiotic stress tolerance (White *et al*., 2013; George *et al*., 2014) . Root hairs increase the root surface; however, few studies support their role in water absorption (Marzec *et al.*, 2015). Among the potentially useful root features, root hairs are regarded as the most important for P absorption by raising the absorbent surface of the root and hence increasing the volume of soil explored by the plant (Clarkson, 1985). The above indications show that root hair is essential to improve crop productivity and stress tolerance in poor soil conditions (Meister *et al.*, 2014).

Several studies have shown the relevance of rhizosheath to nutritional deficiencies. Rhizosheath is also engaged in hard soil tolerance, water deficiency and Al-induced tolerance of acidity (Brown *et al*., 2012; Delhaize *et al*., 2012; Haling *et al*., 2014), in addition to mitigating Zn deficiencies (Haling *et al*., 2013). Greater rhizosheath mass has been revealed to be very significant for enhancing the uptake of mineral in the soil noticeably phosphorus (Haling

et al., 2010; Brown *et al*., 2012; Kwasniewski *et al*., 2015). An extensive and well-established stable rhizosheath may help plants acquire nutrients in dry soil (Watt *et al*., 1994). Additionally, sheaths increase the supply of N to the soil due to high nitrogen fixation associated with sheath (Wullstein *et al.*, 1979). Rhizosheath is also engaged in hard soil tolerance, water deficiency and Al-induced tolerance of acidity (Delhaize *et al*., 2012; Haling *et al*., 2014). Modelling studies on root hair absorption of soil P revealed that an increase in root hair length promotes P absorption (Ma *et al*., 2001; Zygalakis *et al.*, 2011). Long root hairs have been shown to promote the growth of a big root-hair-cylinder surface (rhizosheath) and to allow P to be intercepted by the root (Gahoonia & Nielsen, 1997; Haling *et al*., 2016). In many cases, the root hair cylinder is approximately similar to the rhizosheath (Haling *et al*., 2010).

Initially, rhizosheath was found on semi-arid plants tolerant of drought (Pang & Ryan, 2017). Rhizosheath is described as an important mechanism to improve resistance to drought and to protect roots in arid conditions. Thus, in dealing with other abiotic stresses such as heat stress and drought, rhizosheath has significance (Hartnett *et al.*, 2013). According to North and Nobel (1997), rhizosheaths are efficient in performing these functions as they provide and retain excellent contact at the root and soil interfaces vital for the absorption water. Additionally, larger rhizosheath mass has been shown to enhance plant-water relation among various species by (Watt *et al*., 1994; Young, 1995; George *et al*., 2014; Kwasniewski *et al*., 2015). In grasses, root hairs are essential traits to retain a sufficient amount of water during drought (Marzec *et al*., 2015). Segal *et al.*, (2008) have shown that only the root hair tip domain is directly involved in barley water uptake. Despite the relevance of rhizosheath

in tolerance to edaphic stress, some plant species seem to lack rhizosheath and many others promote only tiny rhizosheaths regardless of their length of root hair (Brown *et al*., 2017), rhizosheath's function in stress tolerance remains uncertain.

Root system architecture (RSA)

Root system architecture (RSA) relates to the organization, the three-dimensional arrangement of the main and lateral roots and other accessory roots in the soil (Ning *et al*., 2012; Smith & De Smet, 2012). Root system architecture (RSA) describes the configuration, shape and structure of a root system in the soil (Dorlodot *et al*., 2007). According to Lynch (1995), RSA does not usually include fine details such as root hairs but are centred typically on the entire root system of an individual plant. Root phenes associated with RSA include root branches, length, biomass, volume, anatomy, and surface area.

In the development of RSA, three main components were described. These are the topology, distribution, and morphology of the root system (Lynch, 1995). Root morphology is related to the internal characteristics of the root axis and may include root hair characteristics, root diameter and secondary roots (Adu *et al*., 2014). The topology, on the other hand, describes how individual roots are branched (Fitter & Stickland, 1991). A description of the topology is essential due to its functional meaning, while the distribution depicts the existence of roots in a spatial context (Gregory, 2008). Root system architecture (RSA) is quite multifaceted and varies between and within plant species (Gregory, 2008).

Role of RSA in tolerance to edaphic stress

The root system architecture is vital for plant productivity under edaphic stress (Lynch, 1995). Root system architecture determines plant ability to exploit spatially heterogeneous distributed soil resources (Lynch and Brown, 2001) hence ensures tolerance of plants to various biotic and abiotic stresses such as salinity, temperature extremes, waterlogging, drought, and nutrient shortage (Lynch and Brown, 2001). The root system architecture is a significant component for the acquisition of soil reserves including N and water (Lynch & Brown, 2001) and is particularly essential for the uptake of highly immobile and limiting nutrients such as P (Lynch, 1995).

According to Miguel (2010), up to 100 per cent of enhanced P acquisition can be found in common bean cultivars since the number of basal root whorls varies between genotypes. Nonetheless, there is some trade-off between P and water absorption, as crops with greater root density in the upper soil and shallower angles have reduced water efficiency since water is generally more abundant in deeper layers under drought circumstances. Root architectural features (root length, density, branching angle, root hair) are essential to enhance the effectiveness of P uptake (Lynch, 2007). Of these characteristics, it has been shown that choosing crops with longer root length and greater root hair density is particularly crucial for enhancing P uptake and plant growth, particularly under P deficient environments (Gahoonia & Nielsen, 1997; Brown *et al*., 2012).

Common bean (*Phaseolus*) with shallow root architecture grow and yield better than genotypes with deep architecture under low P conditions. Similarly, Ao *et al.* (2010) revealed that the most P-efficient genotypes in

soybean had longer and bigger root systems with a bigger share of the root system in the topsoil. More importantly, shallower root systems obtained more P per unit of carbon costs compared to deeper root systems, and shallower root systems obtained more P than deeper root systems in ground layers due to less inter-root rivalry and enhanced root exploration of the upper soil (Rubio, & Lynch, 2000).

Effect of P on development of rhizosheath, root hair and RSA

Genetic and environmental plasticity affect plant root system architecture. Thus, different species develop varying RSA depending on the prevailing soil conditions (Shahzad & Amtmann, 2017). Genetic composition of plants, crop management and environmental factors regulate the growth, development, and penetration of roots in the rhizosphere (Saleem *et al.*, 2018). Roots are the element responsible for the uptake of water and nutrients needed for plant survival, hence plants alter the spatial and temporal development, or architecture, of their root systems in response to a variety of environmental cues (Hermans *et al.*, 2006). Nutrient availability is the most important abiotic factor influencing root system growth besides water (López-Bucio *et al*., 2003). Nitrogen (N) and phosphorus (P) are the two most abundant macronutrients that have the greatest effect on RSA (Osmont *et al.*, 2007).

Low availability of soil P impacts features of the root system (Zhu & Lynch, 2004) such as root lateral branching, root thickness and root hair length (Ma *et al.*, 2001) as well as parenchyma formation (Fan *et al.*, 2003). Also, low P concentrations affect root morphology, delay root growth in plants and reduce the number of root hairs and physiological features connected with P absorption

(Pellerin, Mollier, & Plenet, 2000). Insufficiency of P in common bean has been shown to stimulate shallower basal root growth angles (Liao *et al*., 2001), increased adventitious root production (Miller *et al*., 2003) and overall, it promotes shallow root systems for P-efficient genotypes (Lynch & Brown, 2001). In particular, low tissue P in plant shoots has also been reported to lead to a reduction in photosynthesis and stomatal behaviour, resulting in limited plant growth (Ghannoum & Conroy, 2007). Because of decreased cell division and decreased cell enlargement, phosphorus deficiency leads to stunted shoot and root growth. Insufficient P stimulates the absorption of surplus cations by crops over anions, which in turn enhances the release of protons, which may boost acidification, which may promote the uptake of P (Tang *et al.*, 2001).

According to Hodge (2009), poor P availability results in changes in the distribution among different root types. Study with *Arabidopsis thaliana* and multiple cultivars of rapeseed confirmed that the root system became extremely branched with decreased main root (PR), while the amount and length of lateral roots (LR) increased when crops were grown under low soil P (Akhtar, Oki, & Adachi, 2008; Pérez-Torres *et al*., 2008). It was observed by Schmidt and Schikora (2001) that, P deficiency increased the abundance and length of root hair. With low P status, size reduced and root hair development improved (Zhang, Lynch, & Brown, 2003). A study by Liao *et al*. (2001) in common bean, discovered that the abundance of P altered the shallowness of the basal root length.

It has been reported that root hairs absorb approximately 78% of soil P (Barley & Rovira, 1970) hence, plants increase root hair length and density when P is deficient (Bates & Lynch, 1996; Ma *et al*., 2001). Genotypes develop

longer root hair length in P-deficient conditions (Brown *et al*., 2012). It has been known for some time that low P causes increased extension of root hairs in many plant species (Foehse & Jungk, 1983). Studies by Bates and Lynch, (2000) during his study with Arabidopsis found that root hair length typically exceeds 1mm under P concentration of 1 µM. The authors further concluded that root hair length decreases drastically at high P concentration. Increased root hair proliferation is a major response to early P deficiency (Ma *et al*., 2001; Jain *et al*., 2007). Contrastingly, high levels of soil P does not completely inhibit root hair growth, indicating that high-P plants maintain the potential for plasticity (Bates and Lynch (2000a). Generally, the increase in the root hair density and length in response to P deficiency is a well-researched phenomenon in plant biology (Péret, Clément, Nussaume, & Desnos, 2011).

The size of rhizosheath has been reported to increase with a high concentration of soil P (James *et al*., 2016). Since higher rhizosheath weight was recorded at 2000 mg kg^{-1} P application compared to the control treatment (James *et al*., 2016). This corroborates with previous conclusions that the differences in rhizosheath size of the lines were due to P application (Delhaize *et al*., 2012).

Significance of genetic variability in root hair and RSA traits

Variation in plant genetic resources is a major tool for plant breeders to develop new and improved crops with desirable characteristics (Govindaraj *et al*., 2015). Genetic diversity is the key to the biodiversity and diversity of species and ecosystems (Govindaraj *et al*., 2015). Genetic variations between

and within genotypes in the patterns of development, architecture and response to soil properties have been reported (O'toole & Bland, 1987; Gregory, 2006).

In cowpea, important genetic diversity has been noted for RSA characteristics connected with development in low-nutrient and dry conditions (Singh & Matsui, 2002; Krasilnikoff *et al*., 2003; Matsui & Singh, 2003). Previous research identified cowpea root characteristics that are critical for PUE and PAE (Kugblenu *et al.*, 2014). Under drought conditions, deep root systems are reported to be beneficial (Matsui & Singh, 2003; De Barros *et al.*, 2007; Agbicodo *et al.*, 2009), although some additional maintenance costs may occur for plants investing in deeper roots under limited additional water conditions. Therefore, the plant's uptake of P and efficient use of P is determined genetically and the genetic variation is mainly due to PUE (White *et al*., 2005). Exploiting genetic diversity in root hair and root architecture response to soil resources is, therefore, a promising instrument to improve with plant PAE and PUE (Beebe *et al*., 2006; Hammond *et al*., 2009). Krasilnikoff *et al*. (2003) reported genetic variation in root length and root hair length (RHL) among cowpea genotypes screened during their study.

Therefore, it is necessary to know the genetic variation of root architectural features to identify processes that can be used to grow plants for the acquisition and use of soil P (Liao *et al*., 2001). Differential response of several crops to P nutrition has been reported (White & Hammond, 2008; Hammond *et al*., 2009). For instance, out of 35 cowpea lines evaluated for P response to P-deficient Alfisols soil, P fertilizer considerably increased shooting, root, dry grain weights and nodule weight, with more than 50 per cent of the lines indicating an important response to P (Kolawole *et al.*, 2002).

Genetic diversity and plasticity response variations in cowpea RSA traits are an important breeding tool for enhancing P resource acquisition/use efficiency.

CHAPTER THREE

MATERIALS AND METHODS

Study area

The research was conducted at the Teaching and Research Farm of the School of Agriculture, the University of Cape Coast, Cape Coast (UCC; 5.1155 ^{O} N, 1.2909 ^{O} W). The site is located within the Coastal Savannah Zone, with Acrisol soil type (Asamoah, 1973). The area has a bi-modal rainfall pattern from May to July and August to October with an annual rainfall of 750 to 1000 mm (Asare-Bediako *et al.*, 2014). The experimental area is described by Adu *et al.* (2017) to have the following climatic condition: temperature (24 ^{O}C to 32 ^{O}C), relative humidity (60 to 80%), solar radiation (3151 kJ cm^{-2} day^{-1} to 3804 kJ cm^{-2} day^{-1}) and day length (11.30 to 12.40 hours).

Genetic materials

The research used sixty (60) cowpea genotypes. The genotypes consisted of local genotypes of improved cowpea, newly introduced genotypes of inbred cowpea and landraces. Seeds of cowpea were obtained from the Crop Research Institute, Council for Scientific and Industrial Research (CSIR-Fumesua), Uganda and International Institute of Tropical Agriculture (IITA). Six (6) locally enhanced lines and 54 freshly introduced lines made up the genetic materials.

Experimental design and treatments

Two (2) independent pot experiments were conducted in a greenhouse. Sixty (60) cowpea genotypes were screened for genotypic variation in RSA, rhizosheath and root hair traits among cowpea genotypes under unamended soil condition in Experiment one (1). Twenty (20) selected cowpea genotypes from Experiment 1 were evaluated under P- amended soil condition for the effect of $[P]_{ext}$ and variation in RSA, rhizosheath, root hair traits and biomass production in Experiment 2.

Each experiment was performed twice to check for repeatability or broad-sense heritability of measured traits. Screening of cowpea genotypes was carried out in batches but a common genotype was included in each batch so that variation between batches can be analysed on the common genotype and accounted for as a cofactor.

An 8 × 8 alpha lattice design with four (4) replications was used for both experiments. However, Experiment 1 was composed of single treatment (cowpea genotypes), while cowpea genotypes and external P level ($[P]_{ext}$) were the treatments used in Experiment 2.

Soil preparation

The soil used for the study was collected at a depth of 0-15 cm in an area closer to the Research farm. The soil is classified as a sandy clay loam of the series Benya, a member of the Edina Benya-Udu association, according to Asamoah (1973). The soil was an arable type with haplic acrisol features of the coastal savanna. Soil samples were thoroughly mixed and aired under rain

shelter for three (3) days. Aired soil was sieved with 2mm sieve mounted on a wooden platform to remove coarse materials and vegetative matter.

Initial soil physical and chemical properties analysis

Soil analysis was conducted on soil sample to determine the following physical and chemical parameters including soil pH, bulk density, particle density, cation exchange capacity (CEC), total nitrogen, available phosphorus, exchangeable potassium, magnesium, and calcium. Table 1 shows the results of the soil's initial physicochemical properties used for the first and second experiments.

Table 1: Initial physical and chemical properties of experimental soil

Parameter	Value
Nitrogen (N) %	0.08
Phosphorus (P) ppm	7.00
Potassium (K) cmol/kg	0.05
Calcium (Ca) cmol/kg	2.59
Magnesium (Mn) cmol/kg	0.87
pH	5.43
CEC (cmol/kg)	0.63
Bulk Density (g/cm^3)	1.36
Particle Density (g/cm^3)	2.68

Source: Field research, Opoku (2020)

Estimating air-dried soil required to fill nursery pots

The mean weight of aired-soil required to fill each nursery pot was estimated by filling three (3) empty nursery pots with an air-dried soil sample to about 2 - 3 cm from the top. Filled nursery pots were weighed with a top pan balance and the mean weight of air-dried soil was calculated. The mean

represented the weight of air-dry soil that was needed to fill each nursery pot during the experiment (Table 2).

Table 2: Weight of air-dried soil used in estimating mean soil weight needed to fill each nursery bag

Nursery Pot	Weight of aired soil (g)
Pot 1	1689.47
Pot 2	1717.20
Pot 3	1694.62

Source: Field research, Opoku (2020)

$$Weight\ of\ air-dried\ soil\ to\ fill\ nursey\ pots = \frac{W1+W2+W3}{3}$$

Eqn (1)

$$\frac{1689.47g + 1717.2g + 1694.62g}{3}$$

The total quantity of soil required for each experiment was estimated by multiplying the number of nursery pots needed for the experiment by the weight of air-dried soil needed to fill each nursery pot.

Estimating the amount of water needed to irrigate experimental plants

The amount of water required to irrigate nursery bags filled with soil was estimated using a gravimetric field capacity method. The detailed step used in the estimation is presented in appendix 1. Each nursery pot was irrigated with pipe water at 80% field capacity.

Estimating the total external P source for incubating soil samples for experiment two

Potassium dihydrogen phosphate (KH_2PO_4) was the external P source used for the study. The rates of P for incubating soil for P response curve were 0, *100, 250, 500, 750, 1000 mg P/kg soil*. The total quantity of soil used for each level was estimated by multiplying the number of nursery bags to be used with the weight of soil to fill each nursery bag, as shown in *equation 1* above. The quantity of KH_2PO_4 applied to obtain each level of P applied for soil incubation was estimated as illustrated in (Table 3).

Table 3: Quantity of external phosphorus (KH_2PO_4) source used in incubating soil samples at varying $[P]_{ext}$ level.

100 mg P / kg soil	**250 mg P / kg soil**	**500 mg P / kg soil**	**750 mg P / kg soil**	**1000 mg P / kg soil**
1 kg soil = 100 mg P	1 kg soil = 250 mg P	1 kg soil = 500 mg P	1 kg soil = 750 mg P	1 kg soil = 1000 mg P
17 kg soil = *x*	17 kg soil = *x*	17 kg soil = *x*	17 kg soil = *x*	17 kg soil = *x*
$\frac{100\ mgP\ \times 17\ kg\ soil}{1\ kg}$	$\frac{250\ mgP\ \times 17\ kg\ soil}{1\ kg}$	$\frac{500\ mgP\ \times 17\ kg\ soil}{1\ kg}$	$\frac{7500\ mgP\ \times 17\ kg\ soil}{1\ kg}$	$\frac{1000\ mgP\ \times 17\ kg\ soil}{1\ kg}$
= 1700 mg P	*= 4250 mg P*	*= 8500 mg P*	*= 12750 mg P*	*= 17000 mg P*
Convert mg to g	*Convert mg to g*	*Convert mg to g*	*Convert mg to g*	*Convert mg to g*
$\frac{1700\ mgP}{1000}$ = 1.7 g P	$\frac{1700\ mgP}{1000}$ = *4*.25 g P	$\frac{8500\ mgP}{1000}$ = *8*.5 g P	$\frac{12750\ mgP}{1000}$ = 12.75g P	$\frac{1700\ mgP}{1000}$ = 17 g P
Hence if,	***Hence if,***	***Hence if,***	***Hence if,***	***Hence if,***
136.09 g KH_2PO_4 = *31 g P*	136.09 g KH_2PO_4 = *31 g P*	136.09 g KH_2PO_4 = *31 g P*	136.09 g KH_2PO_4 = *31 g P*	136.09 g KH_2PO_4 = *31 g P*
***x** = 1.7 g P*	***x** = 4*.25 g P	***x** = 8.5 g P*	***x** = 12.75 g P*	***x** = 17 g P*
$\frac{136.09\ g \times 1.7\ g}{31\ g}$ =	$\frac{136.09\ g \times 4.25\ g}{31\ g}$ =	$\frac{136.09\ g \times 8.5\ g}{31\ g}$ =	$\frac{136.09\ g \times 12.75\ g}{31\ g}$ =	$\frac{136.09\ g \times 17\ g}{31\ g}$ =
7.463 g KH_2PO_4	**18.66 g KH_2PO_4**	**37.32 g KH_2PO_4**	**55.97 g KH_2PO_4**	**74.63 g KH_2PO_4**

Source: Field research, Opoku (2020). *The molecular weight of KH_2PO_4 = 136.09 mol/g, Atomic mass of P = 31 g. Hence each 136.09 g of KH_2PO_4 contains 31 g of P. Total soil weight used for each P level = 17 kg.*

Sowing, watering, and harvesting of genetic materials

Polyethene nursery bags of approximately 3300 cm^3 (22 cm deep × 15 cm × 10 cm) were filled with prepared soil samples for the experiment (Plate 1A). Four (4) replicates was used for each cowpea genotype. Each nursery pot was hand-sown with two (2) healthy seeds at a depth of about 2cm below the soil surface. Seedlings were thinned to one stand per pot a week after germination (Plate 1B).

Nursery pots were irrigated with pipe-borne water at a field capacity of 80% determined gravimetrically (Appendix 1). Nursery pots were rotated in the greenhouse to avoid the possible effect of fluctuation in temperature and relative humidity.

Cowpea genotypes were harvested at 4 - 6 leaf growth stage (3 weeks after germination). Harvesting was done by carefully cutting the polyethene pots symmetrically at its two sides (Plate 1C) and systematically shaking each plant by hand until no more bulk soil became attached to roots. The remaining soil attached to root was considered as rhizosheath (Plate 1C).

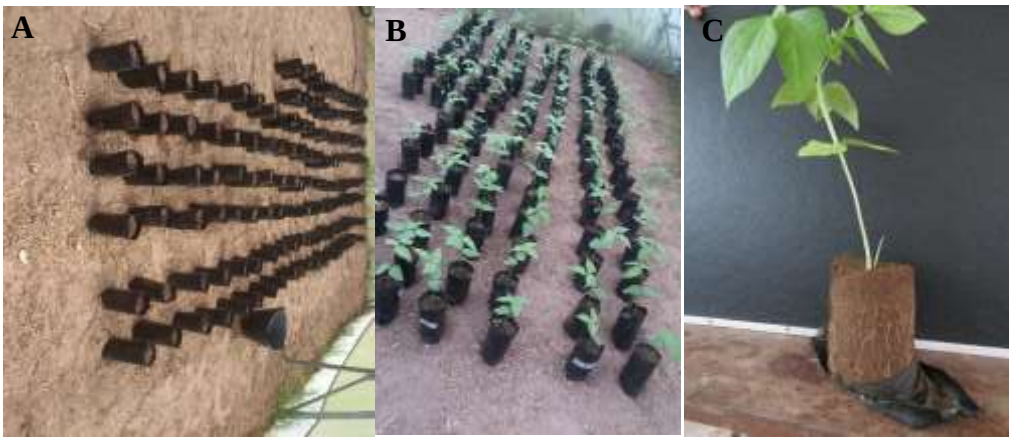


Plate 1: (A) Images of polyethylene nursery bags filled with air-dried soil arranged in greenhouse; (B) Image illustration cowpea seedlings thinned to one sand per bag and (C) Image of 3 weeks old cowpea plants with soil bulk after removal from nursery polybags

Data collection

Rhizosheath

After excavation of cowpea genotypes, rhizosheath mass was determined as described below. Roots of data plants were separated from shoots by cutting the collar region of the stem using a blade. Roots were then shaken carefully to remove any form of lumps and avoid loss of rhizosheath mass. The time and force for shaking remained constant throughout the experimental period to avoid the introduction of error. Roots together with rhizosheath were afterwards placed in a disposable cup with a known weight. The weight of the root and rhizosheath was measured immediately using an electronic pan balance. Using tap water, roots were carefully washed free of rhizosheath mass. Care was taken to avoid damage to the roots of data plants during washing. Washed roots were carefully patted with tissue paper and weighed to obtain a rhizosheath-free weight or root fresh weight (RFW). The difference between RFW and root with soil was calculated to represent absolute rhizosheath weight (ARW) (George *et al*., 2014; Adu *et al*., 2017). Washed soils were oven-dried at 105 ^{0}C for 3 days and weighed to obtain rhizosheath dry weight (RDW). Relative rhizosheath weight (RRW) (g g^{-1} root) was expressed as the quotient of ARW and root dry weight (Adu *et al*., 2017). Specific rhizosheath weight (SRW) was estimated as the quotient of absolute rhizosheath and root hair length.

Root data

Imaging of roots

Washed roots were uniformly spread in a basin with a black background filled with water to approximately 1/4th of its volume. Care was taken to avoid roots overlying on each other. Each root was correctly labelled with its code. A macro – lens digital camera (Canon Power shot SX 730 HS) was held still at a height of 50 cm on a tripod above the sink. Images of the roots were then taken. Recorded images were used for the extraction of total root length - TRL. Macros in *ImageJ* software was used for extracting data on TRL (Adu *et al*., 2017).

For extraction of root hair traits, root hair density (RHD) and root hair length (RHL), the tips of five (5) longest seminal root were cut within 4–6 cm. Severed root tips were placed in labelled Petri glass plates containing water. Root hair images were captured with a computer-connected-AmScope mounted with a compound microscope (2x magnification; Irvine, California USA, *www.amscope.com*). Data on root hair parameters were measured from root images using *ImageJ* software (Adu *et al*., 2017).

Extracting of total root length, root hair length and root hair density using Image J software

Total root length was extracted from root images using macros and root analysing plugins installed in *ImageJ*. Data on root hair length was obtained by using a freehand line feature to trace and measure fifteen (15) completely elongated root hairs on each microscopic image captured. The mean of measured length was calculated to represent RHL for each replication. Data on RHD was obtained by measuring the area of a representative rectangle using

ImageJ. The number of root hairs within the representative area was visually counted. Root hairs counted was divided by the representative area to calculate the RHD (count/mm^{-2}) (Adu *et al*., 2017).

Root and shoot biomass measurements

Leaves of excavated genotypes were carefully cut from the shoot and uniformly arranged flat and end-to-end on a black background board with a scale and genotype code. Images of same captured with a macro – lens digital camera Canon Power shot SX 730 HS held still at a height of 50 cm on a tripod above the leaves. Recorded images were used for the extraction of the total leaf area - TLA. Macros in addition to the binarization/thresholding extraction feature in *ImageJ* software was used for extracting data on TLA (Adu *et al*., 2017). Specific leaf area was calculated as a quotient of TLA and LDW. Thus,

$$\text{Specific leaf area (SLA)} = \frac{Total\ leaf\ area}{Leaf\ dry\ weight} \qquad \text{Eqn (2)}$$

Leaf mass per area was calculated using the formula described below.

$$\text{Leaf mass per area (LMA)} = \frac{1}{Specific\ leaf\ area} \qquad \text{Eqn (3)}$$

The shoots of excavated cowpea genotypes were weighed with an electronic balance to obtain the fresh weight of shoot (SFW). Washed roots were patted with tissue paper and weighed with an electronic balance to obtain root fresh weight (RFW). Root and shoot samples were bagged in labelled envelopes and oven-dried at 80 ^{0}C for 3 d. Oven-dried samples were allowed to cool in a desiccator after which they were weighed to obtain the dry weight of shoot and root (root dry weight – RDW and shoot dry weight – SDW)

Establishing P response curve for experiment two (2)

Incubation of soil

Using a shovel and spade, air-dried soil samples were manually and respectively mixed thoroughly with six different rates (0, 100, 250, 500, 750 and 1000 mg P/kg soil) of external P source (KH_2PO_4) on a cemented platform. Amended soils were covered with a black polyethene sheet and incubated for 28 days. Nursery bags were filled with respective incubated soil after 28 days and arranged in the greenhouse for the planting of cowpea seeds.

Sowing, watering, and harvesting of genetic materials

For each P level, ten (10) nursery pots were used. Each nursery pot was sown with two (2) seeds at a depth of 2 – 3 cm above the soil surface. Cowpea plants were thinned to one plant per pot after germination.

During the growth period, soils were maintained at a field capacity of 80% by irrigating with tap water if necessary. Nursery pots were rotated frequently to decrease the impacts of possible gradients from the environment.

Five (5) randomly selected plants for each P level were harvested 21 days after germination for root and shoot analysis.

Data collection

Data collected included; root and shoot fresh weight, root, and shoot dry weight, tissue P concentration, tissue P content, P efficiency ratio (PER). P uptake efficiency (PUpE), Agronomic P use efficiency (APE), P utilization efficiency (PUtE) and Physiological P use efficiency (PPUE).

Tissue P concentration and content

Three (3) replicates of oven-dried samples for both root and shoot were blended and used for P analysis. Phosphorus concentration in shoots and roots samples were determined using a spectrophotometric protocol as described by Fontaine (1942). One gram (1g) of milled sample material was digested in 5 mL of 18 M H_2SO_4 at 360 °C for 2 hrs, after which digests were diluted to 100 ml. One millilitre (1 ml) of the diluted solution was pipetted into 25 ml beaker and 4 ml of reagent B (ascorbic acid mixture) was added and was toppled to 25 ml mark with distilled water. A set of standard P solutions containing 0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 ppm P was prepared. The set up was allowed to stand for 15 minutes for a blue colour to develop and thereafter phosphorus content determined using a spectrophotometer. The absorbance of each sample was recorded upon reading (Heffernan, 1985). Tissue P content was calculated as the product of tissue P concentration and tissue dry weight.

Experiment one: Evaluation of genotypic variation in rhizosheath, root hair traits and RSA traits among cowpea genotypes

Genetic materials

The sixty (60) cowpea genotypes (*Vigna unguiculata* L.) used for the study were obtained from Uganda and International Institute of Tropical Agriculture (IITA) as well as local improved genotypes used as check varieties (Table 4). Codes, names, and country of origin for each accession are shown in Table 4.

Table 4: Characteristics of sixty (60) cowpea genotypes used for the first experiment

Codes	Genotype	Cultivar type	Source	Seed colour	Growth habit
1	Soronko	Improved	Ghana	Red	Semi-erect
2	Asontem	Improved	Ghana	Red	Semi-erect
3	Agyenkwa	Improved	Ghana	white	Semi-erect
4	Songotra	Improved	Ghana	white	Erect
5	NE 15*WC 35B	Inbred line	Uganda	Brown	Semi-erect
6	Nketewadea	Improved	Ghana	white	Semi-erect
7	Secow 5T	Improved	Uganda	Brown	Semi-erect
8	WC 36	Landrace	Uganda	Brown	Semi-erect
9	IT91	Inbred line	IITA	Brown	Semi-erect
10	Secow 2W	Improved	Uganda	White	Semi-erect
11	IT889	Inbred line	IITA	Mottled	Semi-erect
12	Secow 5T*NE 51	Inbred line	Uganda	Brown	Semi-erect
13	NE 48*Secow 5T	Inbred line	Uganda	Mottled	Semi-erect
14	Alegi*Secow 4WA	Inbred line	Uganda	Mottled	Semi-erect
15	IT97K819	Inbred line	IITA	Brown	Semi-erect
16	F258T2E	Inbred line	Uganda	Brown	Erect
17	WC 64	Landrace	Uganda	Mottled	Semi-erect
18	Ebelate*NE 51	Inbred line	Uganda	Mottled	Semi-erect
19	NE 50	Landrace	Uganda	White	Semi-erect
20	NE 51*NE 50	Inbred line	Uganda	Brown	Semi-erect
21	MU9A(Ama)	Inbred line	Uganda	Brown	Semi-erect
22	Alegi*Secow 4WB	Inbred line	Uganda	Brown	Semi-erect
23	MU9	Landrace	Uganda	Brown	Semi-erect
24	Alegi*Secow 5T	Inbred line	Uganda	Brown	Semi-erect
25	NE 48*WC 10	Inbred line	Uganda	Brown	Semi-erect
26	WC 35B*Secow 5T	Inbred line	Uganda	Brown	Semi-erect
27	WC 35B*WC 66	Inbred line	Uganda	Brown	Semi-erect
28	WC 35B*NE 50	Inbred line	Uganda	Brown	Semi-erect
29	Secow 5T*WC 36	Inbred line	Uganda	Brown	Semi-erect
30	NE 15*Sunshine	Inbred line	Uganda	Brown	Semi-erect

31	ACC 122W*WC 10	Inbred line	Uganda	Brown	Semi-erect

Table 4 contd.

	·cow 5T	Inbred line	Uganda	Brown	Semi-erect
33	NE 50*WC 36	Inbred line	Uganda	Brown	Semi-erect
34	NE 15*NE 50	Inbred line	Uganda	Brown	Semi-erect
35	Alegi*Sunshine	Inbred line	Uganda	Brown	Semi-erect
36	WC 35B*Alegi	Inbred line	Uganda	Brown	Semi-erect
37	ACC 122W*NE 15	Inbred line	Uganda	Brown	Semi-erect
38	NE 15*NE 48	Inbred line	Uganda	Brown	Semi-erect
39	ACC 122W	Landrace	Uganda	Brown	Creeping
40	WC 10	Landrace	Uganda	Brown	Semi-erect
41	WC 10*WC 36	Inbred line	Uganda	Brown	Semi-erect
42	Alegi*ACC 12	Inbred line	Uganda	Brown	Semi-erect
43	Sunshine	Landrace	Uganda	Brown	Semi-erect
44	NE 51*WC 66	Inbred line	Uganda	Brown	Semi-erect
45	ACC 122W*Alegi	Inbred line	Uganda	Brown	Semi-erect
46	ACC 122W*WC 36	Inbred line	Uganda	Black	Semi-erect
47	NE 50*Sunshine	Inbred line	Uganda	Brown	Semi-erect
48	NE 48*Secow 5T	Inbred line	Uganda	Brown	Semi-erect
49	Ebelate	Inbred line	Uganda	Brown	Semi-erect
50	WC 66*NE 50	Inbred line	Uganda	Brown	Semi-erect
51	WC 66*Sunshine	Inbred line	Uganda	Brown	Semi-erect
52	WC 36*Sunshine	Inbred line	Uganda	Brown	Semi-erect
53	WC 35B*NE 48	Inbred line	Uganda	Brown	Semi-erect
54	WC 35B	Landrace	Uganda	Brown	Semi-erect
55	Alegi*Secow 1T	Inbred line	Uganda	Brown	Semi-erect
56	NE 50*WC 10	Inbred line	Uganda	Mottled	Semi-erect
57	NE 48*NE 50	Inbred line	Uganda	Brown	Semi-erect
58	Secow 3B	improved	Uganda	Brown	Semi-erect
59	ACC 122W*NE 48	Inbred line	Uganda	Brown	Semi-erect
60	NE 15*WC 36	Inbred line	Uganda	Brown	Semi-erect

Source: Field research, Opoku (2020)

Data collection

Data was collected on the following root and shoot parameters; shoot (SRW and SDW), root (RFW and RDW), leaf area traits (TLA, SLA and LMA) root hair traits (RHL and RHD), RSA (TRL) and rhizosheath parameters (ARW, SRW and RRW).

Experiment two: Effect of $[P]_{ext}$ on rhizosheath, root hair and RSA among selected cowpea genotypes

Treatments

Two (2) treatments comprising of cowpea and external P were used for the experiment. The cowpea genotypes comprised of twenty (20) genotypes selected from the evaluation of the first 60 varieties in Experiment 1 (Table 5). The selection of twenty cowpea genotypes was done by dividing genotypes into three equal quadrants/groups to ensure uniform distribution of genotypes for a selected trait (Appendix 2). Group I represented group with high value for the parameter of selection, group II represented intermediate whilst group III had the least value for the selection criteria. However, local genotypes were used as checks during the study.

Table 5: Characteristics of twenty (20) cowpea genotypes selected for the second experiment

No.	Codes	Genotype	Cultivar type	Source	Seed colour	Growth habit
1	**1**	Soronko	Improved	Ghana	Red	Semi-erect
2	**2**	Asontem	Improved	Ghana	Red	Semi-erect
3	**3**	Agyenkwa	Improved	Ghana	white	Semi-erect
4	**4**	Songotra	Improved	Ghana	white	Erect
5	**5**	NE 15*WC 35B	Inbred line	Uganda	Brown	Semi-erect
6	**6**	Nketewadea	Improved	Ghana	white	Semi-erect
7	**7**	Secow 5T	Improved	Uganda	Brown	Semi-erect
8	**8**	WC 36	Landrace	Uganda	Brown	Semi-erect
9	**9**	IT91	Inbred line	IITA	Brown	Semi-erect
23	**10**	MU9	Landrace	Uganda	Brown	Semi-erect
24	**11**	Alegi*Secow 5T	Inbred line	Uganda	Brown	Semi-erect
25	**12**	NE 48*WC 10	Inbred line	Uganda	Brown	Semi-erect
28	**13**	WC 35B*NE 50	Inbred line	Uganda	Brown	Semi-erect
30	**14**	NE 15*Sunshine	Inbred line	Uganda	Brown	Semi-erect
35	**15**	Alegi*Sunshine	Inbred line	Uganda	Brown	Semi-erect
41	**16**	WC 10*WC 36	Inbred line	Uganda	Brown	Semi-erect
43	**17**	Sunshine	Landrace	Uganda	Brown	Semi-erect
58	**18**	Secow 3B	improved	Uganda	Brown	Semi-erect
19	**19**	NE 50	Landrace	Uganda	White	Semi-erect
20	**20**	NE 51*NE 50	Inbred line	Uganda	Brown	Semi-erect

Source: Field research, Opoku (2020)

External P level

The levels of external P source used for this experiment were selected based on the results obtained from the P response curve. Three (3) external P levels (0, 250 and 500 mg P/kg soil) were used for the study. Table 6 below describes the quantity of external P (KH_2PO_4) applied to obtain each level of P used in the experiment.

Table 6: Quantity of external phosphorus (KH_2PO_4) used in incubating soil samples at varying $[P]_{ext}$ levels.

0 mg P / kg soil	**250 mg P / kg soil**	**500 mg P / kg soil**
1 kg soil = 0 mg P	1 kg soil = 250 mg P	1 kg soil = 500 mg P
136 kg soil = *x*	136 kg soil = *x*	136 kg soil = *x*
$\frac{0 mgP \times 136\ kg\ soil}{1\ kg}$	$\frac{250\ mgP \times 136\ kg\ soil}{1\ kg}$	$\frac{500\ mgP \times 136\ kg\ soil}{1\ kg}$
= 0 mg P	*= 34000 mg P*	*= 68000 mg P*
Convert mg to g	*Convert mg to g*	*Convert mg to g*
$\frac{0\ mgP}{1000}$ = 0 g P	$\frac{34000\ mgP}{1000}$ = *34* g P	$\frac{68000\ mgP}{1000}$ = *68* g P
Hence if,	***Hence if,***	***Hence if,***
136.09 g KH_2PO_4 = *31 g P*	136.09 g KH_2PO_4 = *31 g P*	136.09 g KH_2PO_4 = *31 g P*
***x** = 0 g P*	***x** = 34* g P	***x** = 68 g P*
$\frac{136.09\ g \times 0\ g}{31\ g}$ =	$\frac{136.09\ g \times 34\ g}{31\ g}$ =	$\frac{136.09\ g \times 68\ g}{31\ g}$ =
0 g KH_2PO_4	**149.26 g KH_2PO_4**	**298.52 g KH_2PO_4**

Source: Field research, Opoku (2020). *The molecular weight of KH_2PO_4 = 136.09 mol/g, Atomic mass of P = 31 g.*

Data collection

Data was collected on the following root hair traits (RHL and RHD), TRL, rhizosheath (ARW, SRW and RRW), tissue P concentration (root and shoot P concentrations), tissue P content (root and shoot P content), P efficiency

ratio (PER). P uptake efficiency (PUpE), Agronomic P use efficiency (APE), P. utilization efficiency (PUtE) and Physiological P use efficiency (PPUE).

Estimating P uptake and use efficiency

Parameters on phosphorus uptake and use efficiencies were estimated using formulas as described by Hammond *et al*. (2009).

Agronomic P use efficiency

Agronomic phosphorus use efficiency (APE) was calculated by the Equation 4;

$$APE = (Y_{high} - Y_{low}) / \Delta Papp \qquad \text{Eqn (4)}$$

Where; Y_{high} *= Dry matter on P amended soil;* Y_{low} *= Dry matter on unamended soil and* $\Delta Papp$ *= difference in the amount of P applied as fertilizer between P amended and unamended soil treatment.*

Phosphorus uptake efficiency

Phosphorus uptake efficiency (PUpE) was calculated by Equation 5.

$$PUpE = [(P_{high} \times Y_{high}) - (P_{low} \times Y_{low})] / \Delta Papp \qquad \text{Eqn (5)}$$

Where; Y_{high} *= Dry matter on P amended soil;* Y_{low} *= Dry matter on unamended soil,* P_{high} *= tissue P concentration on P amended soil treatment;* P_{low} *= tissue P concentration on unamended soil treatment and* $\Delta Papp$ *= difference in the amount of P applied as fertilizer between P amended and unamended soil treatment.*

Phosphorus efficiency ratio

Phosphorus efficiency ratio (PER) was calculated by Equation 6.

$$PER = Y_{high} / (P_{high} \times Y_{high}) \text{ or } Y_{low} / (P_{low} \times Y_{low}) \quad \text{Eqn (6)}$$

Where; Y_{high} = Dry matter on P amended soil; Y_{low} = Dry matter on unamended soil, P_{high} = tissue P concentration on P amended soil treatment; P_{low} = tissue P concentration on unamended soil treatment and ΔPapp = difference in the amount of P applied as fertilizer between P amended and unamended soil treatment.

Phosphorus utilization efficiency

Phosphorus utilization efficiency (PUtE) was calculated by Equation 7.

$$PUtE = (Y_{high} - Y_{low}) / [(P_{high} \times Y_{high}) - (P_{low} \times Y_{low})] \quad \text{Eqn (7)}$$

Where; Y_{high} = Dry matter on P amended soil; Y_{low} = Dry matter on unamended soil, P_{high} = tissue P concentration on P amended soil treatment; P_{low} = tissue P concentration on unamended soil treatment and ΔPapp = difference in the amount of P applied as fertilizer between P amended and unamended soil treatment.

Physiological P use efficiency

Physiological P use efficiency (PPUE) was calculated by Equation 8.

$$Y_{high} / P_{high} \text{ or } Y_{low} / P_{low} \quad \text{Eqn (8)}$$

Where; Y_{high} = Dry matter on P amended soil; Y_{low} = Dry matter on unamended soil, P_{high} = tissue P concentration on P amended soil treatment and P_{low} = tissue P concentration on unamended soil treatment.

Statistical analysis

Statistical analysis was carried out using GenStat Release 12.1 Copyright 2009 (VSN International Ltd). For each of the experiments, data from both trials were combined to determine descriptive statistics. Residual maximum likelihood (REML) procedures were used to estimate variance components for all the selected traits and ANOVA was used to determine variation between genotypes, phosphorus, trials, and interaction effects depending on the experiment. All factors were categorized as random factors in REML so that the proportional contribution of genotype to overall variation in traits could be determined (Adu *et al*., 2019). The following models (Eqn 9 and 10) were used for both REML and ANOVA for Experiment 1 and Experiment 2 respectively.

$$Y_{ij} = \mu + g_i + t_j + gt_{ij} + \varepsilon_{ij} \qquad \text{Eqn (9)}$$

Where: y_{ij} = observation from the ij^{th} genotype and trial, μ = overall mean, gi = effect of the i^{th} genotype, t^j = effect of the j^{th} trial, gt_{ij} = interactive effect of the i^{th} genotype with the j^{th} trial and ε_{ijk} = experimental error.

$$Y_{ijk} = \mu + g_i + t_j + p_k + gt_{ij} + gp_{ik} + pt_{jk} + gtp_{ijk} + \varepsilon_{ijk} \qquad \text{Eqn (10)}$$

Where: y_{ijk} = observation from the ijk^{th} genotype, trial and phosphorus level, μ = overall mean, gi = effect of the i^{th} genotype, t^j = effect of the j^{th} trial, p^k = effect of the k^{th} phosphorus level, gt_{ij} = interactive effect of the i^{th} genotype with the j^{th} trial, gp_{ik} = interactive effect of the i^{th} genotype with the k^{th} phosphorus level, pt_{jk} = interactive effective effect of the k^{th} phosphorus level

and j^{th} trial, gtp_{ijk} = interactive effect of the i^{th} genotype with the j^{th} trial and the k^{th} phosphorus level and ε_{ijk} = experimental error.

Broad-sense heritability (H^2) was calculated as a quotient of genotypic variance and the total phenotype variance (σ^2_g / σ^2_p) (Adu *et al*., 2014). The phenotypic variance was calculated using Equation 11 as applied by Kumar *et al*. (2012).

$$\sigma^2_p = \sigma^2_g + \frac{\sigma^2_{g \times t}}{n} + \frac{\sigma^2_\varepsilon}{rn} \qquad \text{Eqn (11)}$$

where: r is the number of replicates, n is the number of trials, $\sigma^2_g \times t$ is the genotype x trial variance and σ^2_p = phenotypic variance.

Principal components analysis (PCA) was done to identify major traits accounting for most of the variation among the studied cowpea genotypes. The PCA was based on the correlation matrix and the number of significant principal components was determined based on the Kaiser criterion, retaining any component with an eigenvalue greater than one (Tabachnick and Fidell, 1996).

Correlation analysis and cluster analysis were carried out using a statistical package for social science (SPSS) version 23. Clustering analysis was performed using Ward's hierarchical approach based on the minimum variance linking method with Euclidean distance as the similarity measure. The optimal number of clusters was chosen based on a standardized range of (-1 to +1).

CHAPTER FOUR

RESULTS

Establishing phosphorus response for experimental soil

Root and shoot dry weights of cowpea genotype increased to an asymptote with increasing external $[P]_{ext}$ concentration as shown in (Figure 1A and 1B) respectively. Both shoot and root dry weight were highest at soil amended with 500 mg P/kg (Figure 1A and 1B). Tissue P concentration of cowpea showed a similar response. Shoot and root P concentrations increased exponentially in response to an increase in external P application as shown in (Figure 1C and 1D) respectively. This response is illustrated by an exponential rise to a peak where it began to decline. However, cowpea shoot recorded the highest level of P concentrations compared to root P concentrations. Phosphorus treatments were selected based on the point at which maximum biomass weight, as well as tissue P concentration, was high (Figure 1C and 1D).

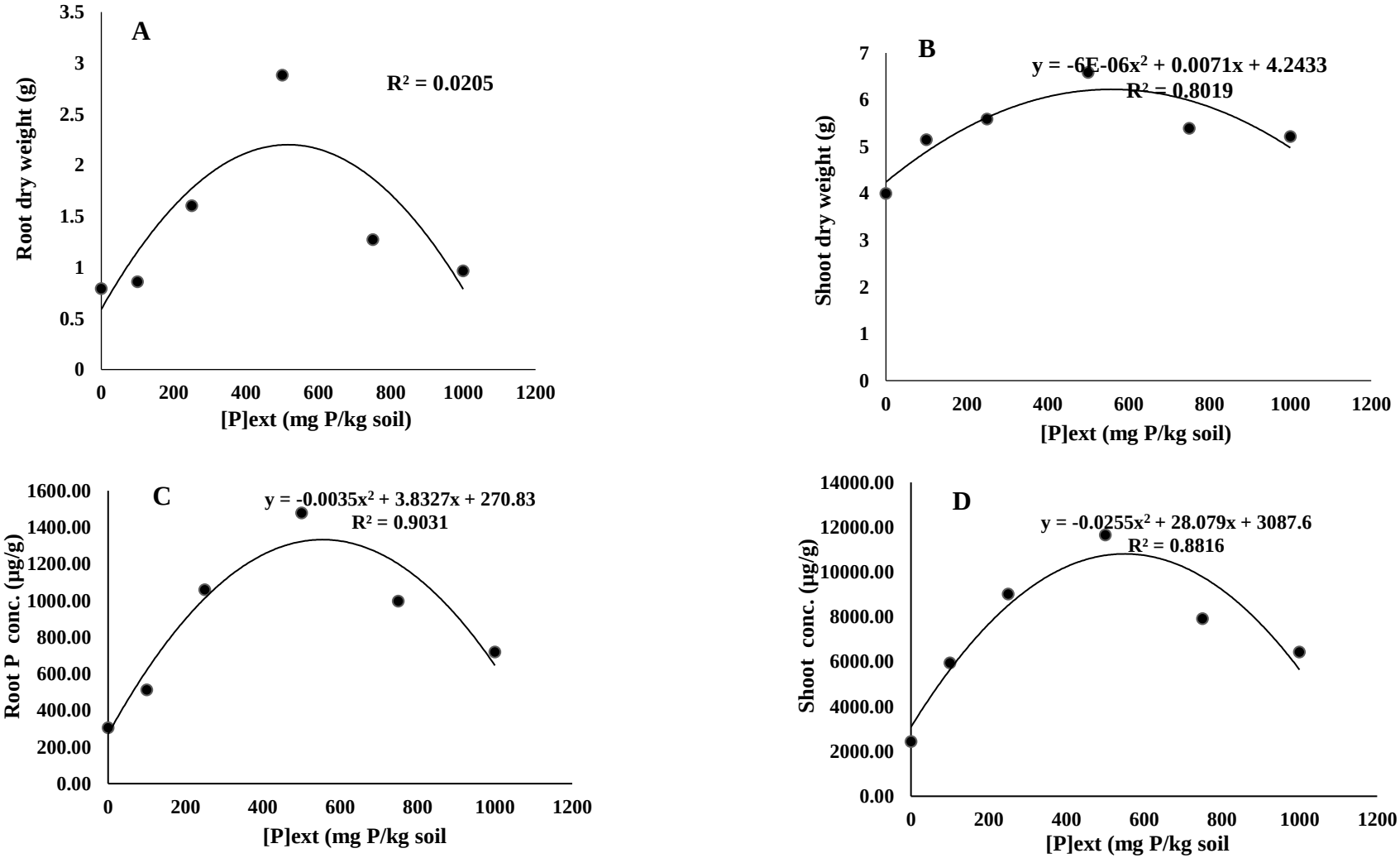


Figure 1: Response of **(A)** Root dry weight **(B)** Shoot dry weight **(C)** Root P concentration (D) Shoot P concentration to varying $[P]_{ext.}$ Values are the mean of five (5) replicates. The amount of P added to obtain maximal growth on this soil was calculated to be 250 and 500 mg P kg^{-1} soil.

Experiment 1: Evaluation of cowpea genotypes for formation of root hair, root system architecture (RSA) and biomass traits.

Biomass parameters

Root dry weight

The effects of genotype accounted for significant ($P < 0.001$) variation in RDW among the cowpea genotypes (Figure 2A). Root dry weight ranged from 0.18 - 0.85g. The topmost five cowpea genotypes with superior RDW in the first screening were NE 48*NE 50: (0.85 g); Alegi*Secow 4WA: 0.78 (g); Secow 2W: (0.75 g); IT97K819: (0.72 g) and F258T2E: (0.71 g) (Figure 2A). On the other half, genotypes Alegi*Sunshine: (0.24 g); NE 15*Sunshine: (0.24 g); NE 15*NE 48: (0.22 g); NE 48*WC 10: (0.22 g) and NE 51*WC 66: (0.18 g) made up the last five genotypes with least RDW (Figure 2A).

Similarly, the genotypic effect was significant ($P < 0.001$) for RDW among cowpea genotypes in the second screening (Figure 2B). The remaining genotypes obtained RDW ranging from 0.34 g - 0.55 g (Figure 2B). The top five genotypes with high RDW distribution were NE 50*WC 10: (0.78 g); NE 48*NE 50: (0.66 g); MU9: (0.64 g); IT91: (0.64 g) and Songotra: (0.63 g). Genotypes with low RDW were ACC 122W*NE 15: (0.30 g); NE 48*WC 10: (0.27 g); Ebelate: (0.25 g); NE 15*NE 50: (0.25 g) and NE 51*NE 50: (0.24 g) (Figure 2B).

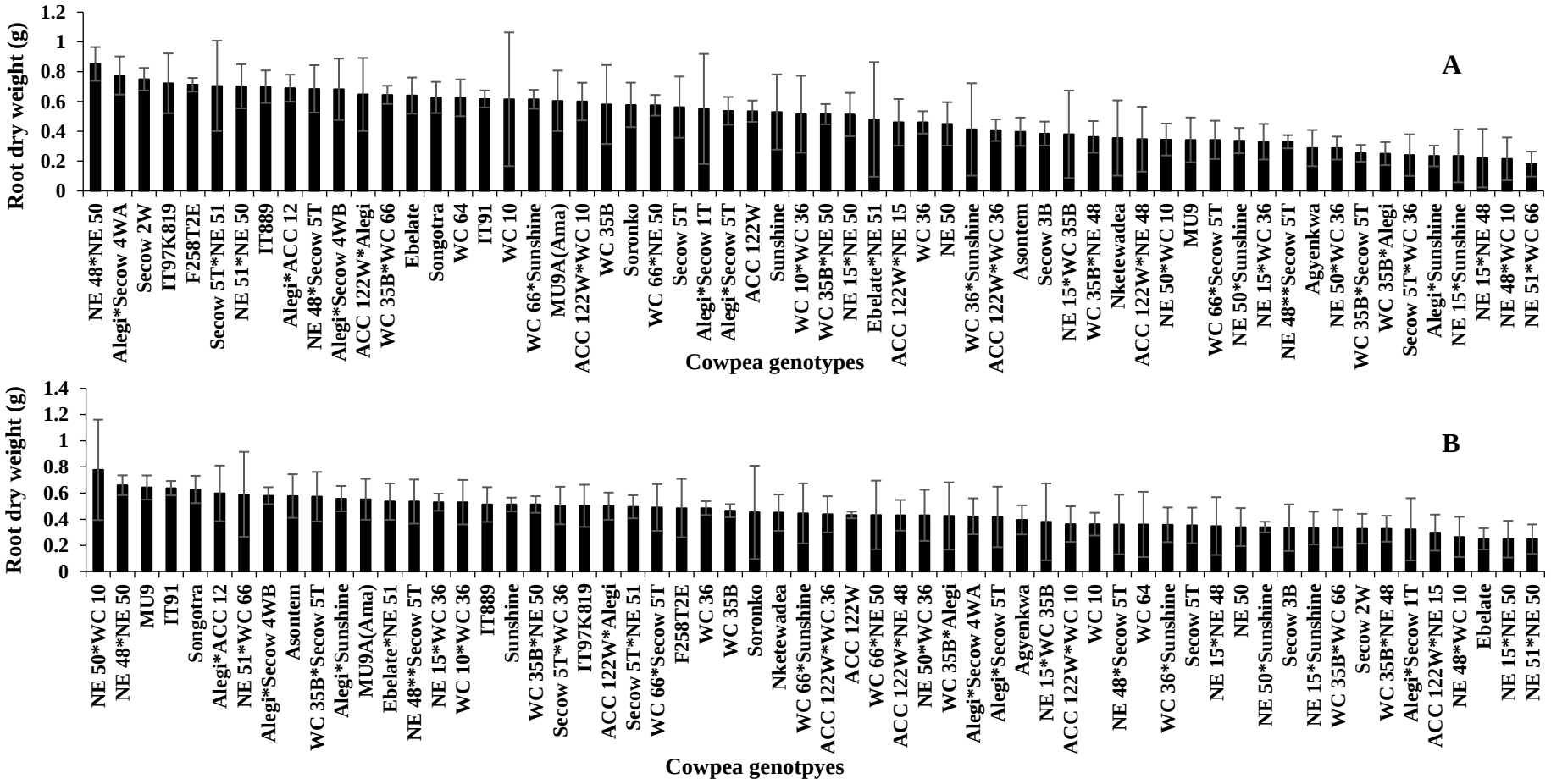


Figure 2: Variation in root dry weight of three (3) weeks old cowpea genotypes grown under greenhouse condition for **(A)** First trial; **(B)** Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Difference between cowpea genotypes was established using ANOVA.

Shoot dry weight

Genotype had a significant effect ($P < 0.001$) on SDW recorded in the first screening (Figure 3A). Shoot dry weight ranged from 0.82 - 5.25 g during the first screening (Figure 3A). Genotypes WC 10: (5.25 g); NE 48*NE 50: (4.645 g); Ebelate: (4.33 g); Secow 5T: (4.14 g); IT889: (4.07 g); WC 66*Sunshine: (3.87 g); Alegi*Secow 4WA: (3.74 g); WC 35B*WC 66: (3.57 g); WC 10*WC 36: (3.47 g) and Alegi*ACC 12: (3.40 g) recorded the highest SDW. However, genotypes NE 48**Secow 5T: (1.74 g); WC 35B*Secow 5T: (1.59 g); NE 50*WC 36: (1.37 g); NE 15*Sunshine: (1.30 g); Alegi*Sunshine: (1.22 g); NE 15*NE 48: (1.21 g); WC 35B*Alegi: (1.12 g); Secow 5T*WC 36: (1.10 g); NE 51*WC 66: (0.92 g) and NE 48*WC 10: (0.82 g) recorded lowest SDW which was significantly different from the remaining genotypes (Figure 3A).

There was significant ($P < 0.001$) variation in SDW obtained by genotypes in the second screening (Figure 3B). Among the distribution, genotypes Alegi*Secow 5T: (5.67 g); F258T2E: (5.04 g); NE 51*WC 66: (3.86 g); NE 15*WC 36: (3.82 g); Songotra: (3.52 g); Alegi*Sunshine: (3.44 g); NE 48*NE 50: (3.42 g); Soronko: (3.38 g); Secow 5T*NE 51: (3.35 g) and WC 66*Secow 5T: (3.34 g) make up the last ten genotypes with high SDW (Figure 3B). Genotypes Secow 2W: (1.94 g); WC 35B*WC 66: (1.85 g); ACC 122W: (1.82 g); NE 15*NE 48: (1.71 g); NE 50: (1.66 g); NE 51*NE 50: (1.64 g); NE 15*NE 50: (1.57 g); Agyenkwa: (1.41 g); Alegi*Secow 4WA: (1.41 g) and Ebelate: (1.37 g) recorded low SDW in the second screening (Figure 3B).

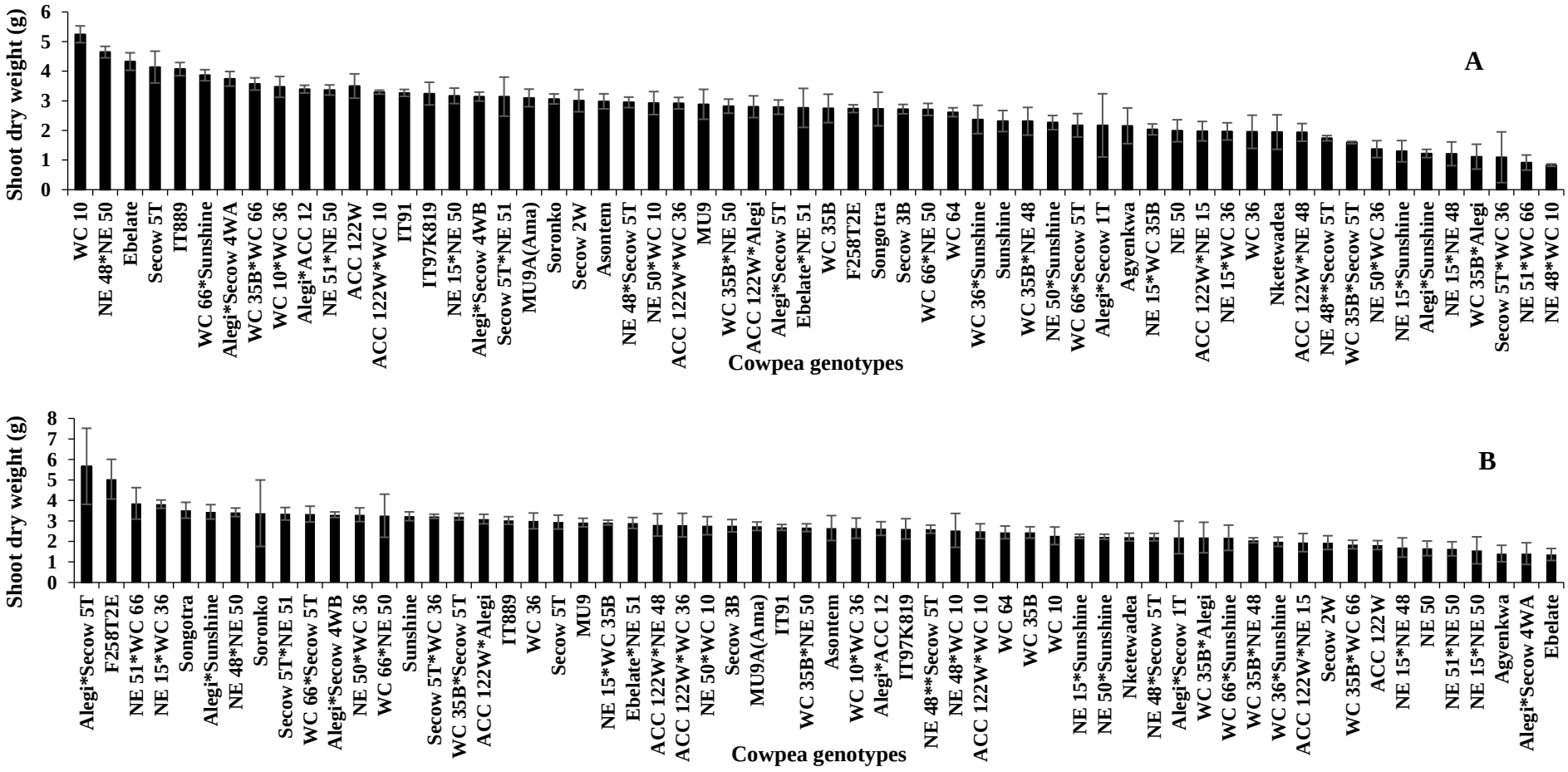


Figure 3*:* Variation in shoot dry weight of three (3) weeks old cowpea genotypes grown under greenhouse condition for **(A)** First trial; **(B)** Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Difference between cowpea genotypes was established using ANOVA.

Total leaf area

The sixty cowpea genotypes screened exhibited significant ($P < 0.001$) variation in TLA during the first trial (Figure 4A). Total leaf area in the first trial ranged from 155.2 - 676.6 cm^2. Genotypes IT97K819, ACC 122W*NE 15, IT889, Secow 5T, NE 48*Secow 5T, Alegi*Secow 5T, Asontem, WC 35B*Secow 5T, ACC 122W*NE 48 and WC 66*Sunshine made up the topmost ten genotypes with the highest TLA (Figure 4A). However, genotypes Songotra, Alegi*Secow 1T, NE 48**Secow 5T, NE 48*WC 10, Nketewadea, ACC 122W*Alegi, Agyenkwa, WC 36, WC 35B*NE 48 and WC 64 recorded lowest TLA which was significantly different from the remaining genotypes (Figure 4A).

There was a significant ($P < 0.001$) variation in TLA obtained by genotypes in the second trial (Figure 4B). Among the genotypes, Alegi*Secow 4WB, F258T2E, Sunshine, Alegi*Sunshine, Soronko, NE 51*WC 66, WC 35B*Secow 5T, WC 35B*NE 50, Secow 5T*WC 36 and ACC 122W*Alegi made up the first ten genotypes with high TLA (Figure 4B). Genotypes NE50, WC35B*Alegi, Alegi*Secow 4WA, Ebelate, NE15*WC35B and WC36*Sunshine recorded least TLA weight in the second trial (Figure 4B).

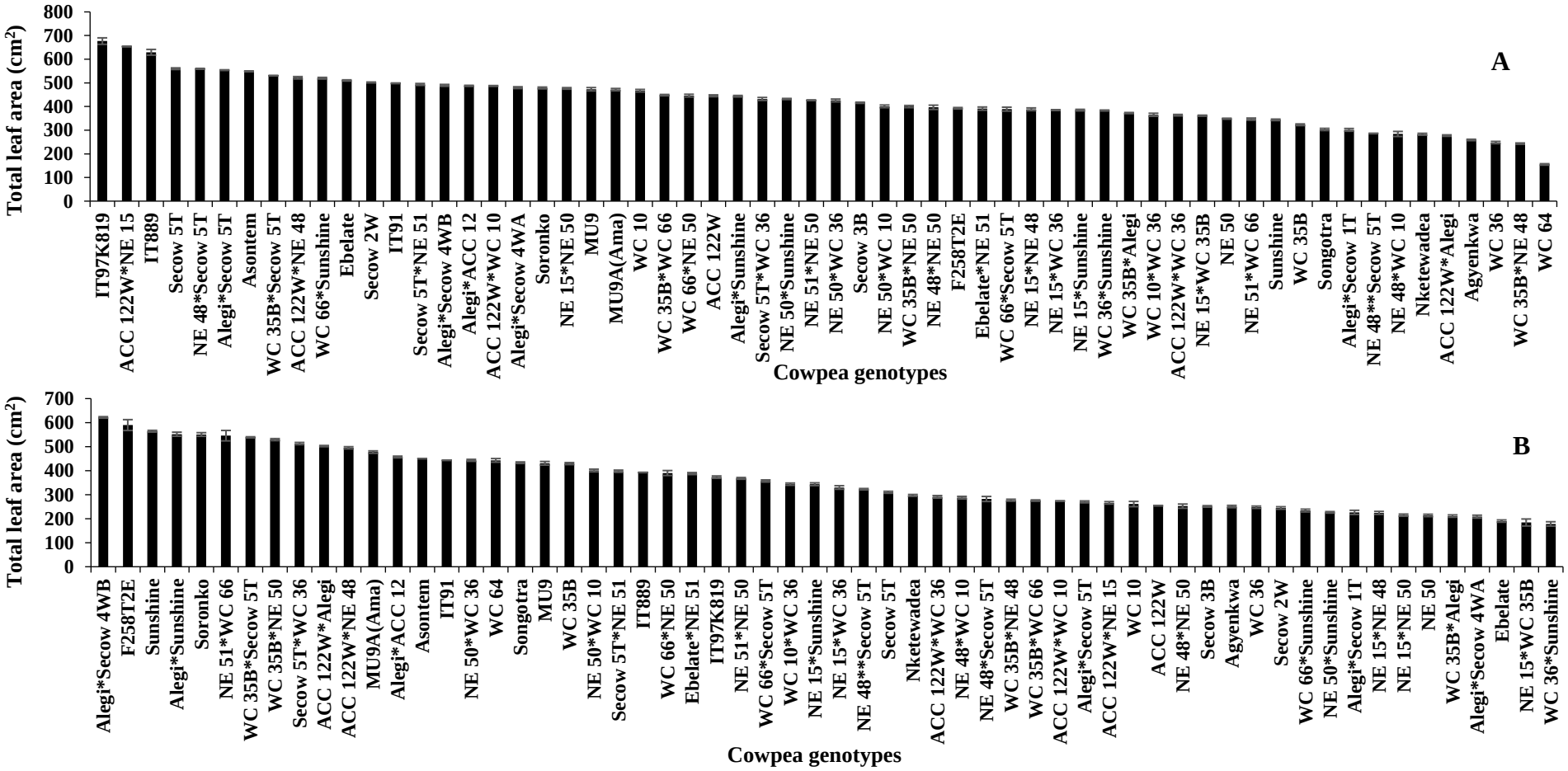


Figure 4: Variation in total leaf area of three (3) weeks old cowpea genotypes grown under greenhouse condition for (**A**) First trial; (**B**) Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Difference between cowpea genotypes was established using ANOVA.

Specific leaf area

The sixty cowpea genotypes evaluated exhibited significant ($P < 0.001$) variation in SLA during the first trial (Figure 5A). Specific leaf area per plant ranged from 138.3 - 1340.3 g cm^{-2} for genotypes WC 10 and WC 35B*Alegi respectively. There was more than one-fold variation in SLA among the sixty screened genotypes with WC 35B*Alegi, Secow 5T*WC 36, NE 48*WC 10, NE 51*WC 66, Alegi*Sunshine, WC 35B*Secow 5T, NE 15*NE 48, NE 50*WC 36, IT97K819 and ACC 122W*NE 48 making up the topmost ten genotypes with the highest specific leaf area (Figure 5A). However, ACC 122W*Alegi, NE 51*NE 50, Ebelate, WC 35B, WC 35B*NE 48, NE 48*NE 50, WC 64, Songotra, WC 36 and WC 10 made up the last then genotypes with the least SLA (Figure 5A).

Similarly, a significant ($P < 0.001$) variation in SLA was obtained by genotypes in the second trial (Figure 5B). Among the distribution of genotypes, Alegi*Secow 4WA, NE 50*WC 10, NE 51*NE 50, Agyenkwa, Alegi*Secow 4WB, Soronko, Asontem, ACC 122W*NE 48, WC 35B*Alegi and WC 35B*NE 50 made up the first ten genotypes with high SLA (Figure 5B). Genotypes, NE 50*Sunshine, Secow 5T, WC 36*Sunshine, NE 15*WC 36, Alegi*Secow 1T, ACC 122W*WC 36, WC 36, NE 48*NE 50, NE 15*WC 35B and Alegi*Secow 5T recorded SLA in the second trial (Figure 5B).

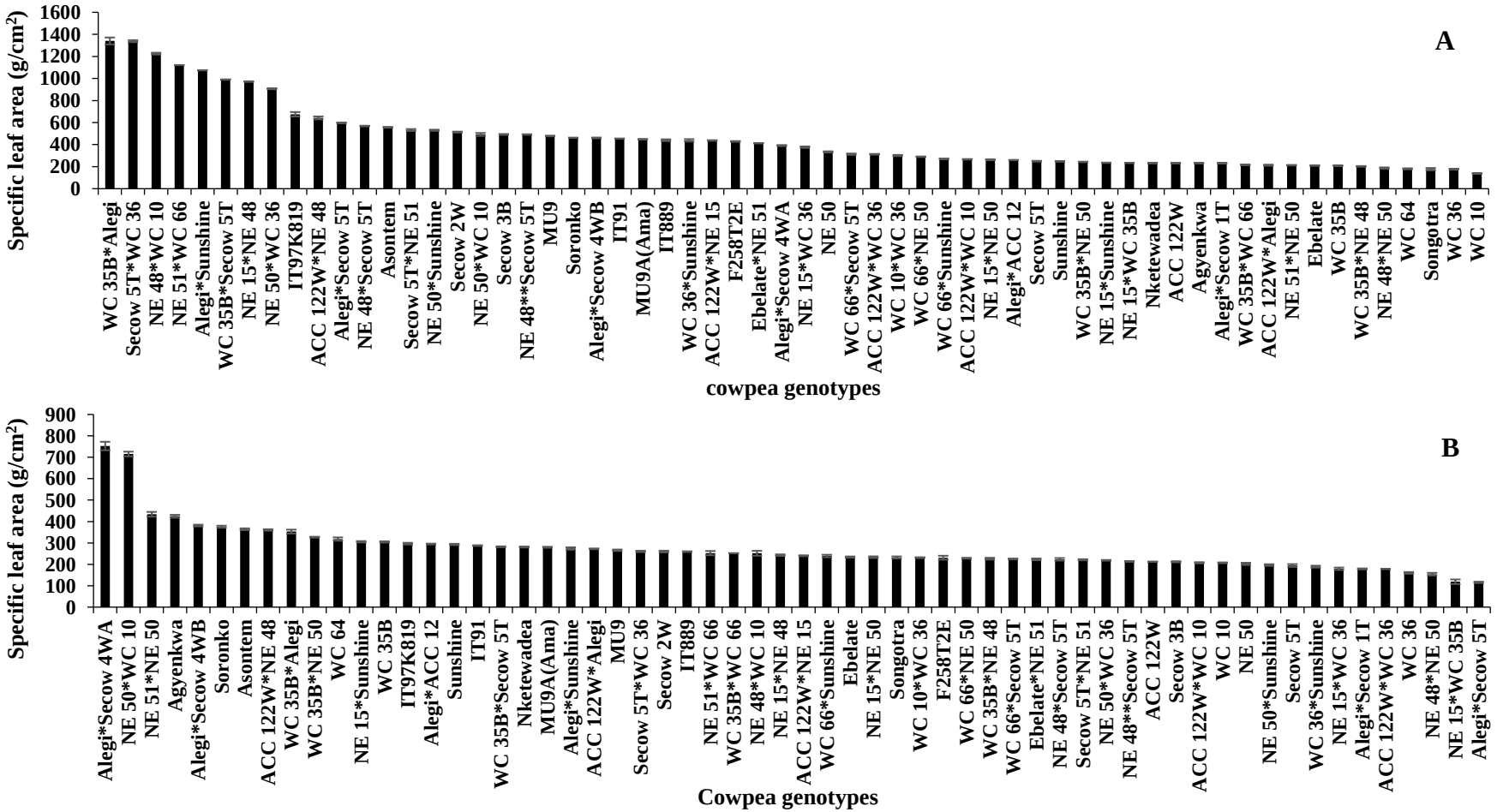


Figure 5: Variation in specific leaf area of three (3) weeks old cowpea genotypes grown under greenhouse condition for (A) First trial; (B) Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Difference between cowpea genotypes was established using ANOVA.

Leaf mass per area

The sixty cowpea genotypes screened exhibited significant ($P < 0.001$) variation in LMA during the first trial (Figure 6A). Leaf mass per area in the first trial ranged from 0.0009 – 0.089 g/cm^2 for genotypes NE 51*WC 66 and NE 48*NE 50 respectively. There was more than one-fold variation in LMA among the sixty screened genotypes with NE 48*NE 50, WC 36, ACC 122W*NE 48, Songotra, WC 10, NE 50*WC 10, NE 15*NE 50, Secow 5T*NE 51, WC 64 and WC 35B making up the topmost ten genotypes with the highest LMA (Figure 6A). However, Alegi*Secow 5T, NE 48**Secow 5T, MU9A (Ama), IT91, NE 15*WC 35B, Soronko, NE 15*NE 48, WC 35B*Secow 5T, Alegi*Sunshine and NE 51*WC 66 made up the last then genotypes with the least LMA (Figure 6A).

Similarly, a significant ($P < 0.001$) variation in LMA was obtained by genotypes in the second trial (Figure 6B). Among the distribution, NE 15*WC 36, WC 35B*Alegi, Alegi*Secow 4WA, WC 66*Sunshine, NE 50*WC 10, NE 15*WC 35B, Alegi*Secow 5T, NE 50 and WC 36 made up the first ten genotypes with high LMA (Figure 6B). Genotypes WC 35B*Secow 5T, WC 10*WC 36, IT91, IT889, ACC 122W*Alegi, Alegi*ACC 12, WC 35B*NE 50, ACC 122W*WC 36, ACC 122W and WC 35B*WC 66 recorded LMA in the second trial (Figure 6B).

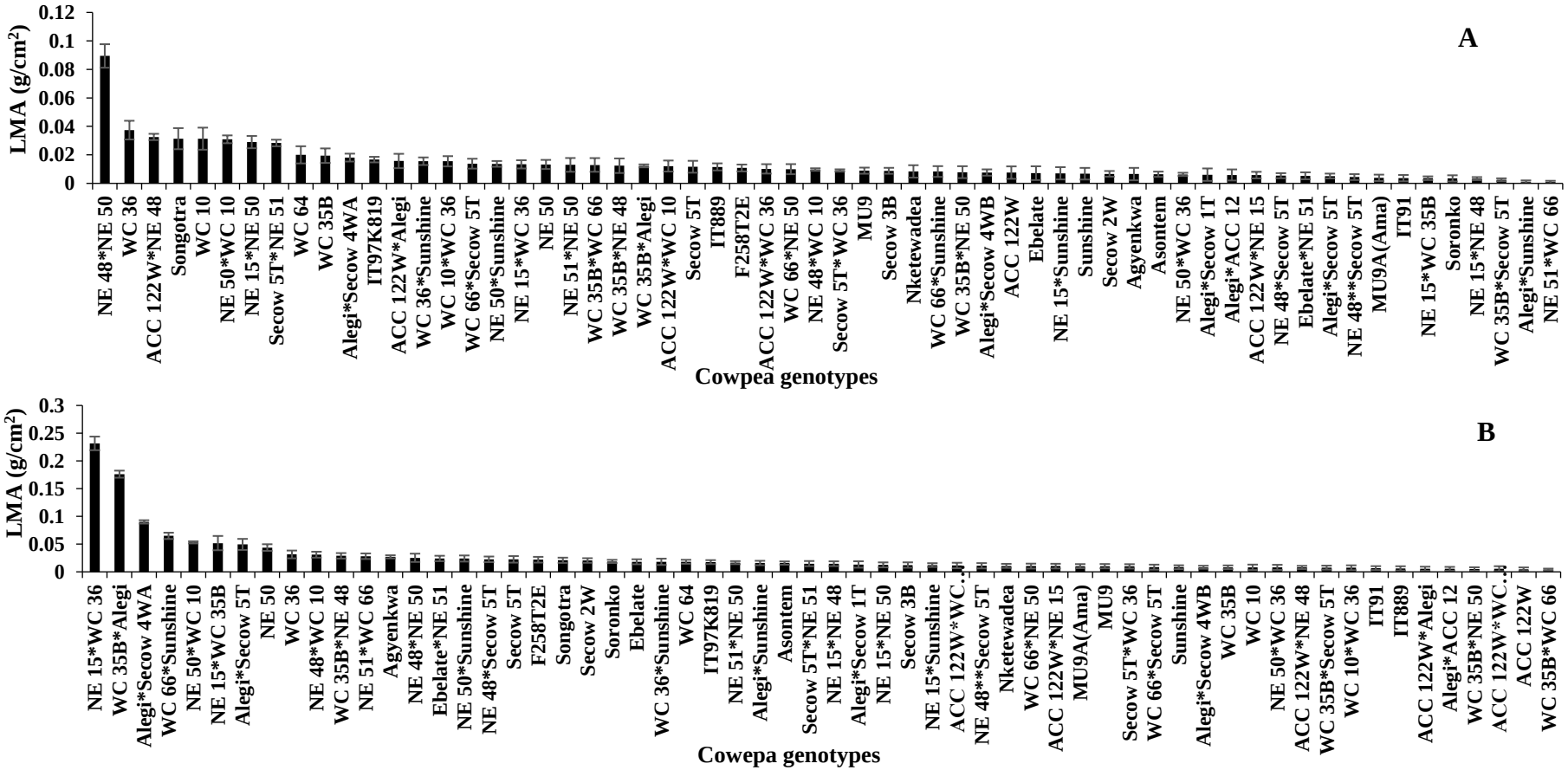


Figure 6: Variation in leaf mass per area of three (3) weeks old cowpea genotypes grown under greenhouse condition for (A) First trial; (B) Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Difference between cowpea genotypes was established using ANOVA.

Rhizosheath parameters

Absolute rhizosheath weight

Genotype had a significant (*P < 0.001*) effect on ARW obtained by cowpea genotypes both in the first trial (Figure 7A). Absolute rhizosheath weight ranged from 3.31g - 6.34g indicating more than one-fold of variation in ARW obtained by cowpea genotypes (Figure 7A). The topmost ten genotypes included WC 10*WC 36, ACC 122W*Alegi, NE 51*NE 50, IT889, NE 15*WC 36, ACC 122W*WC 36, IT91, IT97K819, Secow 5T*NE 51 and ACC 122W with ARW ranging from 6.34g – 4.77g. The genotypes NE 15*WC 35B, NE 50*WC 10, Ebelate, WC 66*Sunshine, Nketewadea, Alegi*Sunshine, WC 10, Alegi*Secow 5T, ACC 122W*WC 10 and NE 15*NE 50 were the ten genotypes with the least ARW ranging from 3.31 - 3.60g. The remaining genotypes obtained 3.60 - 4.72g (Figure 7A).

In the second screening, WC 35B*WC 66, Alegi*Sunshine, NE 15*NE 50, WC 35B*NE 50, WC 66*Secow 5T, NE 15*NE 48, ACC 122W, ACC 122W*WC 10, Ebelate and Secow 5T constituted the ten topmost genotypes with the highest ARW 4.23g - 4.75g (Figure 7B). On the other hand, the last ten genotypes with the least ARW were Alegi*Secow 4WB, F258T2E, WC 10*WC 36, Secow 3B, Alegi*Secow 4WA, IT889, NE 15*WC 36, WC 64, ACC 122W*Alegi and Secow 5T*NE 51 with values ranging from 2.93g - 3.21g (Figure 7B).

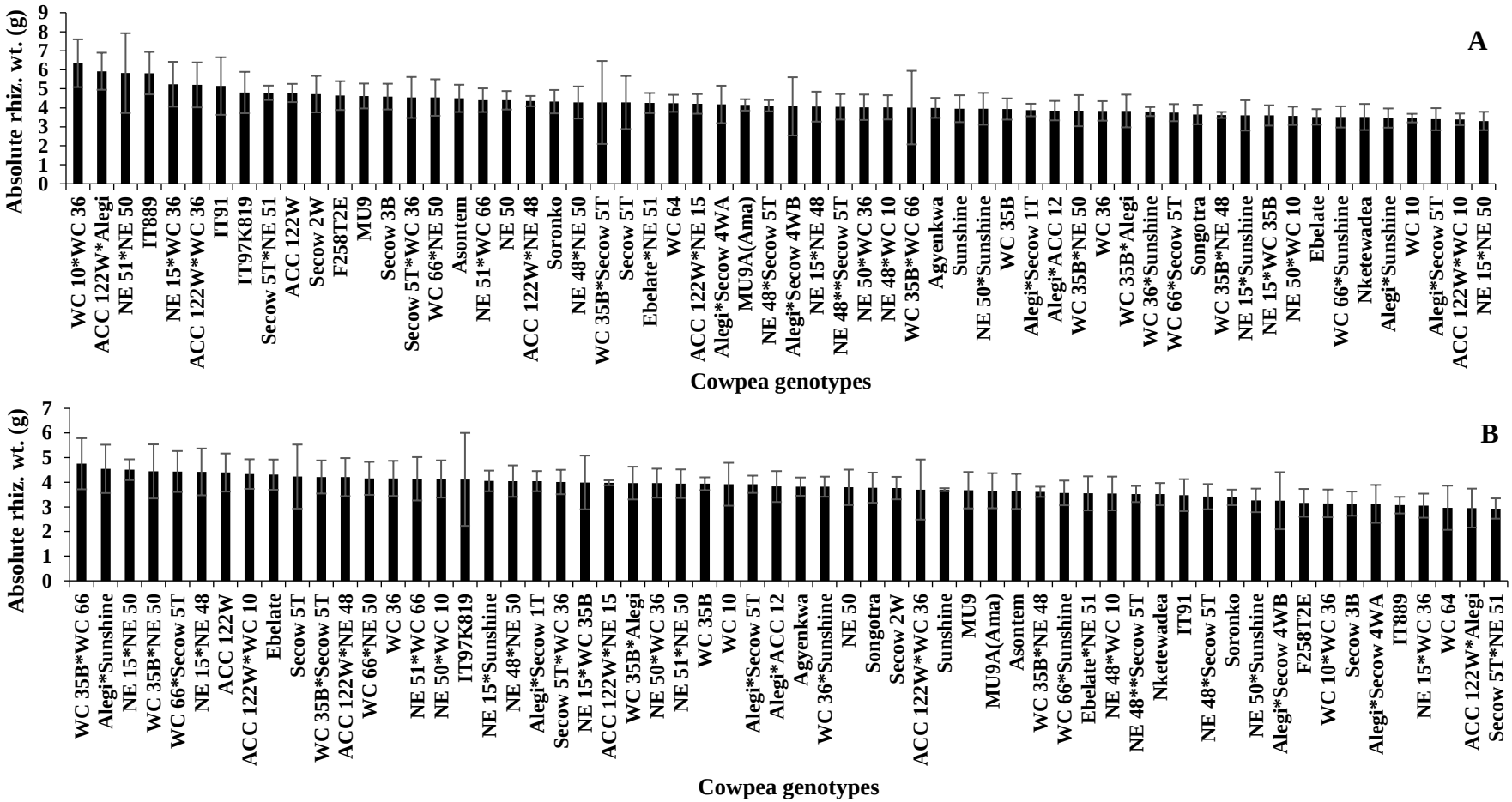


Figure 7: Variation in absolute rhizosheath weight of three (3) weeks old cowpea genotypes grown under greenhouse condition for (A) First trial; (B) Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Difference between cowpea genotypes was established using ANOVA.

Relative rhizosheath weight

Relative rhizosheath weight recorded during both trials was significantly ($P < 0.001$) different among cowpea genotypes (Figure 8A and 10B). Relative rhizosheath weight ranged from approximately 25.63 g to 4.8 g in the first screening (Figure 8A). In the first screening, topmost ten genotypes with the highest RRW were NE 48*WC 10: 25.63 (g/g root); Secow 5T*WC 36: 24.05 (g/g root); NE 51*WC 66: 18.43 (g/g root); Alegi*Sunshine: 15.06 (g/g root); MU9: 15.05 (g/g root); Agyenkwa: 14.8 (g/g root); NE 50*WC 36: 13.88 (g/g root); ACC 122W*NE 48: 13.66 (g/g root); ACC 122W*WC 36: 13.2 (g/g root) and NE 15*NE 48: 12.61 (g/g root). On the other half, genotypes NE 48*Secow 5T: 6.07 (g/g root); Songotra: 6.04 (g/g root); Alegi*Secow 5T: 5.93 (g/g root); ACC 122W*WC 10: 5.76 (g/g); WC 66*Sunshine: 5.76 (g/g root); Alegi*ACC 12: 5.56 (g/g root); Ebelate: 5.44 (g/g root); Alegi*Secow 4WA: 5.29 (g/g root); NE 48*NE 50: 5.01 (g/g root) and WC 10: 4.8 (g/g root) constitute the last ten genotypes with low RRW (Figure 8A).

In the second screening, genotype had a significant ($P < 0.001$) effect on RRW obtained by cowpea genotypes (Figure 8B). Genotypes WC 35B*WC 66: 14.46 (g/g root); WC 66*NE 50: 13.96 (g/g root); Secow 5T: 12.78 (g/g root); NE 51*NE 50: 12.29 (g/g root); ACC 122W*NE 15: 11.62 (g/g root); NE 50: 11.62 (g/g); Alegi*Secow 5T: 11.57 (g/g); Secow 2W: (11.49 g/g root); WC 35B*NE 48: 11.48 (g/g root) and NE 15*NE 50: 11.34 (g/g root) make up the topmost ten genotypes with high RRW (Figure 8B).

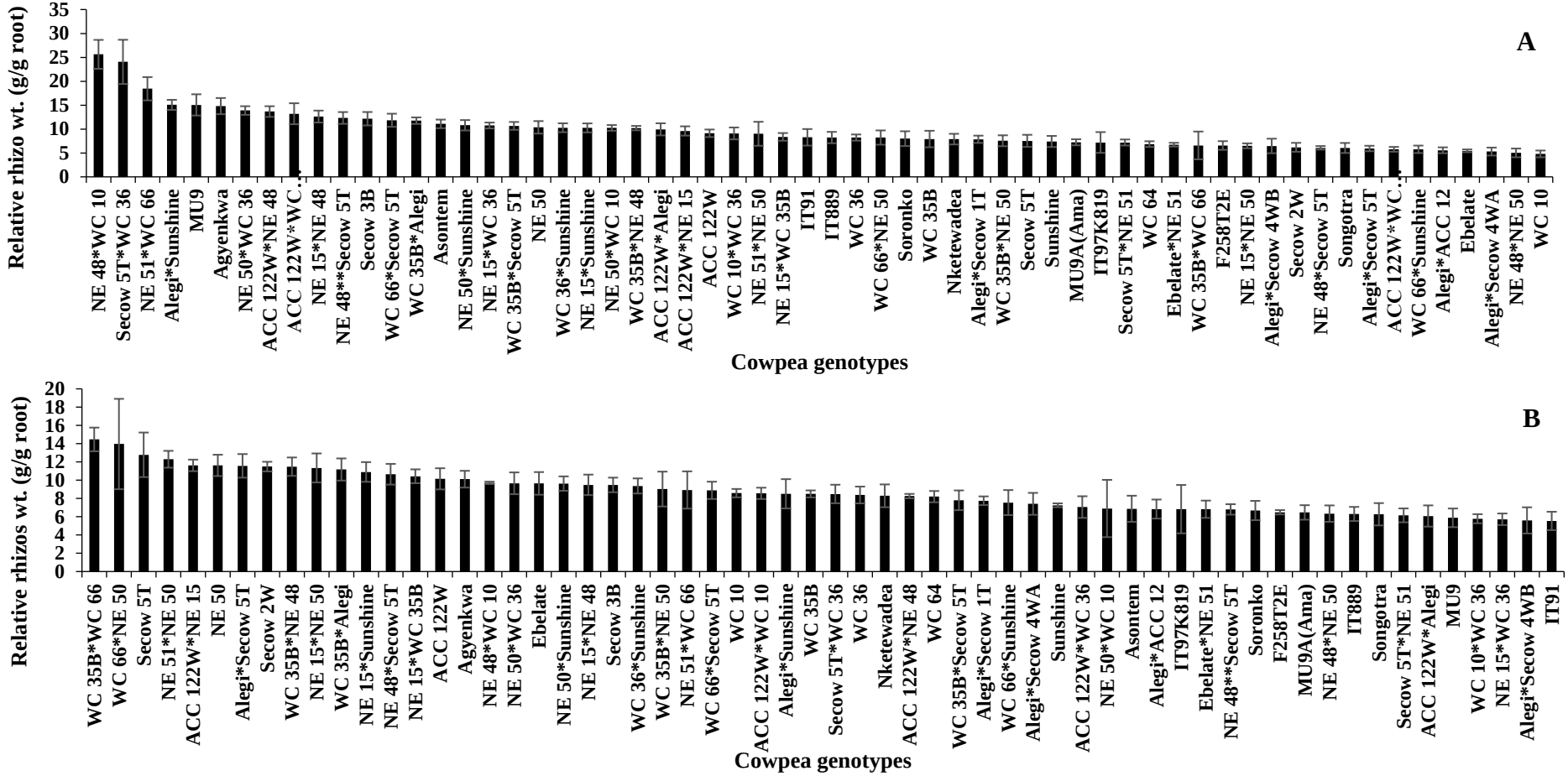


Figure 8: Variation in relative rhizosheath weight of three (3) weeks old cowpea genotypes grown under greenhouse condition for **(A)** First trial; **(B)** Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Difference between cowpea genotypes was established using ANOVA.

Specific rhizosheath weight

In the first screening, SRW ranged from 7.54 - 51.08 (g/cm root). Genotypes screened showed significant ($P < 0.001$) variation in specific rhizosheath weight (Figure 9A). Genotypes with the highest SRW included Songotra, WC 66*NE 50, NE 51*NE 50, Nketewadea and NE 50*WC 10: 42.91 (Figure 9A). Genotypes Ebelate*NE 51, Alegi*Secow 4WA, NE 48*Secow 5T, WC 64, ACC 122W*NE 48, ACC 122W*Alegi, IT91, Secow 3B, NE 15*WC 36 and NE 50*Sunshine constituted the last ten genotypes with low SRW (Figure 9A).

In the second screening, genotype had a significant ($P = 0.001$) effect on SRW obtained by cowpea genotypes (Figure 9B). Genotypes WC 66*Sunshine: (g/cm root); NE*WC 10: 53.59 (g/cm root); ACC 122W: 52.08 (g/cm root); Alegi*Secow 5T: 50.09 (g/cm root); NE 15*Sunshine: 49.47 (g/cm root); WC 66*Secow 5T: 48.00 (g/cm root); NE 50*WC 10: 47.95 (g/cm root); Ebelate: 46.24 (g/cm root); NE 48*NE 50: 46.22 (g/cm root) and Ebelate*NE 51: 44.21 (g/cm root)) were the topmost ten genotypes with high SRW (Figure 9B).

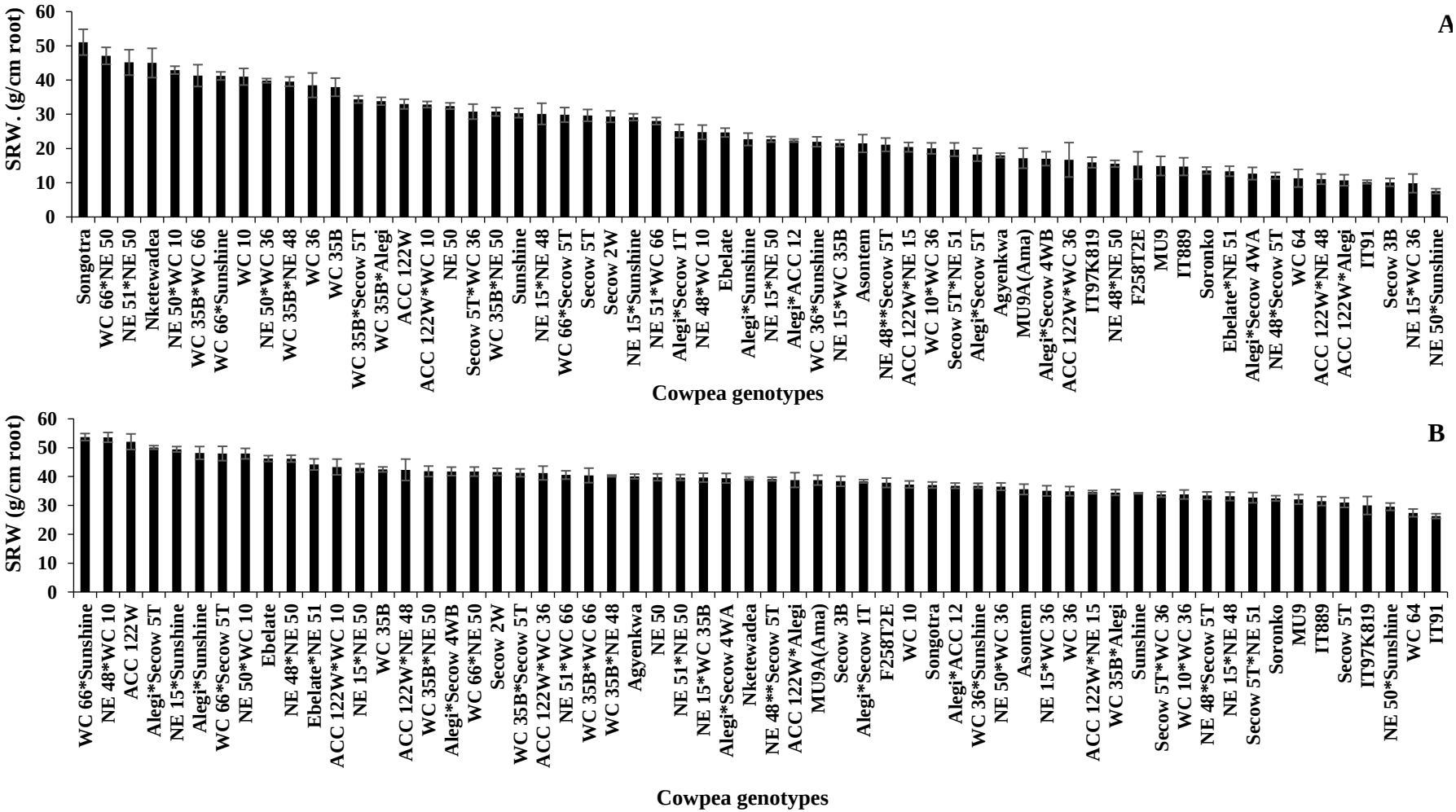


Figure 9: Variation in specific rhizosheath weight of three (3) weeks old cowpea genotypes grown under greenhouse condition for **(A)** the First trial; **(B)** the Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Difference between cowpea genotypes was established using ANOVA.

Root hair parameters

Root hair length

In general, cowpea genotypes evaluated produced substantial variation in root hairs (Figure 11A - 11C). Longer RHL was recorded in the first trial compared to the second trial (Figure 10A and 12B). Results in Figure 10A indicates that RHL obtained by cowpea genotypes were significantly different ($P < 0.001$) from each other. Root hair length ranged from approximately 0.13 mm to 0.06 mm. Genotypes ACC 122W*Alegi, WC 10*WC 36, NE 15*WC 36, ACC 122W*WC 36, NE 50*Sunshine, Alegi*Secow 4WA, Secow 5T*NE 51; Agyenkwa, ACC 122W*NE 48 and Secow 3B obtained the longest RHL ranging from 0.19 - 0.25 mm (Figure 10A). The remaining genotypes obtained RHL values ranging from 0.08 - 0.19 mm (Figure 10A).

Genotype had a significant effect ($P = 0.026$) on RHL obtained by cowpea genotypes in the second trial (Figure 10B). Genotypes IT91; NE 15*NE 48; Secow 5T; IT97K819; WC 36; Secow 5T*WC 36; ACC 122W*NE 15; WC 35B*WC 66; NE 50*Sunshine and WC 35B*Alegi obtained the longer RHL (Figure 10B). Genotypes ACC 122W*Alegi; Alegi*Secow 4WA; Alegi*Secow 4WB; WC 66*Sunshine and NE 48*WC 1 constituted the genotypes with the short RHL distribution. The other genotypes obtained RHL values ranging from 0.08 - 0.11 mm (Figure 10B).

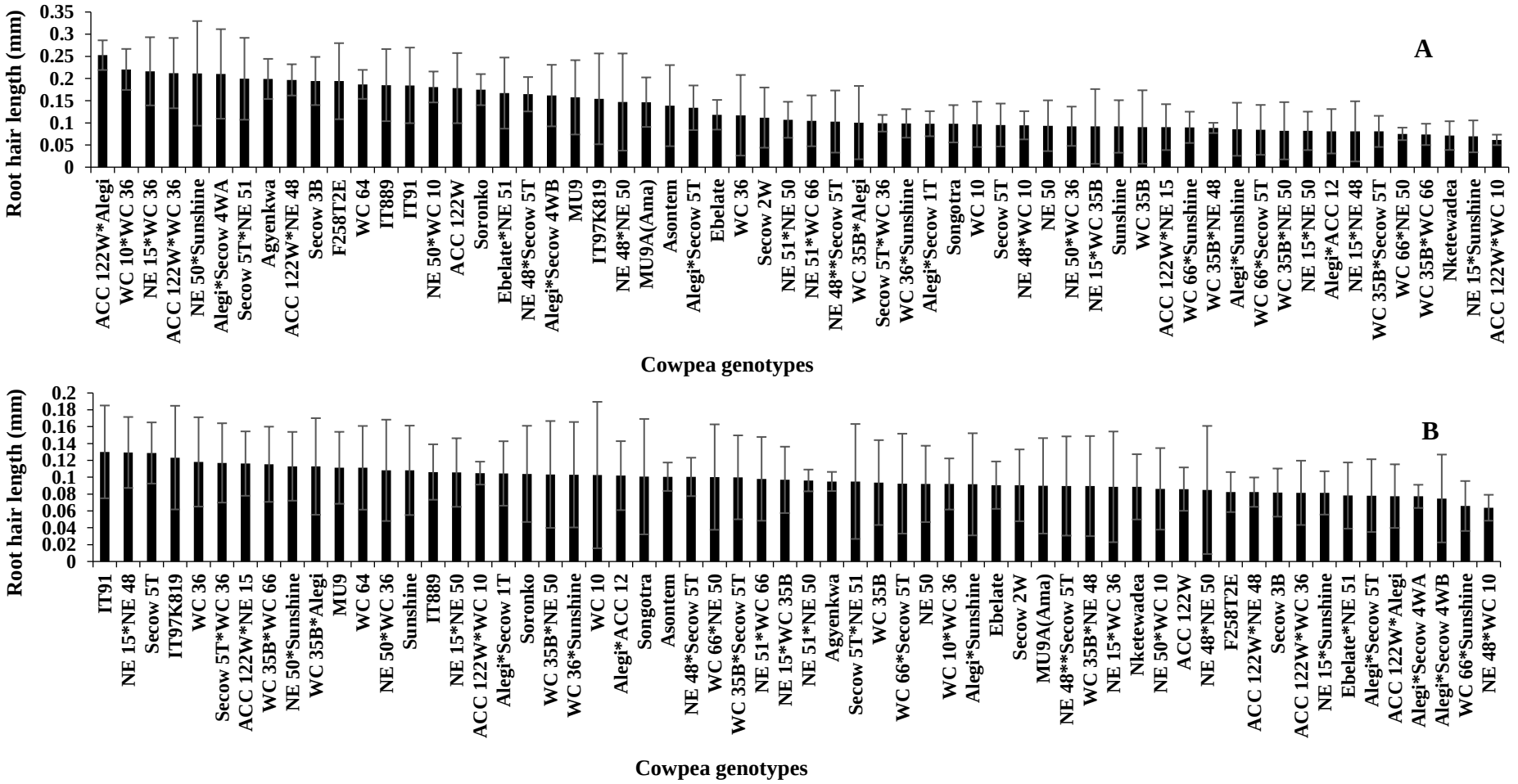


Figure 10: Variation in root hair length of three (3) weeks old cowpea genotypes grown under greenhouse condition for **(A)** First trial; **(B)** Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Difference between cowpea genotypes was established using ANOVA.

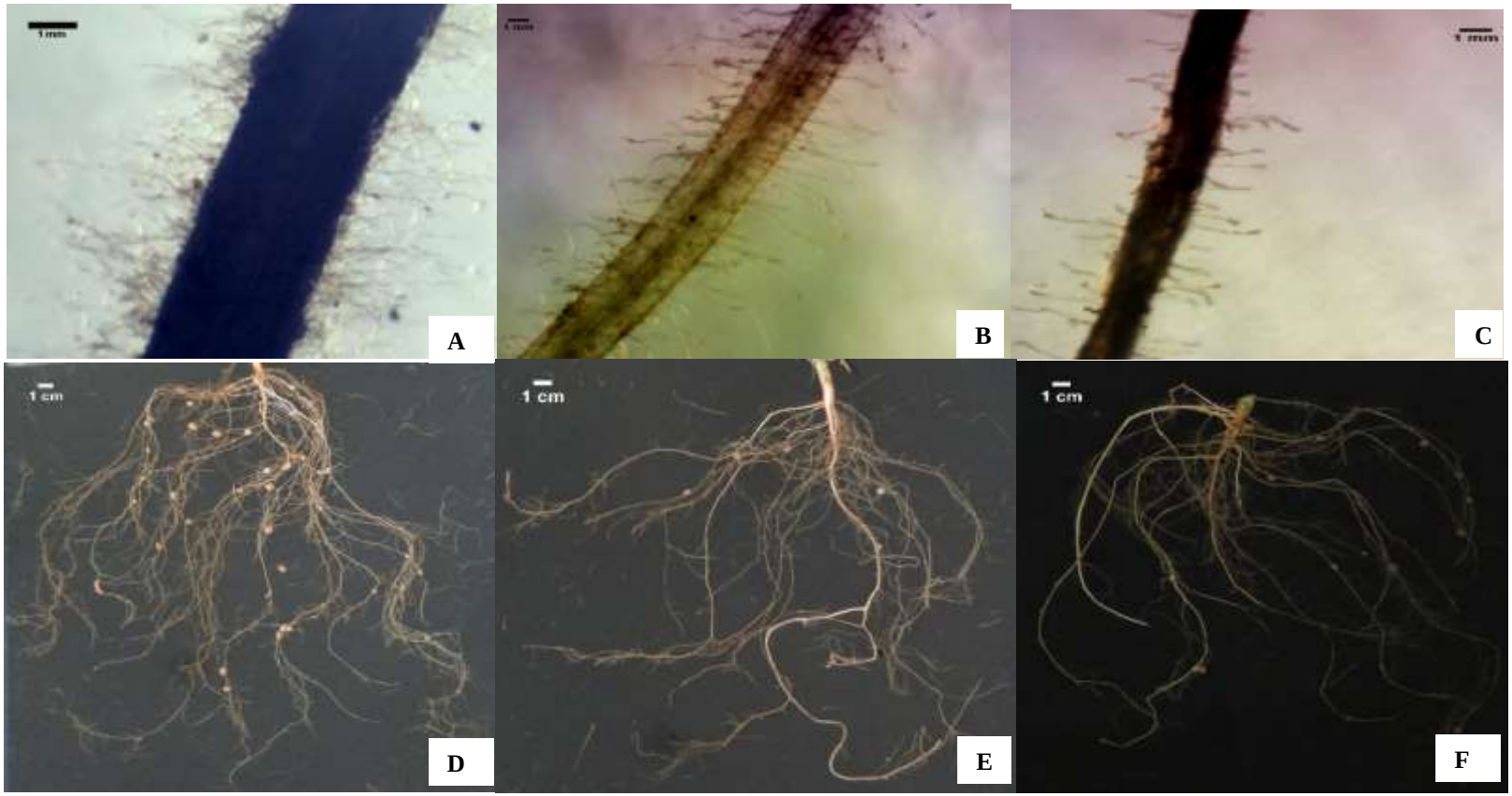


Figure 11: Images of cowpea root hairs showing **(A)** longer and denser root hairs; **(B)** short and less dense root hair; **(C)** Shorter and fewer root hairs. Images of cowpea root system showing **(D)** longer root length; **(E)** Short root length; **(F)** shorter root length. Root hair images were captured at ×2 magnification with a digital camera mounted compound Microsoft connected to computer. Root system images were capture with a digital camera held on tripod 50cm above sink in which root was evenly spread.

Root hair density

Variation in RHD was observed during both trials (Figure 11A and 11B). In the first trial (Figure 12A), RHD per plant ranged from approximately 21 - 33.16 count/mm^2. A significant difference ($P < 0.001$) existed between root hair density obtained by cowpea genotypes. From Figure 12A it is clear that genotypes NE 48*WC 10: 33.16; NE 15*NE 48: 32.9; WC 66*NE 50: 32.77; NE 50*WC 36: 32.16; WC 35B*Secow 5T: 32.05; WC 35B*WC 66: 32.01; WC 35B*NE 50: 31.01; WC 35B*NE 48: 30.97; NE 51*NE 50: 30.93 and Alegi*ACC 12: 30.27 were the topmost ten genotypes with high RHD in the first screening. Genotypes Secow 5T: 24.06; IT889: 24.04; NE 15*WC 35B: 23.98; IT91: 23.94; Alegi*Secow 5T: 23.92; WC 64: 23.83; F258T2E: 23.01; WC 36*Sunshine: 22.7; NE 48**Secow 5T: 22.63 and MU9: 21.79 made up the last ten genotypes with least root hair density. The remaining genotypes obtained RHD ranging from 24.27 - 30.17 count/mm^2 (Figure 12A).

Root hair density per plant in the second trial ranged from 17.46 - 36 count/mm^2 (Figure 12B). Genotype had a significant ($P < 0.001$) effect on RHD obtained by cowpea genotypes. The topmost ten genotypes with high RHD were NE 50*Sunshine: 36; Nketewadea: 35.29; NE 15*Sunshine: 32.69; Secow 2W: 32.55; NE 15*NE 50: 32.19; WC 66*Secow 5T: 31.22; Agyenkwa: 30.71; WC 10*WC 36: 30.27; NE 15*WC 36: 30.09 and ACC 122W*NE 48: 30.01. Genotypes IT97K819: 20.33; MU9: 19.97; Asontem: 19.9; WC 10: 19.17 and Alegi*Secow 5T: 17.46 recorded lowest RHD in the second trial (Figure 12B).

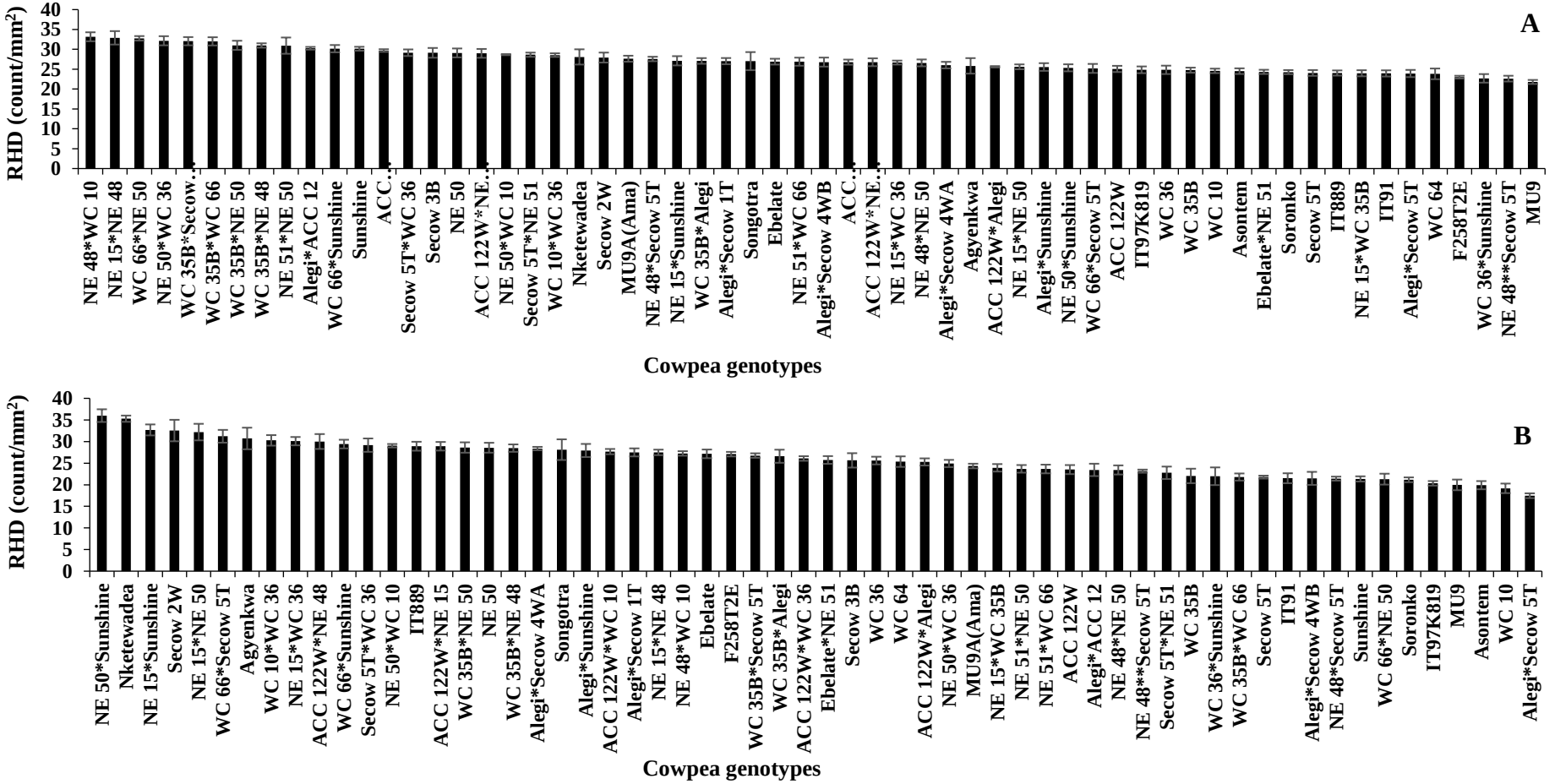


Figure 12: Variation in root hair density of three (3) weeks old cowpea genotypes grown under greenhouse condition for **(A)** First trial; **(B)** Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Difference between cowpea genotypes was established using ANOVA.

Regression between rhizosheath traits and root hair parameters

The relationship between RHD and rhizosheath parameters (Figure 13) revealed that there is a weak correlation between root hair density and rhizosheath parameters. Generally, RHD had a weak but significantly positive ($R^2 = 0.096, P < 0.01$) relationship with ARW (Figure 13A). Thus, an increase in RHD resulted in an increase in ARW at a decreasing trend. Similar trend was observed between RHD and RRW ($R^2 = 0.079, P < 0.01$) and SRW ($R^2 = 0.197, P = < 0.01$) respectively (Figure 13B and 13C).

Plotting rhizosheath and RHL data obtained from cowpea genotypes showed a positive significant ($P < 0.001$) relationship between the variables with ARW ($R^2 = 0.390$), RRW ($R^2 = 0.612$) and SRW ($R^2 = 0.312$) (Figure 14A, 14B and 14C).

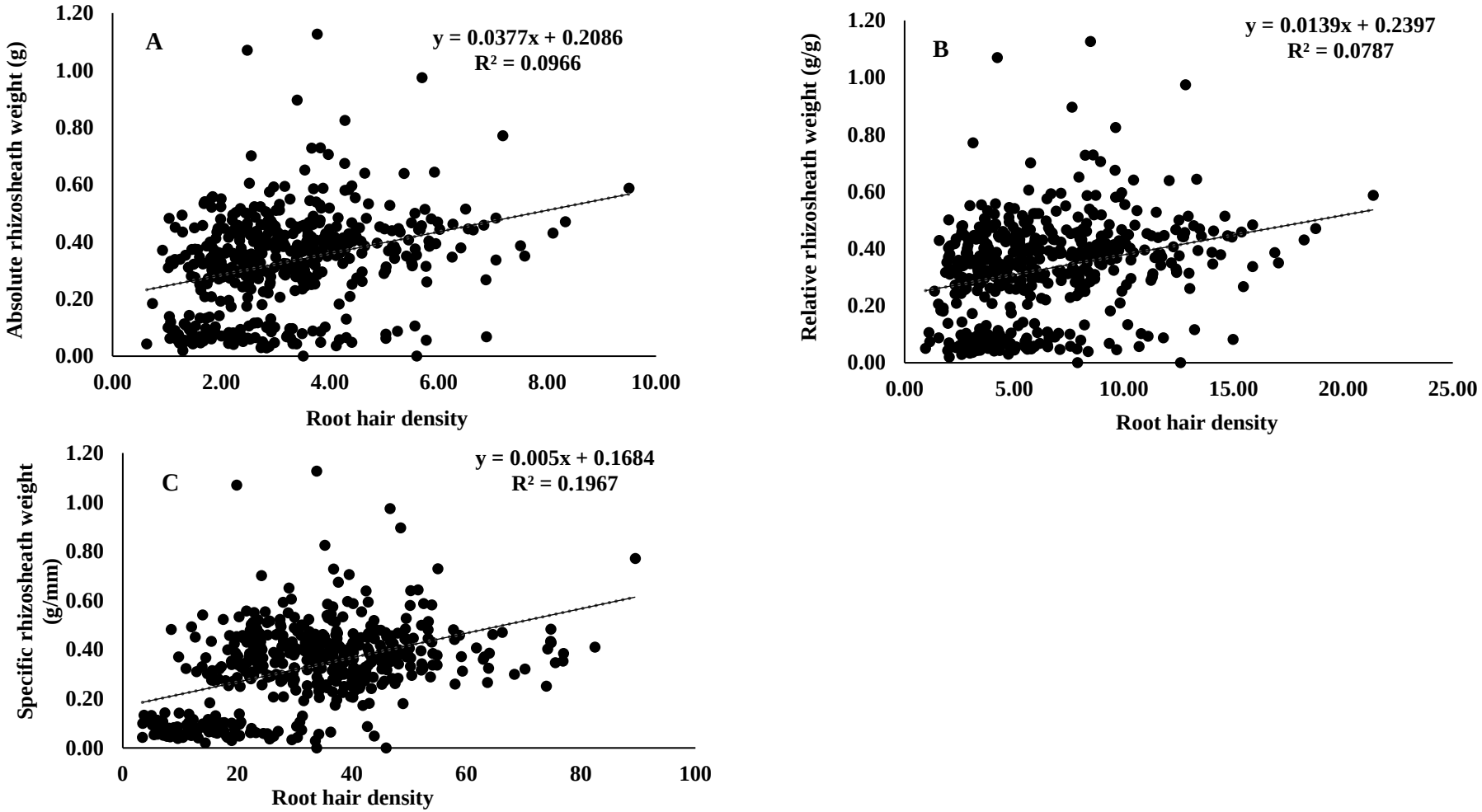


Figure 13: Regression between root hair density of cowpea genotypes and **(A)** absolute rhizosheath weight: **(B)** relative rhizosheath weight and (C) specific rhizosheath weight

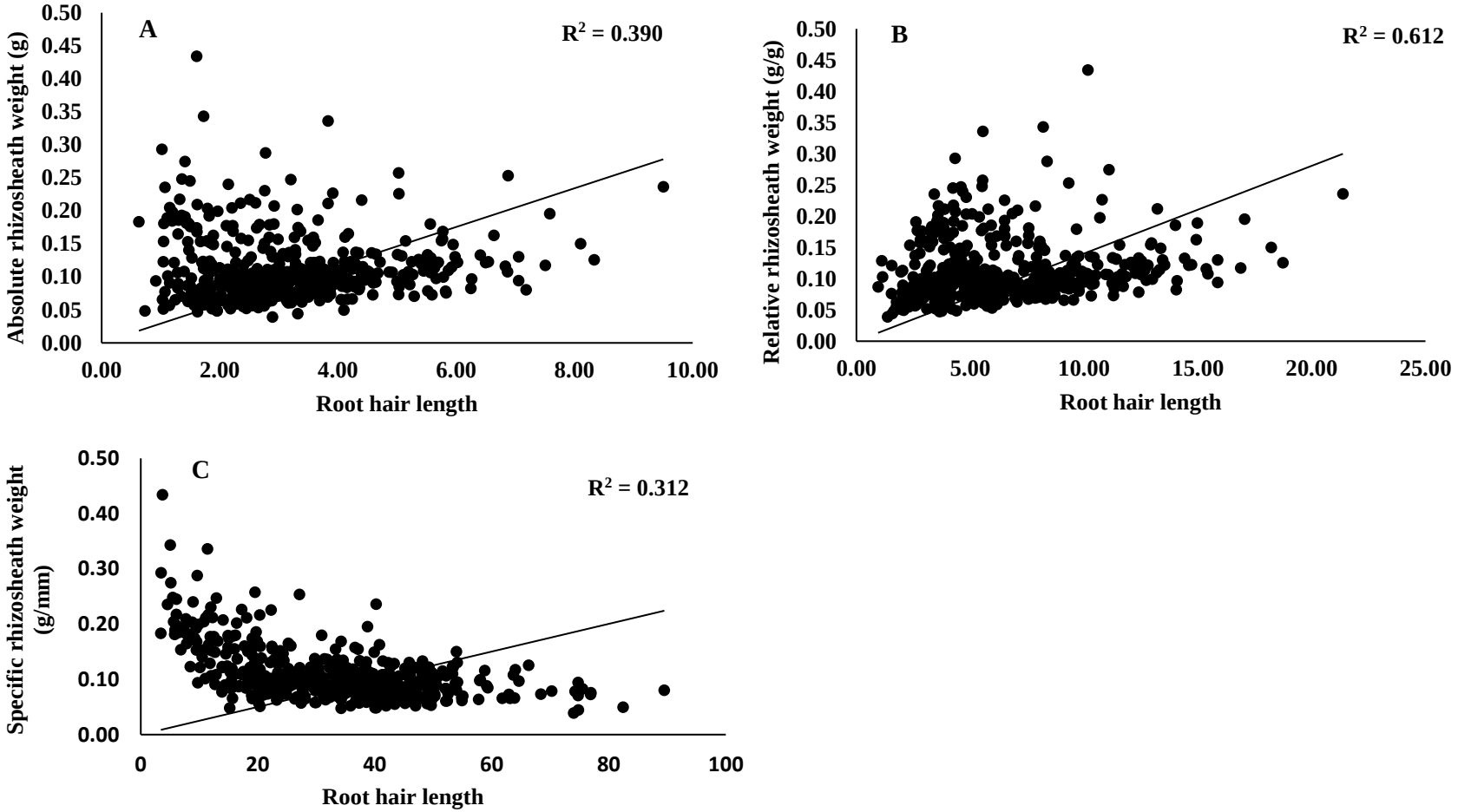


Figure 14: Regression between root hair length of cowpea genotypes and **(A)** absolute rhizosheath weight: **(B)** relative rhizosheath weight and (C) specific rhizosheath weight

Total root length

Variations existed among TRL produced by cowpea genotypes during the study (Figure 11A and 11B). In general, genotypes WC 36, WC 66*NE 50 and WC 10*WC 36 were part of the topmost ten genotypes with longer root length in both first and second screening whilst NE 48*NE 50 obtained short TRL in both screenings (Figure 15A and 17B).

In the first screening (Figure 15A), genotype had a significant ($P < 0.001$) effect on TRL obtained by cowpea genotypes. Total root length ranged from approximately 221.80 - 823.60 cm. Genotypes WC 36; WC 66*NE 50; Alegi*ACC 12; NE 50*WC 10; NE 50; WC 35B*Alegi; NE 15*NE 48; Agyenkwa; WC 35B*NE 48 and WC 10*WC 36 made up the topmost distribution with the highest TRL values ranging from 628.00 - 823.60 cm. The lowest distribution consisted of genotypes ACC 122W*Alegi; IT889; MU9; Secow 3B; NE 15*WC 36; NE 48*NE 50; Secow 2W; IT91; NE 48*Secow 5T and Alegi*Secow 5T with root length ranging from 221.80 - 338.3 cm (Figure 15A).

In the second screening (Figure 15B), genotype had a significant ($P < 0.001$) effect on TRL obtained by cowpea genotypes. Genotypes MU9A(Ama); WC 35B*NE 48; ACC 122W; NE 15*WC 36; WC 36; Secow 5T*NE 51; WC 10*WC 36; Alegi*Sunshine; WC 66*NE 50 and MU9 obtained the longest TRL compared to F258T2E; ACC 122W*WC 36; Ebelate; NE 48*WC 10; NE 48*NE 50; ACC 122W*NE 48; NE 51*WC 66; Secow 3B; WC 66*Secow 5T and WC 35B*Alegi which recorded the short root length. The remaining genotypes obtained root lengths between the upper and lower distribution (Figure 15B).

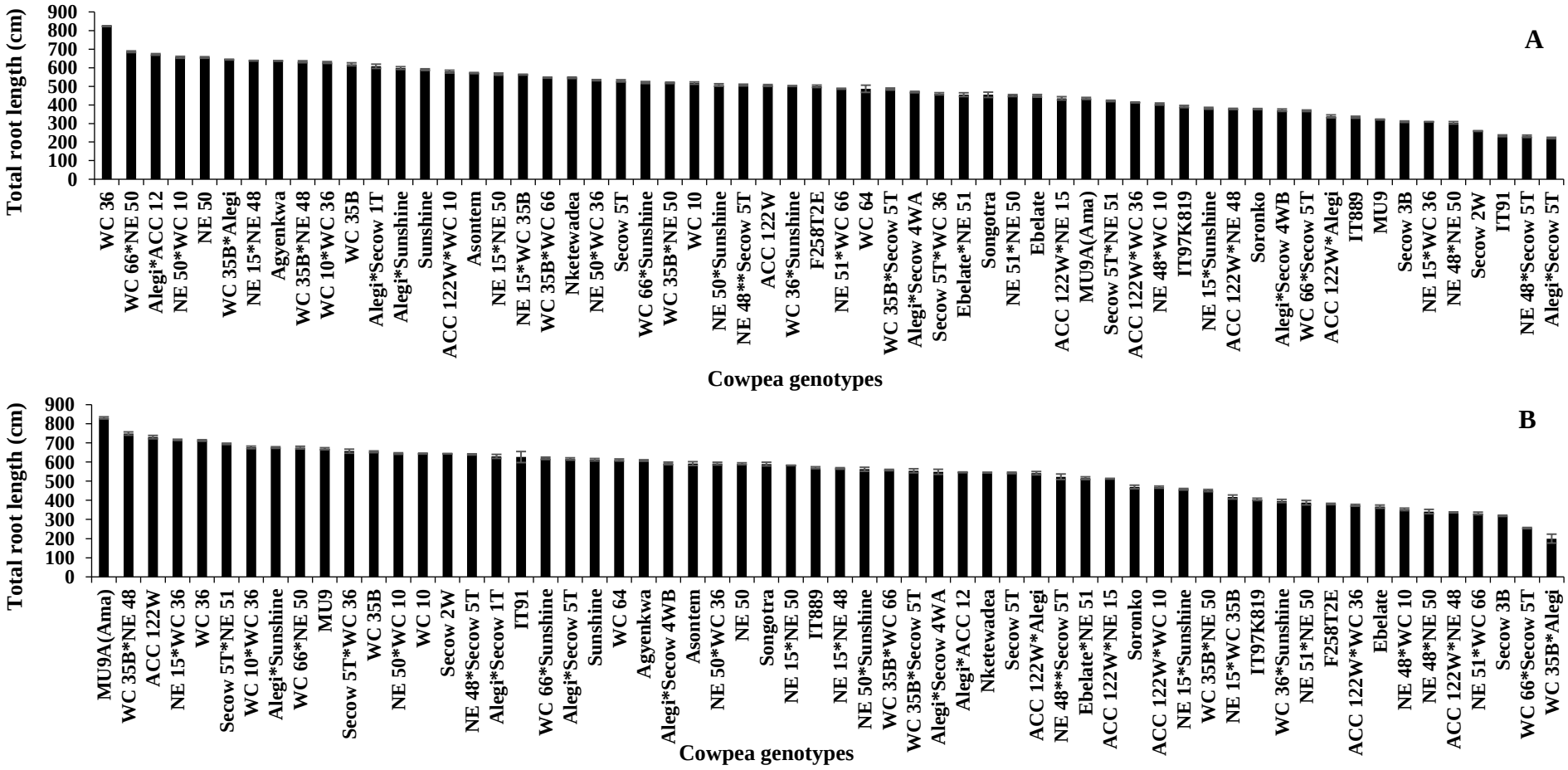


Figure 15: Variation in total root length of three (3) weeks old cowpea genotypes grown under greenhouse condition for **(A)** First trial; **(B)** Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Difference between cowpea genotypes was established using ANOVA.

Summary of plant measurement between trials

Variation in plant measurements obtained during the first and second screening of cowpea genotypes is presented in Table 7. Genotype, run and their interaction had a significant effect ($P < 0.001$) on all biomass parameters measure in both first and second screenings. Higher means were recorded during the second screen for SDW 2.69 g, RFW 3.21 g and shoot fresh weight 21.14 g. Root hair density 27.13 counts/mm^2, on the other hand, obtained higher means in the first trial relative to the second trial 26.04 count/mm^2. Root dry weight (0.492 g) was high in the first screening compared to second screening.

Also, genotype, run as well as their interactions had a significant ($P < 0.001$) effect on RSA traits. Total root length was high in the second screening 544.8 cm compared to the first screening 483.8 cm (Table 7).

Interaction of genotype and trial had a significant effect on RHL but insignificantly ($P = 0.239$) influenced RHD of cowpea genotypes in both screens. Comparing both trials, RHD 27.13 count/mm^2 and RHL 0.1256 mm was higher in the first run. Absolute rhizosheath weight was significantly ($P < 0.001$) affected by genotype in both trials however, the interaction effect was insignificant ($P = 0.31$) (Table 7). Relative rhizosheath weight was significantly affected by genotype ($P = 0.007$) and the interaction between genotype and run ($P = 0.019$). However, the trial had insignificant effect ($P = 0.66$) on relative rhizosheath weight (Table 7).

Table 7: Summary of plant measurement recorded three (3) weeks after germination in nursery bags for cowpea genotypes grown under greenhouse condition

	Measurements						
	Absolute rhizosheath weight (g)	Relative rhizosheath weight ($g^{-1}g^{-1}$)	Total root length (cm)	Root hair length (mm)	Root hair density (count/mm^2)	Root dry weight (g)	Shoot dry weight (g)
Trial 1	4.233	9.60	483.8	0.1256	27.13	0.4915	2.663
Trial 2	3.801	8.68	544.8	0.0972	26.04	0.4530	2.693
L.s.d							
Genotype	1.783	5.390	139.23	0.032	6.098	0.129	0.747
Trial	0.325	0.985	25.42	0.006	1.113	0.024	0.136
Genotype × Trial	2.521	7.629	196.91	0.046	8.625	0.182	1.057
C.v	45.1	60.0	27.5	29.5	23.3	27.8	28.4
ANOVA							
Genotype	<.001	0.007	<.001	<.001	0.003	<.001	<.001
Trial	0.1662	0.066	<.001	<.001	0.055	0.001	0.668
Genotype × Trial	0.31	0.019	<.001	<.001	0.239	<.001	<.001

Source: Field research, Opoku (2020)

Table 7 contd.

	Measurements			
	Leaflet fresh weight (g)	Leaflet dry weight (g)	Root fresh weight (g)	Shoot fresh weight (g)
Trial 1	10.17	1.246	2.906	19.08
Trial 2	11.44	1.441	3.209	21.14
L.s.d				
Genotype	2.811	0.422	0.732	4.636
Trial	0.513	0.077	0.134	0.846
Genotype × Trial	3.975	0.597	1.035	6.557
C.v	26.5	31.9	24.3	23.4
ANOVA				
Genotype	<.001	<.001	<.001	<.001
Trial	<.001	<.001	<.001	<.001
Genotype × Trial	<.001	<.001	<.001	<.001

Source: Field research, Opoku (2020)

Relationship between rhizosheath, root hairs, root system architecture and biomass parameters

A significant positive correlation was observed between biomass parameters measured during the experiment (Table 8). Shoot fresh weight had a strong positive relationship with SDW ($r= 0.722$, $P < 0.01$) and positive but a weak relationship with RFW ($r = 0.441$, $P < 0.01$), RDW ($r= 0.439$, $P < 0.01$), TLA ($r = 0.473$, $P < 0.01$) and LMA ($r = 0.195$, $P < 0.01$). However, SFW negatively correlated with RHL ($r = -0.72$) and SLA (r = -0.278) (Table 8). Root dry weight had a strong positive correlation with RFW ($r = 0.524$, $P < 0.01$) but negatively correlated with rhizosheath parameters and RHD (Table 8).

Total root length negatively correlated with RHL ($r = -0.192$, $P < 0.01$) but had a significant but a weak association with RHD ($r = 0.288$, $P < 0.01$). Total root length had a weak positive association with ARW ($r = 0.91$, $P < 0.05$), RRW (r = 0.008) and SRW ($r = 0.199$, $P < 0.01$) (Table 8).

Root hair density had a weak but positive correlation with ARW ($r = 0.013$, $P < 0.05$), RRW ($r = 0.281$, $P < 0.01$) and SRW ($r = 0.344$, $P < 0.01$) (Table 8). Root hair length had a strong significant correlation with SRW ($r = 0.543$, $P < 0.01$) but a weak positive correlation with ARW ($r = 0.191$, $P < 0.01$) and RRW ($r = 0.311$, $P < 0.01$).

Table 8: Correlations between traits observed in plants grown in soil-filled nursery polybags

SFW	**SFW**												
SDW	.722**	**SDW**											
RFW	.441**	.422**	**RFW**										
RDW	.439**	.594**	.524**	**RDW**									
TRL	.010	.000	.327**	.039	**TRL**								
RHL	-.072	.059	-.119**	.160**	-.192**	**RHL**							
RHD	.058	-.061	.182**	-.171**	.288**	-.440**	**RHD**						
ARW	.021	-.004	.165**	-.050	.091*	.191**	.013	**ARW**					
RRW	.031	-.026	.073	-.080	.008	.311**	.281**	.744**	**RRW**				
SRW	.075	.004	.197**	-.079	.199**	.543**	.344**	.728**	.412**	**SRW**			
TLA	.473**	.435**	.201**	.361**	-.049	.061	-.150**	-.153**	-.159**	-.152**	**TLA**		
SLA	-.278**	-.365**	-.258**	-.168**	-.098*	.060	-.135**	-.170**	-.192**	-.180**	.272**	**SLA**	
LMA	.195**	.292**	.181**	.091*	.101*	-.130**	.200**	.147**	.135**	.195**	-.468**	-.544**	**SLM**
****. Correlation is significant at the 0.01 level.**													
***. Correlation is significant at the 0.05 level.**													

Source: Field research, Opoku (2020)

Traits in matrix are SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight, TRL: total root length, RHL: root hair length, RHD: root hair density, ARW: absolute rhizosheath weight, SRW: specific rhizosheath weight, RRW: relative rhizosheath weight TLA: total leaf area, SLA: specific leaf area and LMA: leaf mass per area.

Variance component and broad-sense heritability estimate

Genotypic variance ranged from 28.8% for shoot fresh weight to 85.8% for SRW (Table 9). Genotype accounted for less than 50% variation in RRW (39.0%), SDW (31.5%), RFW (41.2%), SFW (28.8%), RHD (31.9%), RHL (45.8%), TLA (29.5%) and SLA (34.9%) (Table 9). However, genotype accounted for more than 50% variation in SRW (85.8%), RDW (51.5%), TRL (59.4%) and SLM (62.1%) (Table 9).

Broad-sense heritability ranged from 0.04 for SFW to 0.72 for RDW (Table 9). Except for LMA (0.08), ARW (0.08) and SFW (0.03), broad-sense heritability was higher than 0.10 for the remaining traits (Table 9).

Table 9: Estimates of variance components and broad-sense heritability (H^2) of cowpea genotypes screened under greenhouse condition

Traits	Genotype	Trial	Genotype × Trial	Error	H^2
SRW	85.85	1.17	8.71	4.28	0.17
RRW	38.98	3.74	38.98	10.69	0.21
ARW	13.32	5.17	22.20	59.31	0.08
RDW	51.46	0.95	0.68	46.90	0.72
SDW	31.51	16.51	44.50	5.46	0.39
RFW	41.23	4.34	22.15	11.29	0.29
SFW	28.80	4.06	43.16	23.98	0.04
RHD	31.92	0	37.12	9.96	0.26
RHL	45.80	0	14.05	19.15	0.33
TRL	59.44	4.84	26.29	5.43	0.11
TLA	29.49	10.84	43.76	6.91	0.13
SLA	34.92	14.56	38.97	2.55	0.14
LMA	62.07	12.20	22.03	3.70	0.08

Source: Field research, Opoku (2020)

Principal component analysis

Varimax with Kaiser Normalization principal component analysis (PCA) are shown in Table 10. A Kaiser-Meyer-Olkin measure of sampling adequacy of 0.668 was obtained for measured traits. This makes data obtained robust and suitable for principal component analysis. In all, five (5) distinct principal components were obtained based on components with Eigenvalues (> 1) and factor loadings of ±0.3 explaining 79 % of the total variance (Table 10).

The first principal (PC 1) component contributed to 25% of the total variation observed. This was mainly explained by SDW (g), SFW (g), RDW (g) and RFW (g) (Table 10). Principal component two (2) on the other hand accounted for 22% of the total variation observed among measured traits. The variation was explained by ARW (g) and RRW (g/g) (Table 10). Leaf mass per area (g), SLA (cm/g) and TLA (cm^2) account for 12% of variation explained by the third principal component (PC 3). Root hair length (mm), SRW (g/mm root) and RHD (count/mm^2) explained 12% of variation contributed by the fourth principal component (PC 4). The fifth PC contributed to 8% of the variation among measured parameters (Table 10).

Table 10: Rotated component matrix of five factor model explaining 79% of the total variance for trait

	Components					
Measurements	**PC 1**	**PC 2**	**PC 3**	**PC 4**	**PC 5**	**Communalities**
Shoot dry weight (g)	**.881**	-.023	.225	-.045	-.051	.833
Shoot fresh weight (g)	**.856**	.020	.092	.147	-.086	.771
Root dry weight (g)	**.743**	-.048	.038	-.264	.256	.691
Root fresh weight (g)	**.589**	.124	.105	.088	.579	.716
Absolute rhizosheath weight (g)	.002	**.936**	.070	.154	.055	.907
Relative rhizosheath weight (g/g)	-.033	**.908**	.111	-.077	-.011	.844
Leaf mass per area (g/cm^2)	.110	.051	**.879**	.125	.037	.805
Specific leaf area (g/cm^2)	-.265	-.128	**-.782**	-.039	-.044	.702
Total leaf area (cm^2)	.647	-.101	**-.650**	-.020	-.067	.856
Root hair length (mm)	.009	.177	-.045	**-.929**	-.047	.898
Specific rhizosheath weight (g/cm root)	.031	.607	.089	**.667**	.073	.828
Root hair density (count/mm^2)	-.068	.286	.117	**.645**	.257	.582
Total root length (cm)	-.039	.003	.040	.183	**.882**	.814
Eigen values	3.196	2.903	1.583	1.554	1.010	
Per centage of total variance	24.588	22.327	12.177	11.953	7.769	
Cumulative per centage of variance	24.588	46.916	59.093	71.046	78.815	

Source: Field research, Opoku (2020)

Cluster analysis

Clustering analysis was performed using Ward's hierarchical approach based on the minimum variance linking method with Euclidean distance as the similarity measure (Figure 16). Cluster analysis for measured traits exhibited a clear demarcation between the cowpea genotypes. Based on these traits, the dendrogram divided the genotypes into three main clusters (Figure 16). Cluster I included the genotypes ACC 122W*WC 10, WC 35*WC 66, WC 66*NE 50, NE 51*NE 50, WC 66*Sunshine, NE 15*WC 35B, WC 35*NE 50, Songotra, Alegi*Secow 1T, WC 36*Sunshine, Alegi*ACC 12, NE 15*NE 50, ACC 122W*NE 15, NE 15*Sunshine, WC 35B, WC 36, NE 48*NE 50, WC 10, ACC 122W, Ebelate and Secow 5T. Cluster II was made up of genotypes NE 15*NE

48, NE 48*WC 10, Alegi*Sunshine, NE 50*WC 36, Secow 5T*WC 36, WC 35B*Alegi, WC 35B*Secow 5T, NE 51*WC 66, Nketewadea, NE 50, WC 35B*NE 48, NE 50*WC 10, Agyenkwa, Sunshine, WC 66*Secow 5T and NE 48**Secow 5T (Figure 16). Cluster III included genotypes IT889, MU9, Alegi*Secow 4WA, Alegi*Secow 4WB, IT91, NE 48*Secow 5T, Alegi*Secow 5T, IT97K819, Asontem, WC 10*WC 36, ACC 122W*WC 36, Secow 2W, Secow 5T*NE 51, Soronko, Ebelate*NE 51, MU9A(Ama), F258T2E, WC 64, ACC 122W*Alegi, Secow 3B, ACC 122W*NE 48, NE 50*Sunshine and NE 15*WC 36 (Figure 16).

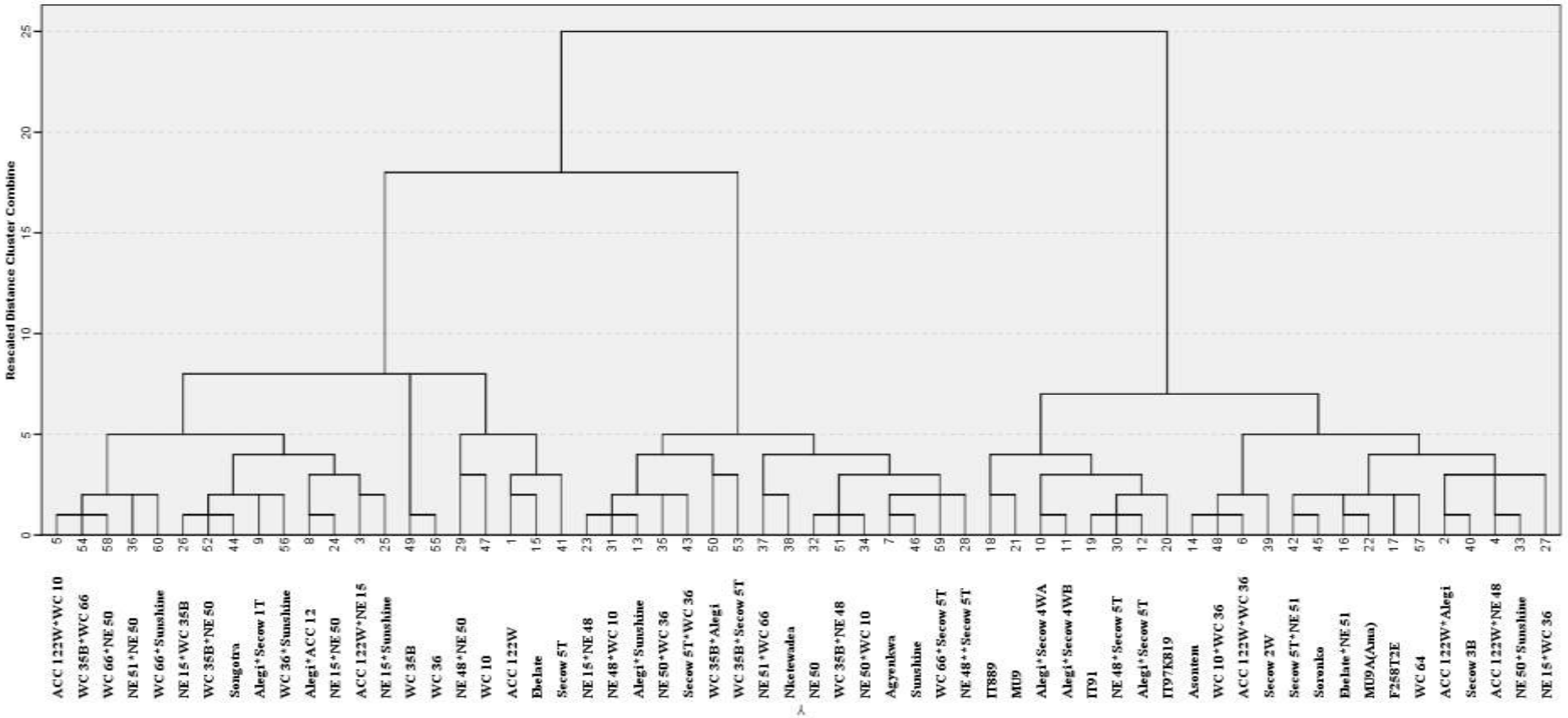


Figure 16: Clustering of sixty (60) cowpea genotypes grown in soil-filled nursery polybags grown for three (3) weeks. Clustering was performed using the Ward's hierarchical approach based on the minimum variance linking method with Euclidean distance as the similarity measure.

Experiment two: Effect of external phosphorus (P) on rhizosheath, root hair traits and RSA among cowpea genotypes

Biomass parameters

Root dry weight

Phosphorus level had a significant effect (*P = 0.013*), but genotype (*P = 0.165*) and interaction of genotype and phosphorus (*P = 0.200*) had no significant influence on the RDW among cowpea genotypes in first trial (Figure 17A). Genotypes NE 48*WC 10, Alegi*Secow 5T, Soronko and WC 35*NE 50 had the highest biomass at 0 mg P/kg soil (Figure 17A). Genotypes Agyenkwa, Alegi*Sunshine, Asontem, MU9, Nketewadea, Secow 3B, Sunshine, WC 10*WC 36 and WC 36 had high RDW at 500 mg P/kg soil. A significantly high RDW was observed for remaining genotypes at 250 mg P/kg soil phosphorus treatment. Root dry weight obtained at 500 mg P/kg was 6 and 29 per cent greater than RDW obtained 250 at and 0 mg P/kg soil respectively (Figure 17A).

Genotypes evaluated in the second trial showed significant difference (*P = 0.022*) for RDW (Figure 17B). Root dry weight of genotypes grown on unamended soil ranged from 0.268 - 0.375 g (Figure 17B). Phosphorus application significantly (*P < 0.001*) increased RDW among cowpea genotypes. Genotypes NE 15*Sunshine, NE 50, NE 51*NE 50, Nketewadea and Songotra recorded high root dry weight at 250 mg P/kg soil. The remaining genotypes had significantly high RDW at 500 mg P/kg soil treatment (Figure 17B). Root dry weight obtained 500 mg P/kg was 6 and 26 per cent greater than RDW obtained 250 and 0 mg P/kg soil respectively (Figure 17B). The interactional effect of genotypes and $[P]_{ext}$ significantly (*P = 0.032*) affected RDW in the

second trial (Figure 17B). In general, all genotypes evaluated increased RDW in response to increasing external P concentration (Figure 17B).

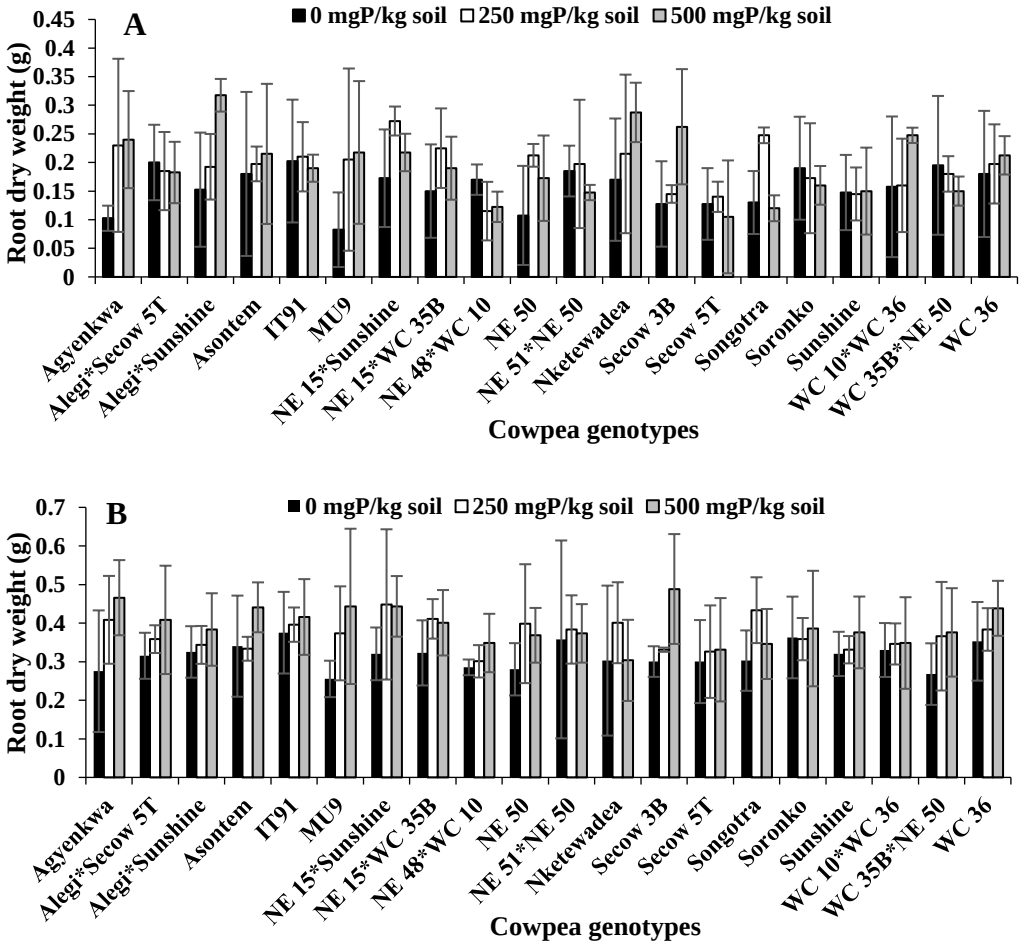


Figure 17: Effect of $[P]_{ext}$ on root dry weight among 3 weeks old cowpea genotypes grown under greenhouse conditions for **(A)** First screening; **(B)** Second screening. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Shoot dry weight

Genotype, $[P]_{ext}$ and their interaction had a significant ($P < 0.001$) effect on SDW in both first and second trials (Figure 18A and 20B). Application of phosphorus significantly improved SDW although cowpea genotypes responded differently to phosphorus concentrations.

In the first trial, an increasing trend in SDW was observed with an increase in phosphorus rates. Shoot dry weight obtained by genotypes grown on soil amended with 250 and 500 mg P/kg soil were 61% and 62% greater than plants grown on unamended soil (Figure 18A). Except for genotype Alegi*Secow 5T and MU9, the remaining cowpea genotypes evaluated during the experiment produced higher SDW at 500 mg P/kg soil in the first screen (Figure 18A).

Similarly, in the second trial (Figure 18B), a significant ($P < 0.001$) increase in SDW was observed with increasing phosphorus level. From Figure 18B, high SDW was observed in genotypes Agyenkwa, Alegi*Secow 5T, MU9, Nketewadea, Secow 5T, Soronko and WC 10*WC 36 at treatment 250 mg P/kg soil. The remaining genotypes obtained high value of SDW at 500mg P/kg soil (Figure 18B)

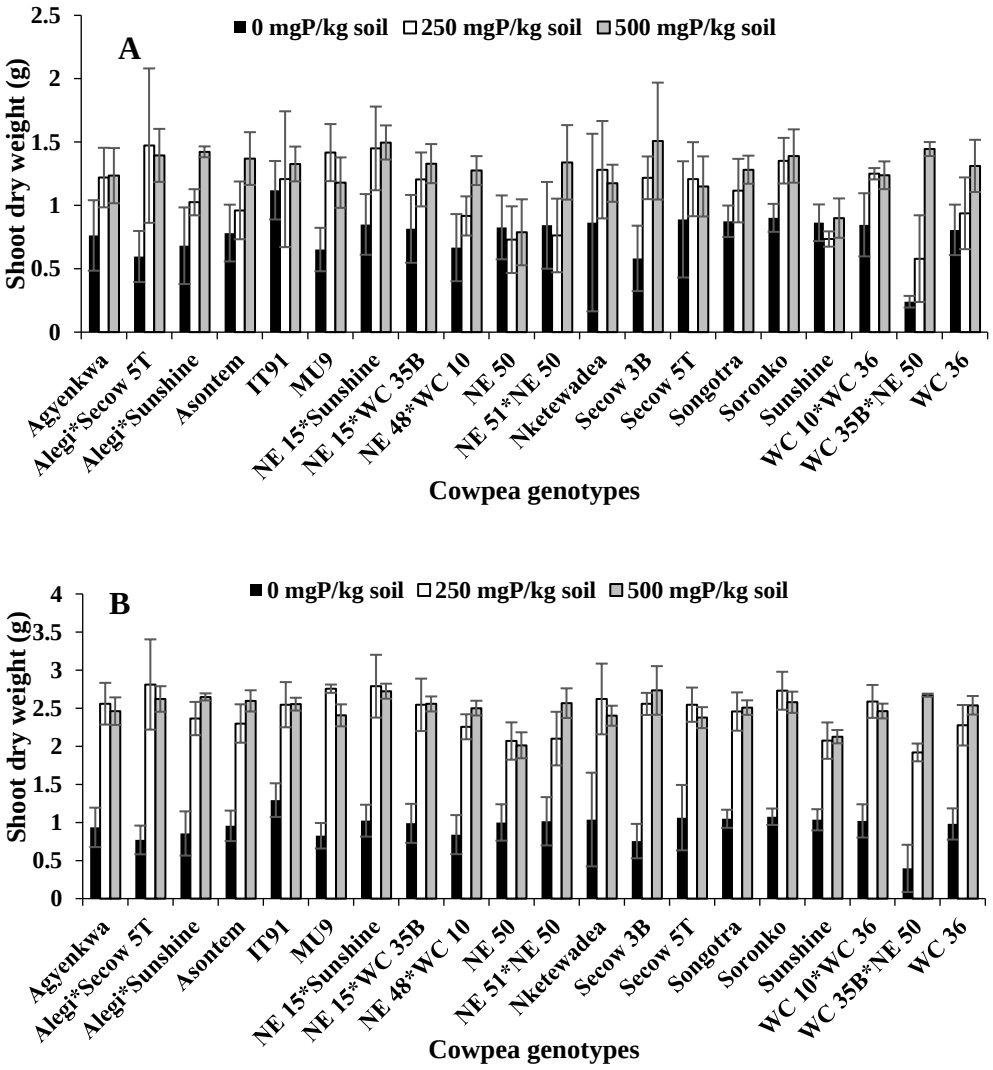


Figure 18: Effect of $[P]_{ext}$ on shoot dry weight among 3 weeks old cowpea genotypes grown under greenhouse conditions for **(A)** First screening; (**B**) Second screening. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Total leaf area

The twenty (20) genotypes screened under varying phosphorus level exhibited significant ($P < 0.001$) variation in TLA in the first trial (Figure 19A). Total leaf area ranged from 83.2 – 218.4 cm^2 for treatment 0 mg P/kg soil, 174.1 – 335.6 cm^2 for treatment 250 mg P/kg soil and 244.4 - 411.8 cm^2 for 500 mg P/kg soil (Figure 19A). There was a significant ($P < 0.001$) variation in TLA among cowpea genotypes grown under different $[P]_{ext}$ soils. The general trend

was that TLA increased with increasing phosphorus application. The leaf area obtained at 500 mg P/kg soil is 23 per cent greater compared that obtained under 250 mg P/kg and 54 per cent greater compared to 0 mg P/kg soil (Figure 19A). Generally, there was an insignificant ($P = 0.110$) interaction effect of genotype and $[P]_{ext}$ on TLA with plants grown under 500mg P/kg recording highest leaf area (Figure 19A).

In the second trial, genotype significantly ($P < 0.001$) affected TLA obtained by cowpea genotypes (Figure 19B). Total leaf area ranged from 77.7 – 230.1 cm^2 for treatment 0 mg P/kg soil, 181.7 - 388.1 cm^2 for treatment 250 mg P/kg soil and 257.0 - 411.8 cm^2 for 500 mg P/kg soil (Figure 26B). There was a significant ($P < 0.001$) variation in TLA between genotypes grown under different $[P]_{ext}$ soils. The general trend was that TLA increased with increasing phosphorus application. The TLA obtained under 500 mg P/kg soil was 16 per cent greater compared to that of 250 mg P/kg and 21 per cent greater compared to that of 0 mg P/kg soil (Figure 19B). Generally, there was an insignificant ($P = 0.110$) interactional effect of genotype and $[P]_{ext}$ on TLA with plants grown under 500mg P/kg recording highest TLA (Figure 19B).

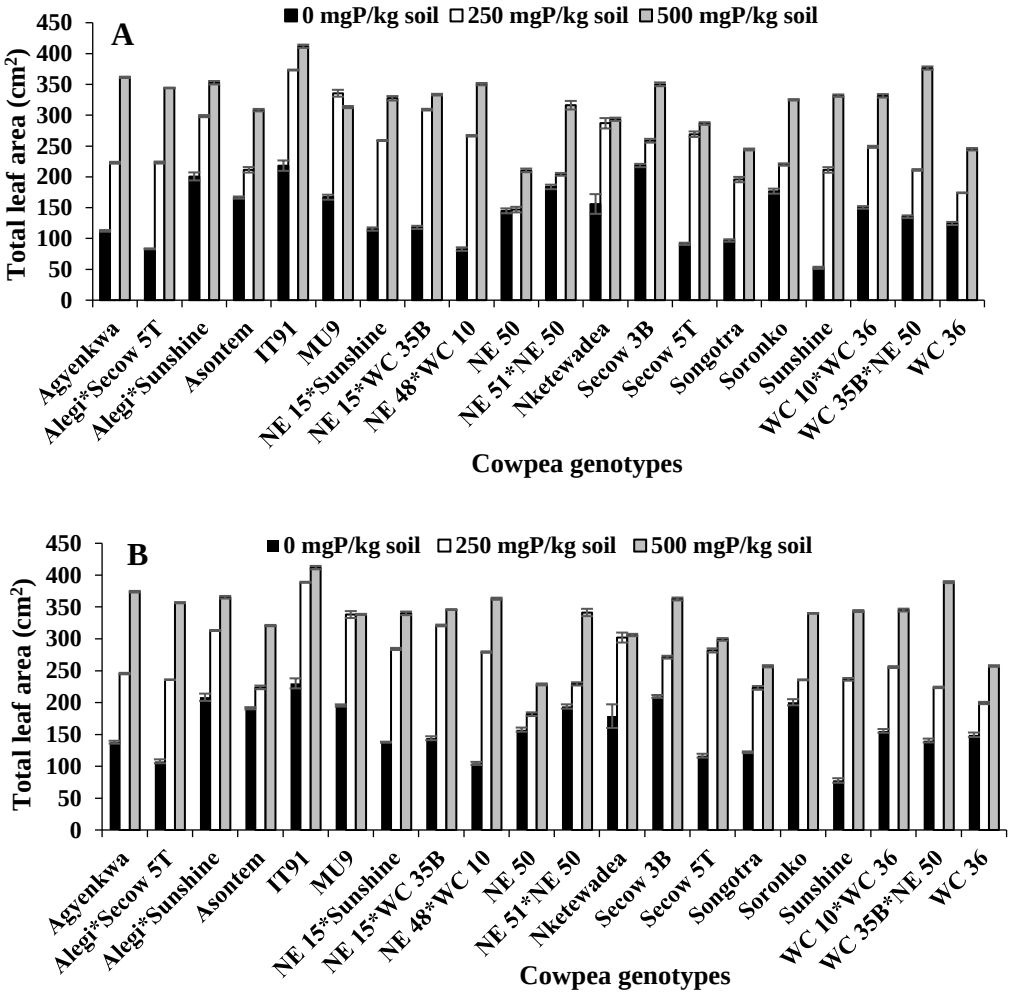


Figure 19: Effect of $[P]_{ext}$ on total leaf area among 3 weeks old cowpea genotypes grown under greenhouse conditions for (**A**) First screening; (**B**) Second screening. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Specific leaf area

The twenty (20) genotypes screened under varying phosphorus level exhibited significant ($P < 0.001$) variation in SLA in the first trial (Figure 20A). The specific leaf area under 0 mg P/kg soil ranged from 131.7 - 640.6 g/cm^2 for genotype Sunshine and WC 35B*NE 50 respectively. At 250 mg P/kg, SLA ranged from 241.6 - 644.7 g/cm^2 for Alegi*Sunshine and 357.4 – 645.9 cm/g for 500 mg P/kg soil (Figure 20A). There was a significant ($P < 0.001$) variation

in SLA of genotypes grown under different $[P]_{ext}$ soils (Figure 20A). The general trend was that SLA increased with increasing phosphorus application. The SLA obtained by under 500 mg P/kg soil is 16 per cent greater compared to 250 mg P/kg and 21 per cent greater compared to 0 mg P/kg soil (Figure 27A). Generally, there was an insignificant ($P = 0.110$) interaction effect of genotype and $[P]_{ext}$ on SLA with plants grown under 500mg P/kg recording highest leaf area (Figure 20A). However, genotypes MU9, NE 51*NE 50, Secow 3B, WC 35B*NE 50 and WC 36 obtained highest SLA under unamended soil treatment (Figure 20A).

In the second trial, genotype significantly ($P < 0.001$) affected SLA obtained by cowpea genotypes (Figure 20B). Specific leaf area ranged from 134.5 - 453.2 cm/g for treatment 0 mg P/kg soil, 212.8 - 420.2 g/cm^2 for treatment 250 mg P/kg soil and 281.4 - 463.4 g/cm^2 for 500 mg P/kg soil (Figure 27B). There was a significant ($P < 0.001$) variation in SLA of genotypes grown under different $[P]_{ext}$ soils (Figure 20B). The general trend was that SLA increased with increasing phosphorus application. The SLA obtained by under 500 mg P/kg soil is 14 per cent greater compared to 250 mg P/kg and 23 per cent greater compared to 0 mg P/kg soil (Figure 20B). Generally, there was a significant ($P = 0.021$) interaction effect of genotype and $[P]_{ext}$ on SLA with plants grown under 500mg P/kg recording highest SLA (Figure 20B). Genotypes MU9, Secow 3B and WC 36 had the highest SLA at unamended soil treatment.

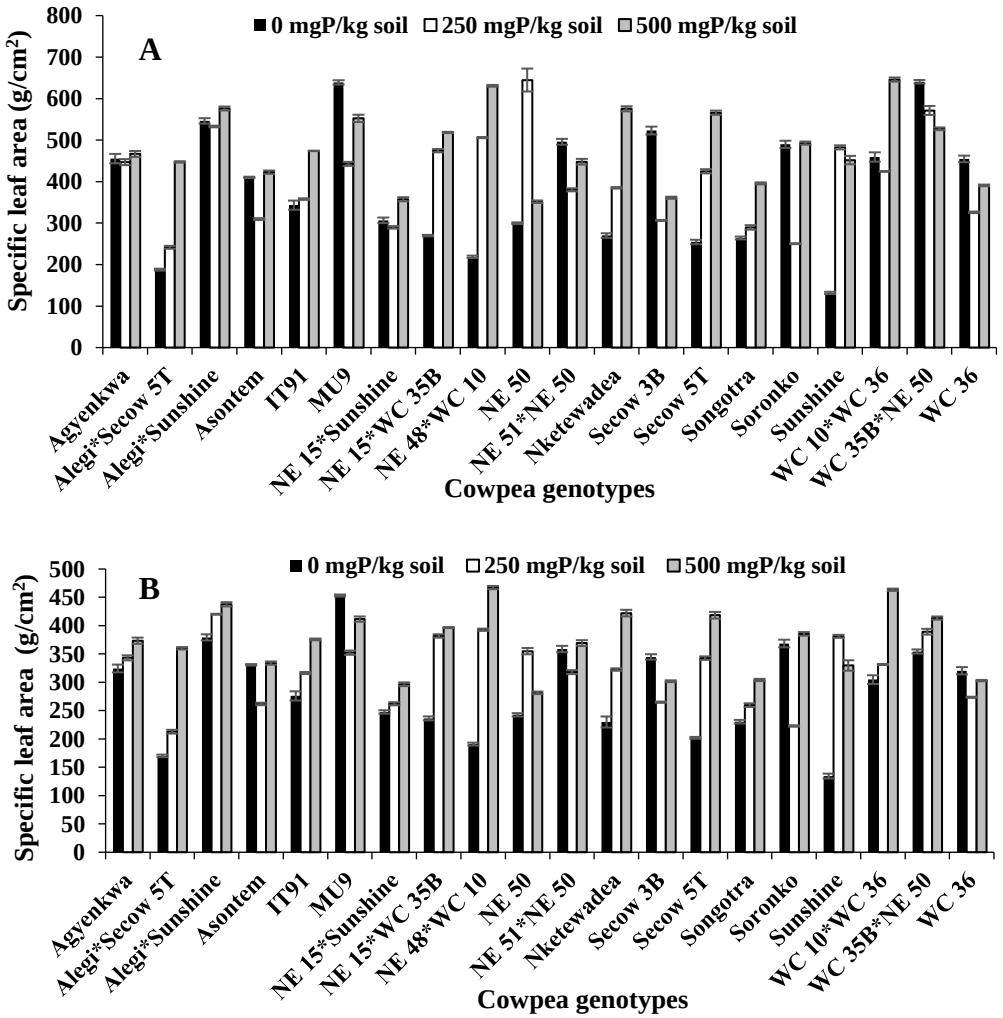


Figure 20: Effect of $[P]_{ext}$ on specific leaf area among 3 weeks old cowpea genotypes grown under greenhouse conditions for (**A**) First screening; (**B**) Second screening. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Leaf mass per area

The twenty (20) genotypes screened under varying phosphorus level exhibited significant ($P < 0.001$) variation in LMA in the first trial (Figure 21A). The LMA at 0 mg P/kg soil arranged from 0.161 - 0.554 g, 0.189 - 0.452 g for 250 mg P/kg, and 0.16 - 0.324 g for 500 mg P/kg soil (Figure 21A). There was a no significant difference ($P = 0.542$) in LMA obtained by cowpea genotypes grown under different $[P]_{ext}$ soils (Figure 21A). The general trend was that LMA

decreased with increasing phosphorus application (Figure 21A). Generally, there was a significant (*P = 0.007*) interaction effect of genotype and $[P]_{ext}$ LMA with plants grown under unamended soil recording highest LMA (Figure 21A). However, genotypes Asontem, Secow 3B, Soronko, WC 35*NE 50 and WC 36 obtained highest LMA under amended soil treatment (Figure 21A).

In the second trial, genotypic effect was significant (*P < 0.001*) for LMA among cowpea genotypes (Figure 21B). Specific leaf area ranged from 0.161 - 0.409 g for treatment 0 mg P/kg soil, 0.198 - 0.401 treatment 250 mg P/kg soil and 0.161 – 0.324 g/cm^2 for 500 mg P/kg soil (Figure 21B). There was a no significant difference (*P = 0.342*) in LMA obtained by cowpea genotypes grown under different $[P]_{ext}$ soils, although the general trend was that, LMA decreased with increasing phosphorus application (Figure 28B). Generally, there was a significant (*P = 0.027*) interaction effect of genotype and $[P]_{ext}$ LMA with plants grown under unamended soil recording highest LMA (Figure 21B). Genotypes Agyenkwa, Alegi*Secow 5T, Asontem, NE 51*NE 50, Secow 3B, Secow 5T and WC 35*NE 50 had the highest LMA at amended soil treatment (Figure 21B).

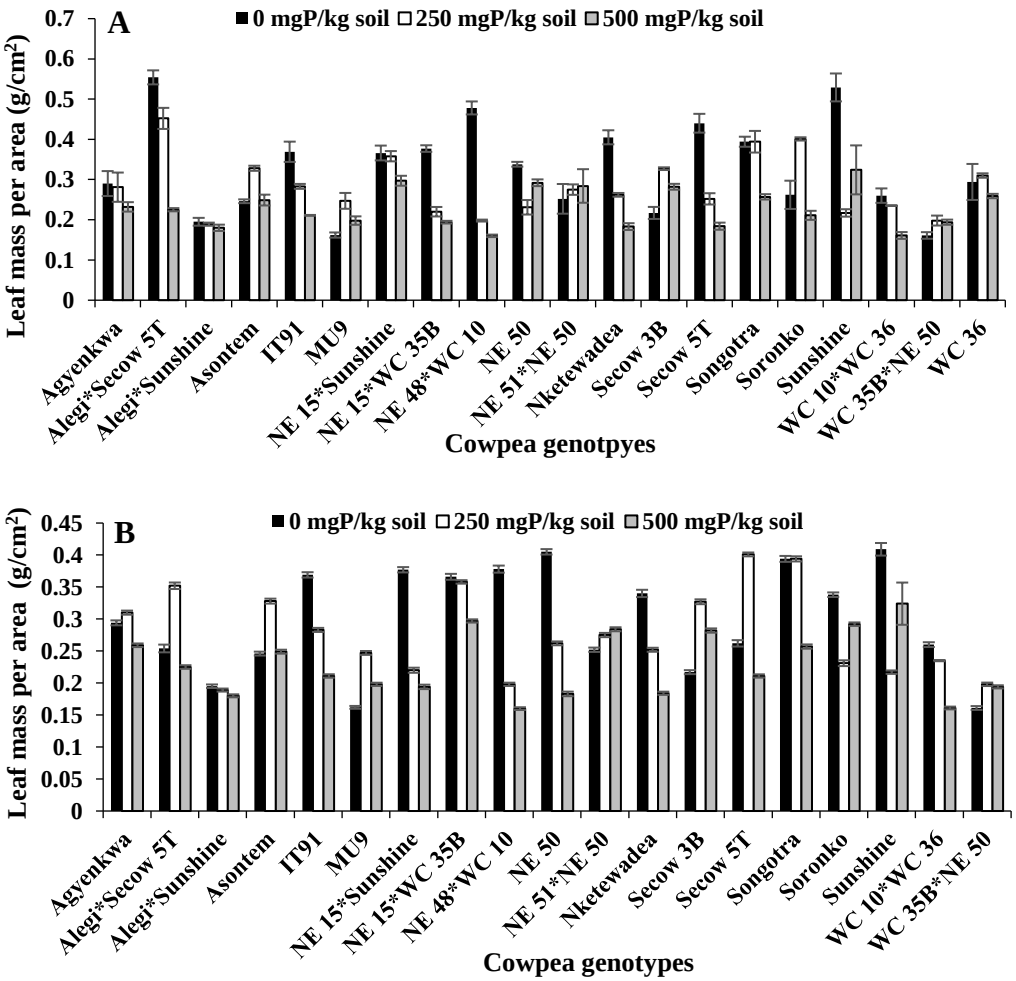


Figure 21: Effect of $[P]_{ext}$ on leaf mass per area among 3 weeks old cowpea genotypes grown under greenhouse conditions for **(A)** First screening; **(B)** Second screening. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Rhizosheath parameters

Absolute rhizosheath weight

Cowpea genotypes evaluated under varying $[P]_{ext}$ exhibited significant variation ($P < 0.001$) for ARW during the first trial (Figure 22A). Absolute rhizosheath weight under 0 mg P/kg soil ranged from 1.28 – 5.31 g with genotype NE 15*WC 35B and IT91 recording the lowest and highest weights respectively. Under 250 mg P/kg soil, ARW ranged from 1.50 - 6.13 g for

genotypes NE 50 and Agyenkwa respectively. However, at 500 mg P/kg, genotype WC 35B*NE 50 (2.58 g) recorded the lowest ARW and Secow 3B (7.11 g) obtained the highest weight. Generally, rhizosheath increased with increasing $[P]_{ext.}$ Absolute rhizosheath of genotypes grown on 250 and 500 mg P/kg soil were 36 and 38% respectively, greater than ARW of genotypes on unamended soil (Figure 22A). Interaction between genotypes and $[P]_{ext}$ had a significant ($P < 0.001$) effect on ARW such that some genotypes obtained the highest weight at high $[P]_{ext}$ whilst others also obtained highest absolute rhizosheath weight at low $[P]_{EXT}$ (Figure 22A). For example, genotypes Agyenkwa, Alegi*Secow 5T, NE 51* NE 50, Secow 5T, Songotra, Soronko, WC 35B*NE 50 and WC 36 recorded high ARW under 250 mg P/kg soil (Figure 22A).

Similarly, genotype, phosphorus and their interaction had a significant effect ($P < 0.001$) on ARW in the second trial (Figure 22B). Absolute rhizosheath weight under 0 mg P/kg soil ranged from 1.41 - 5.45 g with genotype MU9 and WC 36 recording the lowest and highest weights respectively. Under 250 mg P/kg soil, ARW ranged from 1.68 - 6.31 g for genotypes NE 50 and Agyenkwa respectively. However, at 500 mg P/kg genotype WC 35B*NE 50 (2.80 g) recorded the lowest ARW and Secow 3B (7.33 g) obtained the highest weight (Figure 22B). Phosphorus application significantly ($P < 0.001$) affected ARW obtained by cowpea genotypes. An increasing trend was observed with increasing $[P]_{ext}$ (Figure 22B). There was a significant ($P < 0.001$) interaction between genotypes and $[P]_{ext}$ on absolute rhizosheath weight during the second trial (Figure 22B). Highest ARW was obtained at 500 mg P/kg soil by genotypes Alegi*Sunshine, Asontem, NE

15*Sunshine, NE 15*WC 35B, NE 48*WC 10, NE 50, Nketewadea, Secow 3B and Sunshine. The remaining genotypes recorded higher ARW under 250 mg P/kg soil (Figure 22B).

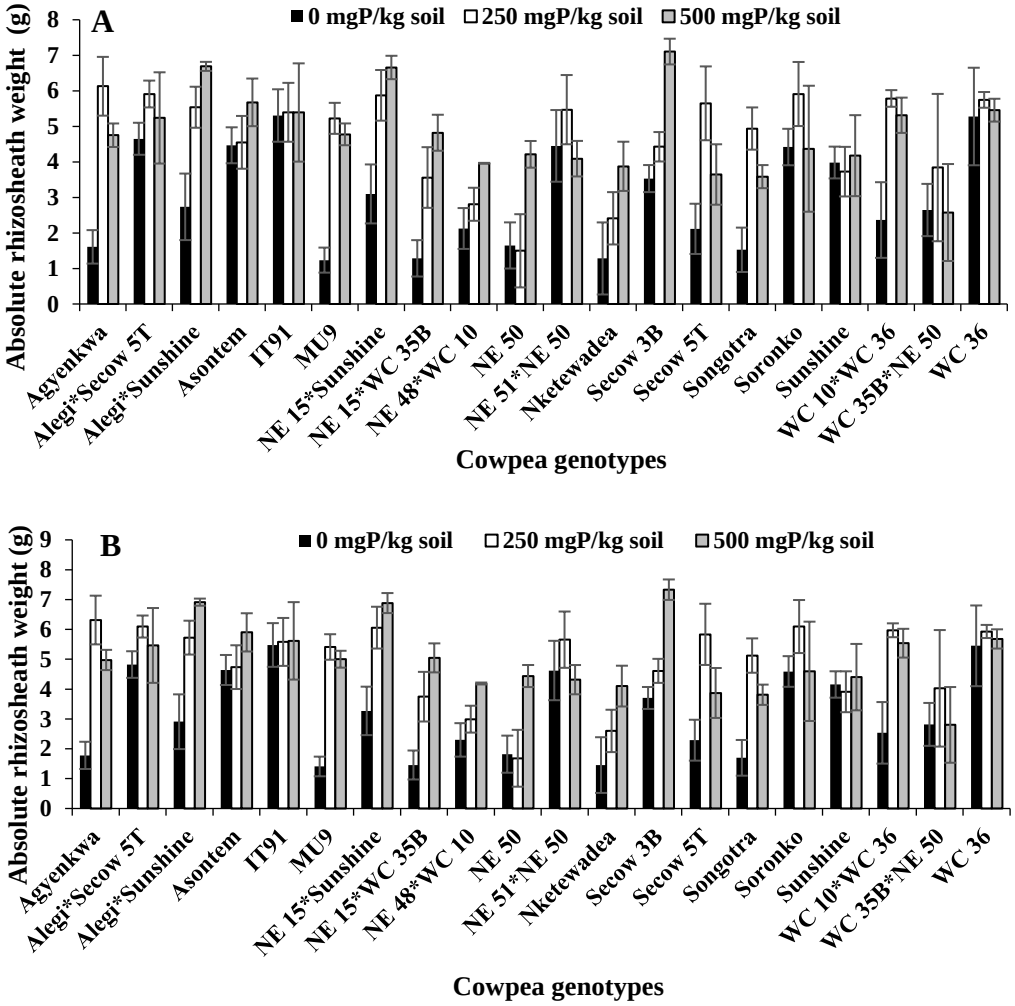


Figure 22: Effect of $[P]_{ext}$ on absolute rhizosheath weight among 3 weeks old cowpea genotypes grown under greenhouse conditions for **(A)** First screening; **(B)** Second screening. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Relative rhizosheath weight

Genotype, $[P]_{EXT}$ and their interaction significantly ($P < 0.001$) affected the RRW in both trails (Figure 23A and 23B). In the first trial, $[P]_{ext}$ significantly ($P < 0.001$) affected RRW obtained by cowpea genotypes (Figure 23A). Majority of cowpea genotypes produced higher RRW with increasing phosphorus rates except genotype WC 35B*NE 50 which recorded significant-high ARW at 0 mg P/kg soil (Figure 23A). Relative rhizosheath weight obtained at 500 and 250 mg P/kg soil was 25 and 23% greater compared to unamended soil treatment (Figure 23A). The interaction between genotypes and $[P]_{ext}$ significantly ($P < 0.001$) affected RRW among cowpea genotypes (Figure 23A). Genotypes Agyenkwa, Alegi*Secow 5T, Alegi*Sunshine, MU9, NE 51* NE 50, Secow 5T, Soronko, WC 10B*WC 36 and WC 36 obtained highest RRW at 250 mg P/kg soil relative to IT91, NE 15* Sunshine, NE 15*WC 35B and NE 48*WC 10 which obtained significantly higher RRW at 500 mg P/kg soil (Figure 23A).

In the second screening (Figure 23B), lower P significantly ($P < 0.001$) decreased overall RRW among cowpea genotypes. Relative rhizosheath weight obtained at amended soil treatments was 24% more compared to unamended treatment (Figure 23B). Genotypes and their interaction with $[P]_{ext}$ were significant ($P < 0.001$) for RRW. Genotypes responded differently for RRW under varying phosphorus rates. Genotypes Agyenkwa, Alegi*Secow 5T, Alegi*Sunshine, MU9, NE 51*NE 50, Secow 5T, Soronko, WC 10*WC 36 and WC 36 produced more RRW at 250 mg P/kg soil compared to genotypes Sunshine which obtained high RRW at 0 mg P/kg soil. The remaining genotypes increased RRW with increased application of P (Figure 23B).

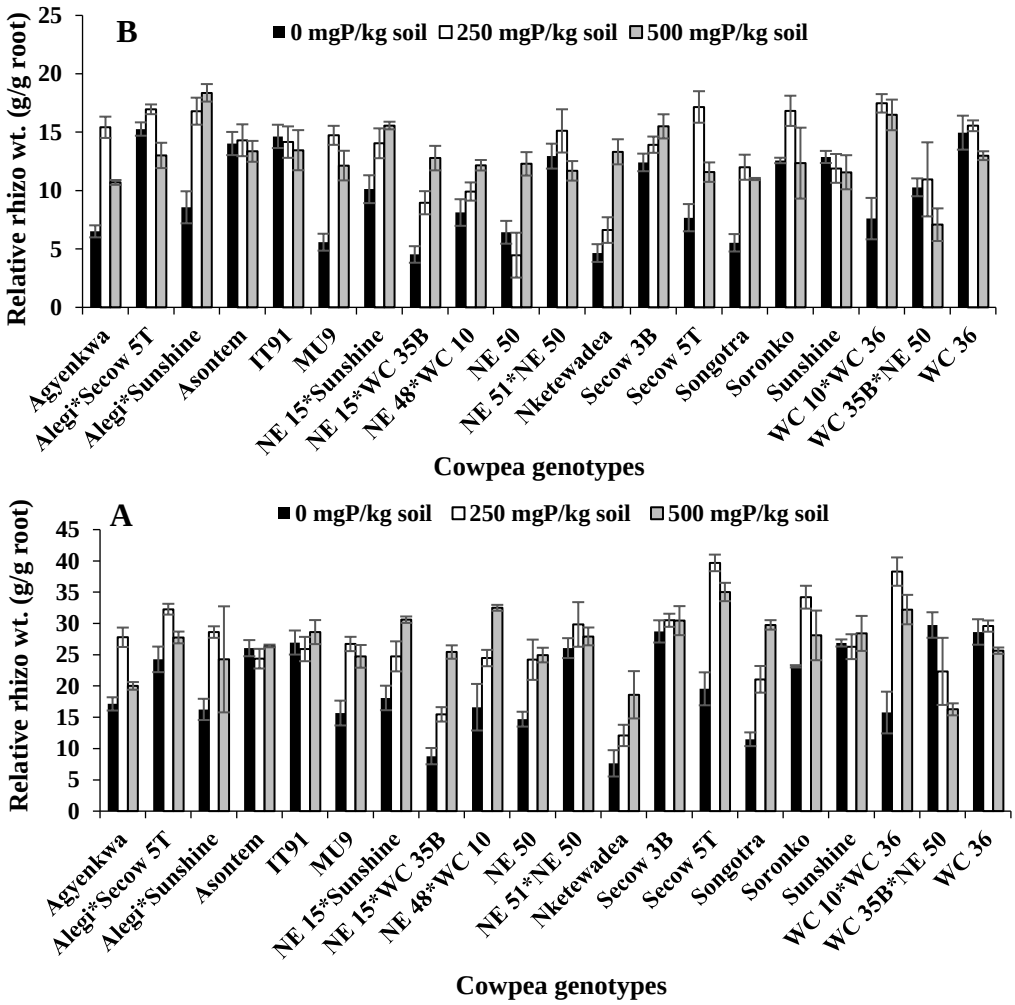


Figure 23: Effect of $[P]_{ext}$ on relative rhizosheath weight among 3 weeks old cowpea genotypes grown under greenhouse conditions for **(A)** First screening; **(B)** Second screening. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Specific rhizosheath weight

There was significant ($P < 0.001$) variation in SRW obtained by cowpea genotypes screened in the first trial (Figure 24A). Specific rhizosheath weight ranged from 4.22 - 19.36 g/mm (0 mg P/kg soil), 7.85 - 34.51 g/mm (250 mg P/kg soil) and 7.60 - 31.77 (500 mg P/kg soil) (Figure 24A). Specific rhizosheath weight obtained at 500 mg P/kg soil was 7% greater compared to

250 mg P/kg soil and 33% compared to 0 mg P/kg soil (Figure 24A). For most of the genotypes, SRW increased on amended soil treatments. The interaction between genotypes and phosphorus level had a significant ($P < 0.011$) effect on SRW (Figure 24A). Certain genotypes such as Asontem and Secow 3B produced highest SRW at 500 mg P/kg soil. Also, genotypes Alegi*Secow 5T, IT91, MU9 and NE 48*NE 10 obtained the highest specific rhizosheath weight at unamended soil treatment. The remaining genotypes recorded highest SRW at 250 mg P/kg soil (Figure 24A).

In the second trial (Figure 24B) significant ($P < 0.001$) variation existed in SRW obtained by cowpea genotypes (Figure 24B). Specific rhizosheath weight ranged from 5.38 - 18.16 g/mm (0 mg P/kg soil) with genotype Songotra obtaining the lowest and WC 36 recording the highest SRW. Genotype Alegi*Secow 5T (6.44 g/mm root) obtained the lowest SRW at 250 mg P/kg soil while WC 10*WC 36 (33.94 g/mm) obtaining the highest. At 500 mg P/kg soil, SRW ranged from 5.31 - 27 g/mm root (Figure 24B). There was no significant variation ($P = 0.24$) in SRW of genotypes grown under varying $[P]_{EXT}$ levels (Figure 24B). The interaction between genotypes and phosphorus level had significant ($P = 0.051$) effect on SRW (Figure 24B). Genotypes such as Asontem, MU9, IT91, and NE 48*WC 10 obtained highest SRW at 0 mg P/kg soil. The remaining genotypes recorded highest SRW under amended soil treatments (Figure 24B).

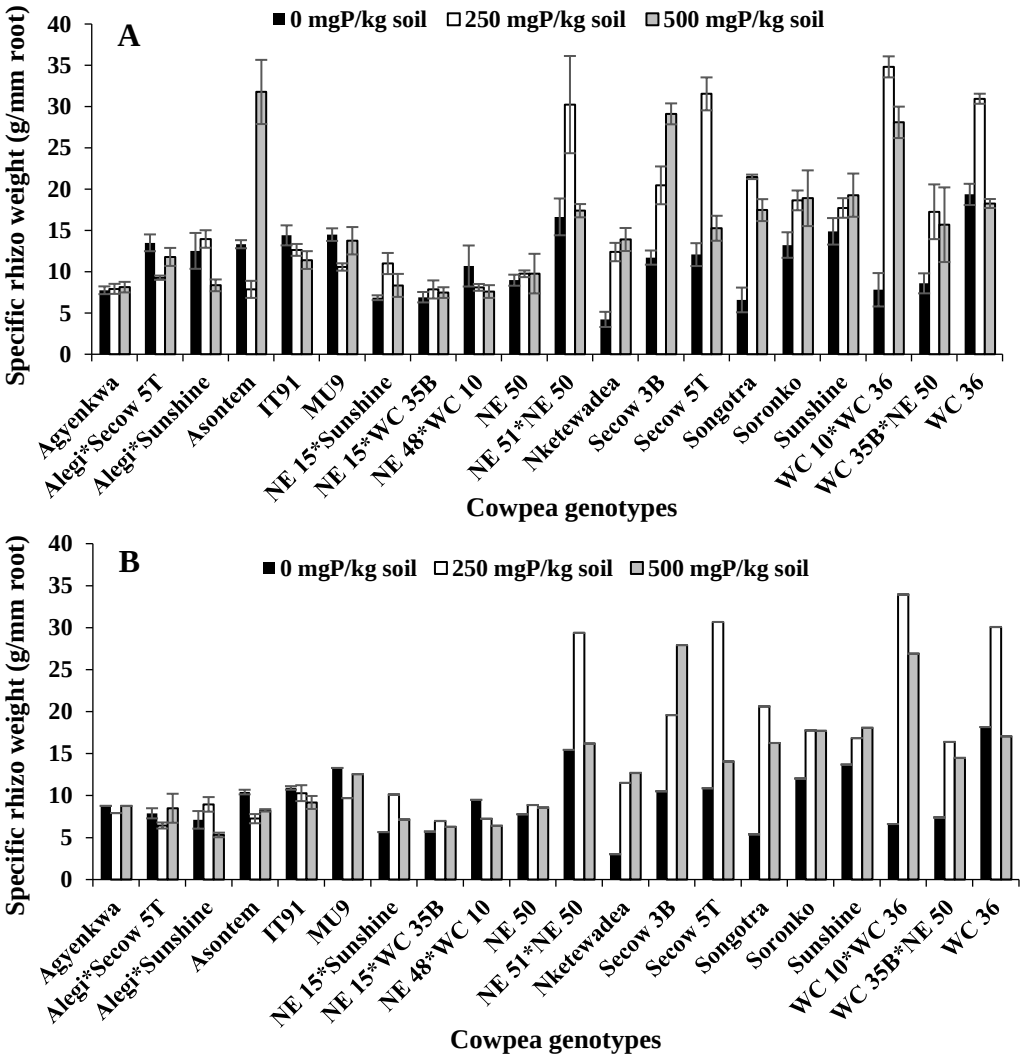


Figure 24: Effect of $[P]_{ext}$ on specific rhizosheath weight among 3 weeks old cowpea genotypes grown under greenhouse conditions for **(A)** First screening; **(B)** Second screening. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Root hair parameters

Root hair length

Root hair length in the first trial was significantly ($P < 0.001$) influenced by genotype, $[P]_{ext}$ and their interactions (Figure 25A). Phosphorus application increased RHL among genotypes, however, genotypes Agyenkwa, MU9, NE 15* Sunshine, NE 51*NE 50, Nketewadea, Secow 3B, Songotra, Soronko and

WC 10*WC 36 obtained significantly (*P < 0.001*) higher RHL at 0 mg P/kg soil compared to amended soils. Example, genotype Asontem produced a higher RHL at 250 mg P/kg soil. Higher values for RHL was also observed in Alegi*Secow 5T, Alegi*Sunshine, IT91, NE 15*WC 35B, NE 48*WC 10, Nketewadea, Secow 5T and WC 36 AT 500 mg P/kg soil (Figure 25A).

In the second trial (Figure 25B), RHL was significantly (*P < 0.001*) affected by genotype, $[P]_{ext}$ and their interactions. Significantly higher RHL was obtained at 0 mg P/kg soil by Agyenkwa, MU9, NE 15* Sunshine, NE 15*WC 35B, NE 51*NE 50, Nketewadea, Secow 3B, Songotra, Soronko, Sunshine, WC 35B*NE 50 and WC 10*WC 36 compared to 250 mg P/kg soil Alegi*Secow 5T and Asontem and 500 mg P/kg soil Alegi*Sunshine and Secow 5T (Figure 25B).

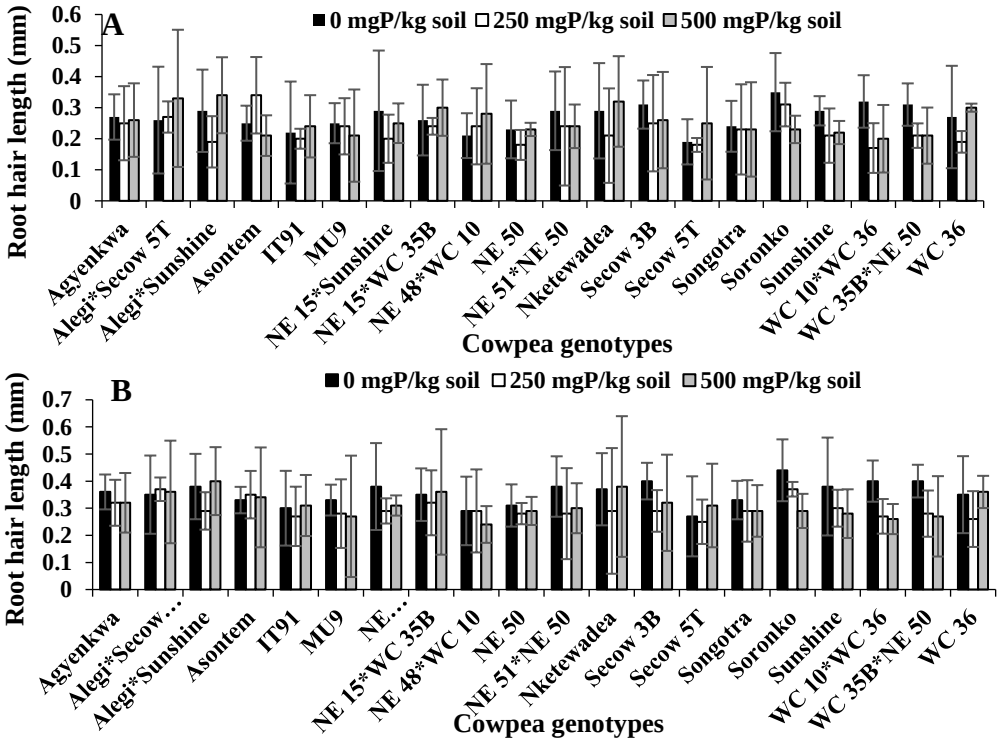


Figure 25:: Effect of $[P]_{ext}$ on root hair length among 3 weeks old cowpea genotypes grown under greenhouse conditions for **(A)** First screening; **(B)** Second screening. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Root hair density

Root hair density was significantly ($P < 0.001$) influenced by cowpea genotypes and their interaction with $[P]_{ext}$ in the first trial. Phosphorus application had a significant effect ($P = 0.013$) on RHD in the first trial (Figure 26A). For most cowpea genotypes screened, phosphorus application increased RHD. Genotypes Agyenkwa, Alegi*Sunshine, IT91, MU9, NE 15*Sunshine, Nketewadea, Secow 5T and Soronko produced significantly ($P < 0.001$) high root hair density at 500 mg P/kg soil. Additionally, significantly high values for root hair density was observed at 250 mg P/kg soil for (Asontem, NE 50, NE 51* NE 50, Songotra and WC 36) (Figure 26A). Genotypes (NE 15*WC 35B, WC 10*WC 36 and WC 35B*NE 50) obtained high RHD at 0 mg P/kg soil (Figure 26A).

Phosphorus application had insignificant ($P = 0.115$) effect on root hair density in the second trial (Figure 26B). However, RHD obtained at amended soil treatment was 8 per cent greater than genotypes evaluated on unamended soil. Genotype and its interaction with $[P]_{ext}$ caused significant ($P < 0.001$) variation in RHD among cowpea genotype in the second trial (Figure 26B). Root hair density ranged from 13.42 – 26.61 count/mm^2 (0 mg P/kg soil), 13.83 - 24.73 count/mm^2 (250 mg P/kg soil) and 13.16 - 30.35 count/mm^2). Genotypes Agyenkwa, Alegi*Secow 5T, IT91, NE 15*WC 35B, NE 48*WC 10, NE 50, NE 51*NE 50, Secow 3B, Sunshine and WC 10*WC 36 had significantly high RHD at 0 mg P/kg soil in the second trial (Figure 26B). High RHD was observed at 250 mg P/kg soil for genotypes Asontem, MU9 and WC 36 and 500 mg P/kg soil for genotypes Alegi*Sunshine, Nketewadea, Secow 5T and Soronko.

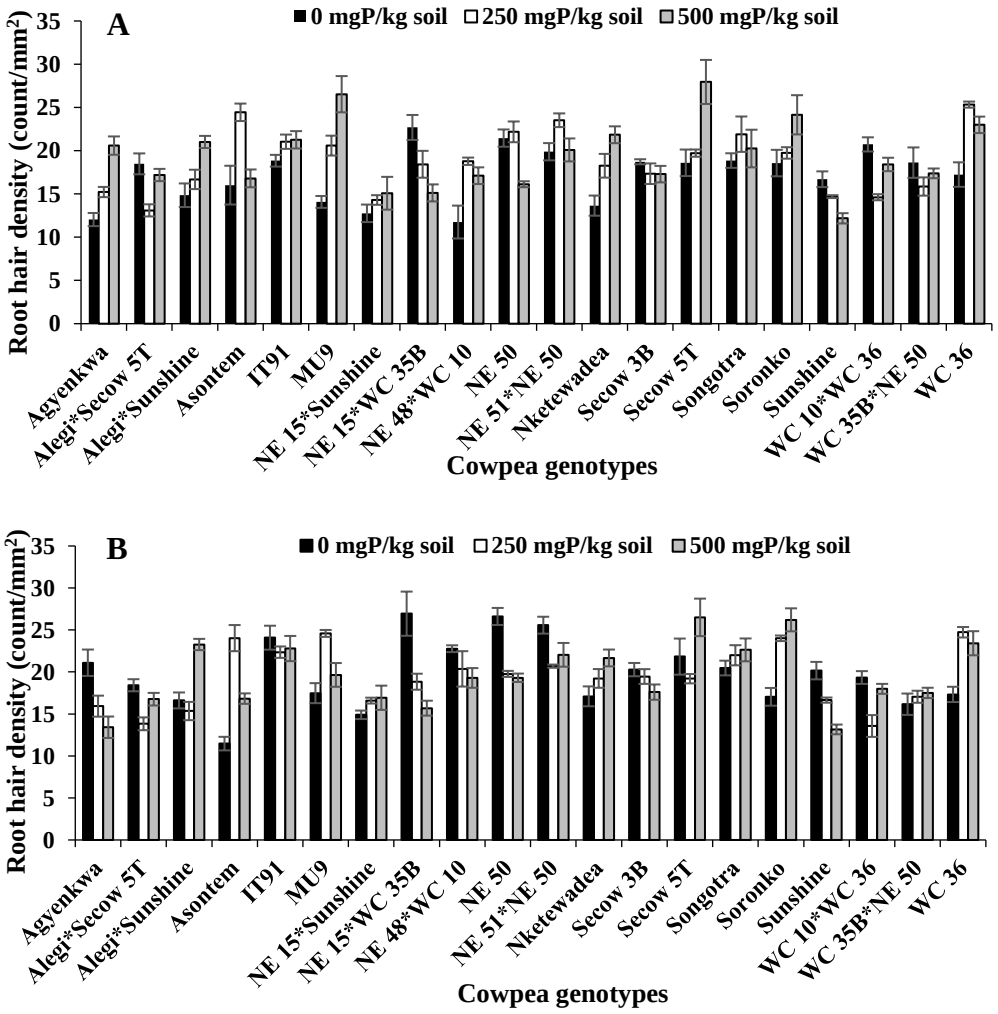


Figure 26: Effect of $[P]_{ext}$ on specific rhizosheath weight among 3 weeks old cowpea genotypes grown under greenhouse conditions for **(A)** First screening; **(B)** Second screening. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Relationship between root hair density, root hair length and rhizosheath parameters

The relationship between RHD and rhizosheath parameters (Figure 27) revealed that, there is a weak correlation between RHD and rhizosheath parameters. Generally, RHD had a weak but significant ($R^2 = 0.143, P = 0.015$) relation with ARW and RRW ($R^2 = 0.139, P = 0.045$) (Figure 27A and 27B). However, RHD had a weak but significantly positive ($R^2 = 0.32, P < 0.001$) relation with SRW (Figure 27C)

When RHL was correlated with rhizosheath parameters, a positive relationship was observed between the traits (Figure 28). Generally, RHL had a positive but insignificant ($R^2 = 0.227, P = 0.139$) relation with ARW (Figure 28A). However, RHL had a weak and significant ($R^2 = 0.431, P = 0.001$) relation with SRW (Figure 30C) and a strong significantly positive RRW ($R^2 = 0.745, P < 0.001$) (Figure 28B).

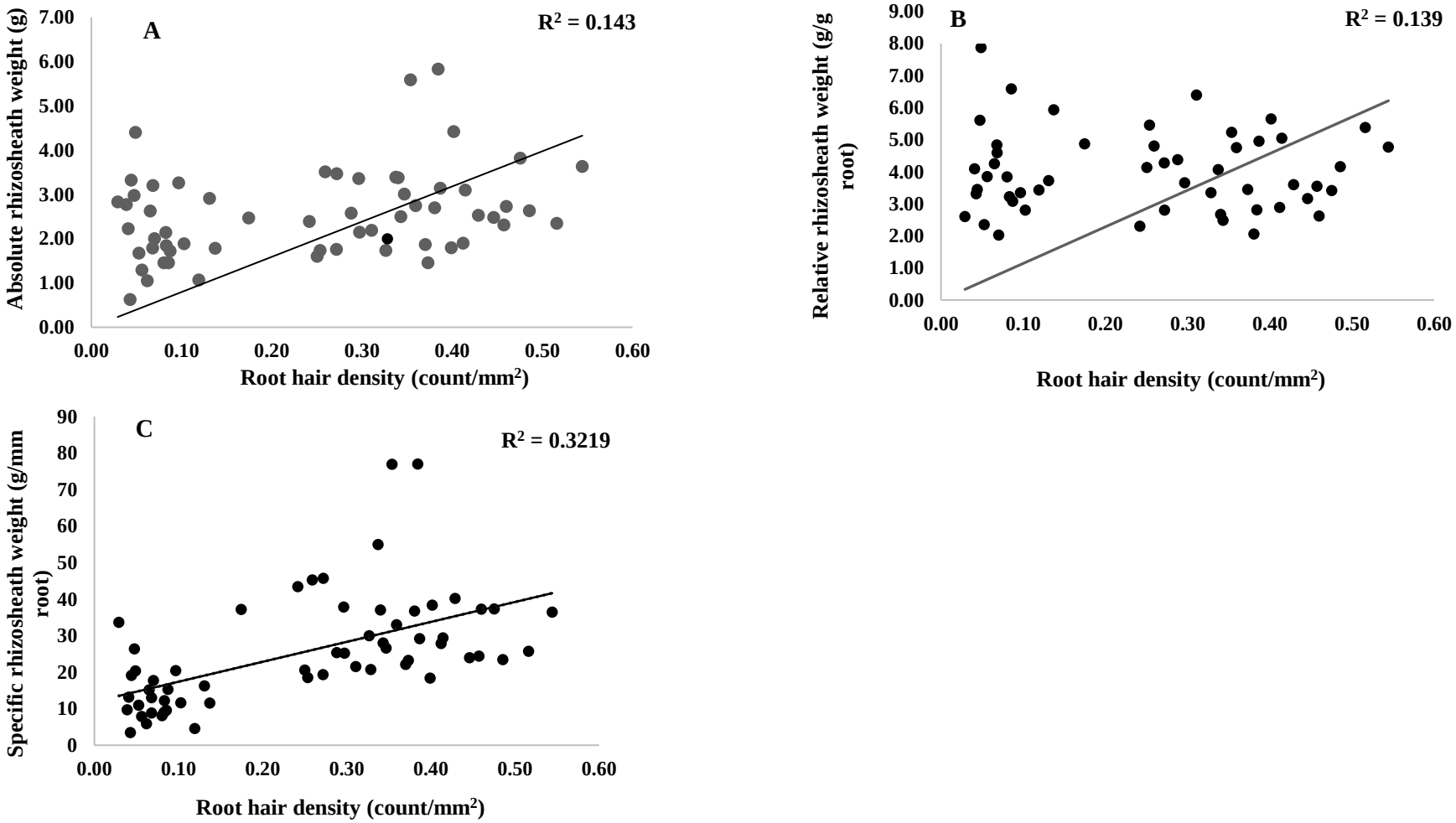


Figure 27: Regression between root hair density of cowpea genotypes and **(A)** absolute rhizosheath weight: **(B)** relative rhizosheath weight and **(C)** specific rhizosheath weight

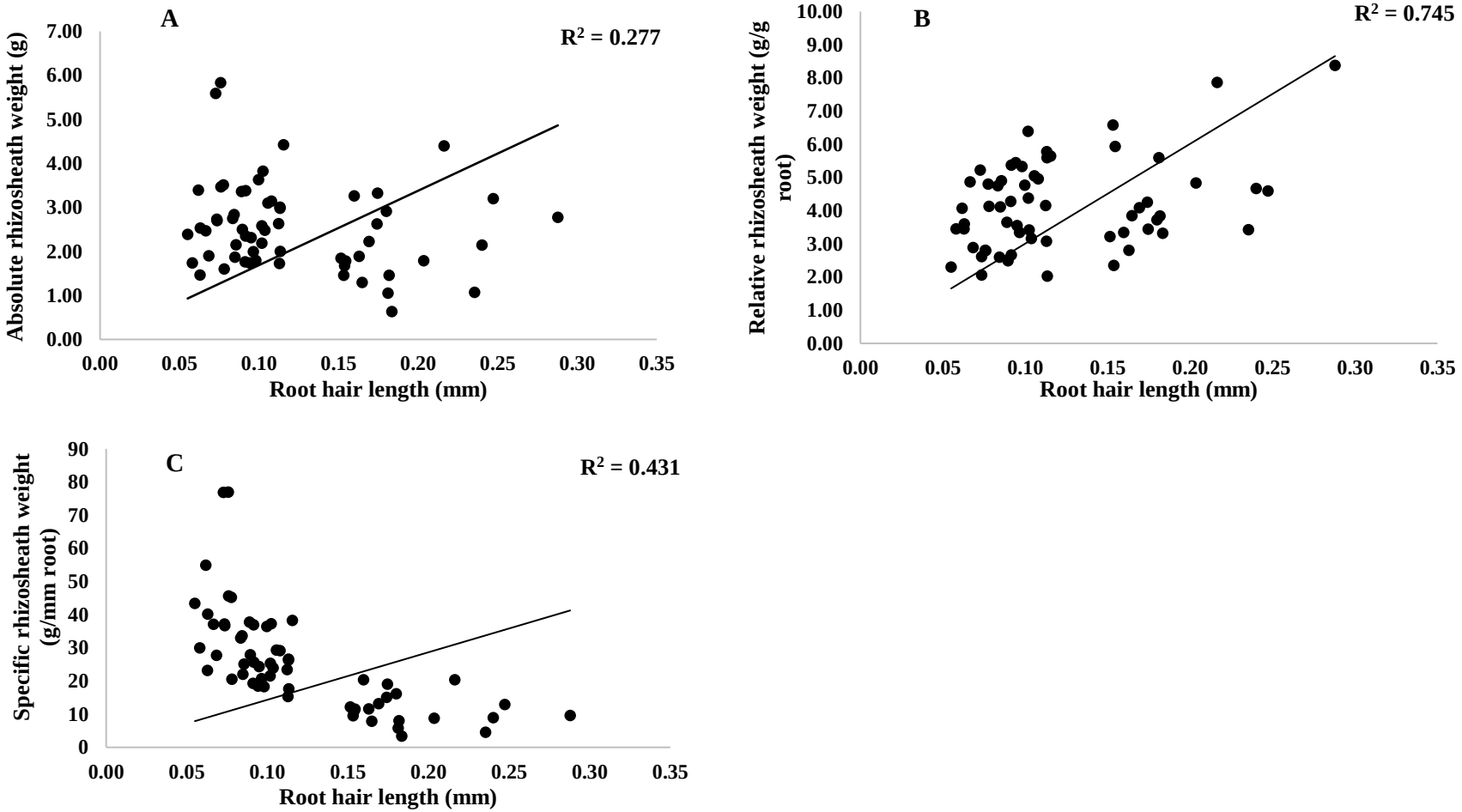


Figure 28: Regression between root hair length of cowpea genotypes and **(A)** absolute rhizosheath weight: **(B)** relative rhizosheath weight and **(C)** specific rhizosheath weight

Total root length

Total root length was significantly ($P < 0.001$) affected by genotype, $[P]_{ext}$ and the interaction of genotypes and $[P]_{EXT}$ in both first and second trial (Figure 29A and 31B). Total root length ranged from 300.60 - 501.1 cm (0 mg P/kg soil) for genotypes Songotra and WC 36, 304.8 - 640.0 cm (250 mg P/kg soil) for genotypes WC 35B*NE 50 and WC 36 respectively. Total root length obtained at 500 mg P/kg soil ranged from 255.5 - 601.90 cm for genotype Alegi*Secow 5T obtaining the highest value and NE 48*WC 10 obtaining the lowest (Figure 29A). Total root length increased with increasing phosphorus application for majority of cowpea genotypes. Total root length obtained by genotypes screened at 500 mg P/kg soil was 19 per cent greater than genotypes screened at 0 mg P/kg soil. Genotypes NE 15*WC 35B, NE 48*WC 10, Secow 5T, Songotra and WC 10*WC36 developed longer root length at 250 mg P/kg soil (Figure 29A). Genotype NE 50 rather produced longer root length at 0 mg P/kg soil. Majority of cowpea genotypes including Agyenkwa, Alegi*Secow 5T, Alegi*Sunshine, Asontem, MU9, NE 15* Sunshine, NE 51*NE 50, Nketewadea, Secow 3B, Soronko, Sunshine, WC 35*NE 50 and WC 36 produced longer root length under 500 mg P (Figure 29A). Thus, with certain genotypes, phosphorus application increased TRL to a point and declined under higher phosphorus application (Figure 29A).

In the second screening, phosphorus application significantly ($P < 0.001$) affected total root length (Figure 29B). Total root length recorded at amended soil treatment of 500 mg P/kg soil was 26 per cent higher than at 0 mg P/kg soil whilst total root length at 250 mg P/kg soil was 19 per cent greater compared to unamended soil treatment (Figure 29B). Genotypes Secow 3B

produced longer root length under unamended soil which was significantly ($P < 0.001$) different from that of 250 and 500 mg P/kg soil treatments (Figure 29B). However, genotypes Agyenkwa, Asontem, IT91, MU9, NE 15* Sunshine, NE 15*WC 35B, NE 48*WC 10, NE 51*NE 50, Nketewadea, Songotra, Soronko, Sunshine and WC 10*NE 36 obtained longer root length at 500 mg P/kg soil. Genotypes Alegi*Sunshine, NE 50, Secow 5T, WC 35B*NE 50 and WC 36 obtained longer root length at 250 mg P/kg soil (Figure 29B).

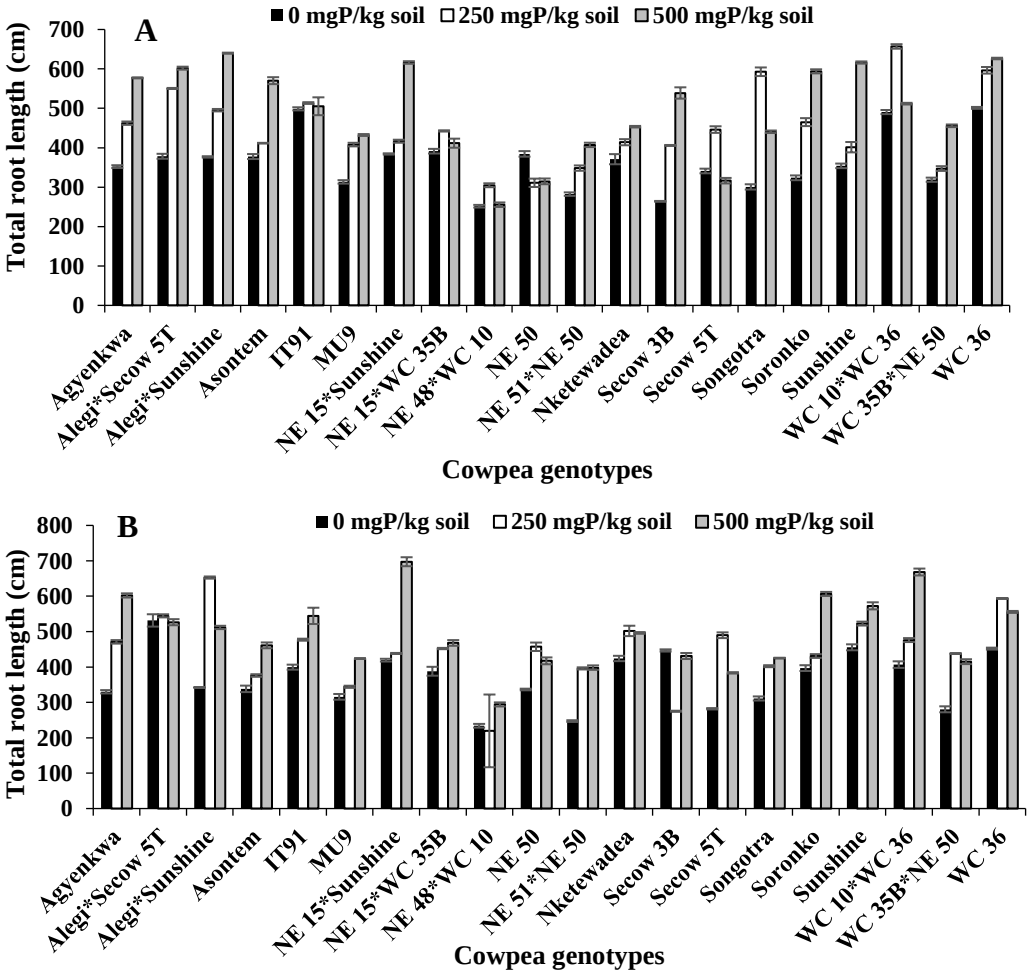


Figure 29: Effect of $[P]_{ext}$ on total root length among 3 weeks old cowpea genotypes grown under greenhouse conditions for **(A)** First screening; **(B)** Second screening. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Genetic variation in the uptake of soil P among cowpea genotypes

Shoot P concentration

Generally, shoot P concentration among cowpea genotypes screened under varying $[P]_{ext}$ was significantly ($P < 0.001$) affected by genotype, $[P]_{ext}$ and interaction between genotypes and $[P]_{ext}$ in both first and second trial (Figure 30A and 30B).

In the first trial, shoot P concentration of cowpea genotypes ranged from 2848 – 9066 µg/g (0 mg P/kg soil), 6772 - 14995 µg/g (250 mg P/kg soil) and 12279 – 19166 µg/g (500 mg P/kg soil) (Figure 30A). A significant ($P < 0.001$) increasing trend of shoot P was observed with increasing P level for all the screened genotypes (Figure 30A). In general, shoot P concentration at treatment 500 mg P/kg was the highest and 76% greater than plants screened under unamended soil (Figure 30A).

In the second trial, a significant ($P < 0.001$) increasing trend of shoot P was observed with increasing P level for all the screened genotypes (Figure 30B). Phosphorus concentration was high in the shoot of cowpea screened at 500 mg P/kg soil (12881 – 19175 µg/g) with genotype Secow 5T obtaining the highest concentration and WC 36 obtaining the lowest P shoot concentration (Figure 30B). Compared to shoot P at unamended soil, shoot P of plants screened at 500 mg P/kg soil had 78 per cent greater phosphorus concentration (Figure 30B).

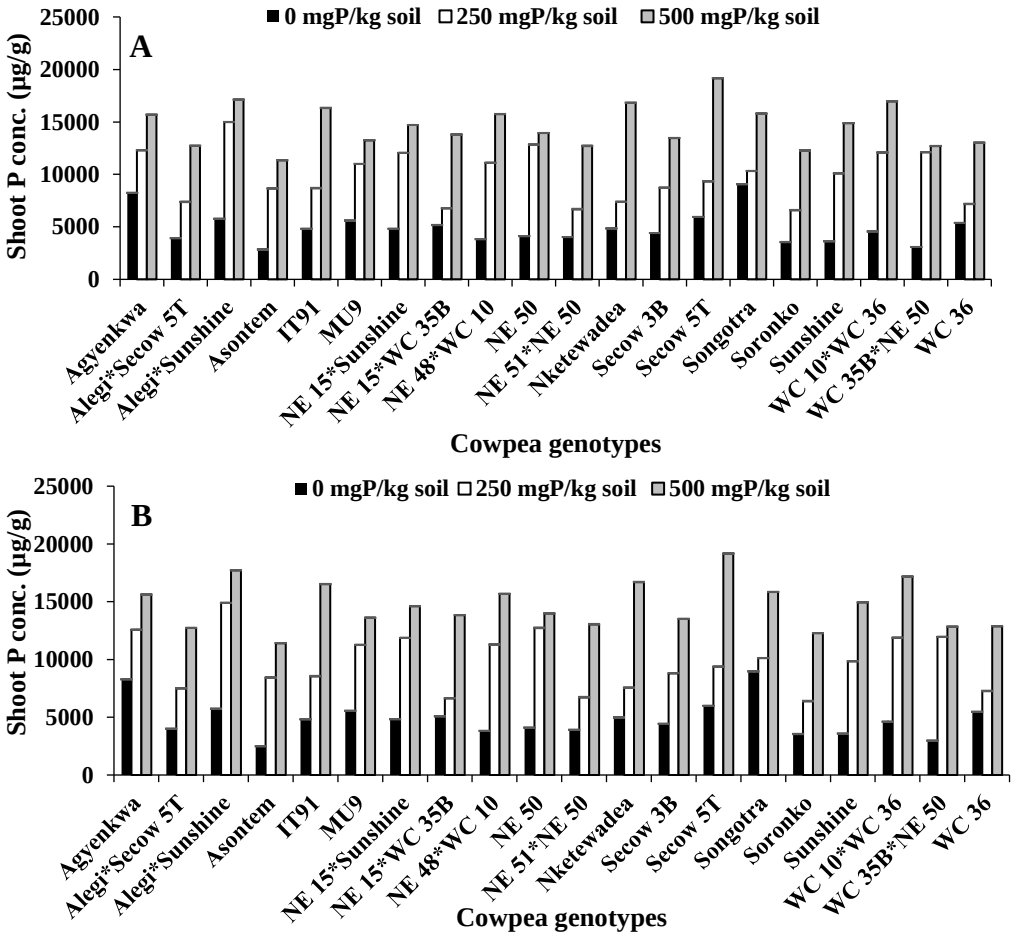


Figure 30: Effect of $[P]_{ext}$ on shoot phosphorus concentration among 3 weeks old cowpea genotypes grown under greenhouse conditions for **(A)** First trial; **(B)** Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Root P concentration

Root P concentration was significantly ($P < 0.001$) influenced by genotype, $[P]_{ext}$ and interaction of $[P]_{ext}$ and genotypes in both first and second trial (Figure 31A and 33B). A significant increase in root P was observed with increasing rates of phosphorus in both the first and second trials. In general treatment 500, mg P/kg soil recorded the highest concentration of P in both root and shoot followed by 250 and 0 mg P/kg soil (Figure 31A).

In the first trial, the highest root P concentration was obtained by genotype Agyenkwa (1840 µg/g) which was significantly different from lowest root P obtained by Asontem (553 µg/g) at 0 mg P/kg soil (Figure 30A). At 250 mg P/kg soil, genotype Alegi*Sunshine (3312 µg/g) had the highest root P concentration which was different from Soronko (1422 µg/g) which had the least root P concentration (Figure 31A). A significant increasing trend in root P concentration was observed with increasing $[P]_{ext.}$ Root P concentration was 84 and 67 per cent greater at 500 and 250 mg P/kg soil respectively than at unamended soil screened genotypes (Figure 31A).

The highest root P concentration was obtained by genotype Agyenkwa (1832 µg/g) which was significantly different from lowest root P obtained by WC 35B*NE 50 (686 µg/g) at 0 mg P/kg soil (Figure 31B). A significant increasing trend in root P concentration was observed with increasing $[P]_{ext.}$ Root P concentration was 75 and 85 per cent greater at 500 and 250 mg P/kg soil respectively compared to P root concentration of genotypes evaluated on unamended soil (Figure 31B).

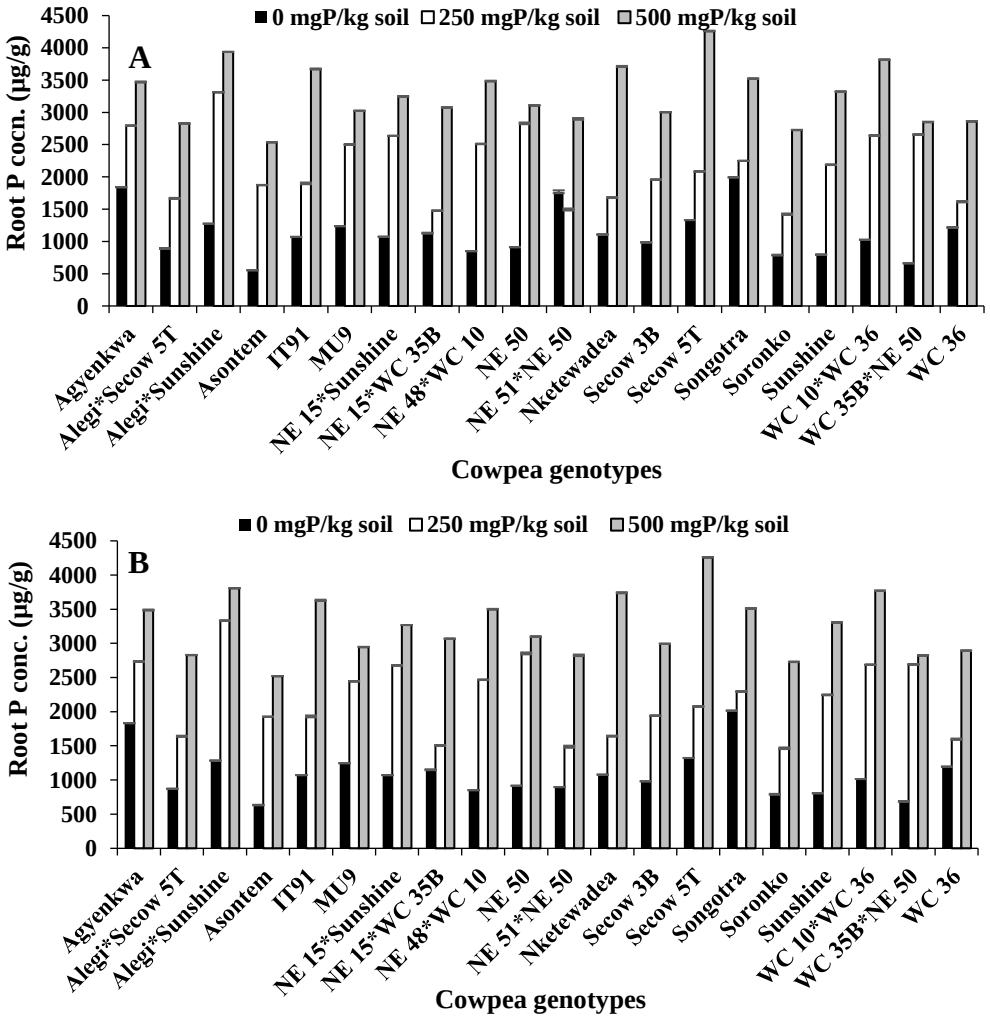


Figure 31: Effect of $[P]_{ext}$ on root phosphorus concentration among 3 weeks old cowpea genotypes grown under greenhouse conditions for **(A)** First trial; **(B)** Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Shoot P content

Cowpea genotypes screened under varying $[P]_{ext}$ exhibited significant ($P < 0.001$) variation in shoot P content in the first trial (Figure 32A). Shoot P content ranged from 2.78 – 8.17 mg/g (0 mg P/kg soil) with Asontem obtaining the lowest shoot P content and Agyenkwa obtaining the highest shoot phosphorus content. Genotype NE 51*NE 50 had the lowest shoot P content

(6.21 mg/g) at 250 mg P/kg soil and Alegi*Sunshine (15.12 mg/g) had the highest. At 500 mg P/kg soil, shoot P content ranged from 12.31 - 18.78 mg/g for genotype NE 51*NE 50 and Secow 5T respectively (Figure 32A). Phosphorus application significantly ($P < 0.001$) affected shoot P content of the screened genotypes. A significantly increasing trend of shoot P content was observed with increasing P application. Shoot P content obtained at 500 and 250 mg P/kg soil was 69 and 31 per cent greater than values obtained in unamended soil treatment respectively (Figure 32A). Genotype and $[P]_{ext}$ interaction significantly ($P < 0.001$) influenced shoot P content, however, for each genotype, highest shoot P content was obtained plants grown on 500 mg P/kg soil treatment (Figure 32A).

In the second trial (Figure 32B) genotype, $[P]_{ext}$ and their interaction significantly ($P < 0.001$) influence shoot P content. Shoot P content ranged from 2.48 - 8.23 mg/g (0 mg P/kg soil). Genotype Soronko had the lowest shoot P content (6.03 mg/g) at 250 mg P/kg soil and Alegi*Sunshine (15.06 mg/g) had the highest. At 500 mg P/kg soil, shoot P content ranged from 11.42 - 18.41 mg/g (Figure 32B). Phosphorus application significantly ($P < 0.001$) affected shoot P content of screened genotypes. A significantly increasing trend of shoot P content was observed with increasing P application. Shoot P content obtained at 500 and 250 mg P/kg soil was 73 and 40 per cent greater than values obtained in unamended soil treatment respectively (Figure 32B). Genotype and $[P]_{ext}$ interaction significantly ($P < 0.001$) influenced shoot P content, however, for each genotype, highest shoot P content was obtained plants grown on 500 mg P/kg soil treatment (Figure 32B).

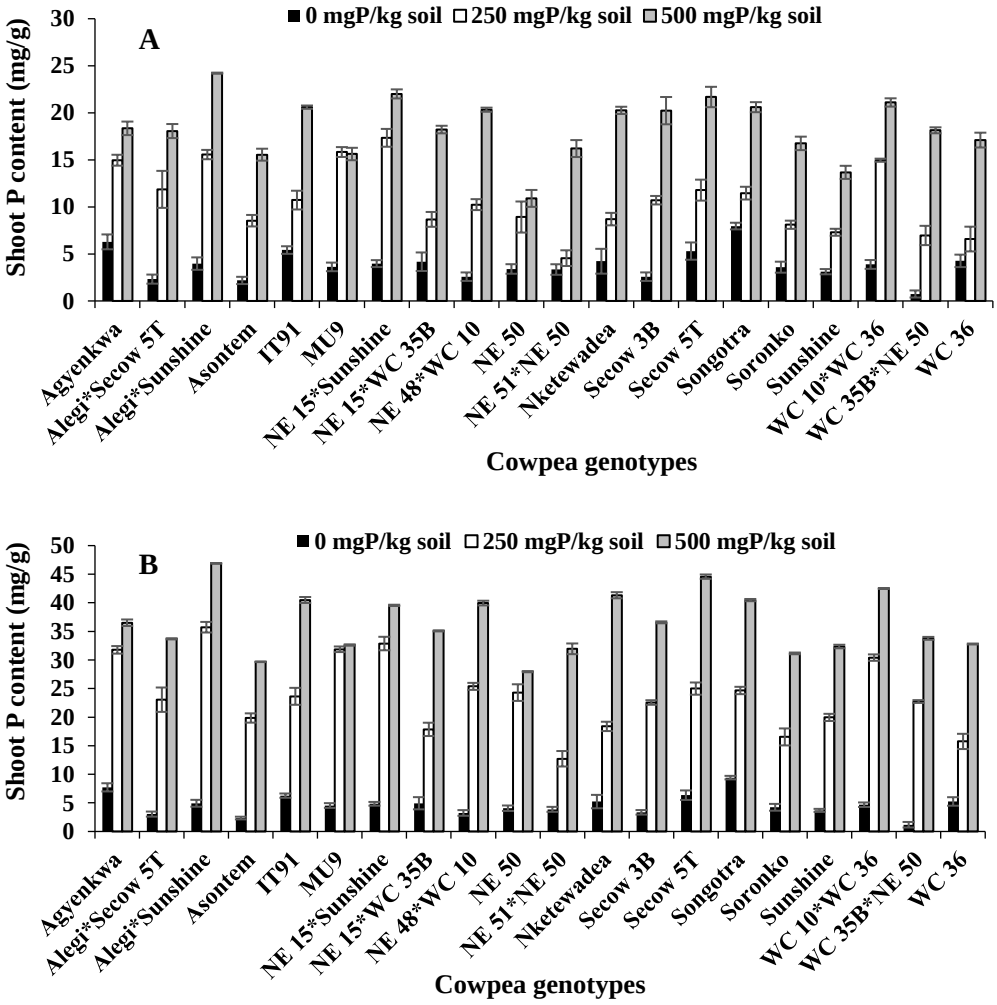


Figure 32: Effect of $[P]_{ext}$ on shoot phosphorus content among 3 weeks old cowpea genotypes grown under greenhouse conditions for **(A)** First trial; **(B)** Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Root P content

In the first trial (Figure 33A) genotype and $[P]_{ext}$ significantly ($P < 0.001$) influenced root P content. Root P content ranged from 0.06 - 0.31 mg/g (0 mg P/kg soil). Genotype Soronko had the lowest root P content (0.23 mg/g) at 250 mg P/kg soil and NE 15*Sunshine (0.71 mg/g) had the highest. At 500 mg P/kg soil, root P content ranged from 0.41 - 1.62 mg/g (Figure 33A). Phosphorus application significantly ($P < 0.001$) affected root P content of

screened genotypes. A significantly increasing trend of root P content was observed with increasing P application. Root P content obtained at 500 and 250 mg P/kg soil was 81 and 63 per cent greater than values obtained in unamended soil treatment (Figure 33A). Genotype and $[P]_{ext}$ interaction had an insignificant ($P = 0.166$) effect on root P content, however, for each genotype, highest root P content was obtained plants grown on 500 mg P/kg soil treatment (Figure 33A).

Cowpea genotypes screened under varying $[P]_{ext}$ exhibited significant ($P < 0.001$) variation in root P content in the second trial (Figure 33B). Root P content ranged from 0.21 - 0.61 mg/g (0 mg P/kg soil) with Asontem obtaining the lowest root P content and Songotra obtaining the highest root P content. Genotype NE 51*NE 50 had the lowest root P content (0.53 mg/g) at 250 mg P/kg soil and NE 15*Sunshine (1.19 mg/g) had the highest. At 500 mg P/kg soil, root P content ranged from 1.03 - 1.45 mg/g for genotype NE 51*NE 50 and Alegi*Sunshine respectively (Figure 33B). Phosphorus application significantly ($P < 0.001$) affected root P content of screened genotypes. A significantly increasing trend of root P content was observed with increasing P application. Root P content obtained at 500 and 250 mg P/kg soil was 80 and 64 per cent greater than values obtained in unamended soil treatment respectively (Figure 33B). Genotype and $[P]_{ext}$ had a significant ($P = 0.008$) influence on root P content however, highest root P content was obtained plants grown on 500 mg P/kg soil treatment.

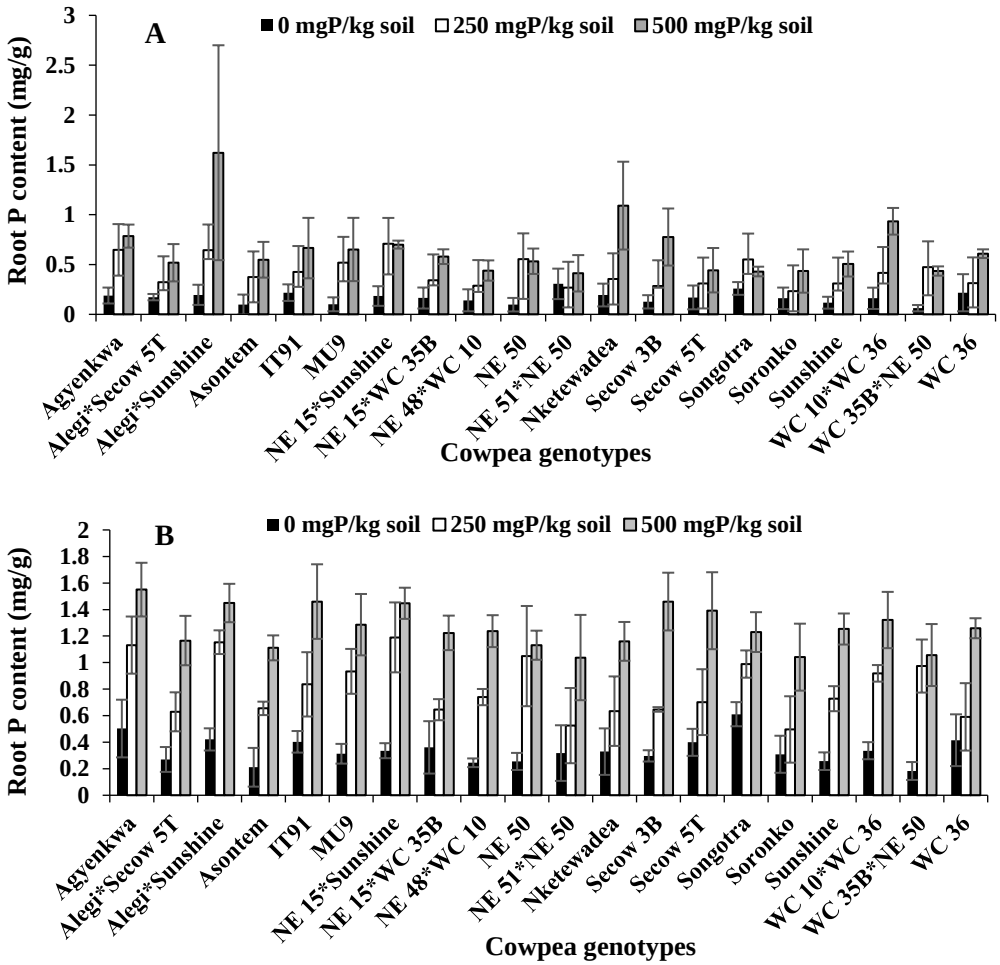


Figure 33: Effect of $[P]_{ext}$ on root phosphorus content among 3 weeks old cowpea genotypes grown under greenhouse conditions for **(A)** First trial; **(B)** Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Phosphorus efficiency parameters

Phosphorus uptake efficiency

Cowpea genotypes screened under varying $[P]_{ext}$ exhibited significant variation ($P < 0.001$) on PUpE during the first trial (Figure 34A). Phosphorus uptake efficiency at 250 mg P/kg soil ranged from 6.72 – 63.19 g DM g^{-1} $[P]_{ext}$ with genotype NE 15*WC 50 and NE*Sunshine recording the lowest and highest weights respectively. Under 500 mg P/kg soil, PUpE ranged from 24.57

- 61.22 g DM g^{-1} $[P]_{ext}$ for genotypes Sunshine and Alegi*Sunshine respectively (Figure 34A). Phosphorus application had a significant (*P = 0.004*) on PUpE of cowpea genotypes (Figure 34A). Interaction between genotypes and $[P]_{ext}$ had a significant (*P < 0.001*) effect on PUpE such that some genotypes obtained high PUpE at high $[P]_{ext}$ whilst others also obtained highest phosphorus uptake efficiency at low $[P]_{ext}$ (Figure 34A). For example, genotypes Asontem, Alegi*Sunshine, NE 15*WC 35B, NE 48*WC 10, NE 51*NE 50, Nketewadea, Secow 3B, Secow 5T, Songotra, Soronko, Sunshine, WC 35B*NE 50 and WC 36 recorded highest PUpE at 500 mg P/kg soil (Figure 34A). The remaining genotypes had the highest PUpE at 250 mg P/kg soil.

Similarly, genotype and genotype and $[P]_{ext}$ interaction had a significant effect (*P < 0.001*) on PUpE in the second trial (Figure 34B). Phosphorus uptake efficiency at 250 mg P/kg soil ranged from 39.40 - 136 g DM g^{-1} $[P]_{ext.}$ At 500 mg P/kg soil, PUpE ranged from 45.21 - 136.43 g DM g^{-1} $[P]_{ext}$ for genotypes WC 36 and Alegi*Sunshine respectively (Figure 34B). There was a significant interaction between genotypes and $[P]_{EXT}$ on PUpE during the second trial (Figure 34B). Highest PUpE was obtained at 500 mg P/kg soil by genotypes IT91, NE 15*WC 35B, NE 51*NE 50, Nketewadea, Secow 5T, Soronko, and WC 36. The remaining genotypes recorded higher PUpE under 250 mg P/kg soil (Figure 34B).

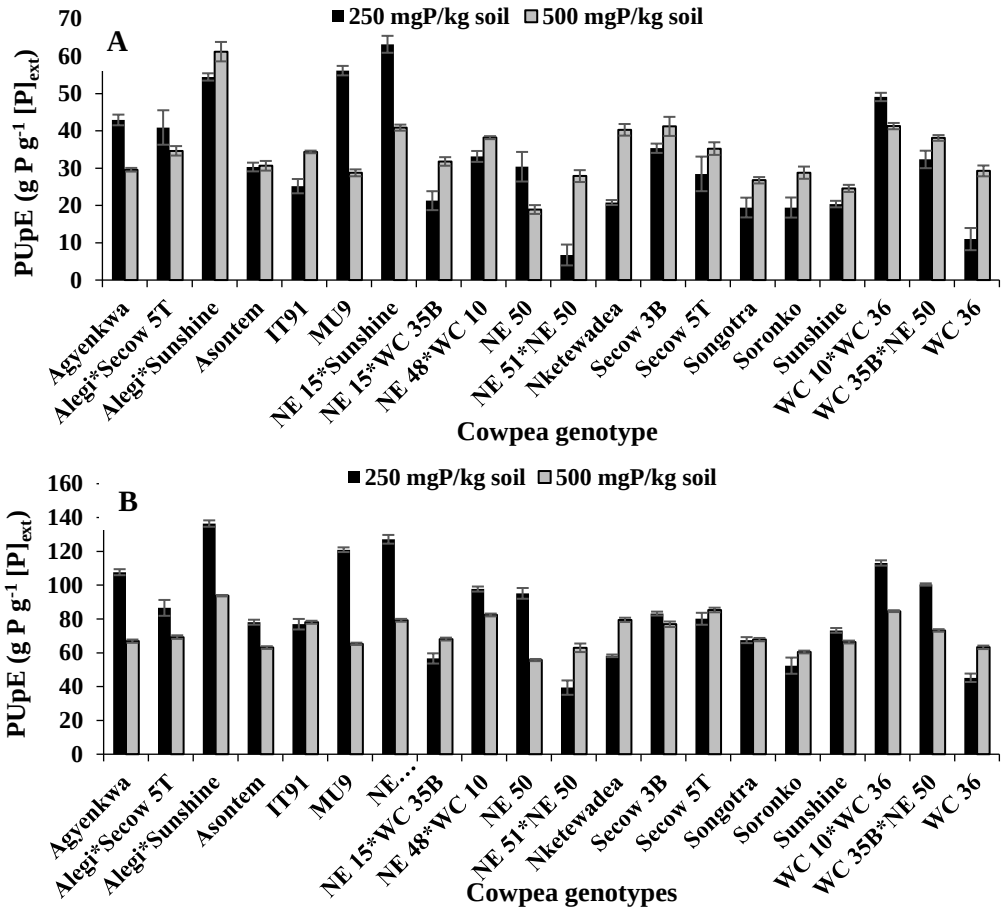


Figure 34: Effect of $[P]_{ext}$ on phosphorus uptake efficiency among 3 weeks old cowpea genotypes grown under greenhouse conditions for **(A)** First trial; **(B)** Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Agronomic P use efficiency

Agronomic P use efficiency was significantly ($P < 0.001$) affected by genotypes, $[P]_{ext}$ and their interactions (Figure 35A). Agronomic P use efficiency of cowpea genotypes screened at 250 mg P/kg soil ranged from 0.59 - 3.55 g DM g^{-1} $[P]_{ext}$ with genotype WC 36 and MU9 obtaining the lowest and highest APE respectively. At 500 mg P/kg soil, APE ranged from 0.27 - 2.52 g DM g^{-1} $[P]_{EXT}$ for genotypes Sunshine and WC 35*NE 50 respectively (Figure 35A). Phosphorus application had a significant effect ($P = 0.001$) on APE of

cowpea genotypes (Figure 35A). Majority of the screened genetic materials increased APE with decreasing $[P]_{ext,}$ however, Alegi*Sunshine, Asontem, NE 48*WC 10, WC 35B*NE 50 and WC 36 had high APE at 500 mg P/kg soil. Interaction between genotypes and $[P]_{ext}$ had a significant ($P < 0.001$) effect on APE such that some genotypes obtained the highest weight at high $[P]_{ext}$ whilst others also obtained the highest APE at low $[P]_{ext}$ (Figure 35A).

Similarly, genotype and genotype and $[P]_{ext}$ interaction had a significant effect ($P = 0.001$) on APE in the second trial (Figure 35B). Phosphorus application significantly ($P < 0.001$) influenced APE in the second trial such that, an increasing trend in APE was observed at low $[P]_{ext}$ for all screened cowpea genotypes. There was a significant interaction between genotypes and $[P]_{ext}$ on APE during the second trial (Figure 35B).

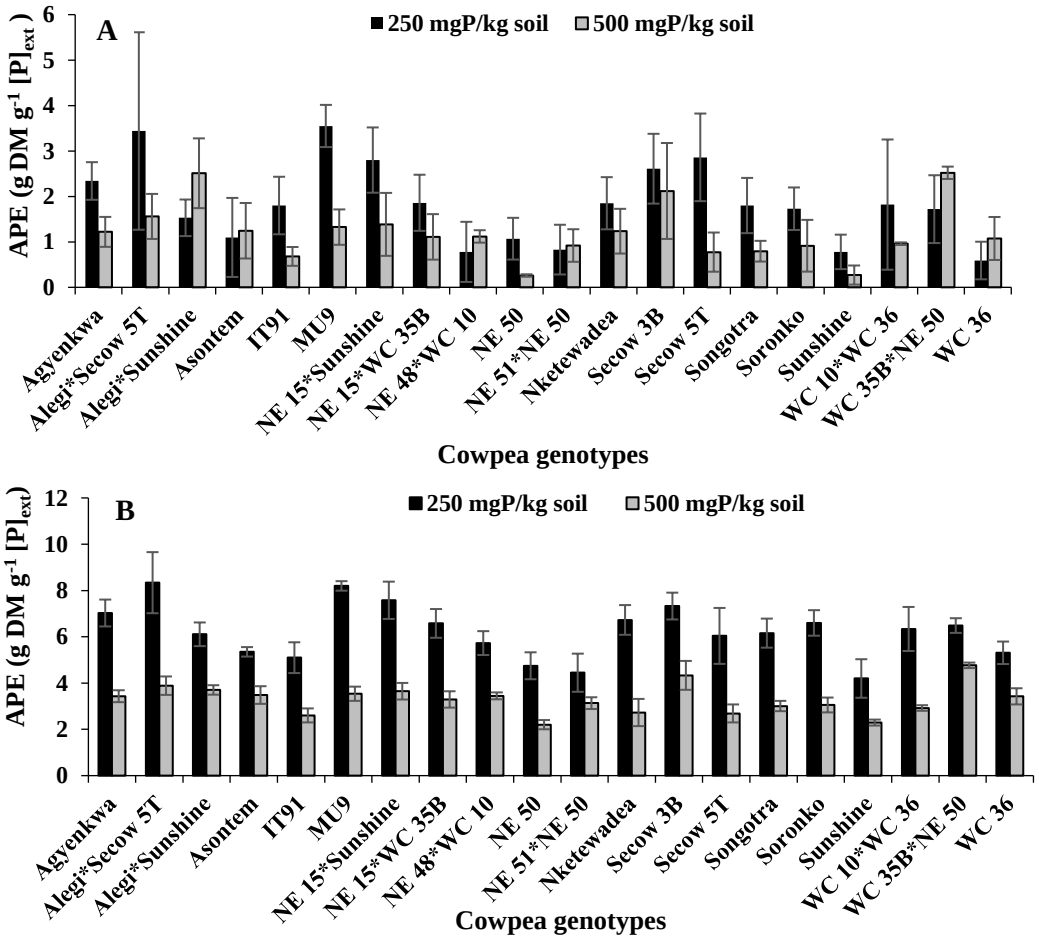


Figure 35: Effect of $[P]_{ext}$ on agronomic phosphorus use efficiency among 3 weeks old cowpea genotypes grown under greenhouse conditions for **(A)** First trial; **(B)** Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Phosphorus utilization efficiency

The twenty (20) genotypes screened under varying $[P]_{EXT}$ exhibited significant ($P < 0.001$) variations in their PUtE in the first trial (Figure 36A). Phosphorus utilization efficiency ranged from 0.11 - 0.37 g DM g^{-1} P at 0 mg P/kg soil, 0.066 - 0. 1179 g DM g^{-1} P at 250 mg P/kg soil and 0.058 - 0.088 g DM g^{-1} P at 500 mg P/kg soil (Figure 36A). Phosphorus application significantly ($P < 0.001$) affected PUtE by genotypes (Figure 36A), with highest PUtE

obtained at unamended soil treatment. Thus, PUtE efficiency decreased with increasing rates of phosphorus. Additionally, the interaction between $[P]_{ext}$ and genotypes significantly affected PUtE in the first trial (Figure 36A).

Similarly, in the second trial genotype, $[P]_{ext}$ and their interactions significantly ($P < 0.001$) affected PUtE among screened genotypes (Figure 36B). Phosphorus utilization efficiency of cowpea genotypes screened at 0 mg P/kg soil ranged from 0.11 - 0.41 g DM g^{-1} P, 250 mg P/kg soil ranged from 0.066 - 0.178 g DM g^{-1} P with genotype Alegi*Sunshine and NE 51*NE 50 obtaining the lowest and highest weights respectively. At 500 mg P/kg soil, PUtE ranged from 0.058 - 0.088 g DM g^{-1} P for genotypes WC 10*WC 36 and Asontem respectively (Figure 36B). Phosphorus application had a significant effect ($P < 0.001$) on PUtE of cowpea genotypes (Figure 36B). The twenty genotypes screened exhibited a decrease in PUtE with increased phosphorus rates.

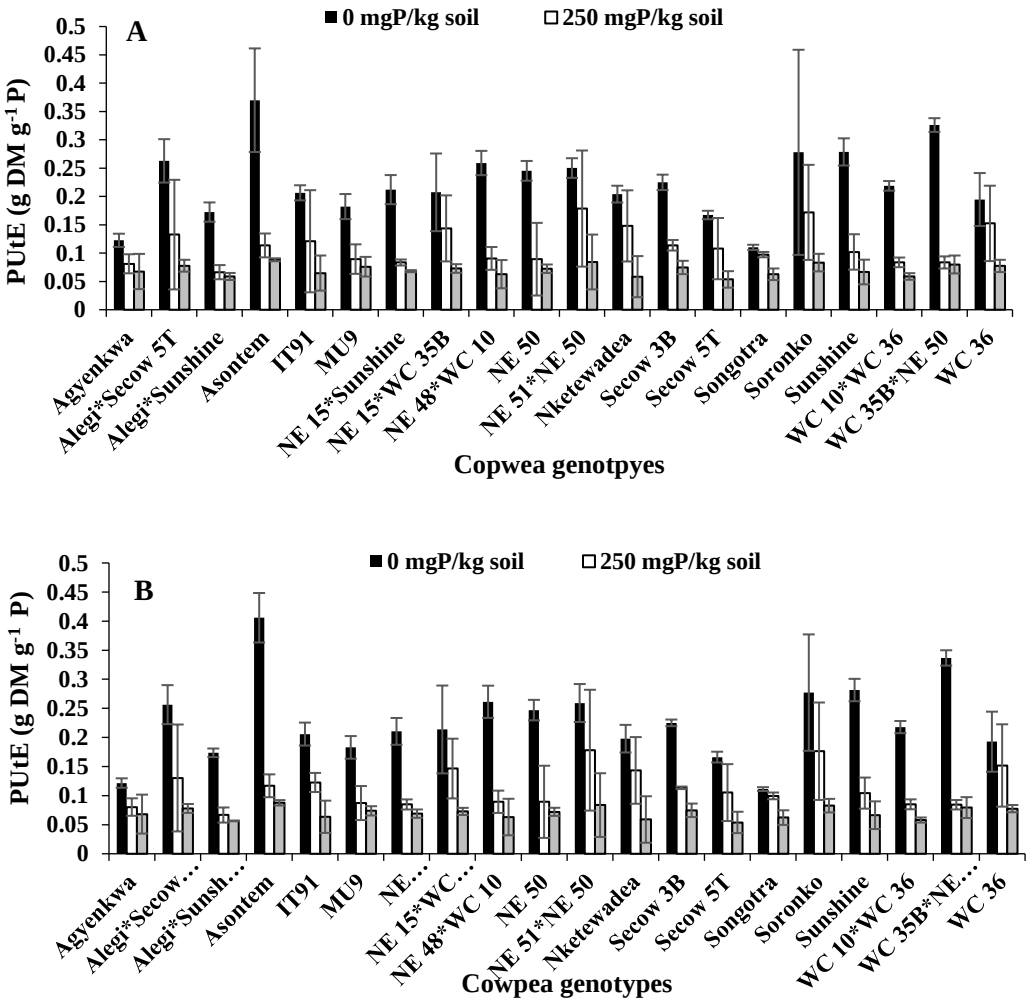


Figure 36: Effect of $[P]_{ext}$ on phosphorus utilization efficiency among 3 weeks old cowpea genotypes grown under greenhouse conditions for **(A)** First trial; **(B)** Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Phosphorus efficiency ratio

The twenty (20) genotypes screened under varying $[P]_{ext}$ exhibited significant ($P < 0.001$) variations in their PER in the first and second trial (Figure 37A and 39B). Phosphorus efficiency ratio ranged from 0.11 - 0.37 g DM g^{-1} P at 0 mg P/kg soil, 0.066 - 0. 1179 g DM g^{-1} P at 250 mg P/kg soil and 0.058 - 0.088 g DM g^{-1} P at 500 mg P/kg soil (Figure 37A). Phosphorus application significantly ($P < 0.001$) affected PER by genotypes (Figure 37A),

with the highest PER obtained at unamended soil treatment. Thus, PER ratio decreased with increasing rates of phosphorus. Additionally, the interaction between $[P]_{EXT}$ and genotypes significantly affected PER in the first trial. However, a decreasing trend in PER was observed among screened cowpea genotypes (Figure 37A).

Similarly, in the second trial genotype, $[P]_{ext}$ and their interactions significantly ($P < 0.001$) affected PER among screened genotypes (Figure 37B). Phosphorus efficiency ratio of cowpea genotypes screened at 0 mg P/kg soil ranged from 0.11 - 0.41 g DM g^{-1} P, 250 mg P/kg soil ranged from 0.066 - 0.178 g DM g^{-1} P with genotype Alegi*Sunshine and NE 51*NE 50 obtaining the lowest and highest weights respectively. At 500 mg P/kg soil, PER ranged from 0.058 - 0.088 g DM g^{-1} P for genotypes WC 10*WC 36 and Asontem respectively (Figure 37B). Phosphorus application had a significant effect ($P < 0.001$) on PER of cowpea genotypes (Figure 37B). The twenty genotypes screened exhibited a decrease in PER concerning increased phosphorus rates.

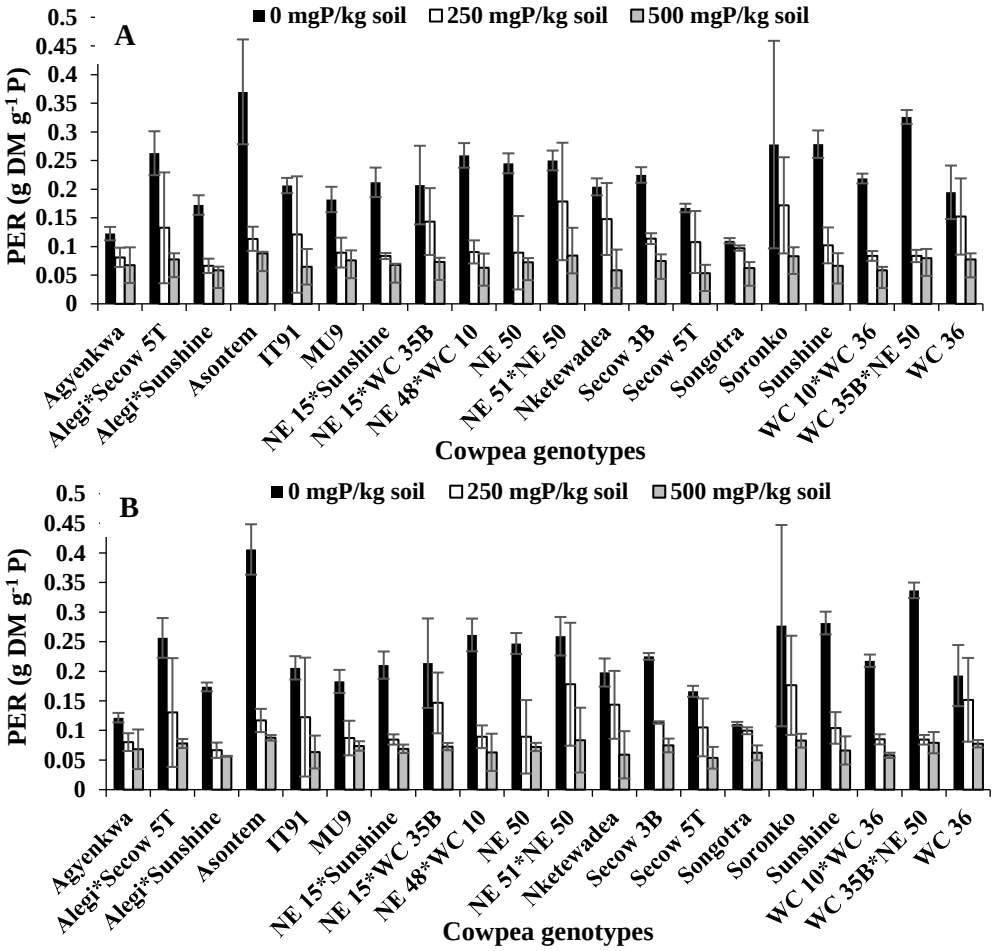


Figure 37: Effect of $[P]_{ext}$ on phosphorus efficiency ratio among 3 weeks old cowpea genotypes grown under greenhouse conditions for **(A)** First trial; **(B)** Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Physiological phosphorus use efficiency

In the first trial (Figure 38A), PPUE obtained by genotypes was significantly ($P < 0.001$) affected. At 0 mg P/kg soil, PPUE ranged from 0.11 - 0.36 g^2 DM g^{-1} P with genotype Asontem obtaining the highest PPUE and genotype WC 35B*NE 50 obtaining the lowest value (Figure 38A). At treatment 250 mg P/kg soil, PPUE ranged from 0.064 - 0.27 g^2 DM g^{-1} P with genotype Soronko having the highest and WC 35B*NE 50 obtaining the lowest PPUE. However, at 500 mg P/kg soil genotype Secow 5T (0.067 g^2 DM g^{-1} P)

had the lowest PPUE (Figure 38A). Phosphorus application significantly ($P < 0.001$) affected PPUE among cowpea genotype. In all, PPUE was greater at unamended soil compared to 250 mg P/kg soil than 500 mg P/kg soil (Figure 38A). The interaction of genotype and phosphorus had a significant effect ($P < 0.001$) on PPUE. Genotypes Alegi*Sunshine, Asontem, IT91, NE 15*Sunshine, NE 48*WC 10, NE 50, NE 52*NE 50, Secow 3B, Secow 5T, Soronko, Sunshine, WC 10*WC 36 and WC 36 had the highest PPUE on unamended soil treatment compared to amended soils (Figure 38A).

In the second trial (Figure 38B), genotype, phosphorus and their interaction caused a significant ($P < 0.001$) in PPUE. Cowpea genotypes responded differently to $[P]_{EXT}$ with genotypes Agyenkwa, Alegi*Secow 5T, IT91, MU9, N 51*NE 50, Nketewadea, Secow 3B, Secow 5T, Songotra, Soronko and WC 36 obtaining significantly high PPUE at 250 mg P/kg soil. However, genotypes Asontem, NE 15*Sunshine, NE 48*WC 10, NE 50, Sunshine and WC 10*C 36 had highest PPUE at unamended soil treatment (Figure 38B).

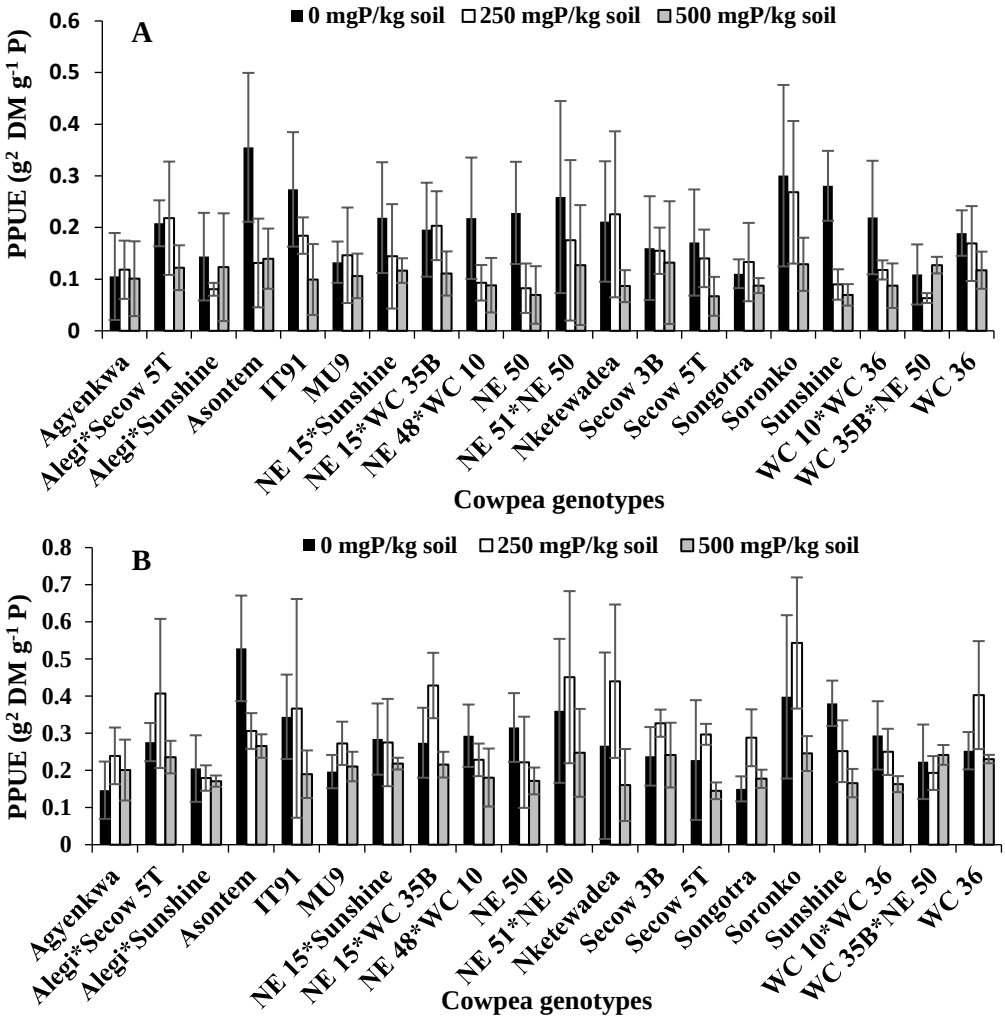


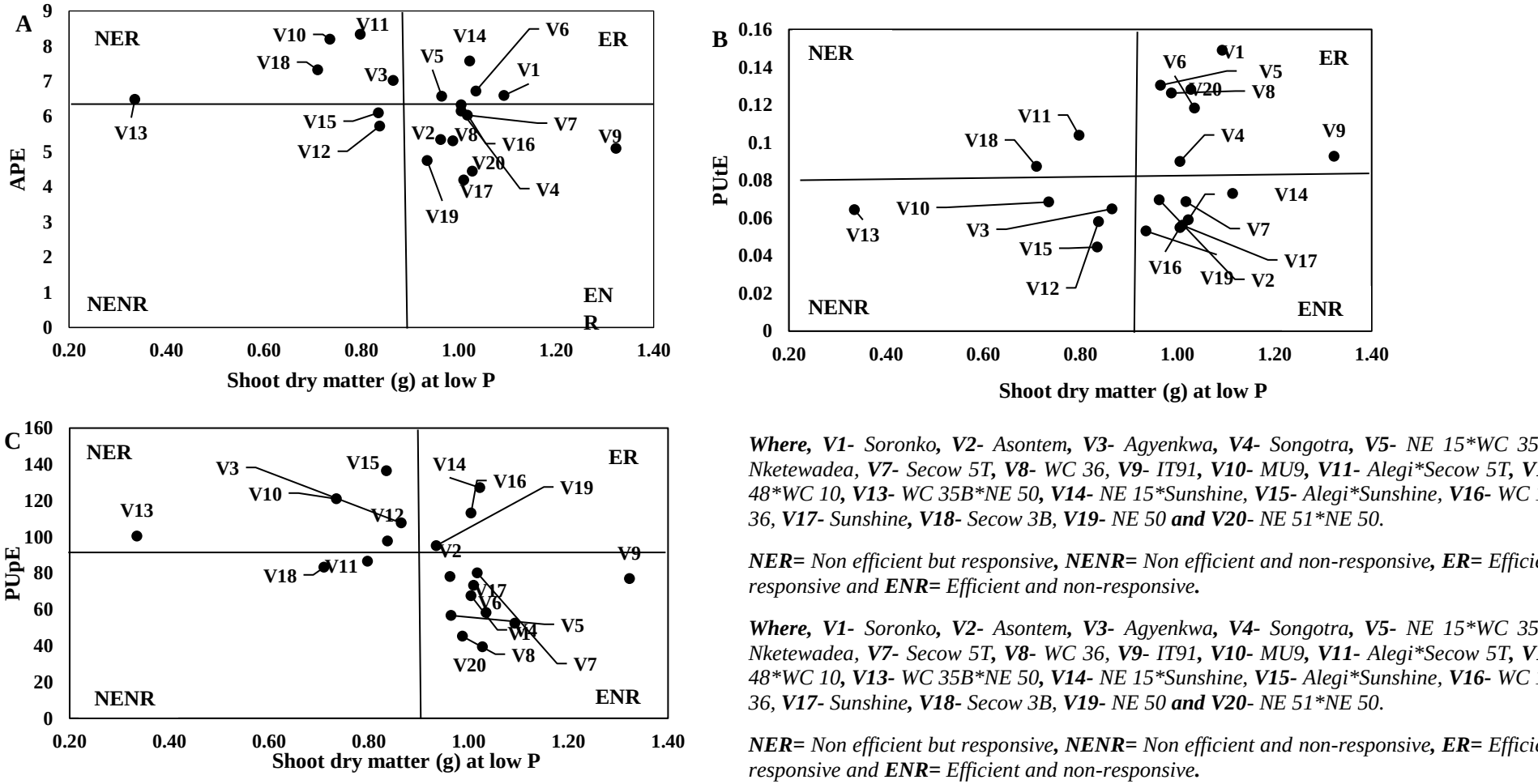
Figure 38: Effect of $[P]_{ext}$ on physiological phosphorus use efficiency among 3 weeks old cowpea genotypes grown under greenhouse conditions for **(A)** First trial**; (B)** Second trial. Data represent mean of four (4) replicates, with error bars representing the s.e.m. Differences between cowpea genotypes was established using ANOVA.

Relationship between dry matter and responsiveness to $[P]_{ext}$

Cowpea genotypes were divided into four (4) groups based on their responsiveness to $[P]_{ext,}$ measured as APE, PUtE, or PUpE, and their dry matter produced at low $[P]_{ext}$ (Figure 39). For responsiveness to $[P]_{ext}$ as measured as APE, four (4) genotypes including MU9, Alegi*Secow 5T, Agyenkwa and Secow 3B were within the group of NER whilst genotypes Asontem, Secow 5T, NE 50, Songotra IT91 and WC 36 were within the ENR group (Figure 39A).

Genotypes Secow 3B and Alegi*Secow 5T were in the NER group in their responsiveness to $[P]_{ext}$ measured in terms of PU_tE but genotypes NE 48*WC 10, MU9, Agyenkwa, WC 35B*NE 50 and Alegi*Sunshine were in the group NENR (Figure 39B). Genotype Asontem, WC 10*WC 36, Secow 5T and NE 15*Sunshine were within the efficient and responsive group (Figure 39B).

Among the twenty (20) cowpea genotypes cultivated under varying $[P]_{ext,}$ five (5) genotypes including Agyenkwa, Alegi*Sunshine, WC 35B*NE 50, MU9 and NE 48*WC 10 were within the NER group when their responsiveness to $[P]_{ext}$ was measured in terms of PU_PE (Figure 39C). However, two (2) genotypes including Secow 3B and Alegi*Secow 5T were within the NENR quadrant. Genotypes such as WC 36, IT91, Secow 5T, NE 51*NE 50, IT91 and Sunshine were within the efficient but non-responsive group. Genotypes Asontem, NE 15*Sunshine, WC 10*WC 36 and NE 50 were within the efficient and responsive quadrant (Figure 39C).



Where, V1- *Soronko,* ***V2-*** *Asontem,* ***V3-*** *Agyenkwa,* ***V4-*** *Songotra,* ***V5-*** *NE 15*WC 35B,* ***V6-*** *Nketewadea,* ***V7-*** *Secow 5T,* ***V8-*** *WC 36,* ***V9-*** *IT91,* ***V10-*** *MU9,* ***V11-*** *Alegi*Secow 5T,* ***V12-*** *NE 48*WC 10,* ***V13-*** *WC 35B*NE 50,* ***V14-*** *NE 15*Sunshine,* ***V15-*** *Alegi*Sunshine,* ***V16-*** *WC 10*WC 36,* ***V17-*** *Sunshine,* ***V18-*** *Secow 3B,* ***V19-*** *NE 50* ***and V20-*** *NE 51*NE 50.*

NER= *Non efficient but responsive,* ***NENR=*** *Non efficient and non-responsive,* ***ER=*** *Efficient and responsive and* ***ENR=*** *Efficient and non-responsive.*

Where, V1- *Soronko,* ***V2-*** *Asontem,* ***V3-*** *Agyenkwa,* ***V4-*** *Songotra,* ***V5-*** *NE 15*WC 35B,* ***V6-*** *Nketewadea,* ***V7-*** *Secow 5T,* ***V8-*** *WC 36,* ***V9-*** *IT91,* ***V10-*** *MU9,* ***V11-*** *Alegi*Secow 5T,* ***V12-*** *NE 48*WC 10,* ***V13-*** *WC 35B*NE 50,* ***V14-*** *NE 15*Sunshine,* ***V15-*** *Alegi*Sunshine,* ***V16-*** *WC 10*WC 36,* ***V17-*** *Sunshine,* ***V18-*** *Secow 3B,* ***V19-*** *NE 50* ***and V20-*** *NE 51*NE 50.*

NER= *Non efficient but responsive,* ***NENR=*** *Non efficient and non-responsive,* ***ER=*** *Efficient and responsive and* ***ENR=*** *Efficient and non-responsive.*

Figure 39: Relationship between shoot dry matter (DM) and responsiveness to $[P]_{ext}$ measured as agronomic P use efficiency (APE) **(A)**, P utilization efficiency (PUtE) **(B)**, and P uptake efficiency (PUpE). Lines for dividing square into quadrants represent the mean value for the axis. Points represent the total number of cowpea genotypes in each quadrant

Relationship between measured traits

Correlational analysis between measured plant traits (biomass, rhizosheath formation, root hair, RSA, P concentration, content, and P efficiency) is shown in (Table 11). Root fresh weight had a strong significant positive correlation with SFW ($r = 0.691$, $P < 0.01$), SDW ($r = 0.667$, $P < 0.01$), but had a weak positive correlation with RDW ($r = 0.339$, $P < 0.01$). Root fresh weight had a significantly strong positive relationship with SDW ($r = 0.770$, $P = 0.01$) (Table 11).

Total root length had a significantly ($P < 0.01$) positive relationship with SFW ($r = 0.344$), RFW ($r = 0.360$), SDW ($r = 0.242$) and RDW ($r = 0.146$). Total root length had a negative correlation with RHL ($r = -055$) but weaker positive correlation with RHD ($r = 0.045$ $P < 0.05$) (Table 11). Total root length positively correlated with shoot P content ($r = 0.233$, $P < 0.01$), root phosphorus P concentration ($r = 0.233$, $P < 0.01$), shoot P content ($r = 0.274$, $P < 0.01$) and root P content ($r = 0.221$, $P < 0.01$). Total root length had a significant negative relation with phosphorus efficiency ratio ($r = -0.234$, $P < 0.001$). Phosphorus utilization efficiency ($r = 0.56$), APE ($r = 0.029$) and PUpE ($r = 0.078$) had a weaker positive but insignificant relationship with TRL (Table 11).

Root hair density had a significantly weak positive correlation with biomass parameters including SFW ($r = 0.045$), RFW ($r = 0.021$), SDW ($r = 0.08$) and RDW ($r = 0.91$, $p < 0.05$). Root hair length has a weak but significant positive association with PPUE ($r = 0.277$, $p < 0.01$), PER ($r = 0.206$, $p < 0.01$), APE ($r = 0.235$, $p < 0.01$) and PUpE ($r = 0.245$, $p < 0.01$). However, RHL negatively correlated with PUtE ($r = 0.517$, $p < 0.01$) (Table 11).

Root phosphorus concentration had positive correlation with shoot phosphorus concentration ($r = 0.984, P < 0.01$), but had a negative insignificant correlation with ARW ($r = -0.062$) and RRW ($r = -0.066$) (Table 11). Shoot P concentration had a significantly strong positive correlation with SFW ($r = 0.517, P < 0.01$) but a weak positive correlation with root fresh weight ($r = 0.380, P < 0.01$), SDW ($r = 0.460, p < 0.01$) and RDW ($r = 0.165, P < 0.01$). Shoot P concentration negatively correlated with RHL ($r = -0.163$) but weakly correlated with RHD ($r = 0.063$) (Table 11).

Absolute rhizosheath weight had a weak positive but significant correlation ($r = 0.094, P = 0.05$) with RHL but negatively correlated with RHD ($r = 0.035$). Relative rhizosheath weight strongly correlated with RHL ($r = 0.652, P < 0.01$), weakly correlated with ARW ($r = 0.182, P < 0.01$) but negatively correlated with RHD. Specific rhizosheath weight had a significantly positive correlation with RHL ($r = 0.455, P < 0.01$) and a weaker positive correlation with RHD ($r = 0.004$) (Table 11).

Physiological phosphorus use efficiency had a significantly weak but positive correlation with SFW ($r = 0.120, P < 0.01$), RFW ($r = 0.308, P < 0.01$), SDW ($r = 379, P < 0.01$) and RDW ($r = 0.398, P < 0.01$). However, had a significantly strong negative correlation with shoot P concentration ($r = -0.530, P < 0.01$) and root P concentration ($r = -0.524, P < 0.01$) (Table 11). Phosphorus efficiency ratio negatively correlated with SFW ($r = -0.521, P = 0.01$), RFW ($r = -0.378, P < 0.01$), SDW ($r = -0.495, P < 0.01$) and RDW ($r = -0.150, P < 0.01$) (Table 11). Agronomic use efficiency on the had hand had a strong positive relationship with RFW ($r = 0.560, P < 0.01$), SDW ($r = 796, P$

< 0.01) and RDW ($r = 0.513$, $P < 0.01$) but a weak positive correlation with SFW ($r = 296$, $P < 0.01$) (Table 11).

Total leaf area had a strong significantly positive correlation with shoot P concentration ($r = 0.632$, $P < 0.01$), root P concentration ($r = 0.625$, $P < 0.01$) and shoot P content ($r = 0.650$, $P < 0.01$) but a weak positive correlation with root P content ($r = 0.459$, $P < 0.01$) (Table 42). Specific leaf area had a positive significant correlation with shoot P concentration ($r = 0.293$, $P < 0.01$) and root P concentration ($r = 0.310$, $P < 0.01$) but a weak positive correlation with root P content ($r = 0.041$) and shoot P content ($r = 0.036$) (Table 11).

Table 11: Correlations between traits observed in plants grown in soil-filled nursery polybags.

SFW	**SFW**																		
RFW	.691**	**RFW**																	
SDW	.667**	.770**	**SDW**																
RDW	.339**	.569**	.554**	**RDW**															
TRL	.344**	.360**	.242**	.146**	**TRL**														
RHL	-0.055	0.087	.095*	.284**	-0.033	**RHL**													
RHD	0.045*	0.021	0.08	.091*	-0.014	-0.076	**RHD**												
ARW	0.07	-0.008	-.170**	-0.063	.157**	.094*	-0.035	**ARW**											
RRW	-0.061	-.166**	-.221**	-.188**	-0.015	.452**	-0.086	.182**	**RRW**										
SP conc.	.517**	.380**	.460**	.165**	.233**	-.163**	0.063	-0.062	-0.063	**SP conc.**									
RP conc.	.509**	.367**	.449**	.156**	.233**	-.157**	0.06	-0.062	-0.066	.984**	**RP conc.**								
SP cont.	.638**	.676**	.860**	.453**	.274**	0.02	0.083	-.155**	-.178**	.797**	.781**	**SP cont.**							
RP cont.	.455**	.551**	.577**	.829**	.221**	.094*	0.074	-0.084	-.171**	.605**	.604**	.710**	**RP cont.**						
PPUE	.120**	.308**	.379**	.398**	-0.029	.277**	0.038	-0.036	-.108*	-.530**	-.524**	-0.07	-0.055	**PPUE**					
PER	-.521**	-.378**	-.495**	-.150**	-.234**	.206**	-0.054	0.076	0.08	-.893**	-.874**	-.716**	-.532**	.525**	**PER**				
PUtE	0.08	0.024	0.085	0.033	0.056	-.113*	-0.036	0.04	-0.098	-0.024	-0.025	0.025	0.009	0.102	-0.008	**PUtE**			
APE	.296**	.560**	.796**	.513**	0.029	.235**	-0.07	-.225**	-.225**	-.232**	-.230**	.443**	.281**	.720**	.176**	0.077	**APE**		
PUpE	.268**	.554**	.763**	.538**	0.078	.245**	-0.09	-.265**	-.227**	.272**	.269**	.766**	.528**	.296**	-.328**	0.03	.784**	**PUpE**	
SRW	0.08	-0.051	-.170**	-.179**	.212**	.455**	0.004	.694**	-0.062	0.05	0.048	-.115*	-.094*	-.183**	-0.067	0.089	-.269**	-.298**	**SRW**
TLA	.699**	.538**	.563**	.241**	.282**	-.057	.053	-.004	-.033	.632**	.625**	.650**	.459**	-.100*	-.598**	.009	.070	.169**	.064
SLA	-.021	-.098*	-.164**	-.206**	-.042	-.165**	-.012	.023	.063	.293**	.310**	.036	.041	-.224**	-.411**	-.061	-.386**	-.237**	.124**
LMA	-.041	-.020	.015	-.008	.046	.018	-.072	.001	.002	.014	.011	.019	-.012	.006	.020	.001	-.014	.012	-.021

SFW																			
RFW																			
SDW																			
RDW																			
TRL																			
RHL																			
RHD																			
ARW																			
RRW																			
SP conc.																			
RP conc.																			
SP cont.																			
RP cont.																			
PPUE																			
PER																			
PUtE																			
APE																			
PUpE																			
SRW																			
TLA	**LA**																		
SLA	.366**	**SLA**																	
LMA	-.014	-.301**	**SLM**																

Source: Field research, Opoku (2020) **** Correlation is significant at the 0.01 level * Correlation is significant at the 0.05 level**

Traits in matrix are SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight, TRL: total root length, RHL: root hair length, RHD: root hair density, ARW: absolute rhizosheath weight, RRW: relative rhizosheath weight, SP conc: shoot phosphorus concentration, RP conc: root phosphorus concentration, SP cont: shoot phosphorus content, RP cont: root phosphorus content, PPUE: physiological phosphorus use efficiency, PER: phosphorus efficiency ratio, PUtE: phosphorus utilization efficiency, APE: agronomic phosphorus use efficiency, PUpE: phosphorus uptake efficiency, SRW: specific rhizosheath weight, TLA: total leaf area, SLA: specific leaf area and LMA: leaf mass per area.

Variance component and broad-sense heritability estimate

The effects of genotype, $[P]_{ext}$ and the interaction between genotype x trial x $[P]_{EXT}$ accounted for most of the experimental variation (Table 12). The effect of genotype ranged from 0.01% for shoot phosphorus concentration to 53.6% for root dry weight. Genotype accounted for less than 10% variation in root P concentration (4.11%), SFW (0%), shoot P concentration (0.01%), PUtE (4.83%), PPUE (4.83%), PUpE (6.48%), PER (4.51%), shoot P content (2.12%) and root P content (7.15%) (Table 12). However, genotype accounted for greater than 20% variation in ARW (24.91%), RRW (23.61%), RDW (53.61%), SDW (42.44%), RFW (20.70%), RHL (32.34%), TRL (52.18%) and SRW (21.23%) (Table 12). Genotype accounted for 18% variation in leaf area, 8% SLA and 0.02% LMA.

Phosphorus application accounted greater than 50% variation in shoot P concentration (99.95%), root P concentration (78.75%), shoot P content (51.59%), PUtE (73.33%), PPUE (73.33%), phosphorus efficiency ratio (89.43%) and root P content (66.24%). It accounted for less than 30% variation in RDW, SDW, RFW, SFW, RHD, RHL, TRL, APE, SRW and PUpE (Table 12). Phosphorus application contributed to 58% of variation observed in leaf area and 13% variation in SLA.

The effect of interaction between genotype and $[P]_{ext}$ ranged from 0.00% for SFW to 41.62% for RHD (Table 12). The interaction accounted for more than 20% variation in RRW (20.37%) and RHD (41.62%) and less than 20% variation in the remaining genotypes (Table 9). Interaction effect of genotype and phosphorus levels accounted for 15% variation observed in TLA, 42% variation in SLA and 36% variation in LMA (Table 12)

Broad-sense heritability ranged from 0.01 for SLA to 0.99 TRL (Table 9). Except for SDW (0.40) and SRW (0.13), broad-sense heritability was larger than 0.50 for the remaining traits (Table 12).

Table 12: Estimates of variance components and broad-sense heritability (H^2) of cowpea genotypes screened under greenhouse condition on varying $[P]_{ext}$

Traits	Genotype	Phosphorus	Trial	Genotype × Trial	Genotype × phosphorus	Phosphorus × Trial	Genotype ×Phosphorus × Trial	Error	H^2
SRW	24.91	0.00	3.99	0.21	2.92	9.05	12.25	46.67	0.82
RRW	23.61	14.21	6.16	0.19	20.37	1.44	13.84	20.19	0.87
RDW	53.61	4.33	0.11	0.39	3.52	0.00	0.00	0.41	0.81
SDW	42.44	11.79	18.73	2.95	9.57	11.20	0.00	38.04	0.40
RFW	20.70	18.65	19.09	0.00	19.36	11.52	0.00	3.32	0.97
SFW	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.94
RHD	19.58	16.58	3.12	0.38	41.62	0.64	0.00	10.67	0.80
RHL	32.34	27.50	12.59	0.00	5.72	0.30	0.00	0.00	0.96
TRL	52.18	27.69	5.33	0.00	7.40	0.00	0.00	18.09	0.99
SPconc	0.01	99.95	0.00	0.00	0.02	0.00	0.00	21.54	0.96
RPconc	4.11	78.75	0.00	0.04	10.01	0.00	0.00	7.40	0.88
RPUE	11.04	0.67	34.28	2.98	2.34	0.64	0.00	0.01	0.36
PUtE	4.83	73.33	0.00	0.02	18.01	0.11	0.00	7.08	0.95
PPUE	4.83	73.33	0.00	0.02	18.01	0.11	0.00	48.04	0.95
APE	17.63	10.31	50.52	0.18	3.78	14.59	0.00	3.70	0.91
PUpE	6.48	4.74	67.73	0.34	11.52	4.19	0.00	3.70	0.82
PER	4.51	89.43	0.00	0.02	0.02	0.14	0.00	2.99	0.92
SPcontent	2.12	51.59	19.38	0.35	3.61	17.45	0.00	4.99	0.75
RPcontent	7.65	66.24	3.40	0.00	5.39	9.04	0.62	5.89	0.94
SRW	21.23	20.75	0.00	13.74	8.00	7.98	8.40	5.50	0.13
TLA	18.24	58.00	1.15	0.15	15.85	0.01	0.00	6.59	0.91
SLA	8.10	12.77	31.08	1.19	41.65	0.05	0.00	5.16	0.61
LMA	0.02	0.00	0.00	0.04	35.96	0.25	0.00	63.73	0.01

Source: Field research, Opoku (2020). Where SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight, TRL: total root length, RHL: root hair length, RHD: root hair density, ARW: absolute rhizosheath weight, RRW: relative rhizosheath weight, SP conc: shoot phosphorus concentration, RP conc: root phosphorus concentration, SP cont: shoot phosphorus content, RP cont: root phosphorus content, PPUE: physiological phosphorus use efficiency, PER: phosphorus efficiency ratio, PUtE: phosphorus utilization efficiency, APE: agronomic phosphorus use efficiency, PUpE: phosphorus uptake efficiency, SRW: specific rhizosheath weight, LA: leaf area, SLA: specific leaf

Principal component analysis (PCA)

Varimax with Kaiser Normalization principal component analysis (PCA) are shown in Table 13. A Kaiser-Meyer-Olkin measure of sampling adequacy of 0.639 was obtained for measured traits. In all, seven (7) distinct principal components were obtained based on components with Eigenvalues > 1 and factor loadings of ±0.3 explaining 78 % of the total variance (Table 13).

The first principal component contributed to 28% of the total variation observed. This was mainly explained by SDW, shoot P content, PUpE, RFW, RDW, APE, root P content and SFW. Principal component two (2) on the other hand accounted for 19% of the total variation observed among measured traits. The variation was explained by shoot P concentration, root P concentration, PER, PPUE and SLA (Table 13). Specific rhizosheath weight, ARW and TRL account for 9% of variation explained by the third principal component. Relative rhizosheath weight and RHL explained 7% of variation contributed by the fourth principal component (PC 4). The fifth principal component explained 6% of the variation observed among measured traits. Principal component five (5) was explained by LMA and TLA. The sixth principal component is explained PUtE and accounts for 6% of the observed total variation (Table 13). The seventh principal component is explained RHD and accounts for 5% of the observed total variation.

Table 13: Rotated component matrix of seven factor model explaining 78% of the total variance for traits

	Components							
Measurements	**PC 1**	**PC 2**	**PC 3**	**PC 4**	**PC 5**	**PC 6**	**PC 7**	**Communalities**
Shoot dry weight	**.901**	-.260	.021	-.025	.042	-.119	-.013	.896
Shoot P content	**.857**	.359	.004	-.017	-.042	-.084	.023	.873
P. uptake efficiency	**.845**	.047	-.082	-.132	-.200	-.024	-.176	.813
Root fresh weight	**.762**	-.193	.255	.034	.109	-.011	-.014	.696
Root dry weight	**.740**	-.116	.012	-.083	-.110	.515	.069	.849
Agronomic P. use efficiency	**.733**	-.470	-.037	-.116	-.067	-.054	-.175	.810
Root P. content	**.691**	.300	.018	-.078	-.148	.503	.074	.855
Shoot fresh weight	**.527**	.023	.460	.196	.375	-.237	.041	.726
Shoot P. concentration	.262	**.938**	-.032	-.002	-.078	-.037	.004	.956
Root P. concentration	.260	**.937**	-.032	-.009	-.078	-.030	.006	.954
Phosphorus efficiency ratio	-.258	**-.908**	.024	.024	.131	.069	.066	.917
Physiological P use efficiency	.470	**-.811**	.021	-.017	.135	-.019	.007	.899
Specific leaf area	-.300	**.500**	-.079	-.163	.494	.161	-.230	.696
Specific rhizosheath weight	-.426	.048	**.753**	-.177	-.163	.169	-.055	.841
Absolute rhizosheath weight	-.320	-.006	**.690**	.251	-.209	.320	-.072	.793
Total root length	.176	.060	**.586**	.124	-.032	-.028	.173	.426
Relative rhizosheath weight	-.230	.091	-.069	**.808**	.087	.105	-.121	.752
Root hair length	.409	-.086	-.255	**.736**	-.009	.208	-.048	.827
Leaf mass per area	.031	.006	.029	.194	**-.564**	-.453	.463	.777
Total leaf area	.384	.316	.307	.156	**.519**	-.304	.074	.733
Root hair density	-.012	.024	-.043	-.241	.357	.252	**.659**	.685
Phosphorus utilization efficiency	.055	-.061	.238	-.244	-.106	**-.406**	-.205	.341
Eigen values	**5.880**	**4.176**	**1.905**	**1.596**	**1.331**	**1.222**	**1.005**	
Per centage of total variance	**26.728**	**18.980**	**8.661**	**7.253**	**6.048**	**5.557**	**4.570**	
Cumulative per centage of variance	**26.728**	**45.708**	**54.368**	**61.621**	**67.669**	**73.226**	**77.796**	

Source: Field research, Opoku (2020)

CHAPTER FIVE

DISCUSSION

Establishing phosphorus response curve

The fresh and dry weight of cowpea biomass increased consistently with increased $[P]_{ext}$ rate to 500 kg P/ha beyond which a decline in biomass was observed at the greatest P rate. Above this, an increase in external $[P]_{ext}$ rate lead to an only marginal increase in the biomass production of cowpea. The marginal increase in biomass at high $[P]_{ext}$ could be attributed to the excess concentration of P in the rhizosphere which tends to become toxic to plants hence impede the efficient absorption and utilization of P for productivity. It was therefore clear that incubating of experimental soil beyond this level, the cost of additional P source to produce extra biomass would likely be greater than the value of additional biomass. Hence, this level of P was selected as high treatment. Additionally, it was clear that experimental soil was responsive to phosphorus application. Thus, experimental soil enhanced the bioavailability of applied phosphorus for plant uptake and utilization. Variation in both biomass weight and tissue phosphorus concentration was mostly due to variation in $[P]_{ext}$ rate used for soil amendment.

Screening for genetic variation in rhizosheath, root hair traits and root system architecture

Biomass parameters

The varietal difference was highly significant for measured biomass parameters during the study in both trials. The significant variations observed in the biomass traits during the first and second trials for all the genotypes could be attributed to inherent genetic variation of the various genotypes as individual genotypes have different genetic makeup. The results indicated that cowpea genotypes have the varying potential for dry matter production hence cowpea production. Although this could be influenced by other prevailing environmental factors such as soil fertility, moisture content, however, dry matter production is predominantly depending on the genetic makeup of cowpea genotypes. Gerrano *et al.* (2015) attributed variation in dry matter production among cowpea genotypes to the genetic makeup of genotypes. Results of the present study confirm the conclusion by Addo-Quaye *et al*. (2011) that cowpea varieties have different capacities for dry matter production.

Rhizosheath, root system architecture and root hair parameters

Cowpea genotypes screened during the study produced a substantial quantity of rhizosheath. However, the amount of rhizosheath produced varied significantly among cowpea genotypes in both trials. This indicates that a considerable amount of genotypic variation exists between cowpea genotypes in the production of rhizosheath (ARW, SRW and RRW). Variation in the quantity of rhizosheath produced between the first and second trials can be attributed to variation in prevailing conditions during the experiment as well as

other factors other than root hair parameters. The production of rhizosheath among cowpea genotypes can be explained by the presence of root hair among genotypes evaluated during the study. Variation in rhizosheath weight among cowpea genotypes during the study can be accounted for by the genetic variation in root hair length among cowpea genotypes screened during the study. Krasilnikoff *et al*. (2003) also reported similar findings among cowpea genotypes during a study on variation in phosphorus use efficiency. Rhizosheath formation is influenced by several factors including root system traits such as root hair length, density and morphology (Haling *et al*., 2010). Variation in plant genetic resources is a major tool for plant breeders to develop new and improved crops with desirable characteristics (Govindaraj *et al*., 2015). The presence of rhizosheath in the fine root of legumes and eudicotyledonous crops have been reported by McCully (1999). Cowpea genotypes with a superior disposition for rhizosheath production serve as a promising tool for future selection and breeding purpose. Rhizosheath enhances hard soil tolerance, water deficiency and Al-induced tolerance of acidity (Brown *et al*., 2012; Delhaize *et al*., 2012; Haling *et al*., 2014).

The significant effect of genotype on total root length among cowpea genotypes suggested that greater genetic variation exists within cowpea genotypes for total root length. The results are in line with Krasilnikoff *et al*. (2003), who concluded that genetic variation existed among cowpea genotypes in terms of root hair length and density. The genetic variation observed thus permits future exploration for breeding superior root traits into modern elite cowpea genotypes because, longer root length is particularly crucial for enhancing P uptake in marginal soils (Brown *et al*., 2012; Gahoonia & Nielsen,

1997) and acquisition of soil resources distributed deeper in the soil such as water and nitrogen (Adu *et al*., 2017). In cowpea, important genetic diversity has been noted for RSA characteristics connected with development in low-nutrient and dry conditions (Krasilnikoff *et al*., 2003; Matsui & Singh, 2003; Singh & Matsui, 2002).

Cowpea genetic materials used for the study produced root hairs and there was significant variation between the lengths of root hairs among cowpea genotypes. Some genotypes produced longer root hair which is advantageous in the acquisition of soil resources such as nutrients and water (Ma *et al*., 2001; Zygalakis *et al*., 2011). Also, longer root hair length has been linked to greater rhizosheath weight (Haling *et al*., 2010). Root hair length is significant in maintaining rhizosheath mass (Haling *et al*., 2014). Contrastingly, Adu *et al* (2017), reported a weak correlation between root hair length and rhizosheath among maize genetic materials. This may be attributed to genotypic variation between genotypes used for the study or maybe due to other factors such as moisture and mucilage which apart from the root hair plays a central role in the formation of rhizosheath (Adu *et al*., 2017). The associated importance of this trait and the presence of genetic diversity among cowpea genotypes for root hair highlighted the need for the exploring genetic basis of the trait and breeding into future cowpea breeding. Exploiting genetic diversity in root hair and root architecture could be a promising instrument to improve plant PAE and PUE (Beebe *et al*., 2006; Hammond *et al*., 2009).

The significant genotypic variation between root hair density among cowpea genotypes indicated that genetic variation existed among genotypes for production of root hair density but the extent to which root hair becomes denser

could be a response to several factors. Some genotypes recorded longer root hair length but recorded less root hair density IT91, Soronko and NE 15*WC 36. However, Nketewadea, ACC 122W*NE 15, WC 66*Secow 5T, ACC 122W and Agyenkwa produced shorter root hair length but were among the topmost ten genotypes with denser root densities. This suggested that root hair density seemed to considerably compensate for root hair length among certain genotypes in the current experiment. With the hypothesis that both root hair length and root hair density are associated to rhizosheath formation (Haling *et al*., 2010b), rhizosheaths observed during the study could be explained by either root hair length or root hair density (Adu *et al*., 2017).

Effect of $[P]_{ext}$ on rhizosheath, root system architecture, root hair and biomass parameters

Biomass parameters

Development of dry matter and its partitioning are the best measure and index of a crop's total production and response to growth conditions (Karikari & Arkorful, 2015). Genotype, $[P]_{EXT}$ and their interaction significantly affected the biomass parameters in both trials. Root dry weight was significantly affected by phosphorus application as well as the interaction of genotype and phosphorus had a significant effect on root dry weight. Root dry weight of cowpea genotypes increased with increasing $[P]_{ext}$ such that root dry weight of plants screened on amended soil was 29 per cent greater compared to root dry weights at unamended soil. Shoot dry weight obtained by cowpea genotypes was significantly ($P = 0.001$) affected by genotype, phosphorus, and their interactions. In general, genotypes recorded high shoot dry weight under

amended soil treatment compared to the genotypes screened under unamended soil treatment. This agrees with Magani and Kuchinda (2009) who observed that dry matter yield of cowpea genotypes increased significantly with levels of phosphorus fertilizer for all the sampling periods. Additionally, Meena *et al*. (2005) using chickpea plants posited that dry matter production increased significantly with each increase in phosphorus levels. Total leaf area genotypes screened under varying phosphorus level exhibited significant ($P < 0.001$) variation in leaf area in the first and second trials. The general trend was that total leaf area increased with increasing phosphorus application. Despite this trend, a remarkable difference existed between total leaf area obtained by genotype. Similar results have been reported by Singh *et al*. (2011) who concluded that leaf area in cowpea is a major plant growth parameter significantly influenced by phosphorus application. Singh and Ahuja (1985) observed that applied P increased leaf area and accumulation of more dry matter in groundnut. The results of the present study suggest that variation in biomass production is dependent on both genotype as well as $[P]_{EXT}$ concentration hence cowpea genotypes have different growth potentials. This corroborates the findings of Karikari and Arkorful (2015) where they observed that phosphorus is a crucial plant nutrient for growth, development and yield of the crops. Furthermore, results imply that different cowpea varieties are likely to possess different strategies to adapt and/or adjust to different phosphorus level. The results support the fact that shoot development, plant leaf area and dry matter of legume plants increase significantly with application and availability of external phosphorus (Tomar & Jajoo, 2014).

Rhizosheath parameters

Rhizosheath parameters (Absolute rhizosheath weight, relative rhizosheath weight and specific rhizosheath weight) of cowpea genotypes screened under varying $[P]_{ext}$ during the study was significantly affected by genotypes, phosphorus level as well their interactions. Such increasing trend could be linked to the role of phosphorus in root hair development. Higher rhizosheath weight observed could be linked to the presence of root hairs among cowpea genotypes. Since a strong correlation has been established between the length of root hair and the weight of rhizosheath (Delhaize *et al*., 2015), which might have contributed to greater rhizosheath weight since phosphorus improves root hair development. The result correlates with Delhaize *et al*. (2016), who stated that, the size of rhizosheath has been reported to increase with high concentration of soil P. Rhizosheath size of the lines were due to differences in the Al^{3+} tolerance and phosphorus application (Delhaize *et al*., 2012).

However, certain genotypes such as WC 35B*NE 50 and Sunshine produced high absolute rhizosheath weight under unamended soil treatment. Also, genotypes Alegi*Secow 5T, IT91, MU9 and NE 48*NE 10 obtained highest specific rhizosheath weight at unamended soil treatment. Among these genotypes, MU9 had longer root hair length at 0 mg P/kg soil compared to amended soil treatments. This indicates two important things, root hair length plays a vital role in the formation of rhizosheath and confirms the hypothesis that other factors aside root hairs play a role in the formation of rhizosheath (Pang *et al*., 2017; Adu *et al*., 2014). Rhizosheath production is related to many factors, including root hairs density, microbial mucilage, soil water content and

mycorrhizal fungi (Moreno-Espíndola *et al*., 2007; Haling *et al*., 2010; Haling *et al*., 2014).

Root hair parameters

Nutrient availability is the most important abiotic factor influencing root system growth beside water (López-Bucio *et al*., 2003). Root hair length (Bates and Lynch 1996) and root hair density (Ma *et al*., 2001) are mostly affected by P availability, which indicates their importance to plants in low P soil. Root hair among cowpea genotypes screened during the study significantly increased under unamended soil compared to amended soil treatment. As a result, most cowpea genotypes screened at unamended soil treatment had long root hairs. This indicates that low phosphorus levels stimulate long root hairs production among cowpea genotypes. Thus, genotypes that produce longer root hairs under P stress condition are advantageous to enhance P acquisition from soil patch. However, the extent to which this occurs varies among species, some showing very dramatic effects. However, certain genotypes Alegi*Secow 5T, Alegi*Sunshine, Asontem, IT91, NE 15*WC 35B, NE 48*WC 10, Nketewadea, Secow 5T and WC 36 produced longer root hairs under higher phosphorus concentrations. This further suggests that genotypes vary in their response to phosphorus concentrations. The present results are in line with Schmidt and Schikora (2001) who concluded that low phosphorus increases the abundance and length of root hair. Additionally, low P increases root hair development (Zhang *et al*., 2003). Also, plants increase root hair length and density when P is deficient (Bates and Lynch, 1996; Ma *et al*., 2001). On the other hand, Vesterager *et al*. (2006) concluded that length of root hairs in pigeon

pea appears not to be affected by soil P level since high levels of soil P does not completely inhibit root hair growth, indicating that high-P plants maintain the potential for plasticity (Bates & Lynch, 2000).s

Phosphorus application had no significant effect on root hair density. However, genotype and its interaction with phosphorus significantly influenced root hair density among cowpea genotypes. In general, higher root hair density was recorded at higher phosphorus concentration in both the first and second screenings. However, genotypes Alegi*Secow 5T, NE 15*WC 35B, NE 50, NE 51*NE 50, Nketewadea, Secow 3B and Sunshine produced high root hair density under low phosphorus concentration. The result of the present study is supported by Ma *et al*. (2001) and Jain *et al*. (2007) who stated that increased root hair proliferation is a major response to early P deficiency. Also, low P concentrations affect root morphology, delay root growth in plants and reduce the number of root hairs and physiological features connected with P absorption (Pellerin *et al*., 2000). Also, plants increase root hair length and density when P is deficient (Bates and Lynch, 1996; Ma *et al*., 2001).

Total root length

Total root length, in general, was significantly affected by genotype, $[P]_{ext}$ and their interaction. An increasing trend in total root length was observed due to increased application of phosphorus. As a result, longer root length was obtained at 500 mg P/kg soil. However, certain genotypes recorded longer root under unamended soil condition. This indicates that genotypes responded differently to phosphorus availability hence, interaction effect existed between genotypes in their response to phosphorus concentration. Results of the present

study are in line with Gerrano *et al*. (2015) who during their study observed that total root length and root surface area were greatest with genotypes grown under phosphorus treatments compared to no phosphorus application during their study on genetic variability among cowpea genotypes. The previous study on the effect of phosphorus on RSA concluded that low availability of soil P impacts features of the root system (Zhu & Lynch, 2004) such as total root length (Ma, Walk, Marcus, & Lynch, 2001). Root architectural features (root length, density, branching angle, root hair) are essential to enhance the effectiveness of P uptake (Lynch, 2007).

Genetic variation in phosphorus uptake and use efficiency

Tissue phosphorus concentration

Results of the present study indicated that phosphorus concentration of both root and shoot increased with increasing phosphorus application. Also, the interaction between phosphorus concentration and genotype significantly affected tissue phosphorus concentration. Low soil P availability resulted in a significant reduction of P concentration and content of the tissues screened. Uptake of phosphorus among cowpea genotypes could be related to root traits such as root hair length, diameter, and total root length and rhizosheath weight among cowpea genotypes. A typical example is genotype Agyenkwa which had longer root hair length under unamended soil treatment was among the genotypes with high tissue phosphorus concentration. This suggested that root hair length plays a paramount role in the uptake of soil resource noticeably immobile phosphorus elements. The results are in line with the hypothesis that, increase in root hair length promotes P absorption (Ma *et al*., 2001; Zygalakis,

Kirk, Jones, Wissuwa, & Roose, 2011). Marschener (1998) posited that these differences in phosphorus uptake could be associated with root size and morphology and/or root physiology. Root architectural features (root length, density, branching angle, root hair) are essential to enhance the effectiveness of P uptake (Lynch, 2007) of which longer root length and greater root hair density are particularly crucial for enhancing P uptake (Brown *et al*., 2012; Gahoonia & Nielsen, 1997).

Variation in phosphorus uptake and efficiency among cowpea genotypes

Cowpea genotypes screened under varying $[P]_{ext}$ conditions exhibited significant variation in phosphorus uptake efficiency. The general trend observed was an increase in phosphorus uptake efficiency with increased phosphorus application. As a result, phosphorus uptake efficiency was high at amended soil treatments compared to unamended treatment. However, genotypes responded differently to $[P]_{ext}$ in terms of phosphorus uptake efficiency, with some genotypes obtaining high P uptake efficiency at 250 mg P/kg soil compared to 500 mg P/kg soil. This indicated that there is species-wide variation within cowpea a genotype for phosphorus uptake efficiency. This finding agrees with results of Vesterager (2006) who also reported genetic variability among cowpea genotype for P-uptake efficiency.

Cowpea genotypes found in the NER quadrant in terms of their responsiveness to $[P]_{ext}$ measured in terms of agronomic P use efficiency included MU9, Alegi*Secow, Agyenkwa and NE 50. These genotypes developed short root hair under unamended P treatments compared to the amended soil treatments. Among these genotypes, NE 50 produced greater

shoot biomass on an unamended soil treatment compared to amended treatment. Additionally, Secow 3B and Alegi*Secow 5T produced longer total root length under unamended soil treatment. Genotypes NE 15*WC 35B, Soronko, Nketewadea, and NE 15*Sunshine were among efficient and responsive genotypes in term of their response to P measured as APE. These group of genotypes produced longer root hair length under low and unamended soil P conditions. This suggests that most efficient cowpea genotypes produce profuse and longer root hairs which are mostly an adaptative mechanism in response to low soil phosphorus conditions. Such genotypes produce longer and well-developed root system to enable efficient exploration of soil patches for the acquisition of unevenly distributed soil resources especially immobile P. Roots of grain legumes adapt to poor soil P conditions by enhancing root development, such as basal and adventitious roots, root architecture alteration (Lynch, 1995). Miller *et al.* (2003) concluded the development of adventitious bean roots is an adaptive mechanism which helps to acquire P by enhancing foraging of plants in marginal soil.

Relationship between rhizosheath, root hair, root system architecture and biomass parameters

Correlation analysis among measured traits showed that a significant positive correlation existed between measured biomass traits (shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, total leaf area, specific leaf area, leaf mass per area, phosphorus use and uptake efficiency, biomass P content and concentration). The present result is supported by the conclusion of Adu *et al*. (2017) who during their studies on maize established a

significant positive correlation between biomass parameters. A similar report was made by Oladiran *et al*., (2012) during their studies on phosphorus response efficiency among cowpea genotypes. Root system architectural trait (total root length) was observed to have a positive but a significantly low correlation with biomass parameters. A similar observation was made between root hair and biomass parameters. This conforms with a conclusion made by Adu et. (2017) who observed a low correlation between biomass and root system architecture during their studies. Also, the root dry weight of cowpea has been reported to have a low and insignificant correlation with other root system parameters (Oladiran *et al*., 2012). Total root length had a weak positive correlation with root hair density and rhizosheath parameters.

Rhizosheath parameters (absolute, relative, and specific rhizosheath weight) had a weak correlation with root hair density during the study. This indicates that, although root hair plays a significant role in rhizosheath formation, however, root hair density is a poor surrogate in predicting rhizosheath weight among cowpea genotypes screened during the study since it poorly correlated with rhizosheath parameters. However, the measured rhizosheath parameter had a positive correlation with root hair length. In the present study, root hair length strongly correlated with specific rhizosheath weight (rhizosheath mass per unit length of root hair) and relative rhizosheath weight (rhizosheath mass per unit weight of dry root). The results are in line with Haling *et al*. (2010) and Brown *et al*. (2012), who observed a positive linear correlation between rhizosheath mass per unit of root hair length. The correlation results suggest that rhizosheath formation in cowpea is influenced strongly by root hair length. This further indicates the significance of

rhizosheath as a surrogate in predicting root hair length among crop varieties. Root hair length and root hair density are associated with rhizosheath formation (Haling *et al*., 2010a). Similarly, a strong correlation has been established between the length of root hair and the weight of rhizosheath (Delhaize *et al*., 2015). Absolute rhizosheath weight poorly correlated with root hair length during the study. Similar results have been reported by George *et al*. (2014) and Brown *et al*. (2017) who observed that root hair length of barley poorly correlated with rhizosheath mass. This indicates that factors other than root hair length substantially influences rhizosheath formation (Adu *et al*., 2017).

Shoot band root phosphorus concentration had a positive correlation with root hair length and density. This suggests that root hair plays a paramount role in the uptake of soil resources importantly immobile phosphorus. Similarly, modelling studies on root hair absorption of soil P revealed that an increase in root hair length promotes P absorption (Ma *et al*., 2001; Zygalakis, Kirk *et al*., 2011). Root hairs are essential traits to retain a sufficient amount of water during drought and ensures nutrient acquisition under nutrient-deficient conditions (Marzec *et al*., 2015; Segal *et al*., 2008).

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

Conclusion

In the present study, polyethene bags (22 cm deep × 15 cm × 10 cm) was adopted to screen for the genotypic variation in rhizosheath, root system architecture, root hair features and phosphorus uptake parameters among sixty (60) cowpea genotypes screened under unamended soil condition (Experiment 1). After experiment 1, twenty (20) cowpea genotypes were selected for further screening under either P amended or unamended soil condition in experiment 2. The experiment was carried out under greenhouse conditions. It was hypothesized that genotypic variation existed among cowpea genotypes in rhizosheath, root hair and root architectural traits. Additionally, these traits display plastic responses to varying soil phosphorus concentrations at various growth stages and can be examined and quantified using a simple approach which is effective and efficient.

A substantial amount of genetic variation existed between cowpea genotypes in terms of root hair, rhizosheath formation, biomass, and root system architectural trait (total root length). This suggests that greater genetic diversity existed among cowpea genotypes hence, serves as a promising tool for selection and breeding of more efficient genotypes. It was observed that positive correlation existed between root hair length and rhizosheath parameters (relative and specific rhizosheath weight) produced by various cowpeas genotypes. Hence, rhizosheath could serve as a proxy in predicting root hair length among cowpea genotypes. Additionally, this indicates the need for

selection of longer root hairs length to improve the acquisition of soil resources, especially immobile phosphorus reserves.

A strong positive correlation was observed between biomass parameters and tissue phosphorus content and concentration during the study but biomass poorly correlated with root and rhizosheath parameters. The results of the present study revealed that root hairs and root system architectural traits exhibit plastic responses to varying phosphorus regimes during the growth of cowpea. The interaction of genotypes and varying phosphorus level significantly influenced root hairs, rhizosheath and root system architectural traits of cowpea genotypes screened during the study. Although phosphorus influenced RSA, biomass and rhizosheath but had no significant effect on both root hair and length among cowpea genotypes despite the variation observed between genotypes. It was observed that biomass increased significantly with increase phosphorus application. Longer root hairs were observed under low phosphorus concentration indicating the importance of root hairs in tolerance to P - deficient soil conditions.

Finally, the results of the study suggest that the use of polyethene bags combined with imaging approach serves as a good method for screening for rhizosheath, root hair and RSA among cowpea and other crops under controlled experimental conditions.

Recommendation

The following recommendations were made based on the results of the study.

1. Various methods for quantifying rhizosheath should be in subsequent works to check for consistencies.
2. Several factors influence the formation of rhizosheath hence, a future study must consider the production of rhizosheath in the consortium of these factors.
3. Future works must focus on establishing the presence of rhizosheath under field conditions.

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APPENDIX

Appendix 1: Estimating field capacity of experimental soil using gravimetric method.

The following parameters were used to estimate field capacity of experimental soil during screening of cowpea genotypes in both Experiment 1 and 2.

i. Per centage (%) moisture content at field capacity
ii. Per centage (%) moisture content of air-dry soil
iii. Weight of oven dry soil in nursery bags
iv. Amount of water in each nursery bag at field capacity
v. Amount of water to add to each soil filled nursery bag to reach field capacity

Per centage moisture at field capacity

Estimation was carried out using three (3) replications with the formula

$$\text{Field capacity per centage moisture} = \frac{Wt\ of\ wet\ soil - Wt\ of\ oven\ dried\ soil}{Weight\ of\ oven\ dried\ soil} \times 100$$

The mean of the three samples or replicates was used to estimate the field capacity

Per centage moisture in air dried soil

$$\text{Air-dried soil per centage moisture} = \frac{Wt\ of\ air-dried\ soil - Wt\ of\ oven\ dried\ soil}{Weight\ of\ oven\ dried\ soil} \times 100$$

Weight of oven dried soil in pot

Weight of oven dried soil to fill nursery bag =

$$\frac{wt\ of\ air-dried\ soil\ to\ fill\ nursery\ bag \times 100}{100 + air-dried\ soil\ \%\ moisture}$$

Water content of nursery bags at field capacity

Amount of water that would be present in each bag at field capacity was also determined as,

The weight of soil and water at field capacity =

$$\frac{[100+\%moisture\ at field\ capacity] \times Wt\ of\ oven-dried\ soil}{100}$$

Water to add to each soil filled nursery pot to reach field capacity

Weight of water to add per nursery bag = Wt. of soil + Water at field capacity – Wt. of air-dried soil.

Appendix 2: Selection criteria for twenty (20) genotypes evaluated under $[P]_{ext}$