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Phenotyping cowpea for seedling root architecture reveals root phenes important for breeding phosphorus efficient varieties

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Abstract

Cowpea (*Vigna unguiculata* L. Walp.) is a key climate-resilient legume for food security, especially in sub-Saharan Africa. Cowpea yields are limited by edaphic stresses including drought and low phosphorus (P) availability. Identifying genotypes with advantageous root phenotypes can facilitate breeding for improved yield in marginal environments. We evaluated 50 elite genotypes from African and U.S. sources for seedling root architecture and root hair length and density. Significant genotypic variation was detected for all phenes, and high heritability was observed for architectural phenotypes including primary root length (77%), basal root number (72%), and taproot branching density (67%). Moderate heritability was detected for root hair length and density among different root classes (34 to 63%), which were positively associated with each other. Principal component analysis identified three clusters, primarily defined by seed dimension and seedling root architecture. Genotypes were identified with longer root hairs (TVu-7778, Vita7, and Sanzi) and longer taproots (IT96D-610, IT98K-111-1, and IT97K-499-35), as potential parents. Root phenotypes, grain, and fodder yield were assessed on a subset of 20 genotypes under contrasting P availability in the field. Some seedling root phenotypes were significantly related to mature plant dry fodder weight (taproot hair density) and to grain yield (lateral root hair density) under low P. Root hairs are positively related to plant productivity under low P. We suggest selection for longer primary roots, as more basal and lateral root roots may be beneficial for cowpea in drought and low P environments. These findings suggest seedling root phenotypes can support cowpea breeding for suboptimal environments.

Abbreviations: ADRN, adventitious root number; BRHD, basal root hair density; BRN, basal root number; LDA, linear discriminant analysis; LRHD, lateral root hair density; LRHL, lateral root hair length; PCA, principal component analysis; PRL, primary root length (in cm); PUpE, phosphorus uptake efficiency; RSA, root system architecture; SSA, sub-Saharan Africa; TBD1, number of first-order lateral roots on the primary root between 2 and 5 cm from the base of the hypocotyl; TBD2, number of first-order lateral roots on the primary root between 5 and 10 cm from the base of the hypocotyl; TRHD, taproot hair density; TRHL, taproot hair length

1 | INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is a grain legume that is an important energy and protein source for millions of people across sub-Saharan Africa (SSA), India, South East Asia, and parts of the Americas (Ojiewo et al., 2018; Snapp et al., 2018). As a nitrogen (N) fixing legume, it serves an important agroecological role, and its nongrain biomass is also an important component of livestock diets. However, the typical yield of the smallholder farmer is below 600 kg ha^{-1} whereas the genetic potential is greater than 3,000 kg ha^{-1} (ICRISAT, 2017). Drought and poor soil fertility, especially low phosphorus (P) availability, constrain cowpea productivity across the vast majority of SSA areas (Adusei et al., 2016; Boukar et al., 2018; Hall, 2012; Sanginga et al., 2000). Drought and low P limitations are expected to increase, as are soil erosion and soil degradation (Godfray et al., 2010; Misra, 2014; Ye et al., 2018). While cowpea is known as a relatively drought-tolerant crop (Agbicodo et al., 2009; Hall, 2012), precipitation timing is increasingly unpredictable (Sultan et al., 2014) and the impacts of seedling-stage drought can be severe (Agbicodo et al., 2009; Hall, 2012). The poor soil fertility problem across most cowpea producing areas is worsened by continuous cropping with little to no addition of inputs and removal of crop residues after harvest, practices common throughout SSA (Bationo et al., 2002).

Cowpea can fix N through biological N fixation (BNF) in association with *Bradyrhizobium* species, thereby contributing to soil fertility. However, this potential is limited in soils with low P availability because of the high P requirements for BNF (Hogh-Jensen et al., 2002; Nisar et al., 2016). Alleviation of this problem by synthetic P fertilizers is problematic because many soils of SSA have high P fixation, and fertilizer use among smallholder African farmers is much less than the global average, due to high cost and limited access (Krasilnikoff et al., 2003; Mbavai et al., 2015; Mohammed et al., 2020). Furthermore, rock phosphate, the raw material for P fertilizers, is a nonrenewable resource; current reserves are being depleted and prices are expected to increase (Kauwenbergh, 2010). Rising costs are likely to make P fertilizer even less accessible for smallholders in the next few decades and will likely lead to reduced yields of farmers that currently use P fertilizers. Consequently, the most sustainable solution to address challenges associated with water deficit and low soil P is through genetic improvement via trait-based selection of phenes associated with drought and low fertility tolerance (Lynch, 2011, 2019). Phosphorus-efficient varieties will help farmers in low-input systems to increase their yield, income, and food security, whereas in the high input systems where intensive P-fertilizers are used, such varieties will help reduce production costs and minimize environmental pollution (Fita et al., 2011; Lynch, 2015; Lynch & Brown, 2012).

Core Ideas

- Significant variation exists for seedling root phenotypes in elite cowpea genotypes.
- Two important seedling root phenotypes were associated with yield under low soil phosphorus conditions.
- Three phenotypic clusters were identified using seed dimension and seedling root architecture phenotypes.
- Seedling root phenotypes can guide cowpea breeding for low phosphorus soils.

Improving crop productivity and resilience is the focus of most breeding programs (Wasson et al., 2012; York et al., 2013) but few directly select for component traits. Trait- or phene-based selection has been successfully implemented in common bean (*Phaseolus vulgaris*) by selecting for root traits including root hair length and density (Burridge et al., 2019). Phenes are the elemental units of plant phenotype, in the same way genes relate to genotype (York et al., 2013). Validation of the utility of specific phene states, and their eventual inclusion as selection criteria, first requires the development of a screening methodology, and quantification of genetic variation and heritability. Genetic variation for root phenes useful for breeding has been found in several crops using both seedling and mature plant phenotyping platforms (Tracy et al., 2020), including maize (*Zea mays* L.; Gao & Lynch, 2016; Mendes et al., 2014; Trachsel et al., 2013), soybean (*Glycine max*; Prince et al., 2018; Vandamme et al., 2016), common bean (De Melo et al., 2016; Faria Vieira et al., 2008; Lynch & van Beem, 1993; Strock et al., 2019), and cowpea (Bucksch et al., 2014; Burridge et al., 2016; Burridge et al., 2017; Matsui & Singh, 2003). Previous studies on cowpea root phenotypes reported evidence of phenotypic variation for root traits and identified some genetic control. However, these reports do not link root phenotypes to crop performance in the field. The current study builds upon the earlier findings by linking seedling root phenotypes and field performance. It used multivariate analysis to identify multiple phenotypes that may be associated with the performance of cowpea under contrasting P soils.

Cowpea employs multiple mechanisms to produce grain with suboptimal water supply (Ehlers & Hall, 1997) including drought escape, avoidance, and tolerance (Agbicodo et al., 2009). These mechanisms employ various morphological, physiological, and biochemical strategies that play important roles individually, or in an integrated fashion to support crop growth and productivity. The idea of nutrient use

efficiency is divided into two components, uptake and use efficiency. This division is important to facilitate our understanding of mechanisms responsible for improved efficiency of nutrients including P, such that if uptake efficiency is the main trait for a crop or set of genotypes, then improvement efforts should be focused on morphology, anatomy, and physiology traits of the root system. Whereas, if use efficiency is more important, then harvest index and biomass production efficiency should be the target of improvement (Ortiz-Monasterio et al., 2012). It is critical to clearly define P efficiency before describing strategies for improving it or managing its deficiency. Phosphorus uptake efficiency (PUpE) refers to the ability of the plant to extract plant available P from the soil, whereas P use efficiency (PUE) is the ability of the plant to convert the absorbed P into grain yield or biomass production (Hammond et al., 2009; Ortiz-Monasterio et al., 2012). Phosphorus uptake is enhanced in genotypes with long and dense root hairs and shallow, highly branched root systems (Bishopp & Lynch, 2015; Lynch & Brown, 2012; Lynch, 2019).

Phosphorus deficiency restricts root growth and plant development. However, plants have adapted to soil P deficiency using various strategies. These include secretion of root exudates such as sugars, oligosaccharides, and organic acids that are capable of inducing soil microbial activity within the rhizosphere to release immobilized P fixed in complex forms (Simpson et al., 2011). The use of soil amendments with biochar has been associated with increased uptake of immobile soil nutrients like P in poor soils and drought-prone areas (Abiven et al., 2015). Furthermore, the association formed between soil mycorrhizal fungi and cowpea in some genotypes is known to enhance uptake of nutrients (Saidou et al., 2012) in soils with low fertility. Similarly, large root surface area has been found in cowpea to be responsible for tolerance to low soil P conditions (Rothe, 2014). The utility of multiple phenotypes integrating root architectural, anatomical, and phenology traits has been identified at the species (Burridge et al., 2020), gene pool (Jochua et al., 2020), and genotype (Klein et al., 2020) levels. A good understanding of these strategies could be used to select and develop resilient varieties that will support adaptation to drought and low P deficiency, the two edaphic factors that are most prevalent in cowpea producing areas (Lobell et al., 2011). Within the context of various drought tolerance and P efficiency strategies, as outlined above, developing an efficient and deep root system favors drought avoidance and efficient use of soil resource without compromising yield potential (Agbicodo et al., 2009; Belko et al., 2014; Hall, 2012; Manschadi et al., 2014).

An obstacle to the use of root traits in crop breeding is the difficulty of phenotyping mature root systems in the field. It is, however, easier and more economical to phenotype roots at the seedling stage. Seedling phenotyping avoids the costs and constraints associated with phenotyping field-grown plants and is associated with the root system architecture (RSA) of mature plants (Strock et al., 2019; Thomas et al., 2016). In legumes and most annual dicot plants, the mature root system is an extension of the seedling root system with all root classes: adventitious, basal, lateral, and taproot roots present at the seedling stage (De Dorlodot et al., 2007; Tuberosa et al., 2002; Zhao et al., 2017). Therefore, it can be expected that seedling root phenotypes should be related to mature plant RSA, as has been demonstrated in soybean (Falk et al., 2020). A relationship between seedling primary root length and lateral root density and performance (seed yield and nutrient capture) was found in field-grown oilseed rape (*Brassica napus*; Thomas et al., 2016). Strock et al. (2019) found seedling basal root number, adventitious root abundance, and taproot length to be related to performance in common bean, a close relative of cowpea. A link between fast taproot growth rate, increased root length density at depth, and increased water acquisition has been found in soybean (Hoogenboom et al., 1987; Kaspar et al., 1984). A relationship between root phenotypes of mature plants and crop performance has been found in multiple crops including wheat (*Triticum aestivum*; Bai et al., 2019; Watt et al., 2013), and *Brassica napus* (Thomas et al., 2016). It is possible that similar links between cowpea seedling root phenes and performance exist.

Root hair length and density (number of root hairs per area) are two of the most important root phenes for efficient uptake of immobile soil resources like P (Bates & Lynch, 2001; Chimungu & Lynch, 2014; Lynch, 2013, 2019) and they can be effectively phenotyped at the seedling stage (Lynch, 2013; Yan et al., 2004; Vieira et al., 2007; Zhang et al., 2018). Root hairs are a subcellular projection from epidermal cells that occur on most vascular plants and support enhanced uptake of water and nutrients (Gilroy & Jones, 2000; Jones & Dolan, 2012), aid in root penetration of compacted soil (Lynch et al., 2014; Paez-Garcia et al., 2015), and increase the surface area explored by the root crown (Paez-Garcia et al., 2015; Segal et al., 2008). Greater root hair length and density are associated with P uptake in common bean (Miguel et al., 2015; Vieira et al., 2007), maize (Zhu et al., 2010) and *Arabidopsis* (Bates & Lynch, 2001; Zhong Ma et al., 2001). The number and length of root hairs are highly influenced by the level of P in the growth medium (Bates & Lynch, 2001; Ma et al., 2001) and plasticity in root hair length has been described (Zhu et al., 2010).

The present study used high-resolution images of root hairs captured using a Nikon camera mounted on a dissecting microscope to measure their length and count the density. Additional methods other than the one used in this study have been deployed to determine the length and density of root hairs including; an agar and moist filter paper platform that permits observation of seedling growth and root elongation (Yazdanbakhsh & Fisahn, 2009), rhizotrons and

minirhizotron facilitating the acquisition of digital images of root sections in situ (Krasilnikoff et al., 2003), acquiring samples via soil coring for field-grown plants (Yan et al., 2004), and a roll-up system using high-resolution digital images of root hairs (Vieira et al., 2007; Yan et al., 2004; Zhu et al., 2005a). Phenotyping root hairs using these procedures are associated with one form of challenge or the other. For instance, the use of roll-ups and agar and moist filter paper is not suitable for the assessment of mature plants. Rhizotrons or mini-rhizotrons require specialized systems to take root images, and therefore, not feasible for all researchers. Soil coring is destructive, time-consuming, laborious procedure and prone to error as root hairs are easily lost or damaged during the coring and washing process (Vincent et al., 2017; Yazdanbakhsh & Fisahn, 2009). However, the roll-up method used by Vieira et al. (2007, 2008), Zhu et al. (2005a), and Strock et al. (2019) is time- and space-efficient and can be deployed to phenotype large collection of root samples within a relatively short period (Bonser et al., 1996; Vieira et al., 2007; 2008; Zhu et al., 2005a ; Strock et al., 2019).

In addition to root phenes, seed quality attributes such as seed size and shape are important criteria in most parts of SSA, with a general preference for large grains (Lucas et al., 2015; Mishili et al., 2009). New climate-resilient varieties that meet farmer preferences regarding seed attributes, are likely to be adopted faster by smallholder farmers (Mishili et al., 2009; Persley & Anthony, 2017). For this reason, and to investigate potential allometric relationships among seedling root phenotypes and seed size, it is important to investigate seed dimension phenotypes.

The present work quantifies the genetic variation and heritability of cowpea seedling root hair and root architectural phenes in 50 genotypes and their relationship to each other and to seed size. A subset of 20 genotypes was compared to mature plant phenes and to performance. We hypothesized that longer and denser root hairs would be associated with performance under low P, and that individual phene utility would depend upon integration with other root architectural and shoot phenotypes.

2 | MATERIALS AND METHODS

2.1 | Plant materials

A cowpea panel consisting of 50 genotypes was kindly provided by the cowpea team at the University of California, Riverside (UCR) and Apache Root Biology Center (ARBC) Arizona, both in the United States. These genotypes represent a wide distribution of elite breeding lines across major cowpea breeding programs in the United States and West African countries of Burkina Faso, Cameroon, Ghana, Nigeria, and Senegal (Supplemental Table S1). Many of these genotypes have been used as parents in biparental or multiparent advanced generation intercross (MAGIC) recombinant inbred lines, and in constructing several genetic maps including cowpea consensus genetic map (Huynh et al., 2013, 2018; Muchero et al., 2009; Muñoz-Amatriaín et al., 2017). They were screened for seedling root system architecture, root hair phenotypes, and seed dimension attributes at the Roots Lab of the Department of Plant Science, Pennsylvania State University.

2.2 | Experimental design and data collection

The cigar roll method of seedling phenotyping was used (Bonser et al., 1996; Miguel et al., 2015; Zhu et al., 2005a, 2005b). Cowpea seeds were surface sterilized with 0.5% sodium hypochlorite (NaOCl) for 1 min, dipped in a 0.1% Captan (*N*-trichloromethylthio-4-cyclohexene-1,2-dicarboximide), 50% wettable powder for 1 min, and then placed onto brown germination paper (Anchor Paper) saturated with 0.5 mM $CaSO_4$ (Supplemental Figure S1). Five randomly selected seeds of each genotype were placed 2 cm from the top of a 37.6-cm-long piece of germination paper, rolled into a moderately tight cigar-roll configuration and placed in a 2 L beaker filled with approximately 6 cm of 0.5 mM $CaSO_4$. Each roll-up constituted a replicate. An average of seven replications was analyzed for each of the genotypes. Each replicate included all genotypes and replicates were sequential over time. The beakers were filled with 10–16 rolls and placed in an incubator chamber for 48 to 72 h set at 32 ˚C and then moved to a light chamber at 28 ˚C with a photoperiod of 16/8 h (light/darkness) for 10 d. After a total of 14 d, the rolls were removed, unrolled, and three healthy and well-developed seedlings were selected from the total of five seedlings for analysis.

2.3 | Seedling root architecture phenotyping

Cowpea genotypes were evaluated for primary root length (PRL; in cm), basal root number defined as the number of first-order lateral roots within 1 cm of the base of the hypocotyl (basal root number [BRN]), number of first-order lateral roots on the primary root between 2 and 5 cm from the base of the hypocotyl (TBD1), and number of first-order lateral roots on the primary root between 5 and 10 cm from the base of the hypocotyl (TBD2) of the taproot length. Sampled seedlings were spread on a flat tray and length was manually measured with a ruler and visual counts were made of the various root classes and root number per zone (see Table 1 for full phene definition).

TABLE 1 List of phene names with their abbreviation and definition

Phene	Definition	Measurement environment
Adventitious root number (ADRN)	Number of 1st order lateral roots emerging from the hypocotyl	Seedling experiment
Adventitious root growth angle (ARGA)	Adventitious root growth angle relative to horizontal (0° is horizontal, 90° is vertical, scored in units of 10°)	Field experiments
Basal root growth angle (BRGA)	Basal root growth angle relative to horizontal (0° is horizontal, 90° is vertical, scored in units of 10°)	Field experiments
Basal root hair density (BRHD, mm)	Number of root hairs in mm^2 on the basal roots	Seedling experiment
Basal root hair length (BRHL, mm)	The length of root hairs measured in mm within the basal root region	Seedling experiment
Basal root number (BRN)	Number of roots in the basal region of the hypocotyl	Seedling and field experiments
Days to first flowering (DFF)	Recorded as the number of days after planting to first flower emergence	Field experiments
Dry fodder weight (DRYFDWT, g)	Weight of dry fodder, per plot	Field experiments
Days to maturity (DMAT)	Recorded as the number of days after planting to 95% maturity of pods in a plot	Field experiments
Grain yield (Yield, kg ha^{-1})	Weight of dried grains per plot in g, and converted to kg ha^{-1}	Field experiments
Primary root length (PRL, cm)	The length of taproot measured in cm from the base of hypocotyl to the tip of the taproot	Seedling experiment
Seed length (SDL, mm)	Distance between the two ends of the seed, in line with the hilum	Seed dimension measurements
Seed thickness (SDTCK, mm)	Distance from one side of the seed to the other, crossing the hilum	Seed dimension measurements
Seed width (SDWTH, mm)	Distance between the hilum and the back side of the seed	Seed dimension measurements
Stem diameter (mm)	Diameter of stem (mm) at soil level	Field experiments
Taproot branching density (TBD1)	Number of first-order laterals emerging from the primary root between 2 and 5 cm of the taproot length	Seedling experiment
Taproot branching density (TBD2)	Number of first-order laterals emerging from the primary root between 5 and 10 cm of the taproot length	Seedling experiment
Taproot hair density (TRHD, mm)	Number of root hairs in mm^2 on the taproot	Seedling experiment
Taproot hair length (TRHL mm)	Root hair length measured on the taproot	Seedling experiment
Taproot lateral root hair density (LRHD, mm)	Number of root hairs in mm^2 on the emerging laterals from taproot	Seedling experiment
Taproot lateral root hair length (LRHL, mm)	Root hair length of a lateral root emerging from the taproot	Seedling experiment
Taproot diameter (TD5, TD10, TD15)	Taproot diameter (mm) 5, 10, and 15 cm below the soil surface	Field experiments
Weight of seeds, g 100 $seeds^{-1}$ (SDWT100, g)	Weight of 100 randomly chosen seeds	Seed dimension measurements

2.4 | Root hair phenotyping

Three 2-cm samples of the basal root, taproot, and taproot laterals were taken from the 14-d-old seedlings in each replicate. Images were taken within the 2-cm segment of the cut sections from the point of emergence of the basal, taproot, and lateral roots. In each replicate, root sections with root hairs were imaged for their root hair length and density using a Nikon Camera (Nikon Digital Sight DS-Fi1, Nikon Corporation Japan) mounted on a dissecting microscope (Nikon SMZ 1500, C-DSS115, Nikon Corporation Japan) set at 30x magnification using an imaging software (NIS-Elements F4.30.01.64-bit; Supplemental Figure S2). A detailed description of this procedure has been provided (Hanlon et al., 2018). Root hair lengths and densities were measured using an open-access image processing software *ImageJ* (https://imagej.nih.gov/ij/) to trace along the edge for length and count the root hairs at the middle of the root section for density. The length of 10 root hairs was traced with the *ImageJ* tool per picture, and a total of four pictures per replicate,

making a total of 24 pictures for each genotype from six replicates, giving an average of 240 root hairs measured per genotype and the same goes for root hair density.

2.5 | Seed dimension measurement

For each genotype, the principal axial dimensions of the seeds (length, width, and thickness) were measured in mm using a digital Vernier caliper (see Table 1; Supplemental Figure S3), whereas a digital weighing balance was used to measure the weight of 100 randomly selected seeds for each genotype.

2.6 | Root architecture phenotyping in the field

Mature plant root architectural phenotype and performance data were obtained from a subset of 20 genotypes screened in the cigar-roll experiment. At least four genotypes from each cluster were selected. For instance, four genotypes were taken from Cluster 1 with high values for lateral root hair density (LRHD); seven from Cluster 2 with greater TBD1, TBD2, and BRN, and longer PRL and high taproot hair density (TRHD); four from Cluster 3 with intermediates attributes partly present in Clusters 1 and 2; and the remaining five were selected based upon their ability to grow normally at the latitudes in question. The field experiments were carried out in 2018 and 2019 at Minjibir, Kano (11°59.475′N 008°40.016′E) and Zaria (11°10′31.7″N 7°36′43.9″E), both in Nigeria. Field soils were inherently low in plant-available P (5.2 and 4.2 mg P kg^{-1} Bray I method, measured at 0- to 20-cm soil depth at Kano and Zaria, respectively). The soil texture of the field soil at Kano was sandy loam, whereas that of Zaria was clay loam with low N (0.13 g kg^{-1}), K (0.68 cmol kg^{-1}) and organic carbon (0.79 g kg^{-1} soil; Supplemental Table S2). The high P treatment was achieved by application of single super phosphate fertilizer (SSP 18% P_2O_5, 11% sulfur, 18% Ca, and 4% moisture; TAK-AGRO) at the rate of 60 kg P_20_5 ha^{-1} based on previous P recommendations for cowpea (Boukar et al., 2018; Mohammed et al., 2021; Sanginga et al., 2000), whereas no SSP was applied to the low P treatments. All the field plots received 30 kg N ha^{-1} (46% N, Urea) and K (30% K_2O, muriate of potash) to avoid any confounding effects of N and K deficiency on the plants. The single fertilizers were applied as dual banding by placing them below the soil surface 5 d after planting. The experiment was conducted between 5 July and 30 Oct. 2018 at Kano, whereas Zaria's experiment was between 25 July and 30 Nov. 2019. Plots were one row of 2 m each with a 1-m unplanted walkway between plots, giving a pot size of 1.5 m^2 and 10 plant stands per plot with an intra- and inter-row spacing of 0.20 and 0.75 m, respectively. Prior to planting, seeds were treated with a broad-spectrum commercial fungicide *Apron star* at a rate of 4 kg seeds to a sachet of 10 g to reduce the incidence of fungal diseases. Plants were protected against insect pests by spraying insecticide (Karate 50 g L^{-1} lambda-cyhalothrin, Syngenta Crop Protection AG). The trial was kept weed-free by hand-hoe weeding. The experiments were arranged in a randomized complete block design in three replications. Four averaged sized plants were excavated and two representative root crowns from each replicate were manually assessed for the following RSA phenotypes: adventitious root number, basal root number, adventitious root growth angle, and basal root growth angle as described (Burridge et al., 2016, 2017), and their aboveground parts were taken as fodder samples. Other data collected from the field experiment include days to first flowering, days to maturity, fodder dry weight (g), and grain yield (kg ha^{-1}). Sampled fodder was air-dried in the screenhouse till constant weight was maintained and weighed with a digital scale (Kerro BL20001). Grain yield was measured as the dry weight of grains per plot in g, and converted to kg ha^{-1} according to the formula described below:

$$\text{Grain yield } \left(\text{kg ha}^{-1}\right) = \frac{\text{Yield per plot (g) } 10,\ 000\ (\text{m}^2)}{\text{Plot size } (\text{m}^2)\ 1,\ 000\ (\text{g})}$$

2.7 | Data analysis

Analysis of variance was first performed on the cigar-roll data using the general linear model of randomized complete block design (RCBD) based on the following model:

$$y_{ijk} = \mu + t_i + b_i + e_{ij}$$

where y_{ijk} is the response from *ij*th experimental unit (cigar-roll), μ is the overall mean, t_i is the effect of *k*th genotype, b_i is the effect of *j*th replication, and e_{ij} is the experimental error.

To generate means of individual genotypes across replications, a linear mixed-effect model using R *lme4* package was used (Bates et al., 2015), and genetic variance components were generated. The response variables (root phenes) were individually modelled by taking the genotypes as a fixed factor, whereas the replications (cigar-rolls) and the number of plants assessed per replication were considered random factors, as described in the best linear unbiased prediction (BLUP) model below

$$\text{BLUP} < -\text{lmer}\left(\text{Trait} \sim -1 + \text{genotype} + (1|\text{replication}) + (1|\text{No\_plant\_assessed}), \text{data} = \text{data\_name}\right)$$

An estimate of broad-sense heritability was computed as a ratio of genetic to phenotypic variances using genetic variance components computed from the mixed model analysis. The equation below was used to estimate the heritability (Falconer & Mackay, 1996).

$$h^2 = \sigma_g[\sigma_g + (\sigma_{e/r})]$$

where h^2 is the estimate of broad-sense heritability, σ_g is the genotypic variance and σ_e is the error variance, and r is the number of replications. In addition, the possible association between seedling RSA, root hair phenes, and seed dimension phenes was investigated with Pearson-product moment correlation.

2.7.1 | Multivariate analysis

Principal component analysis (PCA) was performed to reveal the set of seedling RSA and seed characteristics contributing to the observed variation among the genotypes, and to identify correlated variables. Owing to differences in the unit of measurement of the traits assessed, the data were scaled before PCA analysis, using built-in R functions *prcomp* on the genotype means, and figures were produced using the *factoextra* package (Kassambara, 2017b). Hierarchical clustering on principal components (HCPC) was performed on PCA results using *factoMiner* and *factoextra* packages in R (Kassambara, 2017a) and this helped to group the genotypes based on their shared characteristics. Within HCPC, the PCA step can be considered a denoising step contributing to a more stable clustering. A map of the clusters was formed by grouping the genotypes into three groups based on hierarchical clustering, the grouping was based on the extent of relatedness of the genotypes in relation to the traits measured.

This paper also demonstrates that in multivariate data sets obtained from mature field-grown cowpea plants, structural patterns may exist in the data that may not be revealed by visual observation or simple regression methods. Hence, the use of linear discriminant analysis (LDA) was employed to reveal whether there were differences in the distributions of the shoot and RSA phenotypes among cowpea genotypes previously classified as high or low PUpE based on previous field trials (unpublished data, see Supplemental Table S3). The PUpE was earlier computed as the proportion of total grain P (grain weight in kg ha^{-1} times %grain P) at maturity to the nutrient supplied to the soil. The data used to perform the LDA analysis were dry fodder weight, days to flowering and maturity, and root phenotypes measured in the field experiments (Supplemental Table S3). The PUpE classification data of the genotypes was used as the categorical variable for the LDA grouping. The LDA is often used to predict the probability of individuals belonging to a given group based on some measured predictor variables. This is achieved by using linear combinations of the variables to predict the class of a given individual. In this study, the LDA was computed in R with the *lda()* function of the *MASS package* (R Script available in Appendix 1). Before running the LDA function, the data set was split into the training (80%) and testing (20%) sets using the *set.seed(123)* command in R. Since the LDA assumes that the predictor variables are normally distributed (Gaussian distribution) and the different groups have equal variance and covariance matrices, the data was standardized using the "center", and "scale" functions in R.

3 | RESULTS

3.1 | Summary statistics for seedling root architecture of cowpea genotypes

Root phenotypes differed among genotypes (Table 2). There were wide ranges for some RSA phenes such as PRL (16.8–41.3 cm), BRN (7.0–18 roots), TBD1 (18–37 roots), TBD2 (13–33 roots) and for weight of seeds g 100 $seeds^{-1}$ (9.2–29.1 g). Root hair length of the genotypes varied from 0.2 to 1.20 mm and density ranged from 26 to 161 root hairs mm^{-2}. The broad-sense heritability (repeatability), estimated as the proportion of genetic to the total phenotypic variances, revealed that genetic factors were responsible for observed phenotypic variation in the following amounts PRL (77%), BRN (72%), TBD1 (67%), TBD2 (67%), basal root hair density (BRHD) (34%), basal root hair length (41%), TRHD (43%), taproot hair length (TRHL) (63%), LRHD (44%), and lateral root hair length (LRHL) (46%; Table 2), though these estimates are still preliminary especially considering they are based on a single seedling experiment.

3.2 | Multivariate analysis

The principal component analysis (PCA) identified the major components accounting for most of the variation in seedling RSA and root hair phenotypes. At an eigenvalue of greater than or equal to 1, five PC explained over 70% of the variability of the data sets, with PC1 and PC2 accounting for 23.3 and 19% of the total variability, respectively (Table 3; Supplemental Figure S4). Principal Component 1 was associated with seed dimension (seed length, width, thickness, and weight of seeds g 100 $seeds^{-1}$) and PC2 was related to seedling primary root length, basal root number, and taproot branching density. Principal Component 3, PC4, and PC5 were associated with root hair phenotypes (length and density of root hairs on basal, taproot, and lateral roots; Table 3). The PCA helped identify patterns of correlated variables. The correlation circle

TABLE 2 Summary statistics for cowpea seedling root architecture and seed dimension phenes

Phene	Min[a]	Max	SD	Median values	F prob. for genotype effect	h^2
Primary root length, cm	16.8	41.3	4.9	28	***	77
Basal root number, No.	7.0	18.0	2.0	11	***	72
Taproot branching density, No. roots	18.0	37.0	4.2	27	***	67
Taproot branching density, No. roots	13.0	33.0	4.3	24	***	67
Basal root hair density, No. roots mm^{-2}	39.0	132.0	20.0	79	***	34
Basal root hair length, mm	0.4	0.7	0.1	0.5	***	41
Lateral root hair density, No. roots mm^{-2}	59.0	161.0	20.1	94	***	43
Lateral root hair length, mm	0.4	0.9	0.1	0.5	***	63
Taproot hair density No. roots mm^{-2}	26.0	79.0	13	52	***	44
Taproot hair length, mm	0.2	1.2	0.2	0.8	***	46
Seed length, mm	5.6	10.9	1.1	8.6	***	NA
Seed width, mm	4.9	8.1	0.6	6.5	***	NA
Seed thickness, mm	3.6	6.3	0.6	5.0	***	NA
Weight of seeds, g 100 $seeds^{-1}$	9.2	29.1	4.4	18.5	***	NA

Note. Data from seedling root experiment and seed dimension measurements were used. See Table 1 for phene definitions Values are genotype best linear unbiased predictors.

[a]Min, minimum; Max, maximum; SD, standard deviation; h2, broad-sense heritability; NA, heritability not very informative.

***Significant at the .001 probability level.

shows seed dimension phenotypes were positively correlated and were projected on the same PC plane (Figure 1) and were not related to vectors of the seedling root phenotypes. Root hair phenotypes were positively correlated, and seedling RSA phenotypes were positively correlated, with all positioned on the same direction of the PC plane. The quality of projection of the genotypes to the first two PC was inferred using the squared cosine (cos^2) values (Supplemental Figure S5).

Hierarchical clustering of principal component results analysis produced three clusters (Figure 2), in accord with Ward's criterion that based cluster creation on minimizing the squared Euclidean distance between points, and indicated quantitative variables associated with each cluster (Figure 3; Supplemental Table S4). Cluster 1 consisted of genotypes (n = 17) that are characterized by high values of LRHD and below-median values for BRHD, BRN, SDL, SDWTH, and SDWT100 compared to genotypes in other clusters (Figure 3). The top five genotypes associated with Cluster 1 are Danila-B, Tvu-1438, IT98K-1092-1, Tvu-2731, and Tvu-6443 (Supplemental Table S5). Cluster 2 (n = 20) was composed of genotypes that had greater taproot branching densities (TBD1 & TBD2), greater BRN, longer PRL, and high TRHD (Figure 3), and the following genotypes being representative of the cluster; IT90K-76, IT99K-573-2-1, IT99K-494-6, IT93K-503-1, and IT82E-18 (Supplemental Table S5). Cluster 3 (n = 13) contained genotypes with attributes partly present in Clusters 1 and 2, such as seed dimension phenes SDTK, SDWT100, SDWTH, and SDL. However, higher values in Cluster 3 for seed dimension phenes differentiate it from Cluster 1. Greater seed dimension phenes distinguish Cluster 3 from Cluster 1. Lower values for PRL, TBD1, and TBD2 differentiate Cluster 3 from Cluster 2 (Figure 3). The top five genotypes with features representative of Cluster 3 are IT98K-205-8, UCR779, CB46, IT97K-568-18, and IT00K-1463 (Supplemental Table S5).

3.3 | Relationship of seedling root phenotypes, root hairs, and seed dimensions in cowpea

In addition to the pattern of correlated variables revealed by PCA plots in Figure 1, correlation analysis revealed medium to high correlation (.3–.8) between several pairs of phenes (Table 4). All seed dimension phenotypes were positively associated at $p < .001$ ($r = .5 - .8$) but were not correlated with seedling root hair phenotypes. Lack of correlation between seed weight (g 100 $seeds^{-1}$) and seedling root traits indicates that seedling root architecture does not have an allometric relationship with seed weight. Significant positive correlations were observed between BRN and PRL ($r = .3$, $p < .01$), PRL and TBD2 ($r = .6$, $p < .0001$), and BRN and TBD1 ($r = .6$, $p < .0001$; Table 4; Supplemental Table S6). The number of basal roots was significantly related to the number of laterals on the taproot ($r = .53$, $p < .0001$). The root hair length on all root classes were positively associated with each other, especially root hairs on basal and taproot ($r = .65$,

TABLE 3 Important principal components (PC) and loadings of the variables to the components

Phene[a]	PC1	PC2	PC3	PC 4	PC5
PRL	0.18	**21.02**	3.09	3.12	**11.58**
BRN	1.40	**16.52**	0.17	8.14	0.70
TBD1	0.00	**24.32**	2.43	1.37	0.01
TBD2	1.78	**25.86**	2.25	0.21	0.06
BRHD	0.39	4.72	0.65	1.34	**41.95**
BRHL	1.31	0.14	**14.30**	25.41	**11.45**
LRHD	0.73	0.24	0.36	**18.37**	**21.99**
LRHL	0.34	0.34	**19.77**	**16.59**	0.08
TRHD	1.22	0.26	**27.16**	**12.63**	6.88
TRHL	0.15	3.81	**28.24**	6.88	2.84
SDL	**17.89**	1.79	0.67	5.73	1.35
SDWTH	**22.91**	0.04	0.29	0.15	0.04
SDTCK	**22.84**	0.69	0.00	0.00	1.07
SDWT100	**28.86**	0.26	0.62	0.05	0.00
Eigenvalue	3.22	2.66	1.66	1.28	1.15
Variance, %	22.99	19.00	11.88	9.15	8.23
Cumulative variance, %		41.99	53.86	63.01	**71.24**

Note. BRHD, basal root hair density; BRHL, basal root hair length; BRN, basal root number; LRHD, lateral root hair density; LRHL, lateral root hair length; PRL, primary root length; SDL, seed length; SDTCK, seed thickness; SDWT100, Weight of seeds, g 100 seeds^{-1}; SDWTH , seed width; TBD1, number of first-order lateral roots on the primary root between 2 and 5 cm from the base of the hypocotyl; TBD2, number of first-order lateral roots on the primary root between 5 and 10 cm from the base of the hypocotyl; TRHD, taproot hair density; TRHL, taproot hair length. Loadings over 10 are in bold. Data from the seedling root experiment and seed dimension measurements were used.

[a]See Table 1 for phene abbreviation.

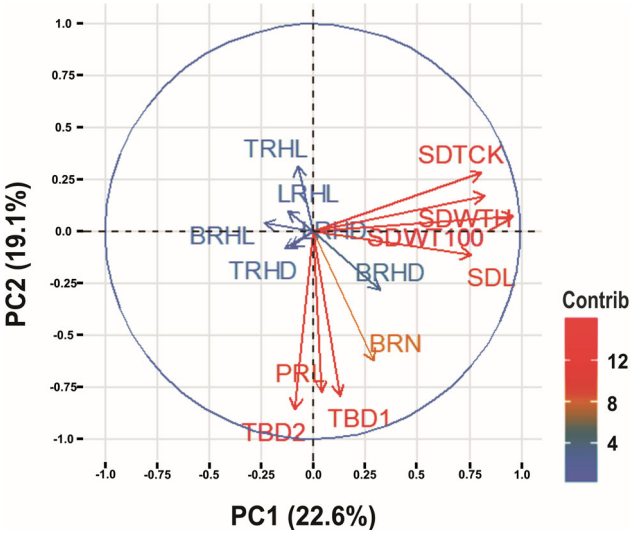

FIGURE 1 Principal component (PC) analysis of root phenotypes and seed dimensions in cowpea genotypes. Red arrows indicate a high contribution of the variable to the PC, and blue indicates the low contribution of the variable to the current PC. See Table 1 for phene abbreviations. Data from the seedling root experiment and seed dimension measurements were used

$p < .0001$), thus providing a basis to limit root hair phenotyping to just one root class. Taproot hair length was inversely related to primary root length ($r = -.39$, $p < .005$), suggesting root hair length and early root depth are associated with different root growth strategies.

3.4 | Seedling root phenotypes related to field root phenotypes and performance

Root hair phenotypes such as BRHD, LRHL, LRHD, and TRHD measured at the seedling stage in cigar-roll papers were associated with basal root number (BRN), adventitious root number (ADRN), and grain yield in low P soils (Table 5). Most of the seedling RSA phenotypes did not show a significant correlation with those of adult plants across the locations (Supplemental Tables S7 and S8). There were significant differences among field phenotypes across locations (Supplemental Table S9). A significant positive correlation was found between lateral root hair density and yield under low P at Kano (Figure 4; .57). Taproot hair density correlated with dry fodder weight in Kano under both low (.45) and high (.55) soil P (Table 5). Some root hair phenotypes showed a correlation with architectural phenotypes of mature plants (Table 5; Supplemental Table S7 and S8). A negative relationship (−.59) between PRL and dry fodder weight found under high P at Kano (Table 5) is suggestive of a tradeoff. No significant relationships between seedling root phenes and mature root phenes or yield were found using the Zaria data (Supplemental Table S8). Several RSA phenotypes such as BRN, ADRN, basal root growth angle, and adventitious root growth angle showed significant association under low and high P conditions at Zaria (Supplemental Table S8). These findings using Zaria data are merely suggestive of phene utility and further investigations are needed to draw a conclusion.

3.5 | LDA distinguishes phenotypes responsible for P uptake in cowpea

The LDA was used to discriminate between different groups of cowpeas shoot and RSA phenes under varying soil P concentrations. It helped answer the question of whether there are differences in the shoot and RSA phenotypes of cowpea grown on high versus low P soil, and between genotypes with high and low P uptake. The LDA results on the data grouped as high versus low P uptake efficient genotypes, based upon field phenotypes in high versus low P soil are presented in Figure 5. Figure 5 reveals modest differences between

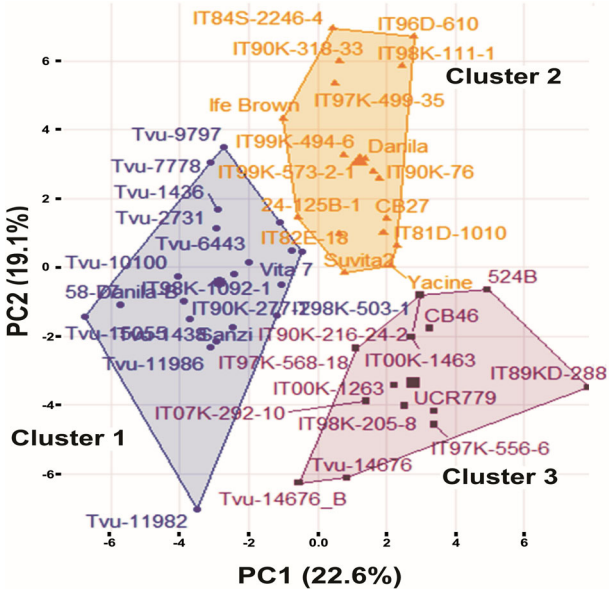

FIGURE 2 Clustering based on principal component (PC) results of the cowpea genotypes evaluated for seedling root phenotypes. PC1 loaded primarily with traits related to seed size and PC2 is related to primary root length, the number of basal roots, and basal root length. Data from the seedling root experiment and seed dimension measurements were used

genotypes with high and low PUpE (red and sky blue) on high P soil, and high and low PUpE (green and light pink) on low P soil. The LDA plots on Figures 5a, 5b, and 5c representing data for 2018, 2019, and pooled data of both years revealed clearer differences between the four groups. The linear discriminate scores for the four groups (high and low PUpE on high P soil, and high and low PUpE on low P soil) were plotted as density plots for the three discriminant axes.

The density plots and the corresponding variable loadings for the three linear discriminant axes that resulted from applying LDA to all the four PUpE groups and P combinations are shown in Figure 6 for 2018, 2019 and the pooled data of the 2 yr. The first linear discriminants (LD1), shown in Figure 6 A, D and G, clearly discriminate between high and low PUpE genotypes (sky blue and grey) on high P soil, and high and low PUpE (orange and light pink) on low P soil for 2018, 2019, and both years, respectively. The second linear discriminants (LD2), shown in Figure 6 B, E, and H, similarly differentiate between the high and low P soil treatments for the high and low PUpE genotypes in 2018, 2019, and the two trials, whereas the third linear discriminant (LD3), as seen in Figure 6c, f & i, had little discriminatory power. The separation of the genotypes in the density plot of LD1 and LD2 indicated that performance (grain and fodder yield and days to flowering and maturity) of the genotypes was influenced by P availability for the low-PUpE genotypes more than the high-PUpE genotypes. Furthermore, the LDA reveals differences in the contribution of the shoot and root phenotypes in cowpea performance under varying soil P concentrations. The loadings of the variables were used to assess the phenotypic contribution to the discriminant functions for the four PUpE and P levels combinations. The mean loadings of the variables for the field trials are presented in Supplemental Table S10. For LD1 and LD2, which had more clear differences between the four groups (Figures 5 and 6), both shoot and root phenotypes such as days to first flowering, days to maturity, basal root number, and tap root lengths had positive loadings on the LD1, whereas fodder dry weight, days to first flowering, adventitious root growth angle, and tap root length contributed to the LD2. The variable loadings for the pooled data of 2018 and 2019 data are presented in Supplemental Table S11. Variables with positive loadings indicate high contribution to the discriminant functions, similarly, a negative loading means minimal effect of the variable to the discriminant function.

4 | DISCUSSION

4.1 | Seedling root architecture

Cowpea genotypes varied for seedling root architecture, which likely has agronomic impact. For instance, PRL, an indicator of early rooting depth, had an average length of 28 cm but some genotypes had values greater than 41 cm (Supplemental Table S12). Although the connection between greater tap root length and drought tolerance was not a focus of the current study, the large genotypic variance coupled with high broad-sense heritability (77%) suggests selection and breeding for longer seedling tap roots in cowpea is feasible. Heritability from actual field measurements might be much smaller. Measuring PRL at the seedling stage enables recovery of the whole taproot length. One of the few studies on early vegetative stage root length used a pin board technique to compare two cowpea genotypes and found that greater deep root length distribution was related to drought tolerance and proposed root length profile as a drought screening criterion (Matsui & Singh, 2003). A study in common bean found greater yield under drought to be associated with longer seedling taproot length (Strock et al., 2019). In *Brassica napus*, PRL was found to be correlated with seed yield in field experiments (Thomas et al., 2016). Water and nitrate tend to be more available in deeper soil layers over time which confers an advantage to genotypes with deeper root phenotypes (Lynch, 2019). With the increased prevalence and severity of drought, drought tolerance achieved by deeper root length profile distributions, which may enhance deep water acquisition, may become even more important (Lynch, 2019; Lynch & Wojciechowski, 2015; Manschadi et al., 2014; Wasson et al., 2012). Therefore, cowpea

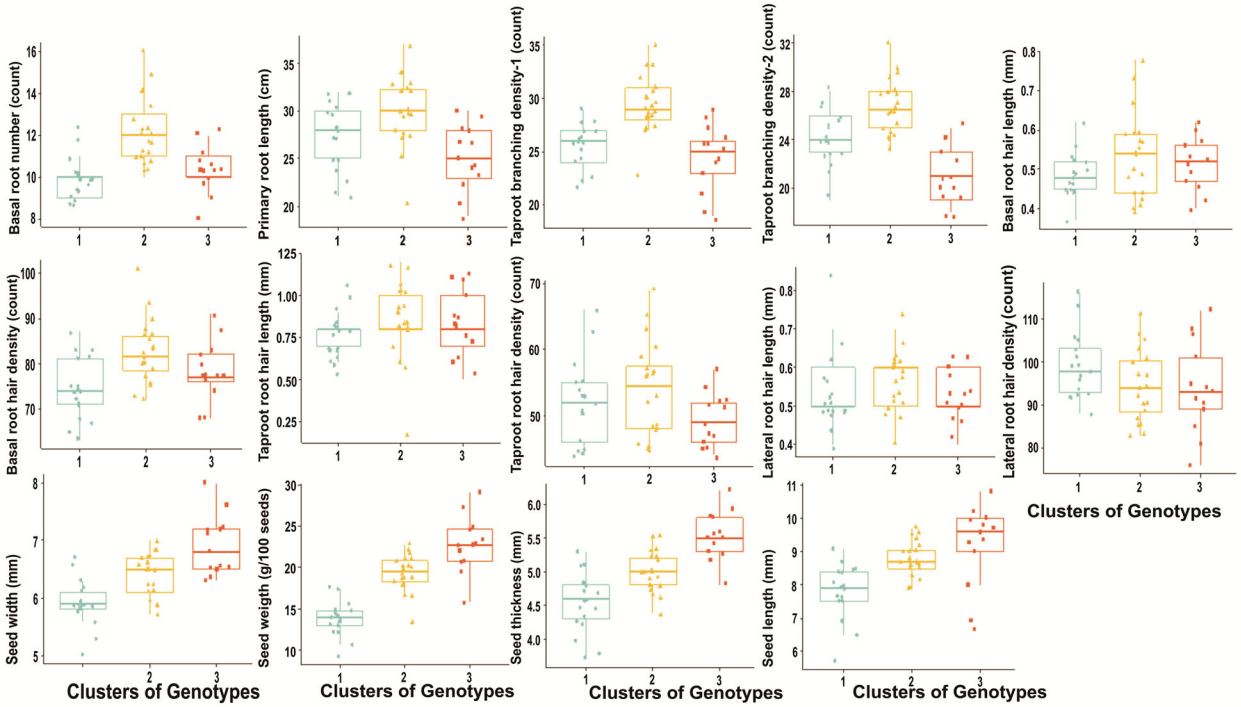

FIGURE 3 Phenotypic clusters. Box plots show median, 25 and 75 percentiles, and whiskers indicate 1.5 times the interquartile range of the variables assessed within each cluster. The *x*-axis shows the cluster numbers as presented in Figure 2. Each point represents genotype best linear unbiased predictor. Data from the seedling root experiment and seed dimension measurement were used

TABLE 4 Pearson's correlation coefficients of seedling root system architecture (RSA), root hairs, and seed dimension phenotypes in cowpea

Phenes[a]	PRL	BRN	TBD2	SDL	SDWTH	SDWT100	BRHL	TRHD
BRN	.3*	–	.4	–	–	–	–	–
TBD1	.4	.6***	.6	–	–	–	–	–
TBD2	.6***	–	–	–	–	–	–	–
SDWTH	–	–	–	.5***	–	.8***	–	–
SDTCK	–	–	–	.5***	.7***	.8***	–	–
SDWT100	–	–	–	.8***	–	–	–	–
TRHL	−.4***	–	–	–	–	–	.7***	.3*

Note. Metrics without significant correlations are omitted (–). BRHL, basal root hair length; BRN, basal root number; PRL, primary root length; SDL, seed length; SDTCK, seed thickness; SDWT100, Weight of seeds, g 100 seeds^{-1}; SDWTH , seed width; TBD2, number of first-order lateral roots on the primary root between 5 and 10 cm from the base of the hypocotyl; TRHD, taproot hair density; TRHL, taproot hair length.

[a]See Table 1 for phene abbreviation. Data from the seedling root experiment and seed dimension measurements were used.

*Significant at the .05 probability level. ***Significant at the .001 probability level.

genotypes with longer PRL may be well-positioned to access water and nitrate in deeper soil horizons in the event of drought.

Seedling RSA phenes such as BRN and TBD1 also had wide ranges that were statistically significant and are likely to be agronomically significant. Genotypes with high scores for these phenes indicate more extensive shallow soil exploration which would favor uptake of P and K (Klinsawang et al., 2018; Lynch, 2019). Genotypes with extreme values for the different phenes could be valuable as parents to create mapping populations for the identification of markers and quantitative trait loci, which could then promote advances involving marker-assisted selection. The utility of phenotypes related to shallow soil exploration, P acquisition and drought tolerance has been demonstrated in common bean (Ho et al., 2005; Miguel et al., 2013). Genotypes with a greater number of shallow basal and adventitious roots were related to enhanced P acquisition (Lynch, 2011). These reports align with the observations of Strock et al. (2019), that greater BRN was related to performance under low fertility conditions

TABLE 5 Pearson's correlation coefficients of seedling root phenotypes (leftmost column) and field derived phenotypes (top row) under contrasting soil phosphorus conditions in 2018 at Kano, Nigeria

	Grain yield		DRYFDWT[b]		ADRN		BRN	
	Soil phosphorus level							
Field derived traits[a]	LP	HP	LP	HP	LP	HP	LP	HP
Seedling RSA phene								
PRL	–	–	–	−0.59*	–	–	–	–
BRHD	–	–	–	–	0.54*	–	–	–
LRHL	–	–	–	–	0.52*	0.51*	0.71**	–
LRHD	0.65**	–	–	–	–	0.48*	0.75**	–
TRHD	–	–	0.45*	0.55*	–	–	–	–

Note. Metrics without significant correlations are omitted (–). Correlations involving seedling basal root number (BRN) and number of first-order lateral roots on the primary root between 2 and 5 (TBD1) and between 5 and 10 cm (TBD2) were not significantly correlated with field phenotypes or performance and are omitted

[a]See Table 1 for phene abbreviation.

[b]DRYFDWT, dry fodder weight; ADRN, adventitious root number; LP, low phosphorus; HP, high phosphorus; RSA, root system architecture; PRL, primary root length.

*Significant at the .05 probability level. **Significant at the .01 probability level.

in more cases than high BRN was related to performance under control, drought, or high-temperature conditions. High BRN may also confer a level of tolerance to biotic stress via root redundancy and be related to performance in compacted or acidic soil conditions that restrict the penetration of the root length into deeper soil horizons (Strock et al., 2019). Similar to Burridge et al. (2016), we observed some cowpea genotypes had well-defined basal roots, usually longer than other first-order lateral roots and found within the 0- to 1-cm region of the base of the hypocotyl (see Supplemental Figure S6), whereas other genotypes did not have a clear distinction between basal and lateral roots. This observation is similar to the description of basal roots by Kahn and Stoffella (1987), one of the earliest reports regarding cowpea root architecture.

One of the objectives of this study was to assess relationships among seedling root phenotypes and mature root phenotypes in the field. Limited correlations were found among seedling and mature phenotypes, but interesting correlations were found involving grain yield and root hair phenotypes (Table 5).

There was substantial variation among root phenotypes and performance across locations. It is likely that variation in root phenotypes and performance was attributable to contrasting climatic and edaphic factors between the locations, especially the amount of rainfall, relative humidity, and temperature. In the present instance, the two locations (Kano and Zaria) where the field trials were conducted had differences in terms of soil type and meteorological indices: rainfall, temperature, and relative humidity. Kano falls in the Sudan savannah with much lower monthly rainfall and relative humidity, and higher monthly temperature compared to Zaria which is in the northern Guinea savannah zone (Supplemental Table S13).

4.2 | Relationships among seedling root phenotypes and seed dimension

Genotypes in Cluster 1 had below-average values for seed dimensions and intermediate seedling root architecture phenotypes (Supplemental Table S12). These small-seeded genotypes are less suitable for breeding programs targeting large seed size, a preferred trait among many cowpea consumers in West Africa (Lucas et al., 2013; Mishili et al., 2009). Genotypes in Cluster 2, with longer PRL and higher BRN, TBD1, TBD2, and intermediate seed size, are prime candidates for breeding programs desiring large-seeded genotypes with root phenotypes related to drought and low fertility tolerance (Strock et al., 2019). Genotypes in Cluster 3 had the highest values for seed dimensions but lower PRL, TBD1, and TBD2. Crossing large-seeded material from Cluster 3 with materials possessing longer PRL and greater branching density may enable co-optimization of deep and shallow soil exploration while retaining desirable seed size.

There are strong preferences for grain size and color among consumers in SSA (Huynh et al., 2015; Ojiewo et al., 2018), and most buyers will pay a premium for large-seeded grains (Lucas et al., 2015; Mishili et al., 2009). New varieties that meet farmer preferences, especially seed attributes, are likely to be adopted faster by smallholder farmers (Mishili et al., 2009; Persley & Anthony, 2017). Seed dimension phenotypes were positively correlated with each other and with seed weight of 100 seeds (Table 4), but no seed dimension phenotype showed correlation with seedling root hair phenotypes, which bodes well for improvement efforts. These finding contrasts with other work which reported an allometric relationship between seed size and early seedling vigor and development (Singh et al., 2017). Seed size was reported to be associated with tolerance to low P conditions (Rothe, 2014)

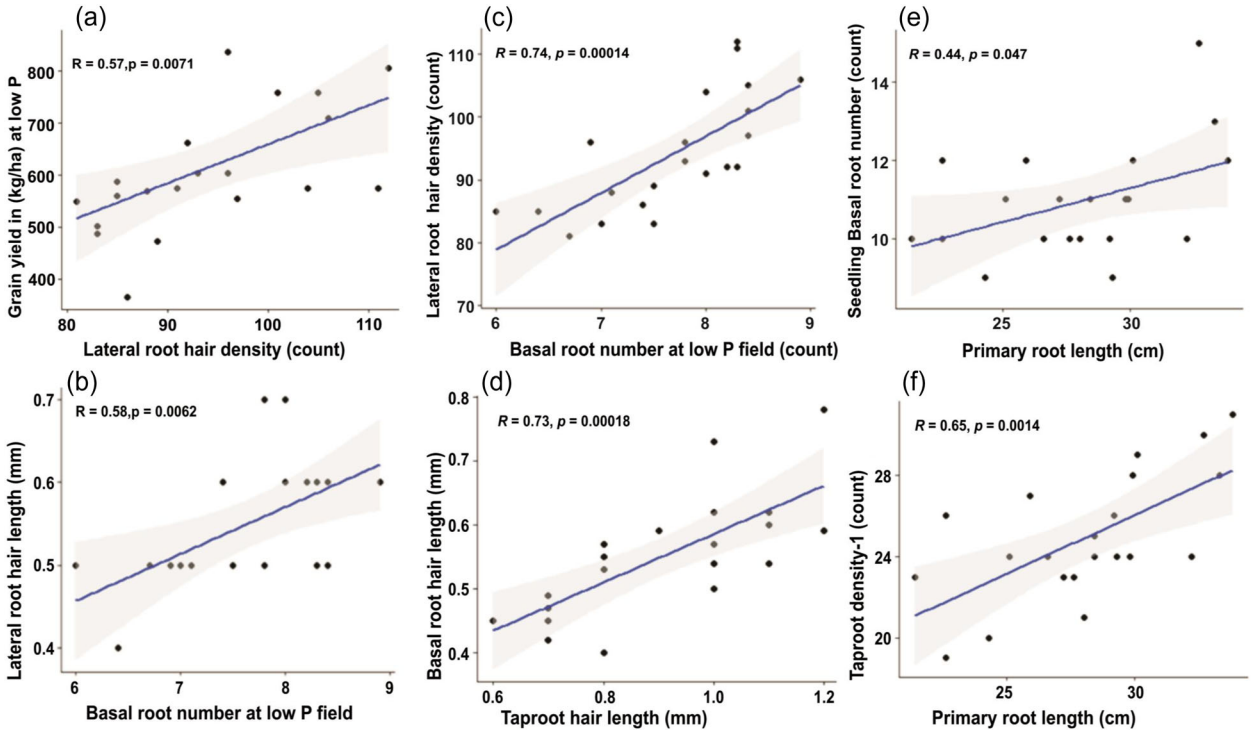

FIGURE 4 Scatterplot showing relationship between pairs of phenotypes from seedling and field phenotyping. Lateral root hair density at seedling stage and grain yield in kg ha^{-1} of field grown plants on low phosphorus (P) field, $R = .57$; $P = .0071$ (a); basal root number of field grown plants on low P field and lateral root hair length (mm) at seedling stage, $R = .58$, $P = .0062$ (b); basal root number of field grown plants on low P field and lateral root hair density at seedling stage, $R = .74$, $P = .00014$ (c); taproot hair length (mm) and basal root hair length (mm) both measured at seedling stage, $R = .73$, $P = .00008$ (d); primary root length (cm) and basal root number both measured at seedling stage, $R = .44$, $P = .047$ (e); primary root length (cm) and taproot density (5–10 cm) both measured at seedling stage, $R = .65$, $P = .0014$ (f). The shaded areas along the regression line indicate the confidence interval, whereas each point represents an average of a genotype. Data from the seedling root experiment and field measurements were used

in cowpea. The differences between these findings may be attributed to difference in the genotypes used and growth conditions. In common bean, seed size and composition has been found to influence the early growth and vigor of seedlings (Singh et al., 2019).

4.3 | Relationship among root hair phenotypes, mature root phenotypes, and yield in low P conditions

For cowpea breeding programs, the highest priority traits that should be used in breeding resilient cultivars for drought-prone and nutrient-deficient environments are greater root hair length and density. It is well established that root hairs play an important role in enhancing P uptake by increasing the absorptive area of the root rhizosphere, and greater root hair length and density has been proposed as a key breeding target (Holz et al., 2018; Lynch, 2011, 2019; Mendrinna & Persson, 2015; Richardson et al., 2011; Simpson et al., 2011). Furthermore, the length and number of root hairs may play a direct role in water acquisition (Segal et al., 2008), enhanced P acquisition (Miguel et al., 2015), may support subsequent deep root growth, and have no observable fitness tradeoffs (Bates & Lynch, 2001). Our results with root hairs are in agreement with earlier work (Krasilnikoff et al., 2003) showing significant variation in root length and root hair length among eight cowpea genotypes grown on low P soil in a pot experiment. Observed root hair length of 0.2–1.2 mm overlaps and exceeds the 0.23- to 0.38-mm length reported earlier; the discrepancy may be due to different germplasm or soil conditions (Krasilnikoff et al., 2003). Root hair density observed in this study ranged from 26 to 161 root hairs mm^{-2} (Supplemental Table S14), similar to wheat and barley cultivars with 20–90 root hairs mm^{-2} (Gahoonia et al., 1997). Root hairs may be related to increased plant–microbial interactions and resultant enhanced mobilization of nutrients from this interaction (Holz et al., 2018). Thus, increasing root hair length and density, especially in environments with limited soil resources, should be a principal breeding objective (Lynch, 2019).

The significant correlation of root hair phenes with BRN, ADRN, and grain yield in low P indicates root hairs may support the growth of other roots in P deficient soils.

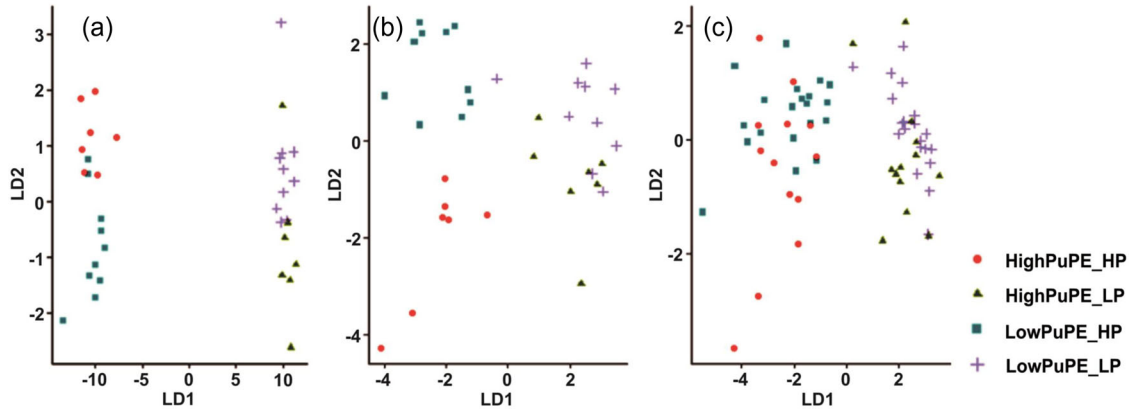

FIGURE 5 Plot of first two linear discriminant (LD) axes separates genotypes by two grouping criteria: phosphorus (P) uptake efficiency and P concentrations of the soil in 2018 field trial (a), 2019 field trial (b), and pooled data of 2018 and 2019 trials (c). PUpE, phosphorus uptake efficiency; HP, high soil phosphorus treatment; LP, low soil phosphorus treatment. Data from field experiments (above and below ground measurements) were used

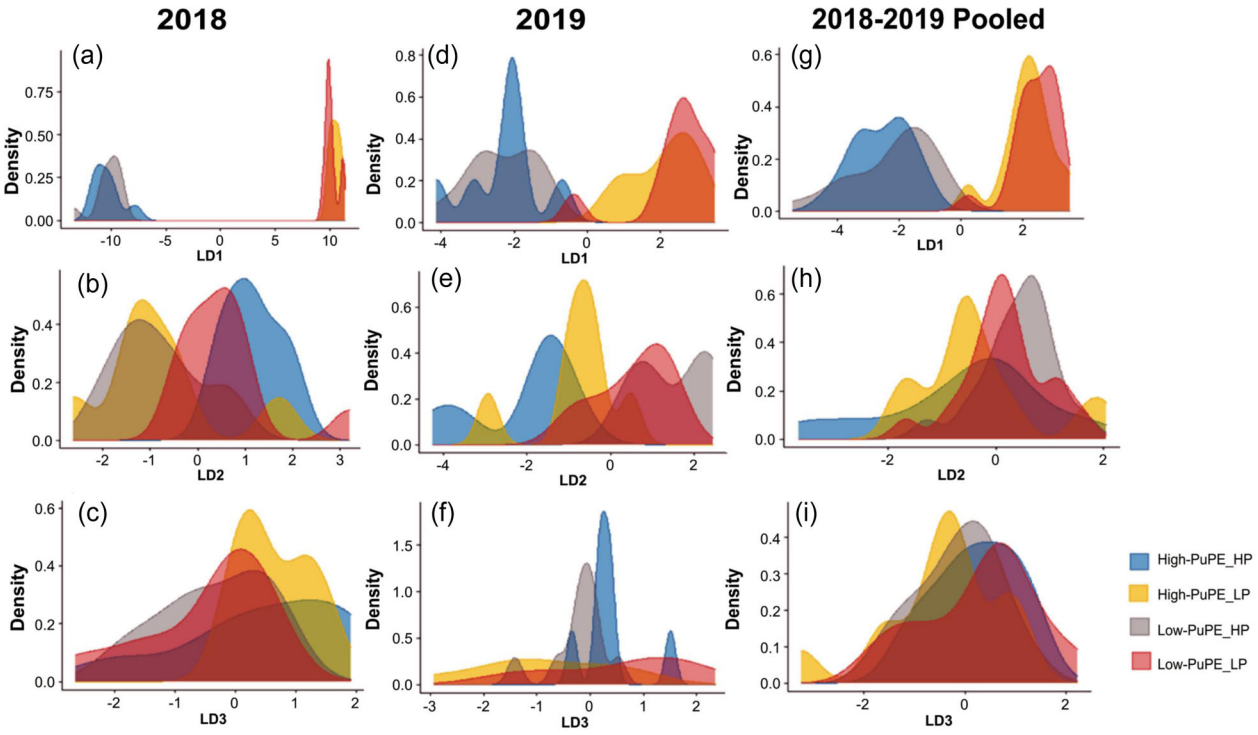

FIGURE 6 Density plots of scores on the three linear discriminants (LD 1–3) using the four groups of high and low phosphorus uptake efficiency (PUpE) genotypes on high and low P soil in 2018 LD1 (a), 2018 LD2 (b), 2018 LD3 (c), 2019 LD1 (d), 2019 LD2 (e), 2019 LD3 (f), 2018–2019 pooled LD1 (g), 2018–2019 pooled LD2 (h), and in 2018–2019 pooled LD3 (i). HP, high soil phosphorus treatment; LP, low soil phosphorus treatment. Data from field experiments (above and below ground measurements) were used

Lateral root hair density was associated with grain yield in low phosphorus, with an R-value of .57 (Figure 4), suggesting genotypes such as Tvu-7778, Sanzi, Danila, IT89KD-288, and IT90K-2772 (see Cluster 1) with high LRHD will be appropriate for increasing grain yield in low P environments. Genotypes such as Vita7, *Yacine*, Tvu-7778, CB27, IT82E-18, IT84S-2246-4, Suvita2, and IT97K-499-35, with high TRHD, more BRN and ADRN in low P soil, and optimum dry fodder weight in both low and high P, may also support increased yields. A negative relationship between PRL and TRHL suggests different parents may have to be used to combine these traits but genotypes such as Vita7, which have greater PRL as well as longer root hair length, are prime candidates for including in breeding programs.

Root hair phenes such as LRHD, LRHL, and BRHD showed correlations with BRN, ADRN, and grain yield under low P conditions suggesting that these phenes may play a crucial role in P acquisition under suboptimal P soils. However, these phenes showed no correlations with mature root phenotypes under nonlimiting P conditions in the same environment. This finding indicates that root hairs are more beneficial for plants under limited P conditions than under nonstressed conditions (Bates & Lynch, 2001). Root hairs have heritability values that could be useful due to positive relationship to yield under low P, low metabolic cost, and straightforward phenotyping protocol making them a promising selection target (Lynch, 2019).

The LDA reveal how cowpea agronomic and RSA phenotypes contribute to P uptake efficiency under different soil P scenarios. The LDA used the linear combinations of the original variables (Supplemental Table S10) to discriminate between the different groups of genotypes (high versus low PUpE on high versus low soil P) and revealed clear differences in the distributions of the linear discriminant scores between high- and low-PUpE genotypes (Figure 5). It is important to note that differences between genotypes with different P uptake efficiencies may not be due to only shoot and root morphological phenotypes, such as root growth angles and taproot length that were measured in this study but may be as a result of differences in other phenes, such as number and density of root hairs, that were measured only at the seedling stage in this study. The differences observed in the distributions of the linear discriminant scores in Figure 6 are clear and highlight structural variances in the data corresponding to high- and low-PUpE genotypes. The variables that emerged as important in the LDA were days to flowering and maturity and root architectural phenotypes (basal root number, adventitious root growth angle, and taproot diameters).

5 | CONCLUSIONS

This study found significant genetic variation in seedling root architecture and root hair phenotypes among cowpea genotypes that may relate to differences in the uptake of water and immobile soil resources like P. Selection for longer PRL and greater BRN, TBD1, and TBD2 may be beneficial for cowpea under drought-prone and low soil P environments. The high degree of diversity for root hairs, moderate to high broad-sense heritability (34 – 77%), and correlation with grain yield in low P soil suggests selecting for greater root hair length and density is feasible and is hereby recommended. Due to the high correlation between root hairs on tap, basal, and lateral roots, we recommend phenotyping root hairs on taproots for breeding programs targeting high P uptake varieties. This study demonstrates that seedling RSA can be effectively phenotyped using cigar rolls, and cowpea RSA is not associated with seed size. Phenotyping cowpea for RSA at the seedling stage offers an opportunity to identify genotypes with desired root architecture for improving water and P uptake for marginal soils.

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AUTHOR CONTRIBUTIONS

Saba B. Mohammed: Conceptualization; Investigation; Formal analysis; Writing-original draft. James D. Burridge: Conceptualization; Formal analysis; Methodology; Writing-review & editing. Mohammad F. Ishiyaku: Investigation; Writing-review & editing. Ousmane Boukar: Investigation; Writing-review & editing. Jonathan P. Lynch: Conceptualization; Funding acquisition; Project administration; Supervision; Writing-review & editing.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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SUPPORTING INFORMATION

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APPENDIX I
R SCRIPTS USED FOR THE LINEAR DISCRIMINANT ANALYSIS

```
install.packages("devtools")
library(devtools)
install.packages("tidyverse")
install.packages("caret")
library(tidyverse)
library(caret)
theme_set(theme_classic())
install.packages("FactoMineR")
library(FactoMineR)
#Load data
ldadata ← read.csv("data_name.csv", header = T)
# Split data into training (80%) and test set (20%)
set.seed(123)
training.samples ← lda_data$Group % > %
createDataPartition(p = 0.8, list = FALSE)
train.data ← ldadata[training.samples,]
test.data ← ldadata[-training.samples,]
# Estimate preprocessing parameters
preproc.param ← train.data % > %
preProcess(method = c("center", "scale"))
# Transform the data using the estimated parameters
train.transformed ← preproc.param % > % predict(train.data)
test.transformed ← preproc.param % > % predict(test.data)
# Fit the model to compute LDA
library(MASS)
model ← lda(Group~., data = train.transformed)
model
plot(model)
#make predictions
predictions ← model % > % predict(test.transformed)
names(predictions)
# Model accuracy
mean(predictions$class = = test.transformed$Group)
###Inspect the results as follows with first 6 members
# Predicted classes
head(predictions$class, 6)
# Predicted probabilities of class memebership.
head(predictions$posterior, 6)
#Linear discriminants
head(predictions$x, 3)
#create the LDA plot using ggplot2:
lda.data ← cbind(train.transformed, predict(model)$x)
lda.data
#Save output to current working dir
write.csv(lda.data, "2018_lda_results.csv")
ggplot(lda.data, aes(LD1, LD2)) +
geom_point(aes(color = Group,shape = Group))
```