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Research article

Analysis of Seedling Root Architecture of *Vigna unguiculata* (Sampea 1-20) Using Cigar-Roll Method under Controlled Condition

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Abstract
Cowpea (*Vigna unguiculat*a) varieties, including the improved SAMPEA series in Nigeria, remain crucial for food security, particularly in sub-Saharan Africa. However, their yields are constrained by edaphic stresses such as low phosphorus (P) and drought. Phenotyping cowpea seedling root architecture using the cigar-roll method is crucial for identifying root phenes needed for breeding phosphorus-efficient and drought-tolerant varieties. This study evaluated the Root System Architecture (RSA) of 20 elite cowpea genotypes (SAMPEA 1 to 20) obtained from the Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria, using the cigar-roll method. Significant ($p<0.05$) phenotypic variation was detected for all phenes evaluated. Significant variation was observed in architectural traits, including primary root length and lateral root density in the first 5cm of the root. Root hair length and density were positively associated with lateral root density in both the first 5cm from the base of the hypocotyl and the lateral roots between 5 and 10 cm from the base of the hypocotyl. Genotypes with longer root hairs (SAMPEA 14, 20, 12 and 13) and a longer taproot (SAMPEA 10) were identified as potential parents for breeding. The identified phenes could guide in breeding efforts for cowpea varieties suited to low phosphorus and water-limiting soils. Our results suggest that the root phenotype plays an important role in cowpea growth in suboptimal environments and can serve as a marker to facilitate the development of improved varieties with enhanced yield, particularly in sub-Saharan Africa, where soils with low phosphorus and drought are predominant.

Keywords: Cowpea; Root phenotyping; Phosphorus efficiency; Drought tolerance; Root

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) plays a vital role as a food legume, serving as a crucial element in cropping systems within arid regions and marginal areas across Asia and Oceania, the Middle East, southern Europe, Africa, southern USA and Central and South America (Snapp *et al*., 2018; Ojiewo *et al*., 2018). Nigeria is the largest producer of cowpea in the world, accounting for 45% global cowpea production. In 2021, Nigeria produced 3.63 million metric tonnes of cowpea across 4.7 million hectares (Chibuzo *et al*., 2024). With a grain protein content of approximately 25%, cowpea serves as a key nutritional source for urban and rural populations with limited access to meat and dairy products (Bialostosky *et al*., 2002). Given its favourable marketability and extended shelf life, cowpea holds the potential to significantly impact sustainable livelihoods and food security in Nigeria. Moreover, cowpea contributes substantially to sustainable agriculture and agroecosystem productivity through biological nitrogen (N) fixation (Martins *et al*., 2003). Nevertheless, cowpea yields in Nigeria face significant challenges from abiotic stresses, particularly drought (Sakariyahu *et al*., 2023), heat (Indabo *et al.*, 2023) and poor soil fertility (Ahmed *et al*., 2023) and biotic stresses such as aphid

(Aliyu *et al.*, 2023). These challenges are exacerbated by factors such as soil degradation, population pressure, and the effects of climate change (Yadav *et al.,* 2018). In the realm of African cowpea cultivation, a notable genetic diversity prevails. However, imperative strides must be taken to elevate crop performance, as a substantial gap persists between the current and potential yields (Gabriel *et al*., 2021). Plant root systems are associated with changes in root exploitation of the soil volume and nutrient-rich patches (Lynch, 2007). Access to soil water and phosphorus of the plant is determined by root system architecture (Lynch, 2013; Mohammed *et al*., 2021). However, less consideration is given to the root morphological features and their relationships with economic yield and morphological characters under soil moisture stress conditions in cowpea. Hence, an understanding of the root system's contribution to the diversity and performance of cowpea by shovelomics—an under-exploited trait-based selection breeding strategy focused on linking specific root traits to efficient resource acquisition—is essential (Cattivelli *et al*., 2008).
Genotyping is essential for crop improvement by adopting the cigar roll method. The cigar-roll method reduces the cost and constraint associated with phenotyping root system architecture of field-grown plant (Thomas *et al*., 2016; Strock *et al*., 2019). It serves as an alternative to shovelomics, offering a controlled laboratory approach for studying seedling root architecture. This method involves rolling the seeds on a substrate such as germination paper, filter paper or even cloth, which allows for the visualization and measurement of the developing root system. It allows for the assessment of root length, root hair length and density, tap root length and lateral root development. This technique provides a more controlled and precise environment compared to shovelomics (Iyer *et al*., 2010; Clark *et al*., 2013; Adu *et al*., 2014). This phenotyping method provides valuable insights into the genotypic and phenotypic variability of cowpea root system architecture (Mohammed *et al*., 2021). The study therefore aimed to phenotype cowpea seedling root architecture using the cigar-roll method, with the goal of elucidating the variation in root systems among the selected cowpea genotypes. This information would be vital for breeding programs aiming to enhance yield in marginal environments.

Materials and Methods

Sample Collection and Study Area

Twenty cowpea improved varieties (SAMPEA 1-20) were obtained from cowpea unit laboratory, Department of Plant Science, Institute for Agricultural Research (IAR), Ahmadu Bello University (ABU), Zaria (11°9'7"N 7°38'18"E). The experiment was conducted in the same laboratory.

Seed Treatment and Cigar-Roll Application

The Miguel *et al*. (2015) "cigar-roll "method of seedling phenotyping was used. Cowpea seeds were surface sterilized with 0.5% sodium hypochlorite (NaClO) mixed with 0.1% Captan (fungicide), for one minute, decanted, and rinsed twice with distilled water. To prepare 10 litres of the stock solution which is the growing medium, 0.86 g of $CaSO_4$ was dissolved in 10 litres distilled water. A moist tan 'regular weight' seed germination paper was placed on a sterile tray and wetted with $CaSO_4$ solution. Five (5) seeds were randomly selected from the bulk and arranged from the top of 37.6 cm long piece germination paper with the micropyle of the seeds facing up; Afterwards, the germination paper was rolled into a moderately tight "cigar-roll" configuration and placed in a beaker containing 2 L $CaSO_4$ solution. The beaker was placed in an incubator chamber for 72 hours at a temperature of 30°C after which it was moved into a locally constructed light chamber made in Nigeria. After a period of 14days, the rolls (diameter of 6 mm and cross-sectional area of approximately 28.27 mm²) were removed and unrolled. Healthy and well-developed seedlings were selected from the total number of seedlings for root system analysis. The following traits were measured; primary root length, basal root number and lateral root density.

Seedling Root Architecture Phenotyping

The cowpea genotypes were assessed for Primary Root Length (PRL), Basal Root Number (BRN) defined as the count of first-order lateral roots within 1 cm of the base of the hypocotyls, the number of first-order lateral roots on the primary root between 1 and 5 cm from the base of the hypocotyls which is known as the Lateral Root

Density 1 (LRD1) and the number of first-order lateral roots on the primary root between 5 and 10 cm from the base of the hypocotyl of the tap root also known as the Lateral Root Density 2 (LRD2). The seedlings were spread on a flat tray and the length was manually measured with a ruler, while visual counts were made of the various root classes and root number per zone.

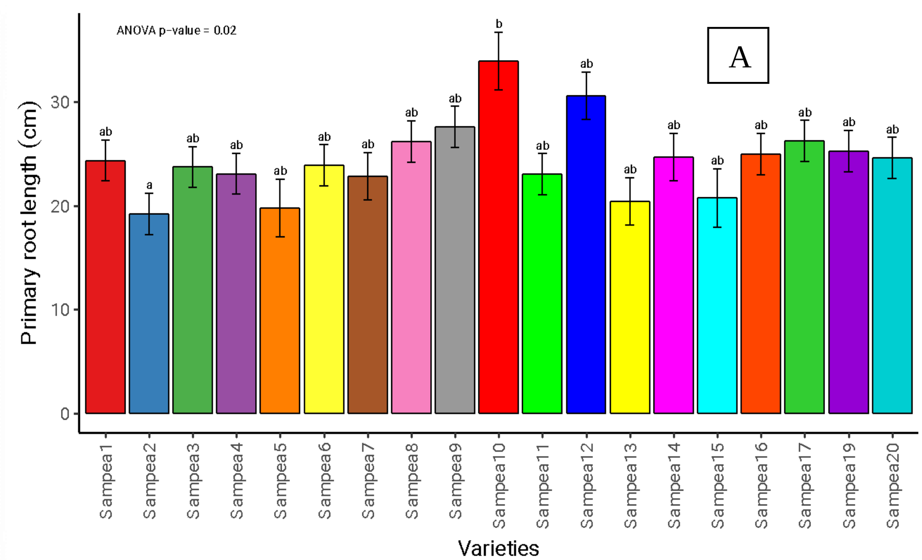
Data Analysis

One way Analysis of Variance (ANOVA) was used to compare the means of genetic parameters of the seedling root traits (PRL, BRN, LRD1, and LRD2). Where significant differences were observed, Tukey's Honestly Significant Difference (HSD) test was used to separate the means. Additionally, Pearson's correlation coefficient was employed to analyze the relationships between selected root traits.

Results

Root phenotypes showed significant variation among the cowpea genotypes ($p < 0.05$). There were wide ranges for some root system architecture phenes such as Primary root length (19.23 to 33.95cm), Basal root number (21.67 to 31.29 roots), Lateral root density 1 (21.67 to 39.71 roots), Lateral root density 2 (17.29 to 34.50 roots). The 20 cowpea genotypes evaluated at early stage for root characteristics showed significant variations for parameters like Primary Root Length ($p = 0.02$) and Lateral Root Density at first 5 cm of the root ($p = 0.00$). Figure 1 and 2 shows the significant variation present among the varieties (SAMPEA1-20) for Primary Root Length (PRL) and Lateral Root Density at first 5 cm of the root ($p = 0.00$). The average PRL among the lines is 24.61cm and ranged from SAMPEA2 (19.23cm) to SAMPEA10 (33.95cm). LRD1 has an average of 39.71 and ranged from SAMPEA5 (21.67) to SAMPEA14 (39.88). There was no significant difference among the cowpea genotypes for Basal Root Number (BRN) from 5 to 10 cm of the root ($p = 0.34$).

Figure 3 shows the phenotypic correlation coefficient among the seedling root traits, which explains the association between any two traits. In a breeding programme, there is a need to know how traits are associated, to determine whether the traits can be bred for simultaneously or independently, traits that are positively correlated may be bred for simultaneously while traits that are negatively correlated may be bred for independently. The result revealed positive and significant ($p \leq 0.05$) correlation between LRD1 and LRD2 ($r = 0.48$), LRD1 and BRN ($r = 0.35$). There was no significant association among the other traits, so they were not reported.



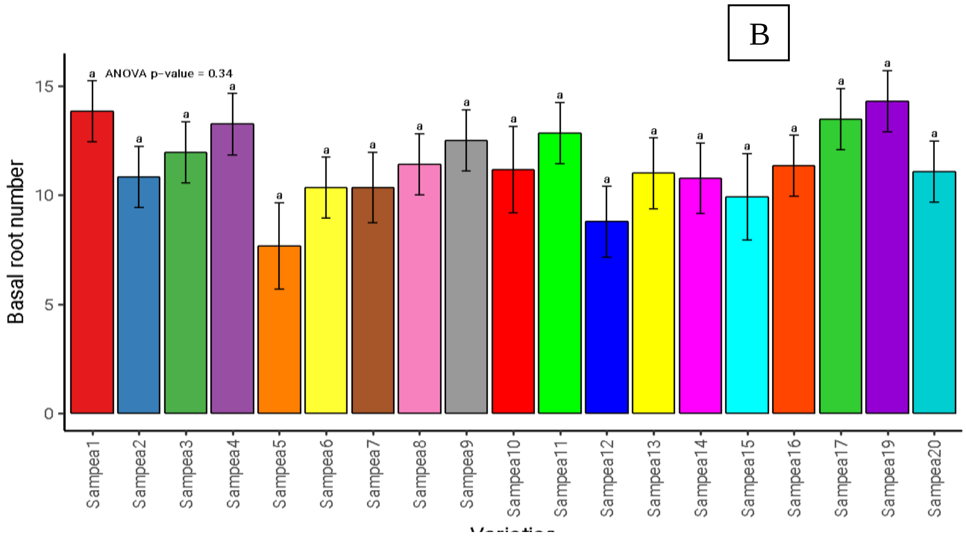
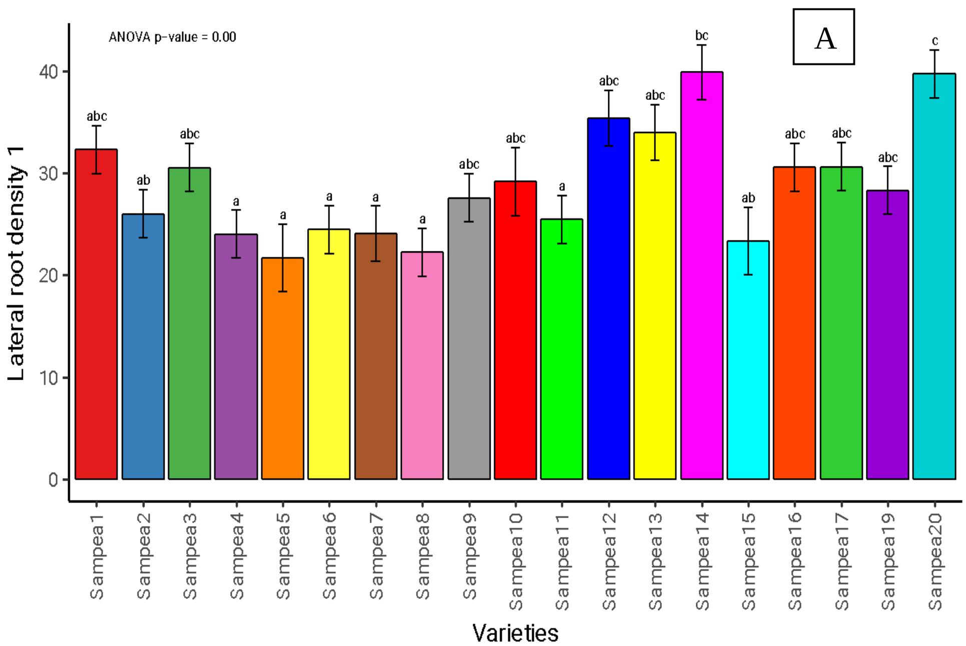


Figure 1: Primary Root Length (A) of SAMPEA (1-20) improved cowpea genotypes
Basal Root Number (B) of SAMPEA (1-20) improved cowpea genotypes



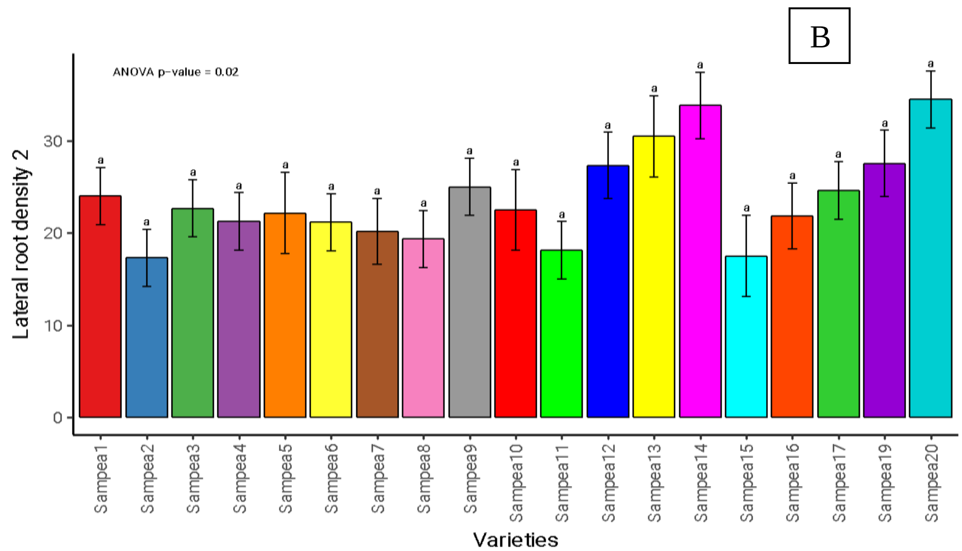


Figure 2: Lateral Root Density 1 (A) of SAMPEA (1-20) improved cowpea genotypes
Lateral Root Density 2 (B) of SAMPEA (1-20) improved cowpea genotypes

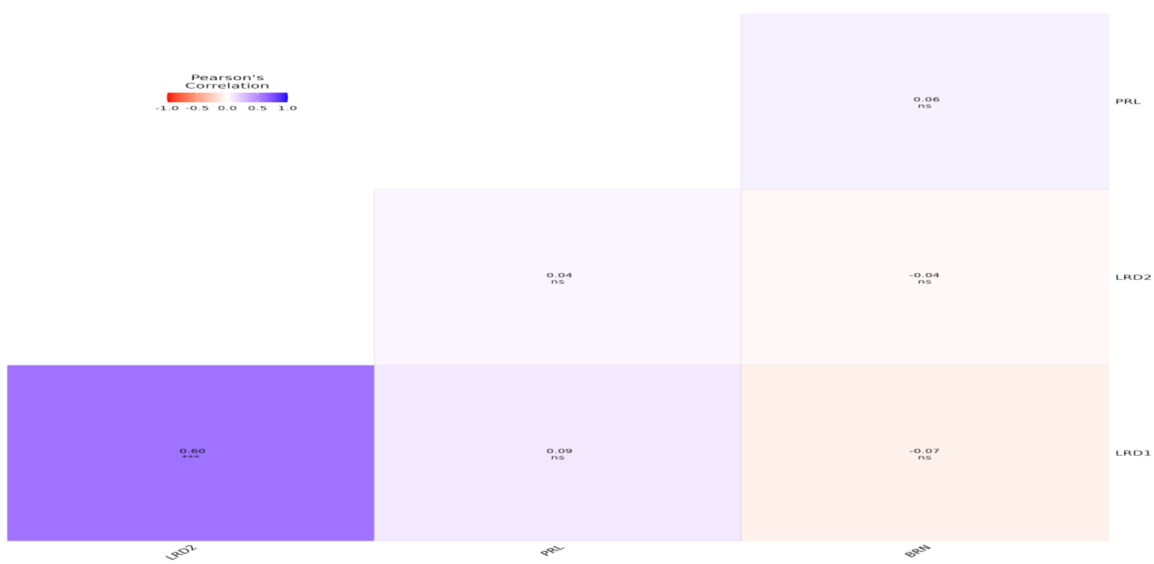


Figure 3: Phenotypic correlation coefficients among the seedling root traits obtained using the cigar-roll method in SAMPEA 1-20 cowpea genotypes

DISCUSSION

Root architecture plays a crucial role in plant growth and development. It influences a plant's ability to anchor itself in the soil, absorb water and nutrients, and store energy reserves. In particular, root architecture is critical for plant stress tolerance, especially in the face of drought and nutrient deficiencies (de Dorlodot *et al*., 2007). In this study we observed that root architectural phenes can be rapidly phenotyped at the seedling stage in cowpea. These architectural phenes are under some degree of genetic control. Significant genetic variation ($p \leq 0.01$) exists for root architectural phenotypes in cowpea and gene pools have distinct root architecture.

Plants with deeper and more extensive root systems such as SAMPEA10 and SAMPEA12 are generally more drought-tolerant (Strock *et al*., 2019; Lynch, 2019). This is because deeper roots can access water from deeper soil layers, even when the surface is dry (Mohammed *et al*., 2021). Additionally, a higher density of lateral roots increases the surface area for water uptake, and enhances drought tolerance (Tatsumi *et al*., 2019). Phosphorus is often in low abundance in soils, making it a limiting factor for plant productivity. Plants with higher lateral root densities are more efficient at extracting phosphorus from the soil. This is because lateral roots have a larger surface area for nutrient uptake, allowing them to scavenge phosphorus from even the most depleted soils (Tatsumi *et al*., 2019).

Previous studies have highlighted essential cowpea root traits crucial for efficient soil phosphorus (P) acquisition and utilization (Kugblenu *et al*., 2014). Similarly, the advantages of deep root system have been noted in mitigating drought conditions (Agbicodo *et al*., 2009; Matsui and Singh, 2003). A study on common beans revealed that increased drought resistance is linked to longer seedling taproot length (Strock *et al*., 2019). Deeper soil layers tend to provide more water and nitrate over time, giving an advantage to genotypes with extended root phenotypes (Lynch, 2019). Given the escalating frequency and intensity of drought, the importance of drought tolerance through extended root profiles, facilitating deep water absorption, is likely to grow (Wasson *et al*., 2012; Manschadi *et al*., 2014; Lynch and Wojciechowski, 2015; Lynch, 2019). Consequently, cowpea genotypes with longer Primary Root Length (PRL) may have an advantageous position for accessing water and nitrate in deeper soil layers during drought.

Seedling Root System Architecture (RSA) traits like BRN and LRD1 show significant variability, indicating potential agronomic importance. High scores for these traits suggest extensive shallow soil exploration, favoring phosphorus (P) and potassium (K) uptake (Klinsawang *et al*., 2018; Lynch, 2019). Genotypes with extreme values for these traits could serve as valuable parents for creating mapping populations to identify markers and quantitative trait loci, facilitating advances through marker-assisted selection. The efficacy of phenotypes linked to shallow soil exploration, P acquisition, and drought tolerance has been demonstrated in common beans (Ho *et al*., 2005; Miguel *et al*., 2013). Genotypes (SAMPEA 14 and SAMPEA 20) with a higher number of shallow basal and adventitious roots have been associated with improved P acquisition (Lynch, 2011), aligning with findings that greater LRD1 is more consistently linked to performance under low fertility conditions (Strock *et al*., 2019).

Conclusion

In conclusion, this study revealed significant variations in root system architecture among cowpea varieties, with SAMPEA 10 exhibiting the longest primary root length (PRL) and SAMPEA 2 showing the shortest. The longer PRL of SAMPEA 10 suggests enhanced drought tolerance, enabling it to access water from deeper soil layers during water-scarce periods. Meanwhile, SAMPEA 14 demonstrated the highest lateral root density (LRD1), indicating efficient phosphorus uptake from shallow soil layers. These findings highlight the potential of SAMPEA 14 for developing cowpea varieties with improved phosphorus acquisition efficiency. The 'Cigar-rolls' method proved effective in phenotyping seedling root system architecture, offering a valuable tool for identifying desirable root traits in cowpea

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