Identifying key contributing root system traits to genetic diversity in field-grown cowpea (*Vigna unguiculata* L. Walp.) genotypes

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ABSTRACT

Cowpea is a grain legume that is grown extensively as an alternate protein and income source for many smallholder farmers. Characterising cowpea root phenotypes could provide the basis for developing genotypes with root system architecture (RSA) traits that increase soil resource acquisition. Measuring RSA traits of any field crop is a demanding task, in terms of expediency, time, cost, and competence. Targeted root phenotyping strategies focusing on a few relevant traits might aid in side-stepping some of the challenges associated with phenotyping roots of field crops. The objectives of this study were to (i) measure genotypic variation for RSA and shoot traits of cowpea genotypes; and (ii) identify candidate variables and genotypes that contribute the largest share of variance. Sixty cowpea accession were grown in field trials at the University of Cape Coast, Ghana. Seventy variables, mostly quantitative RSA traits were measured. Multivariate analysis was used to determine major traits contributing to variation. There were significant differences ($P < 0.05$) for the majority of traits evaluated. Fifty-nine traits had coefficients of variation of ≥ 0.3 among genotypes and were selected for further examination. Broad-sense heritability (H^2) estimates were generally intermediate to high and ranged from 0.11 to 0.96. The Shannon-Weaver diversity index (H') was variable among traits and ranged from 0.00 to 0.88. Shoot and root biomass correlated closely and positively with count- and length- and diameter-related traits. Cluster analysis identified three homogeneous genotype groups and identified groups of cowpea genotypes that could be exploited in breeding programs to improve the genetic basis of root traits. The first nine principal components explained over 74% of total genotypic variation for the twenty-nine traits included in the PCA. Sixteen traits contributed more than other traits to the variability in PC1 and PC2. Soil and root tissue angle-related traits, shoot and root diameter-related traits, root biomass, hypocotyl root length, root count and lateral root density -related traits were among the top 50% of the most important traits contributing to variation and thus warrant consideration in efforts to breed for improved genotypes in cowpea. The workflow presented offers a robust, cost-effective and simple approach to identifying focal root traits that contribute to diversity in grain legumes. The results could potentially facilitate the characterization of specific traits suitable for targeted genotype selection and breeding of new cowpea varieties for efficient RSAs.

1. Introduction

Food legumes such as cowpea (*Vigna unguiculata* (L) Walp) are major food security and income generation crops in several regions of the tropics and sub-tropics (Carlos, 2000; Tharanathan and Mahadevamma, 2003). In Ghana and many areas of sub-Saharan Africa (SSA), cowpea offers affordable source of proteins, especially to the poor. It has been reported that the consumption of legumes, especially

cowpea, is increasing in Ghana (Legume market analysis Ghana, 2012). Due to its good marketability and shelf life, cowpea can be crucial to sustainable livelihoods and food security in these regions. In addition, cowpea can contribute substantially to sustainable agriculture and agroecosystem productivity through biological nitrogen (N) fixation (Martins et al., 2003; Ehlers and Hall, 1997).

Even though there is a wide genetic diversity in cowpea in Africa, there is a need for improvement in crop performance because the gap

between actual and potential yield is still large. In Ghana for instance, the average farm yield of 0.4-0.6 t ha^{-1} is still lower than the 1.6-2.5 t ha^{-1} recorded on research fields (Yirzagla et al., 2016). Plant root systems are critical to the acquisition of soil resources. Root architectural changes are associated with changes in root exploitation of the soil volume and nutrient-rich patches (Lynch, 2007). Hence, an understanding of the root system's contribution to the diversity and performance of cowpea genotypes is essential for crop improvement. Although the impact of genetic and phenotypic variation of cowpea RSA traits on performance has been sparsely explored (Burridge et al., 2016, 2017; De Barros et al., 2007), considerable genetic diversity in cowpea for RSA traits related to growth in nutrient-poor and dry environments have been reported (Krasilnikoff et al., 2002, 2003; Matsui and Singh, 2003; Singh et al., 2002). Previous studies have identified cowpea root traits that are crucial for soil phosphorus (P) acquisition and use efficiency (Kugblenu et al. 2014). Similarly, deep root systems have been reported to be beneficial under drought conditions (Agbicodo et al., 2009; De Barros et al., 2007; Matsui and Singh, 2003), albeit there might be some additional construction and maintenance costs to plants which invest in deeper roots under conditions of limited additional water (Hall, 2012). Exploration of these genetic diversities and attendant variations in plasticity responses in RSA traits among cowpea could support breeding for improved resource acquisition/use efficiency.

Visualizing and measuring root systems for further exploitation of RSA traits in conventional breeding is challenging. Root systems are dynamic so, many phenotypic RSA features are transiently expressed leading to extreme variability in RSA for the same genotype (Orman-Ligeza et al., 2014). Environmental factors strongly influence and determine many aspects of RSA, frequently leading to significant genotype-by-environment (G x E) and genotype-by-environment-by-management interactions (G x E x M) (McCully, 1999; Orman-Ligeza et al., 2014). Soils are heterogeneous and opaque and as a result, field screening of many aspects of RSA is currently not possible. Moreover, appropriate phenology is linked with agro-climatic studies (Gregory, 2008). Thus, the measurement and exploitation of RSA traits in breeding must be well-targeted and relevant to specific environments to improve effectiveness and reduce cost (de Dorlodot et al., 2007). Considerable strides have been made in quantifying RSA of grain legumes. "Shovelomics", the excavation of the root crown from the soil (Burridge et al., 2016; Trachsel et al., 2011), has been instrumental, albeit the approach may be limited by some loss of fine roots. In previous studies that have quantified shoot and RSA traits in cowpea and other grain legumes, diverse variables were measured including: collar diameter, leaf area, shoot and root biomass, root-shoot ratio, and root volume (Ogbonnaya et al., 2003), root surface area, root-diameter root length and growth (Adepetu and Akapa, 1977; Krasilnikoff et al., 2003), root growth rate (Horst et al., 1983), root hair length (Krasilnikoff et al., 2003), branching frequency or root length density (Petrie and Hall, 1992), root growth angles and gravitropism (Bonser et al., 1996; Liao et al., 2006). Others have measured traits such as basal root growth angle (BRGA), basal root whorl number (BRWN) and hypocotyl root number (Burridge et al., 2016; Ho et al., 2005; Liao et al., 2006), and nodulation (Kopittke et al., 2007). More recently, robotic imaging tools and analysis pipelines have also enabled high-throughput visualization, quantification and analysis of diverse RSA traits of many crops including grain legumes (Galkovskyi et al., 2012).

Among the array of traits that have been explored to study genetic diversity in grain legumes, it is not clear which traits contribute most to the phenotypic variation among genotypes or the differences in root phenotypes that exists in a population. It is possible that many of the traits that have been measured are proxies of others measured during the same studies or are mutually correlated and thus measure the same construct (Adu et al., 2018; Bodner et al., 2013). Whilst plant breeders in resource-limited jurisdictions could benefit from a low-cost, field-based phenotyping platforms in understanding G x E and G x E x M

interactions, this benefit could be eroded by measuring multiple non-pertinent RSA traits. If most of the variability of RSA could be accounted for by a few traits identified through robust statistical approaches (de Dorlodot et al., 2007), then it would be unnecessary to measure several traits which are essentially redundant (Adu et al., 2018; Bradshaw et al., 2009; Wishart et al., 2013). The current study aims to contribute to addressing this problem for a collection of field-grown cowpea genotypes by (i) assessing genetic diversity in RSA traits and (ii) identifying candidate traits that contribute the largest share of the variance in the original, multivariate dataset.

2. Materials and methods

2.1. Plant material, soil and environmental conditions

Seeds of sixty cowpea (*Vigna unguiculata* (L) Walp) genotypes were obtained from the International Institute of Tropical Agriculture (IITA, Nigeria) and used in this study. The passport data of these genotypes can be found in Supplementary Table S1).

Field trials were conducted at two adjacent sites (about 5 m apart) at the Teaching and Research Farm of the School of Agriculture, University of Cape Coast (UCC; 5º 06 N, 1º 15' W) between June and September in 2016 and 2017 under rain-fed conditions. The seeds were sown on 7th June 2016 and on 9th June 2017. The study sites experience two seasons of rainfall with a peak in May to June and the minor in October, with dry periods (harmattan) experienced between November and February. Precipitation recorded were 631.2 and 437.6 mm for the 2016 and 2017 cropping periods, respectively. The average temperatures were 23.8 and 25.8 °C and the average relative humidity was 88.6 and 84.4% for the 2016 and 2017 cropping periods, respectively. Day length at the experimental site ranges from approximately 11.30 to 12.40 h while solar radiation ranges from 3151 kJ cm^{-2} day^{-1} to 3804 kJ cm^{-2} day^{-1}, respectively (Adu et al., 2017). The soil was a haplic acrisol with a sandy loam textural class, composed of 30.2, 56.3 and 13.5% clay, sand and silt, respectively and was typical of arable soils of the coastal savannah agro-ecological zone of the Central region of Ghana. The soil had a pH of 5.7, 1.31% organic carbon, 0.11% total nitrogen (N), and 51.1 µg phosphorus (P) g^{-1}. In addition, the soil had a cation exchange capacity (CEC) of 6.23 cmolc kg^{-1}, 3.33 cmolc kg^{-1} calcium (Ca), 0.89 cmolc kg^{-1} magnesium (Mg), and 0.62 cmolc kg^{-1} K. Vegetables, including cucumber (*Cucumis sativus* L.) and bell pepper (*Capsicum annuum*) had previously been cropped on the site but had been lying fallow for a year before the current experiment.

2.2. Field establishment and root excavation

Two independent trials were conducted and were each arranged in Randomized Complete Block Design (RCBD) with two blocks. In both trials, each plot consisted of a two 1.2 m rows per genotype. Seeds were sown to a non-ridged experimental field ploughed and harrowed to a depth of about 30 cm. The seeds were manually sown with a long wooden seed dibber at approximately 5 cm below the soil surface, 40 cm apart in row and 70 cm between rows. Excavation of root crowns followed the protocol of Burridge et al., (2016). Root crowns were excavated from both experiments at anthesis, occurring primarily between 45 and 60 days after planting (DAP) for most genotypes. Four plants per plot were dug with a standard spade at about 25 cm around the stem and at a depth of 30 cm, being careful not to disrupt the soil cylinder agglutinated to the excavated root while lifting into a basin filled with water. The plants together with the roots were soaked in water for approximately 5–8 minutes. They were then removed and massaged carefully in a clean tap water, and shaken cautiously to remove soil still adhered to the roots. The plants were finally moved into a large basin of clean water to rinse the roots of remaining soil particles and other debris. Root crowns from two representative samples of the

four excavated samples were subsequently imaged using a non-reflective black background with a Canon EOS 70D DSLR camera (https://www.usa.canon.com/) held stationary on tripod 0.6 m above the roots.

2.3. Evaluation of root traits

Two types of data from the excavated roots, were taken in this study: data measured directly on the roots; and data determined from the root images using image analysis software. For the direct measurements, eleven traits including shoot diameter (SDIAM), shoot dry weight (SDW), root dry weight (RDW), hypocotyl root length (HRL), lateral root number (LRNo), lateral root diameter (LRDIAM), lateral root branching density (LRBDens), primary root diameter (TRDIAM), primary root branching density (TRBDens), diameter of nodules (NodDIAM) and number or abundance of root nodules (NodAbun) were measured on the root crowns of the two representative samples of each genotype for each of the two trials. Shoot DW and RDW were recorded after oven-drying of shoots and roots at 70 °C for 72 h. Diameters of various root categories were from a mean of three (3) randomly selected roots and were measured with digital calipers 2 cm from the origin of respective roots. Diameter of root nodules were from a mean of three (3) randomly chosen nodules and was measured with a caliper along the widest point of the nodule if the nodule was not symmetrical. Shoot diameter was measured 2 cm above the root-shoot junction. Branching density for each category of root was determined within 3–5 cm distance and 1 cm from the origin of the root and it was assumed that the branching density was constant for the rest of the main axis of the root. Hypocotyl RL was determined using a mean of three randomly chosen roots measured with a ruler. All root nodules were manually counted to determine NodAbun. Units of the nine manually measured RSA traits and the two shoot-related traits, (i.e. SDIAM and SDW) are presented in Table S2. For analysis of the root images, the DIRT platform (Bucksch et al., 2014) was used. The DIRT platform enables the extraction of traits that cannot be assessed with the 'Shovelomics' approach (Burridge et al., 2016) such as root tip diameter, root tissue angle (RTA), soil tissue angle (STA), the so-called D values (cumulative width at a certain fraction of the maximal root depth), and their associated DS values (the slope of the tangent at each D value), among many others (Bucksch et al., 2014). The shovelomics root trait estimation procedures described in Trachsel et al. (2011) assume that root angles of excavated mature field-grown plants are preserved. Table S2 summarizes and defines abbreviations for all traits extracted from root images using the DIRT platform.

2.4. Statistical analysis

The data for the two trials were combined to determine descriptive statistics, including mean ($\bar{x}$), standard deviation (σ) and the coefficient of variation (CV). General analysis of variance was performed for genotype main effects; non-significant variation in a trait between genotypes was used as an initial basis to exclude traits from further analysis. Also, only traits with CV values ≥ 0.3 were selected for further analysis (Chen et al., 2016). The $\bar{x}$ and σ of each trait were used to classify genotypes intro three different categories (n) according to their trait values including: (i) genotypes with low shoot/root trait values of less than or equal to the difference between the mean for that trait and its standard deviation ($\leq \bar{x} - \sigma$); (ii) genotypes with average shoot/root traits values of less than or equal to the sum of the mean for that trait and its standard deviation ($\leq \bar{x} + \sigma$); and (iii) genotypes with high shoot or shoot/root trait values of greater than or equal to the sum of the mean for that trait and its standard deviation ($\geq \bar{x} + \sigma$) (Kumar et al., 2012). The frequency data of the low, medium and high trait values was used to calculate the Shannon–Weaver diversity index (H') for each selected trait as described by Kumar et al. (2012) (Eq. (1)).

$$H' = \sum_{i=1}^{n} P_i \log P_i \tag{1}$$

where: P_i is the ratio of individuals in the ith class of an n–category and n is the number of phenotypic classes for a given character, three (3) in the present study (Kumar et al., 2012). Traits with H' values ≥ 0.3 were selected for principal component analysis (PCA) to identify determinants of root architecture variability across the cowpea genotypes.

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, blocks, trials, and selected interaction effects. All factors were classed as random factors in REML so that the proportional contribution of genotype to overall variation in traits could be determined (Adu et al., 2018). Both REML and ANOVA employed the following model (Eq. (2)):

$$y_{ijk} = \mu + g_i + t_j + gt_{ij} + tb_{jk} + gtb_{ijk} + \varepsilon_{ijk} \tag{2}$$

where: y_{ijk} represents the observation from the ijk^{th} genotype, trial and block, μ is the overall mean, g_i is the effect of the ith genotype, t_j is the effect of the jth trial, gt_{ij} is the interactive effect of the ith genotypes with the jth trial, tb_{jk} is the interactive effect of the jth trial with the kth block, gtb_{ijk} is the interactive effect of the ith genotype with the jth trial and the kth block and ε_{ijk} is the experimental error.

Broad-sense heritability (H^2) across trials was estimated as the quotient of the estimated variance associated with the genotypic effect and the total phenotypic variance for the trait (σ_g^2 / σ_p^2) (Adu et al., 2014). The phenotypic variance was calculated using Eq. (3) as applied by Kumar et al., (2012).

$$\sigma_p^2 = \sigma_g^2 + \frac{\sigma_g^2 \times t}{n} + \frac{\sigma_\varepsilon^2}{rn} \tag{3}$$

where: r is the number of replicates, n is the number of trials and $\sigma_g^2 \times t$ is the genotype x trial 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 (Kaiser, 1960; Tabachnick and Fidell, 1996). Following the PCA, the cos² (squared cosine) was computed which gave the quality of representation of the variables on the factor map and the total contribution of individual traits (contrib). Cos² demonstrates the importance of a component for a given observation and are important in identifying which component to make inference on (Abdi and Williams, 2010; Adu et al., 2018). For a given trait, the sum of the cos² on all dimensions is equal to 1, whereupon if the trait is perfectly represented by only two dimensions (PC1 and PC2), the sum of the cos² on these two dimensions is one (Kassambara, 2017). The contrib, explaining the variations retained by two PCs (PC1 and PC2), is given by contrib = [(C1 x Eig1) + (C2 x Eig2)]/ (Eig1 + Eig2), where: C1 and C2 are the contributions of the variable on PC1 and PC2, respectively and Eig1 and Eig2 are the eigenvalues of PC1 and PC2, respectively (Kassambara, 2017). A 95% confidence ellipse based on country of origin of the cowpea genotypes was constructed in order to observe spread in the data for genotypes that originated from the same country. To identify groups of genotypes with similar key RSA traits, cluster analysis was performed using traits identified by the PCA as key contributing variables to genetic variation. Mean data for selected traits were used for the clustering to generate relatively homogeneous groups of the studied cowpea genotypes. Hierarchical clustering was executed using the Ward's criterion with the Euclidean distance as the similarity measure (Kassambara, 2017; Manschadi et al., 2008). The optimal number of clusters was chosen based on the 'elbow-criterion' which compares the Sum of Squared Differences (SSD) for different cluster solutions (Thorndike, 1953).

The REML analysis and ANOVA were performed using GenStat

(GenStat Release 12.1, VSN International, Oxford, UK). Three packages, including the FactoMineR and the 'corrplot packages in the *R* software, the Language and Environment for Statistical Computing (Kassambara, 2017; R Core Team, 2013; Wei and Simko, 2017) were used for PCA, cluster analysis and graphics. The package Factoextra was used for the visualization of the PCA results (Kassambara, 2017).

3. Results

3.1. Descriptive data and analysis of variance

Descriptive statistics were determined for seventy traits consisting of two shoot-related traits and 68 root traits (Table S3). The ranges for other traits were 40–1465 [(Number of Root Tip Path (RTP count)], 1.9–17.7 mm (tap root diameter), 13.4–61.5 [(Median RTA (RTA_Med)], 3.7–77.4 [1st dominant STA at 25% of the RTP (STA_25_I)], and 0–55.9 [2nd dominant RTA (RTA_Dom _II)]. Coefficients of variation (CV) for the measured traits ranged from 0 for some traits including Median and mean tip diameters of all tips (TD_Med and TD_Avg) to 5.2 for RTA_Min. Fifty-nine traits had CVs ≥ 0.3. Traits including average root density, STA_75_II, STA_90_II, shoot DW, root DW, hypocotyl root length, lateral root diameter as well as all the D-values [i.e.: Cumulative width at 10 to 90% of the maximal depth (D10- D90)] had CVs greater than 0.5. Eleven traits whose CVs were less than three and were not included in subsequent analysis included Dia_Stm, TD_Med, TD_Avg, Ang_Top, STA_Range and STA_Max. The others were STA_Med, RTA_Range, RTA_Max, AR Angle and BR angle (Table S3). These traits were also not considered in ensuing multivariate analysis and included Ang_Btm, STA_Dom_I, STA_Dom_II, Advt_Count, Hyp_Dia, CP_Dia75, number and diameter of basal roots. The remaining traits differed significantly among genotypes at $P \leq 0.001$ for 32 traits, and at $P \leq 0.05$ for fifteen traits.

3.2. Variance components, broad-sense heritability estimates and trait distribution

The effects of genotype, and the interaction between genotype x trial x block accounted for most of the experimental variation (Table 1). The effect of genotype alone ranged from 0.1% for STA_25_I to 87.8% for RTA_DOM_I, and accounted for greater than 40% of the variation in DS50 and less than 30% for most traits (Table 1). Broad-sense heritability (H^2) estimates were generally intermediate to high and ranged from 0.11 to 0.96 (Table 1). The H^2 was largest (> 0.60) for root dry weight, DS50, RTA_MIN and RTA_DOM_I, intermediate (0.25–0.60) for over 30 traits including ADVT_ANG, AR_Bdens, AR_Diam, AR_No, Avg_Dens, BASAL_ANG, BASAL_COUNT and D10-D80 (Table 1). The H^2 estimate was smallest (< 0.25) for traits such as CP_DIA25, CP_DIA50, D90, DS90, number of root nodules, RTA_DOM_II, STA_25_I, STA_50_I, STA_75_I, STA_MIN and TAP_DIA (Table 1). Frequency distribution and Shannon–Weaver diversity index (H') for traits measured in the field-grown cowpea genotypes are presented in Table 1. The number of the cowpea genotypes with superior root traits (≥ $x + \sigma$) were ADVT_ANG [10 (16%)], AR_Bdens [10 (16%)], AR_Diam [9 (15%)], AR_No [11 (18%)], Avg_Dens [7 (11%)], BASAL_ANG [10 (16%)], BASAL_COUNT [8 (14%)], CP_DIA25 [5 (9%)] and CP_DIA50 [5 (8%)]. (Table 1). The number of genotypes with high (≥ $x + \sigma$) Hypo_RL, Nodule_Diam, Nodule_abund, RTA_DOM_II, RTA_DOM_I, RTA_MED, RTP_COUNT and Root_DW ranged from 10% to 23%. The number of genotypes with high or desirable STA-related traits ranged from 6% for STA_MIN to 20% for STA_25_II. The number of genotypes with high (≥ $x + \sigma$) Shoot_DW, Stem_Diam, TAP_DIA, TR_Bdens and TR_diam were 13 (21%), 9 (15%), 1 (2%), 5 (9%) and 7 (12%), respectively (Table 1). No genotype showed superiority in the D values and the number of genotypes with high (≥ $x + \sigma$) DS values were less than three ranging between 1% for DS90 and 4% for DS10 (Table 1). With the exception of RTA_MIN

($H' = 0.01$), STA_MIN ($H' = 0.22$), TAP_DIA ($H' = 0.17$), as well as, all the D values (which presented monomorphism - $H' = 0.00$) and the DS values (which presented H' values of 0.08 – 0.24), all traits measured showed intermediate to high level of diversity with most of the traits having H' values greater than 0.75 (Table 1). The highest values for the Shannon–Weaver index ($H' \geq 0.8$) were noted in ADVT_ANG, AR_B-dens, AR_Diam, AR_No, BASAL_ANG, BASAL_COUNT, Hypo_RL and RTA_DOM_II. Other traits that obtained higher H' values were RTA_MED, STA_25_I, STA_50_I, STA_90_I, Shoot_DW and, TR_Bdens (Table 1). The traits that recorded H' values of less than three were not included in subsequent multivariate analysis.

3.3. Principal component analysis

The first nine, principal components (PCs) with an eigenvalue greater than one explained 74.28% of the total variation among the cowpea genotypes studied for the two shoot and twenty-seven root system traits included in the PCA (Supplementary Table S4). The relative magnitude of eigenvectors for PC1 was 20.76%, explained mostly by the STA-related traits. However, twenty traits including STA_90_I, RTA_DOM_I, STA_90_II, RTA_DOM_II, RTA_MED, STA_75_II, SDIAM, STA_75_I, STA_25_I, STA_50_II, RDW, STA_50_I, and CP_DIA25 ($P \leq 0.001$) were significantly correlated to the first dimension. The others were SDW, ADVT_ANG, STA_25_II, TRBDens, NodAbun, TRDAIM and RTP_COUNT ($P \leq 0.05$). The relative magnitude of eigenvector for the second PC was 17.07%, explained mostly by diameter-related traits, root-count traits (LRNo and RTP_COUNT), as well as RDW and HRL (Supplementary Table S4). Fifteen traits were significantly correlated to the second dimension and included LRDIAM, HRL, LRNo, LRBDens, RDW, RTP_COUNT, NodAbun, SDIAM, STA_25_II, STA_50_I, STA_75_I, TRDAIM and STA_50_II ($P \leq 0.001$), as well as STA_75_II, NodDiam ($P \leq 0.05$). The third PC (PC3, 6.82% of the variation) accounted for the CP-related traits (CP_DIA25 and CP_DIA50) and NodDiam (Supplementary Table S4) but eight traits including CP_DIA50, CP_DIA25, NodDiam, BASAL_COUNT, STA_90_II and NodAbun ($P \leq 0.001$), as well as STA_75_II and BASAL_ANG ($P \leq 0.05$) were significantly correlated to the third dimension. Each of the remaining components contributed approximately 6% or less of the total variation (Supplementary Table S4).

3.4. Relationships between variables

Variable correlation plots, showing relationships among all variables included in the PCA are shown in Fig. 1. For better visibility, trait vectors and objects are shown on separate panels. Fig. 1A and B contain the trait vectors and Fig. 1C contains the location of the cowpea genotypes (objects) according to their principal component (PC) scores. According to the PC scores, although four distinct groups of variables can be identified in the biplot of PC1 and 2, two main positively correlated groups are seen (Fig. 1A). In the first group, the supposed STA- and RTA-related roots traits were positively associated. In the second group, root and shoot biomass traits are positively associated with HRL, root-nodule-related traits, and lateral root-related traits, including LRNo, LRBDens and LRDIAM. Variables that seemed to be negatively associated with these two groups included AVG_DENS, ADVT_ANG BASAL COUNT, BASAL_ANG and TRBDens (Fig. 1A). In the plot of variables of PC2 and PC3, two groups with a positive or negative PC2 are revealed (Fig. 1B). Strongly associated traits with positive correlation to PC2 included the STA-traits, RDW, HRL, SDW, SDIAM and LRDIAM. The CP-traits (i.e: CP_DIA 25 and 50) are strongly associated and correlated positively with PC2 but are negatively correlated with traits such as TRBDens and AVG_DENS (Fig. 1B). Other traits which were strongly associated and had negative correlation to PC2 included RTP COUNT, LRNo, and the diameter and number of root nodules (Fig. 1B). In the quadrants at the left side of the biplot in Fig. 1B (i.e.: negative PC2) were traits such as TRBDens and AVD_DENS.

Table 1

Estimates of variance components and broad-sense heritability (H^2) (A); Frequency distribution and Shannon–Weaver diversity index (H') (B) for traits measured in field-grown cowpea plants.

Trait	A. Variance components						H^2	B. Frequency[1]			H'
	Line	Trial	Line x Trial	Trial x Block	Line x Trial x Block	Error		Small	Med.	Large	
ADVT_ANG	6.6**	0.5*	0.0	0.9	20.5**	71.5	0.27	0.18	0.66	0.16	0.88
AR_Bdens	17.3**	0.5	2.9**	0.0	24.4**	55.0	0.53	0.16	0.68	0.16	0.84
AR_Diam	14.7**	7.8**	0.8*	4.6**	19.7*	52.3	0.52	0.18	0.67	0.15	0.86
AR_No	12.8**	2.3*	0.0	0.0	25.8**	59.1	0.46	0.16	0.66	0.18	0.88
Avg_Dens	6.3	0.2	1.5	33.5	0.0	58.5	0.29	0.03	0.85	0.11	0.49
BASAL_ANG	8.6*	0.0	0.0	0.0	0.0	91.4	0.27	0.17	0.68	0.16	0.86
BASAL_COUNT	13.5**	2.8*	0.0	0.0	4.7	79.0	0.41	0.16	0.70	0.14	0.82
CP_DIA25	3.9*	0.4	0.0	0.0	8.7	87.0	0.15	0.01	0.90	0.09	0.35
CP_DIA50	5.2**	0.0	0.0	0.0	14.7*	80.1	0.21	0.02	0.90	0.08	0.37
D10	13.0**	3.9**	0.1	0.0	0.0	83.0	0.38	0.00	1.00	0.00	**0.03**
D20	12.2**	4.1**	0.0	0.0	0.0	83.7	0.37	0.00	1.00	0.00	**0.00**
D30	10.5**	4.1**	0.0	0.0	0.0	85.4	0.33	0.00	1.00	0.00	**0.03**
D40	9.0**	3.9**	0.0	0.0	1.7	85.4	0.30	0.00	1.00	0.00	**0.00**
D50	8.9**	3.3**	0.0	0.0	6.7	81.1	0.31	0.00	1.00	0.00	**0.00**
D60	8.9**	2.7**	0.0	0.0	10.9	77.4	0.32	0.00	1.00	0.00	**0.00**
D70	8.4**	2.4*	1.2	0.0	12.5*	75.6	0.30	0.00	1.00	0.00	**0.00**
D80	7.2**	2.3*	2.8*	0.0	11.5	76.2	0.26	0.00	1.00	0.00	**0.00**
D90	5.2**	1.2*	0.0	0.0	17.9*	75.7	0.21	0.00	1.00	0.00	**0.00**
DS10	9.2**	2.3*	5.8	0.0	1.0	81.8	0.28	0.00	0.96	0.04	**0.19**
DS20	7.6**	43.2	0.0	0.0	8.4*	40.9	0.43	0.00	0.95	0.04	**0.19**
DS30	4.2**	71.3	0.0	0.0	4.2*	20.3	0.45	0.01	0.95	0.04	**0.23**
DS40	3.5**	71.2	0.0	0.0	3.3*	22.0	0.39	0.01	0.95	0.04	**0.22**
DS50	42.8**	0.2	0.0	0.0	6.4*	50.7	0.77	0.00	0.96	0.04	**0.19**
DS60	11.9**	0.5	0.0	0.0	0.0	87.5	0.35	0.01	0.95	0.04	**0.24**
DS70	16.6**	0.2	0.0	0.0	0.0	83.3	0.44	0.00	0.97	0.03	**0.16**
DS80	6.3**	57.7	0.0	0.0	0.8	35.3	0.42	0.01	0.96	0.03	**0.17**
DS90	6.3**	3.5	0.0	0.0	0.0	90.2	0.22	0.00	0.99	0.01	**0.08**
Hypo_RL	12.4**	4.1**	1.5*	4.6**	20.9**	56.5	0.45	0.19	0.66	0.15	0.88
Nodule_Diam	12.6**	7.0**	0.0	0.0	22.4**	58.0	0.47	0.10	0.80	0.10	0.65
Nodule_abund	2.3**	32.0**	0.0	0.0	32.3**	33.4	0.22	0.09	0.77	0.14	0.69
RTA_DOM_II	0.2*	97.7	0.0	0.0	0.2	1.9	0.23	0.13	0.65	0.23	0.88
RTA_DOM_I	87.8**	0.0	1.5	0.0	0.0	10.8	0.96	0.09	0.75	0.16	0.73
RTA_MED	16.7**	0.7	0.0	0.0	13.2*	69.4	0.49	0.17	0.67	0.16	0.86
RTA_MIN	29.8	0.0	0.3	0.0	0.0	69.9	0.63	0.00	1.00	0.00	0.01
RTP_COUNT	20.7**	4.0**	5.8*	0.0	7.0*	62.5	0.53	0.13	0.72	0.14	0.78
Root_DW	13.1**	17.5**	0.0	9.9**	27.7**	31.8	0.62	0.07	0.81	0.12	0.61
STA_25_I	0.1**	97.3	0.2*	0.0	0.3	2.2	0.11	0.19	0.65	0.16	0.89
STA_25_II	15.2**	0.8	2.6	0.0	0.0	81.4	0.41	0.00	0.80	0.20	0.49
STA_50_I	0.4*	92.5	0.2	0.0	0.0	7.0	0.17	0.16	0.68	0.15	0.84
STA_50_II	4.7**	41.3	1.1	0.0	3.1	49.9	0.27	0.00	0.86	0.14	0.42
STA_75_I	3.3*	0.0	7.9	0.4	0.0	88.4	0.11	0.18	0.68	0.14	0.85
STA_75_II	13.9**	0.0	4.2	0.0	0.0	82.0	0.38	0.04	0.81	0.16	0.58
STA_90_I	0.3*	96.3	0.0	0.0	0.2	3.2	0.28	0.15	0.67	0.18	0.86
STA_90_II	4.788	70.6	0.9*	0.0	0.0	23.8	0.42	0.07	0.77	0.16	0.68
STA_MIN	3.1	1.0	0.0	0.0	3.5	92.5	0.12	0.00	0.94	0.06	**0.22**
Shoot_DW	6.5**	0.0	13.1**	0.2*	30.1**	50.1	0.25	0.12	0.67	0.21	0.85
Stem_Diam	8.8**	21.1**	6.5**	5.6**	27.8**	30.3	0.45	0.17	0.68	0.15	0.86
TAP_DIA	4.1*	1.0	1.5*	0.8	10.6	81.9	0.16	0.01	0.97	0.02	**0.17**
TR_Bdens	7.6**	3.6	0.0	10.4**	16.0*	62.3	0.33	0.24	0.67	0.09	0.83
TR_diam	11.7**	0.0	0.0	4.8**	26.2**	57.4	0.45	0.14	0.74	0.12	0.75

$H' < 0.3$ appear in bold and traits with $H' < 0.3$ were not included in subsequent multivariate analysis. *: $P \leq 0.05$; **: $P \leq 0.01$.

Juxtaposing Fig. 1A onto Fig. 1C reveals that genotypes TVu-8588, 8596, 8598, 1000, 235, 236, among others, are all found in the upper left quadrant in the direction of root and shoot biomass, as well as length and diameter-related traits. Similarly, when Fig. 1A is compared to Fig. 1C, it is seen that genotypes TVu-8601, 8599, 8600, 8600, 399, 7719, 7717, etc. are found in the upper right quadrant of the plot in the direction of the STA- and RTA-related traits. Preliminary analysis revealed that the germplasm could be fairly grouped by country of origin (Fig. 1D) but could not be grouped based on other traits in the passport data including: plant growth habit, growth vigour, and seed size, etc. (Supplementary figure S1). Following the PCA, 95% confidence ellipses were accordingly determined for the mean ($\bar{x}$) of genotypes which originated from each of the five countries (Fig. 1D). All five ellipses overlapped in one way or the other, suggesting that there are some similarities in the values obtained for the measured traits among all the sixty genotypes. The $\bar{x}$ of genotypes from various countries however resolved at different quadrants, indicating that genotypes from different countries might be superior for certain traits. For instance, there was 0.95 probability that the population $\bar{x}$ of genotypes which originated from Togo would fall in the top right quadrant in the direction of superior RTA- and STA-related traits, while genotypes from Ghana would likely be in the top left quadrant, and might be superior in shoot and root biomass, as well as number and size of root nodules. Similarly, there was 0.95 probability that the population $\bar{x}$ of genotypes from Nigeria would be in the bottom left quadrant in the direction of greater TRBDens and AVG_DENS (Fig. 1A and 1D).
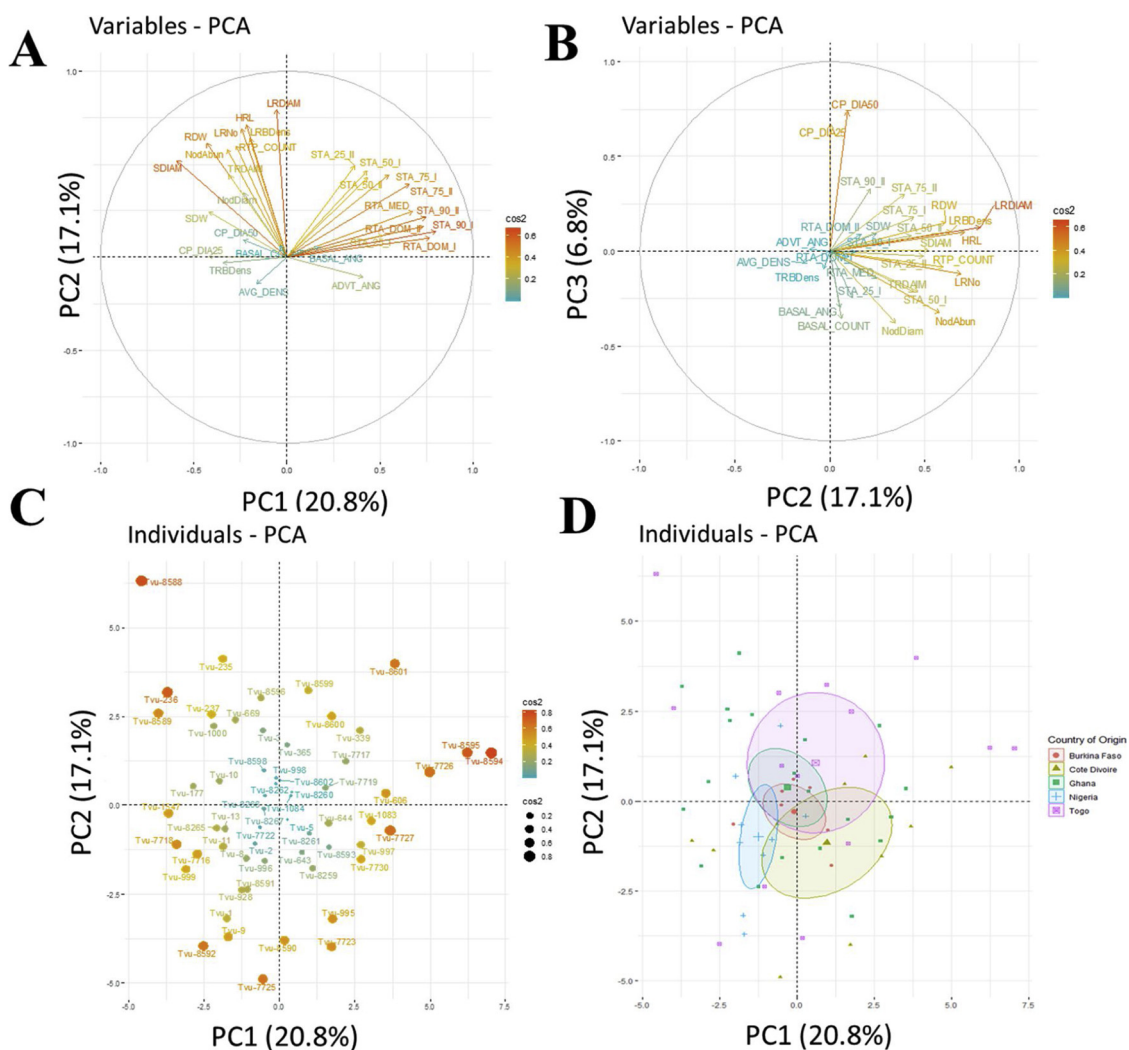
Fig. 1. Variable correlation plots showing relationships between all variables for (A) PC1 and PC2, and (B) PC2 and PC3. Variables are coloured by their quality of representation on the factor map and scale adjacent the plot indicate $\cos^2$ values of corresponding variables; (C) Plot of individuals by their $\cos^2$ values, also showing individual cowpea genotypes that are similar and are therefore grouped together on the plot. Point size and colour scales adjacent the plot indicate $\cos^2$ values of corresponding individual cowpea genotypes; (D) 95% confidence interval ellipses generated by PCA for cluster groups of genotypes which originated from five countries. For each country of origin, the centroid of the 95% confidence ellipse is indicated by a bigger marker.

3.5. Quality of representation of the variables on the factor map

Figs. 1A-C also show the $\cos^2$ (squared coordinates) representing the quality of representation of the variables (Figs. 1A and B) and individuals (Fig. 1C) on the factor map but for better visibility, the variable and individual $\cos^2$ are presented in Fig. 2. Traits including SDIAM, STA and RTA traits were well represented on PC1 with $\cos^2$ between 0.45 – 0.64 (Fig. 2A). Root biomass, HRL and lateral root traits were well represented on PC2, whilst CP_DIA traits and BASAL_COUNT were well represented on PC3 and PC4, respectively (Fig. 2A). Total $\cos^2$ of variables on PC1 and PC2 is shown in Fig. 2B. Here, the highest $\cos^2$ recorded was ≥ 0.6 and the ranking of the top five well represented variables on the factor map was in the order: STA_90_I > LRDIAM > SDIAM > STA_90_I > RTA_DOM_I (Fig. 2B). Fig. 2C shows the quality of the representation for individuals on the PCs and suggests that genotypes TVu-8594, 8588 and 8595 recorded the three highest $\cos^2$ values of ≥ 0.75 and the genotypes TVu-8262, 8260 and TVu-5 recorded the least $\cos^2$ values of ≤ 0.1.

3.6. Contribution of the variables to PCs

The contribution of the variables to the PCs is presented in Fig. 3.

Nine traits comprising of STA_90_I, II, 75_I, II, 25_I, RTA_DOM_I, II, MED and SDIAM contributed above the average cut-off point to the variability in PC1 (Fig. 3C; Supplementary figure S2 A). Thirteen traits contributed above average to the variability in PC2 and were ranked in the order LRDIAM > HRL > LRNo > LRBDens > RDW > RTP_COUNT > NodAbun > SDIAM > STA_25_II > STA_50_I > STA_75_I > TRDIAM > STA_50_II (Fig. 3C; Supplementary figure S2B). Sixteen traits, however, contributed to the variability in the first two dimensions (PC1 and PC2). In ranking order, these comprised of STA_90_I, LRDIAM, SDIAM, STA_90_II, RTA_DOM_I, STA_75_II, RTA_DOM_II, and RDW. The others were HRL, LRNo, RTA_MED, STA_75_I, LRBDens, NodAbun, RTP_COUNT and STA_50_I (Fig. 3A). Few traits recorded higher contributions on the remaining components (i.e.: PC3 to 9; Fig. 3B). These included CP_DIA25 (PC3), BASAL_COUNT (PC4), SDW, TRDIAM and AVG_DENS (PC5), TRDIAM, NoDIAM and STA_50_II (PC6), TRBDens and ADVT_ANG (PC7), STA_25_I (PC8) and BSAL_ANG (PC9) (Fig. 3B). In the present study, twenty-three out of the sixty cowpea genotypes recorded contributions larger than the cut-off point and could be considered as important in contributing to the first two dimensions (Fig. 3C). The top ten of these genotypes included TVu-8588, 8594, 8595, 8601, 7726, 7725, 236, 8589, 8592 and TVu–235 (Fig. 3C).
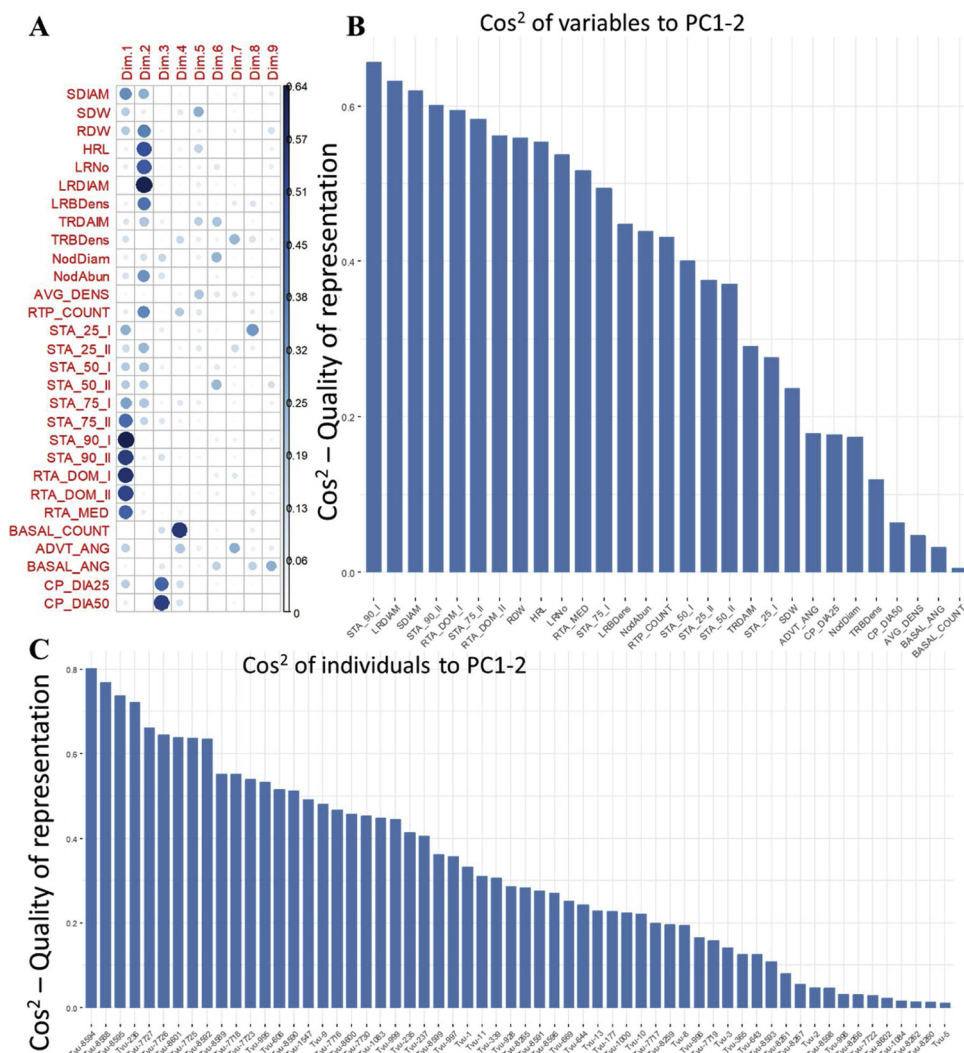
Fig. 2. (A): Plot of quality of representation of the variables (cos^2 of variables) on the factor map for all dimensions considered significant following the PCA. Components with eigenvalues greater than one were considered significant in PCA; (B) Bar plot showing quality of representation of the variables (cos^2 of variables) on the factor map; (C) A bar plot of the quality of representation (cos^2) of individual cowpea genotypes on the factor map.

3.7. Cluster analysis

The dendrogram from the cluster analysis suggested a three-cluster solution (Fig. 4A). Individuals identified under cluster one included TVu-235, 236, 237, 177, 669, 999, 1000, 1547, 7716, 7718, 8588 and 8589 (Figs. 4A and B). Individuals which clustered into the second group included TVu-1, 2, 5, 8, 9, 11. 13, 7725, 7730, 8261, 8590, 8591, 8592, among others (Figs. 4A and B). Examples of individuals categorized under cluster three were TVu-3, 339, 608, 1083, 7726, 7727, 8262, 8594, 8595, 8598, etc. (Figs. 4A and B). Superimposing the individuals on the principal component map (Fig. 4B) suggested that cluster one was largely negative to PC1; cluster 2, largely negative to PC2 and cluster three was mainly positive to PC1. Therefore, individuals in clusters one, two and three were associated with the variables that mapped to the respective quadrants of the factor map. The variables RTP_COUNT, LRNo, HRL, LRDIAM, NodAbun, SDIAM, NodDiam, RDW, BASAL_COUNT, LRBDens, TRDAIM and RTA_DOM_II were most significantly associated with the cluster one (Fig. 4B and Supplementary Table S5). Cluster two was significantly associated with variables such as TRBDens, NodDiam, NodAbun, LRBDens, RTA_-DOM_II, STA_75_I, RTA_DOM_I, STA_90_I, STA_90_II, STA_75_II and RTA_MED. Other variables which associated with cluster two were LRNo, HRL, STA_50_I, STA_50_II, STA_25_II, and LRDIAM (Fig. 4B;

Supplementary Table S5). Fig. 5 displays sample root system images of genotypes under the three identified clusters.

4. Discussion

Plant root systems are critical to improving soil resource acquisition. Breeding for RSA has the potential to support the development of intensified crop production systems on limited arable land and conditions of suboptimal soil resources and a changing climate (de Dorlodot et al., 2007; Kell, 2011; Lynch, 1995; Orman-Ligeza et al., 2014). Consequently, there has been an upsurge in root system studies lately. Some of the current protocols of root system studies, especially those involving robotic root imaging and analysis pipelines, enable the collection of large multi-variate datasets and subsequent processing to analyse and classify the diversity of rooting architectures and develop desired root ideotypes. What is still missing, to a large degree, is the ability to link architectural traits with function, something that is needed to ensure that selected root traits effectively lead to desired functional characteristics. However, a robust characterization of the diversity in RSA utilizing a few, non-correlated, non-redundant root traits, might be essential for various purposes including crop improvement and prediction of crop adaptation under biotic and abiotic stresses (Bodner et al., 2013). There has been limited work
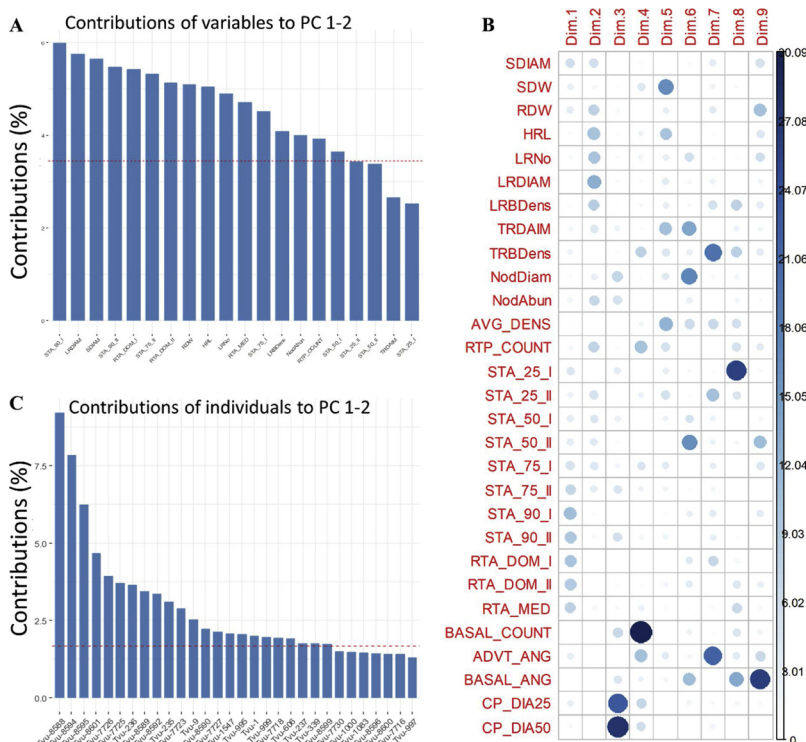
Fig. 3. (A) Plot showing total contribution of variables in accounting for the variability in PC1 and PC2. The red dashed line on the graph indicates the expected average contribution and variables with a contribution greater than this expected average could be considered as important. Contribution of the first twenty variables are shown here; (B) Plot highlighting the most contributing variables for each dimension considered significant after PCA. Components with eigenvalues greater than one were considered significant in PCA; (C) Plots showing the contribution of individual cowpea genotypes to the first two principal components. The red dashed line on the graph indicates the expected average contribution and individual with a contribution greater than this expected average could be considered as important. Contribution of the first 30 individuals are shown here. (see Table S2 for trait descriptions) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

investigating the contribution of various multivariate root system data and to identify the few key contributing traits to genetic variation in cowpea germplasm.

Here, we present a robust approach for reducing trait data dimensionality to identify focal traits useful for characterization of genotypic diversity for breeding purposes. It is anticipated that replacing redundant and correlated variables by a subset of representative variables can reduce noise and simplify analysis of root system data. The present study presents a method of revealing the most important, non-correlated and non-redundant root traits in a multivariate root
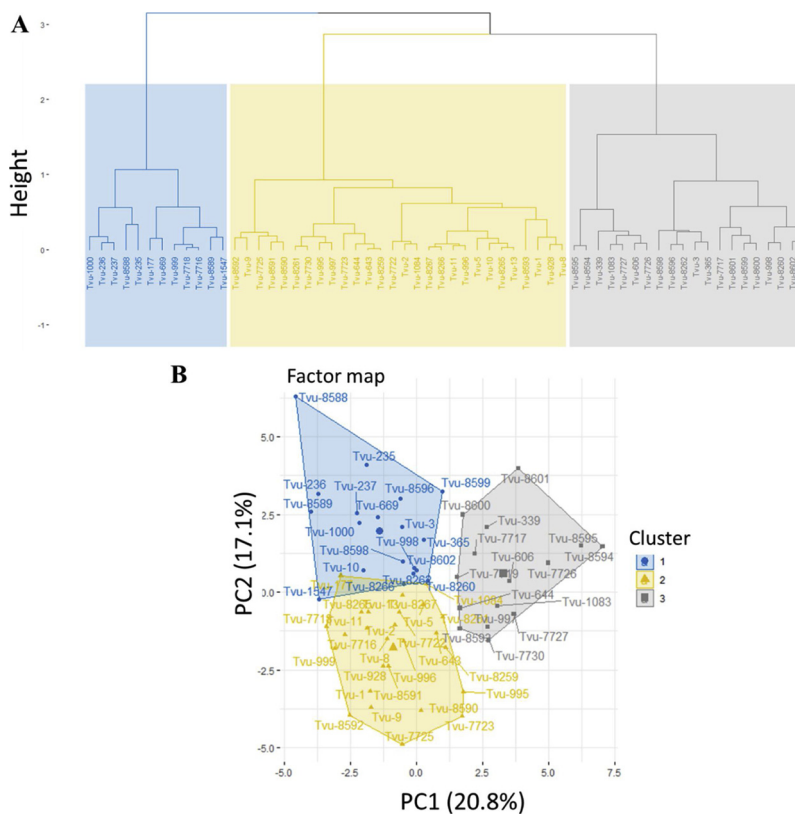

Fig. 4. (A) Dendrogram showing clustering patterns of 29 selected traits with CVs ≥ 0.3 among 60 field-grown cowpea genotypes. (B) individual field-grown cowpea genotypes on the principal component map grouped and coloured according to their assigned group following cluster analysis. (See Table S3 for CV values).

Fig. 5. Photographs of cowpea root crowns showing examples of genotypes that were grouped together following multivariate and cluster analysis.

system dataset. Some limitations could however be cited for the manual excavation of root crown used in this study. In some cases, the root excavation resulted in variation in root sizes and depths among samples, especially in areas where the soil was compacted. The data set generated in DIRT (Bucksch et al., 2014) were based on the images taken of root systems from one direction and as a result, the data of some traits and interpretation of some aspects of the results could be compromised. Sampling size and depth affects traits such as tip diameter, number of root tip path, tap root diameter among others, and imaging roots from different directions may have also influenced the results for soil and root tissue angle.

4.1. Quantitative variations in shoot and RSA traits in cowpea germplasm

The coefficient of variation (CV) provides a measure of relative variability for quantitative traits (Zanklan et al., 2018). Here, the descriptive statistics of seventy traits showed that CVs for quantitative shoot and root traits varied considerably, ranging from 0 (TD_Med, TD_Avg, STA_Range, STA_Max, RTA_Range and RTA_Max), to 2.2 (STA_Min), and 5.2 (RTA_Min) (Supplementary Table S3). Vagaries in experimental conditions could be implicated in the inexplicably large within genotype CVs recorded for traits like RTA_Min. This indicates that, to efficiently exploit the available genetic diversity in these traits,

the confounding effects of the environment must appropriately be delineated. Given the well-known difficulties associated with phenotyping plant roots, whenever many genotypes (such as mapping populations) are involved, decisions on the root parameters to use for comparison are strongly influenced by measurement constraints, including: the need for increased replication or specific expertise, the requirement for bulky or costly equipment, the necessity to phenotype mature plants and to achieve high throughput and resolution at an economical cost per plant sample (i.e.: economical costs for infrastructure and staff) (Dupuy et al., 2017; Chloupek et al., 2010). The large variation in some of the traits could be managed by increasing the number of replications (Adu et al., 2014). Parameters which relate to biomass production could serve as proxies for yield (i.e.: SDW and RDW), and interestingly obtained analogous CVs (0.7), giving a swift insight on traits that might be highly correlated in our dataset. The current results suggested that quantitative traits related to root angles recorded somewhat reduced variation. Two-thirds (67%) of root angle related traits obtained a CV range of 0-0.3, with the implication that the majority of these traits were not included in the multivariate analysis. In the end, the results in the present study suggest the existence of large variations in quantitative root traits among cowpea genotypes that can be exploited to breed for enhanced resource acquisition. Larger between genotype CV values observed for some root traits, for instance, indicates plasticity that could enable a

root phenotype to respond quickly to environmental conditions at a given place and time (Burridge et al., 2016; Zhu et al., 2005).

In the present study, the Shannon–Weaver diversity index was calculated for each trait to compare the phenotypic diversity index (H') among traits. High H' values suggest an even distribution of frequency classes and wide variation in individual traits, while unbalanced frequency classes lead to low H' values and suggests minimal or a lack of genetic diversity in individual traits (Kumar et al., 2012; Upadhyaya et al., 2002). Here, the diversity values were variable among traits. The diversity index was largest (> 0.60) for traits such as nodule_Diam and nodule_abund, RTA_DOM_I, RTP_COUNT, Shoot_DW, Hypo_RL and STA_25_I, indicating a wide range of variation in these traits. On the other hand, the H' values were intermediate (0.25–0.60) for CP_DIA25, CP_DIA50, STA_50_II, Avg_Dens, STA_25_II and STA_75_II and smallest (< 0.25) for traits such as RTA_MIN, TAP_DIA and the majority of the supposed D and DS traits (Table 1), indicating moderate and low variation in these traits, respectively. Further, the measured traits could coarsely be categorized into groups and the H' values averaged across groups. For example, the traits could be categorized into (i) count and root density-related traits, (e.g.: AR_Bdens and BASAL_COUNT), (ii) diameter and root length related traits (e.g.: AR_Diam and Hypo_RL), (iii) D and DS related traits (e.g.: D10 and DS10), (iv) root tissue angle related traits (e.g.: RTA DOM I and II), (v) soil tissue angle related traits (e.g.: STA_25 I and II), (vi) other root angle related traits (ADVT_ANG and BASAL_ANG), and (vii) biomass related traits (Shoot DW and Root DW). In addition to the variation in H' among traits, the results also revealed that the diversity values were variable among the three groups of genotypes categorised based on x and σ, suggesting that the diversity within these groups depended upon the traits. The D and DS related traits had the lowest H' values and the biomass related traits had the highest H' values. The mean H' values recorded for groups (i) to (vii) were respectively 0.76, 0.61, 0.10, 0.62, 0.65, 0.73 and 0.87, pointing to notable variation for quantitative traits in this germplasm of cowpea.

Broad-sense heritability was: highest for root biomass and root tissue angle traits (> 0.60); intermediate (0.25–0.60) for the majority of traits, including shoot biomass, root diameter and density-related traits, BASAL and ADVT_ANG, Hypo_RL, AR_No, RTA_MED, RTP_ and BASAL_COUNT, as well as most of the D, DS and soil tissue angle traits; and lower (< 0.25) for other root traits (Table 1). The low heritability recorded for certain traits could be indicative of strong environmental influences on these (Wang et al., 2004). The H^2 estimates here are largely comparable to other root traits reported for some legumes, including chickpea where heritability estimates were 0.51 to 0.54 for root length density (Kashiwagi et al., 2005) and peanuts, whose H^2 ranged from 0.27 to 0.59 for root dry weight, length, volume and surface area (Painawadee et al., 2009). Shoot biomass traits reportedly have larger broad-sense heritability than root biomass traits (Adu et al., 2014; Arraouadi et al., 2012; Bouteillé et al., 2012). In white clover for example, Caradus and Woodfield (1990) reported that the heritability of leaflet width, petiole length and stolon diameter were all high ($> 0 .5$), while those for root traits were lower, ranging from 0.22 to 0.36. In the present study, the H^2 of root biomass was larger than that of shoot biomass (Table 1), indicating that there may have been a stronger influence of the environment on shoot biomass than on root biomass. The high CV and heritability values for RTA and root DW suggest that these traits would normally require less replication to screen for them. This is because traits with high hereditability require less replication to detect significant differences between genotypes and vice versa (Adu et al., 2014).

Reliable estimation of heritability and variance components are crucial for selection gain and strategies for improving quantitative traits (Kumar et al., 2012). In the present study, the effects of genotype, and interaction between genotype x trial x block accounted for most of the experimental variation. The effect of block alone was not included in the model because a preliminary analysis revealed that effect of block

accounted for very little variation (data not shown). Even so, the analysis suggested that some of the variation in all the traits examined could be attributed to vagaries in experimental conditions between the two trials. Although variation attributable to trial was small, it was unexpectedly large for some traits (Table 1). Variance component estimations show that for a large number of traits, the residual proportion (σ_ε^2) was larger than the genotypic (σ_g^2) or the proportion for the interactive effect of the genotype and other environmental factors (i.e.: σ_{gt}^2 or σ_{gtb}^2) (Table 1), indicating perhaps that within-genotype variation was rather high and/or further probing to obtain a more parsimonious model might be necessary. Moreover, the present study was conducted on adjoining sites with homogenous soil conditions and planting date was approximately the same but climatic conditions recorded for the two cropping seasons varied slightly. For example, the 2016 cropping season recorded a higher precipitation, and may have influenced the soil water content. This suggests that climatic conditions could be the source of some of the variation observed and highlights the importance of replicated field experiments for understanding the genetic basis of population differences in RSA. The genotype x environment interactions were reflective of the low heritability recorded for some traits in this study. Low heritability normally suggests a strong influence of the environment (Wang et al., 2004). Thus, it might be difficult to use such traits directly as a selection criterion, in which case, it might be more reliable to identify molecular markers for these particular traits that could be used in marker-assisted (Wang et al., 2004). Meanwhile, the significant genetic variances and moderate to high heritability values found in this study for some root parameters could facilitate phenotypic selection (Tuberosa et al., 2003).

4.2. Multivariate analysis - correlation between traits, PCA and cluster analysis

Variable plots of PCA suggested correlations between a number of traits (Fig. 1), which would be relevant to cowpea breeding. The positive correlation between LRNo and RTP_COUNT and root length and diameter traits suggest that these traits can be improved simultaneously (Fig. 1). The positive correlation between soil and root tissue angles confirmed that these traits are inverses of each other and their correlation was therefore predictable. The minimal correlations between angle-related traits and other root traits observed here agree with previous studies that have reported of minimal or lack correlation between growth angle and other root traits in legumes and other crops species (Manschadi et al., 2008; Vieira et al., 2008). These results indicate a limited number of traits might account for most of the variability of RSA of field-grown cowpea (de Dorlodot et al., 2007). In the present study, soil and root tissue angles, shoot and root diameter and root biomass were the most important traits contributing to variation. Relatively easy-to-measure counterparts of correlated traits which mapped to the same quadrant of the factor map (Fig. 1), such as biomass or count traits, could be used as indirect traits of diameter-related traits that are more difficult to measure.

The clusters identified in this study can have implications for foraging performance, but are likely to be associated with some trade-offs. Root phenotypes of cluster one were mainly of increased root biomass, length, root number and diameter; that of cluster two were mainly of increased branching density, with cluster three characterised by distinctive root angle, STA and RTA-related traits. It was not surprising that, we found that RTP and RDW were both associated with cluster 1, as increased RTPs could lead to increased RDW. Interestingly, RTPs have been reported to be linearly dependent on and correlated with the number of root nodules and third order basal roots in cowpea (Bucksch et al., 2014), suggesting that genotypes in cluster one might not only be superior in biomass and hence yield, but also in fixation of atmospheric nitrogen in the soil. While the relatively bigger root biomass of cluster one could be advantageous for the acquisition of relatively immobile

soil resources such as phosphorus (P), it may limit plant growth due to the increased respiratory burden of root tissue (Lynch and Ho, 2005; Nielsen et al., 1998, 2001). Similarly, root phenotypes in cluster two are likely to enhance deep soil foraging for water and nitrogen acquisition but may incur trade-offs for P acquisition and spatial competition (Lynch, 2013).

Although the sixty genotypes were grouped into three clusters (Figs. 4 and 5), there was no clear correlation between genetic relationship and plant characteristics such as growth habit (data not shown). There was also no clear correlation between genetic relationship and geographic origin (Fig. 1D) indicating large diversity in the genetic materials even within the same country of origin. Geographic origin of crops could influence crops' rooting characteristics if the geographic locations have different climatic and soil conditions (e.g.: dry vs humid conditions) (Narayanan et al., 2014). All the genetic materials used in the present study originated from neighbouring West African countries with comparable climatic conditions. It was therefore unsurprising that the genetic materials were not grouped based on geographic origin of the germplasm. Nevertheless, the results in the present study highlighted genotype groups that have similar or contrasting features, or contribute strongly to quality of representation on the factor map or to variation and could accordingly be explored to identify the genetic basis of specific traits.

4.3. Quality of variable representation on factor map and contribution of traits to variability

Similar to the observation of the PC loadings on PC1 and PC2, the results of quality representation showed that soil and root tissue angles, diameter related traits and root biomass were well represented on the factor map and thus warrant consideration in efforts to breed for improved genotypes in cowpea (Figs. 1 and 2). It may perhaps be observed that roots represent a large carbon cost for plants and so when carbon costs are considered along with soil resource acquisition, an increased root biomass, for example, may not always be beneficial. Moreover, given that adaptations conferring drought tolerance in crop plants may involve higher ratios of root to shoot growth or an avoidance/escape strategy prior to drought conditions (Kooyers, 2015), paybacks of investing in greater root biomass would depend on intrinsic drought tolerance strategies of plants. For an extremely short season genotype characterized by a drought escape strategy, greater root biomass would likely not be beneficial. Rather, greater root biomass may be more advantageous for long season genotypes as a larger root system may provide a cost-effective advantage later in the cropping season. The results also suggest that certain genotypes including TVu-8588, 8594 and 8595 might have certain superior root features, including greater root biomass, that warrant further probing. Incidentally, the top three genotypes that contributed high cos^2 values are materials from Togo and exhibits semi-erect growth habit with branches more or less perpendicular to main stem but do not touch ground (TVu-8588) or intermediate growth where most lower branches touch the ground growth (TVu-8594 and 8595).

The contributions of traits in accounting for the variability in a given PC are (%) = (cos^2 of the traits x 100) / (total cos^2 of the PC) (Kassambara, 2017). In this study, if the contribution of the variables were uniform, the expected value would be 1/number of variables included in the PCA = 1/29 = 3.4%. Thus, the average contribution of a variable for PC1 and PC2 = (3.4* Eig1) + (3.4 * Eig2) and a variable with a contribution larger than this cut-off point could be considered as important in contributing to the first two axes (Adu et al., 2018; Kassambara, 2017). The most important traits in explaining the variability in a given dataset are those that are correlated with PC1 and PC2 (Adu et al., 2018; Kassambara, 2017). Variables that do not correlate with any PC have low contribution and could be classified as redundant and removed to simplify the overall analysis. In this present study, sixteen traits contributed above average to the variability in PC1 and

PC2. Approximately, 31, 19, 13, 13, and 25% of the traits that contributed highly to the variation in PC1 and PC2 were soil tissue angle traits, root angle traits, diameter related traits, root biomass and hypocotyl root length and count and density-related traits, respectively, and thus, warrant consideration in efforts to breed for drought stress tolerance and efficient nutrient uptake in cowpea. The results here somewhat agree with that of Bucksch et al. (2014) who had previously used relative phenotypic variation [(RPV), the quotient of the variance of the trait of all roots of a dataset and the mean trait variance per genotype], to differentiate cowpea genotypes. According to the authors, traits are more likely to be useful in differentiating genotypes when their RPV greater than 1. They reported that D and DS values obtained relatively lesser RPVs and that second dominant root tissue angles (RTA_DOM_II) showed stronger differentiation potential with RPV of 3.2. The results of the present study suggest that soil and root angle traits, diameter and root biomass traits, as well as root count and lateral root density-related traits are sufficient to differentiate cowpea genotypes. Similar to the contribution of variables, if the contribution of the individuals (the cowpea genotypes) were uniform, the expected value would be 1/number of genotypes (%) = 1/60 = 1.7%. This would be the expected average contribution of genotypes and for a given PC, an accession with a contribution larger than this cut-off point could be considered as important in contributing to the PC (Kassambara, 2017). In this present study, twenty-three genotypes, with the top three being TVu-8588, 8594 and 8595, contributed above average to the variability in PC1 and PC2.

5. Conclusion

Analysis of plant traits have every so often been made using univariate statistical procedures. These approaches do not normally consider that measurements taken on plant traits might be connected by biological networks and hence the traits may be correlated. The present study presents a multivariate approach to analysis of root system data, which is able to document the relative amount of variability in root traits among field-grown cowpea genotypes, detect redundant traits and reveal the most important traits which explains the variability in the dataset. Sixty cowpea genotypes were used to identify which principal traits are sufficient to differentiate genotypes even when comparing plants of varied growth habits and whose days-to-flowering differ up to 28 d. The most important traits contributing to variation in the cowpea genotypes included soil and root tissue angles, diameter related traits, root biomass, hypocotyl root length and root count and lateral root density-related traits. The results here could serve as a basis for further exploration of genotypic variability in soil exploration that may ultimately reflect resource acquisition under field-conditions. This study offers a path to rapid screening of large cowpea samples/population in field experiments for breeding or crop improvement purposes. Robust identification of focal phenotypic root traits could reduce the costs associated with phenotyping roots of field crops. The quantitative approach employed here can underpin further, well-targeted and in-depth field-based study of cowpea root system traits to support breeding strategies and programs, especially in resource-poor jurisdictions.

Declarations of interest

None.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.fcr.2018.12.015.

References

Abdi, H., Williams, L.J., 2010. Principal component analysis. Wiley Interdiscip. Rev. Comput. Stat. 2, 433–459.

Adepetu, J.A., Akapa, L.K., 1977. Root growth and nutrient uptake characteristics of some cowpea varieties[1]. Agron. J. 69, 940–943.

Adu, M.O., Chatot, A., Wiesel, L., Bennett, M.J., Broadley, M.R., White, P.J., Dupuy, L.X., 2014. A scanner system for high-resolution quantification of variation in root growth dynamics of *Brassica rapa* genotypes. J. Exp. Bot. 65, 2039–2048.

Adu, M.O., Asare, P.A., Yawson, D.O., Ackah, F.K., Amoah, K.K., Nyarko, M.A., Andoh, D.A., 2017. Quantifying variations in rhizosheath and root system phenotypes of landraces and improved varieties of juvenile maize. Rhizosphere 3, 29–39.

Adu, M.O., Asare, P.A., Asare-Bediako, E., Amenorpe, G., Ackah, F.K., Afutu, E., Yawson, D.O., 2018. Characterising shoot and root system trait variability and contribution to genotypic variability in juvenile cassava (*Manihot esculenta* Crantz) plants. Heliyon 4, e00665.

Agbicodo, E.M., Fatokun, C.A., Muranaka, S., Visser, R.G.F., Linden van der, C.G., 2009. Breeding drought tolerant cowpea: constraints, accomplishments, and future prospects. Euphytica 167, 353–370.

Arraouadi, S., Badri, M., Abdelly, C., Huguet, T., Aouani, M.E., 2012. QTL mapping of physiological traits associated with salt tolerance in *Medicago truncatula* recombinant inbred lines. Genomics 99, 118–125.

Bodner, G., Leitner, D., Nakhforoosh, A., Sobotik, M., Moder, K., Kaul, H.P., 2013. A statistical approach to root system classification. Front. Plant Sci. 4, 292.

Bonser, A.M., Lynch, J., Snapp, S., 1996. Effect of phosphorus deficiency on growth angle of basal roots in Phaseolus vulgaris. New Phytol. 132, 281–288.

Bouteillé, M., Rolland, G., Balsera, C., Loudet, O., Muller, B., 2012. Disentangling the intertwined genetic bases of root and shoot growth in Arabidopsis. PLoS One 7, e32319.

Bradshaw, J.E., Dale, M.F.B., Mackay, G.R., 2009. Improving the yield, processing quality and disease and pest resistance of potatoes by genotypic recurrent selection. Euphytica 170, 215.

Bucksch, A., Burridge, J., York, L.M., Das, A., Nord, E., Weitz, J.S., Lynch, J.P., 2014. Image-based high-throughput field phenotyping of crop roots. Plant Physiol. 166, 470–486.

Burridge, J.D., Jochua, C.N., Bucksch, A., Lynch, J.P., 2016. Legume shovelomics: high—throughput phenotyping of common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* subsp, *unguiculata*) root architecture in the field. Field Crops Res. 192, 21–32.

Burridge, J.D., Schneider, H.M., Huynh, B.L., Roberts, P.A., Bucksch, A., Lynch, J.P., 2017. Genome-wide association mapping and agronomic impact of cowpea root architecture. Theor. Appl. Genet. 130, 419–431.

Caradus, J.R., Woodfield, D.R., 1990. Estimates of heritability for, and relationships between, root and shoot characters of white clover. I. Replicated clonal material. Euphytica 46, 203–209.

Carlos, G., 2000. Cowpea: Post Harvest Operation. Food and Agricultural Organization of United Nations, Rome, Italy.

Chen, Y., Ghanem, M.E., Siddique, K.H., 2016. Characterising root trait variability in chickpea (*Cicer arietinum* L.) germplasm. J. Exp. Bot. 68, 1987–1999.

Chloupek, O., Dostál, V., Středa, T., Psota, V., Dvořáčková, O., 2010. Drought tolerance of barley varieties in relation to their root system size. Plant Breed. 129, 630–636.

De Barros, I., Gaiser, T., Lange, F.M., Römheld, V., 2007. Mineral nutrition and water use patterns of a maize/cowpea intercrop on a highly acidic soil of the tropic semiarid. Field Crops Res. 101, 26–36.

de Dorlodot, S., Forster, B., Pagès, L., Price, A., Tuberosa, R., Draye, X., 2007. Root system architecture: opportunities and constraints for genetic improvement of crops. Trends Plant Sci. 12, 474–481.

Dupuy, L.X., Wright, G., Thompson, J.A., Taylor, A., Dekeyser, S., White, C.P., Thomas, W.T., Nightingale, M., Hammond, J.P., Graham, N.S., Thomas, C.L., 2017. Accelerating root system phenotyping of seedlings through a computer-assisted processing pipeline. Plant Methods 13, 57.

Ehlers, J.D., Hall, A.E., 1997. Cowpea (*Vigna unguiculata* L. walp). Field Crop. Res. 53, 187–204.

Galkovskyi, T., Mileyko, Y., Bucksch, A., Moore, B., Symonova, O., Price, C.A., Harer, J., 2012. GiA Roots: software for the high throughput analysis of plant root system architecture. BMC Plant Biol. 12, 116.

Gregory, P.J., 2008. Plant Roots: Growth, Activity and Interactions With the Soil. Blackwell Publishing, John Wiley and Sons, Oxford UK.

Hall, A., 2012. Phenotyping cowpeas for adaptation to drought. Front. Physiol. 3, 155.

Ho, M.D., Rosas, J.C., Brown, K.M., Lynch, J.P., 2005. Root architectural tradeoffs for water and phosphorus acquisition. Funct. Plant Biol. 32, 737–748.

Horst, W.J., Wagner, A., Marschner, H., 1983. Effect of aluminium on root growth, cell-division rate and mineral element contents in roots of *Vigna unguiculata* genotypes. Zeitschrift für Pflanzenphysiologie 109, 95–103.

Kaiser, H.F., 1960. The application of electronic computers to factor analysis. Educ. Psychol. Measur. 20, 141–151.

Kashiwagi, J., Krishnamurthy, L., Upadhyaya, H.D., Krishna, H., Chandra, S., Vadez, V., Serraj, R., 2005. Genetic variability of drought-avoidance root traits in the mini-core germplasm collection of chickpea (*Cicer arietinum* L.). Euphytica 146, 213–222.

Kassambara, A., 2017. Practical Guide to Principal Component Methods in R: PCA, M (CA), FAMD, MFA, HCPC, Factoextra Vol. 2. STHDA.

Kell, D.B., 2011. Breeding crop plants with deep roots: their role in sustainable carbon, nutrient and water sequestration. Ann. Bot. 108, 407–418.

Kooyers, N.J., 2015. The evolution of drought escape and avoidance in natural herbaceous populations. Plant Sci. 234, 155–162.

Kopittke, P.M., Dart, P.J., Menzies, N.W., 2007. Toxic effects of low concentrations of Cu on nodulation of cowpea (*Vigna unguiculata*). Environ. Pollut. 145, 309–315.

Krasilnikoff, G., Gahoonia, T.S., Nielsen, N.E., Vanlauwe, B., Diels, J., Sanginga, N., Merckx, R., 2002. Phosphate uptake from sparingly available soil-P by cowpea (*Vigna unguiculata*) genotypes. In: Vanlauwe, B., Diels, J., Sanginga, N., Merckx, R. (Eds.), Integrated Plant Nutrient Management in Sub-Saharan Africa: From Concept to Practice. CAB International, Wallingford, UK, pp. 239–250.

Krasilnikoff, G., Gahoonia, T., Nielsen, N.E., 2003. Variation in phosphorus uptake efficiency by genotypes of cowpea (*Vigna unguiculata*) due to differences in root and root hair length and induced rhizosphere processes. Plant Soil 251, 83–91.

Kumar, B., Abdel-Ghani, A.H., Reyes-Matamoros, J., Hochholdinger, F., Lübberstedt, T., 2012. Genotypic variation for root architecture traits in seedlings of maize (*Zea mays* L.) inbred lines. Plant Breed. 131, 465–478.

Legume market analysis Ghana, 2012. A Preliminary Report by the Bill and Melinda Gates Foundation. Sourced from:. : accessed on 22/08/18. https://agriknowledge.org/downloads/9s161620q.

Liao, H., Yan, X., Rubio, G., Beebe, S.E., Blair, M.W., Lynch, J.P., 2006. Corrigendum to: genetic mapping of basal root gravitropism and phosphorus acquisition efficiency in common bean. Funct. Plant Biol. 33 207-207.

Lynch, J., 1995. Root architecture and plant productivity. Plant Physiol. 109, 7.

Lynch, J.P., 2007. Roots of the second green revolution. Aust. J. Bot. 55, 493–512.

Lynch, J.P., 2013. Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. Ann. Bot. 112, 347–357.

Lynch, J.P., Ho, M.D., 2005. Rhizoeconomics: carbon costs of phosphorus acquisition. Plant Soil 269, 45–56.

Manschadi, A.M., Hammer, G.L., Christopher, J.T., 2008. Genotypic variation in seedling root architectural traits and implications for drought adaptation in wheat (*Triticum aestivum* L.). Plant Soil 303, 115–129.

Martins, L.M., Xavier, G.R., Rangel, F.W., Ribeiro, J.R., Nevesm, M.C., Morgado, L.B., Rumjanek, N.G., 2003. Contribution of biological nitrogen fixation to cowpea: a strategy for improving grain yield in the semi-arid region of Brazil. Biol. Fertil. Soils 38, 333–339.

Matsui, T., Singh, B.B., 2003. Root characteristics in cowpea related to drought tolerance at the seedling stage. Exp. Agric. 39, 29–38.

McCully, M.E., 1999. Roots in soil: unearthing the complexities of roots and their rhizospheres. Annu. Rev. Plant Biol. 50, 695–718.

Narayanan, S., Mohan, A., Gill, K.S., Prasad, P.V., 2014. Variability of root traits in spring wheat germplasm. PLoS One 9, e100317.

Nielsen, K.L., Bouma, T.J., Lynch, J.P., Eissenstat, D.M., 1998. Effects of phosphorus availability and vesicular-arbuscular mycorrhizas on the carbon budget of common bean (*Phaseolus vulgaris*). New Phytol. 139, 647–656.

Nielsen, K.L., Eshel, A., Lynch, J.P., 2001. The effect of phosphorus availability on the carbon economy of contrasting common bean (*Phaseolus vulgaris* L.) genotypes. J. Exp. Bot. 52, 329–339.

Ogbonnaya, C.I., Sarr, B., Brou, C., Diouf, O., Diop, N.N., Roy-Macauley, H., 2003. Selection of cowpea genotypes in hydroponics, pots, and field for drought tolerance. Crop Sci. 43, 1114–1120.

Orman-Ligeza, B., Civava, R., de Dorlodot, S., Draye, X., 2014. Root system architecture. In: Morte, A., Varma, A. (Eds.), Root Engineering, Vol 40 of the Series Soil Biology. Springer, New York, pp. 39–56.

Painawadee, M., Jogloy, S., Kesmala, T., Akkasaeng, C., Patanothai, A., 2009. Heritability and correlation of drought resistance traits and agronomic traits in peanut (*Arachis hypogaea* L.). Asian J. Plant Sci. 8, 325–334.

Petrie, C.L., Hall, A.E., 1992. Water relations in cowpea and pearl millet under soil water deficits. II. Water use and root distribution. Funct. Plant Biol. 19, 591–600.

R Core Team, 2013. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Singh, B.B., Matsui, T., 2002. Cowpea varieties for drought tolerance. In: Fatokun, C.A., Tarawali, S.A., Singh, B.B., Kormawa, P.M., Tamo, M. (Eds.), Challenges and Opportunities for Enhancing Sustainable Cowpea Production. International Institute of Tropical Agriculture (IITA), Nigeria, pp. 287–300.

Tabachnick, B.G., Fidell, L.S., 1996. Using Multivariate Statistics. Harper Collins, Northridge. Cal.

Tharanathan, R.N., Mahadevamma, S., 2003. Grain legumes—a boon to human nutrition.

Trends Food Sci. Technol. 14, 507–518.

Thorndike, R.L., 1953. Who belongs in the family? Psychometrika 18, 267–276.

Trachsel, S., Kaeppler, S.M., Brown, K.M., Lynch, J.P., 2011. Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. Plant Soil 341, 75–87.

Tuberosa, R., Salvi, S., Sanguineti, M.C., Maccaferri, M., Giuliani, S., Landi, P., 2003. Searching for quantitative trait loci controlling root traits in maize: a critical appraisal. Roots: the Dynamic Interface Between Plants and the Earth. Springer, Dordrecht, pp. 35–54.

Upadhyaya, H.D., Ortiz, R., Bramel, P.J., Singh, S., 2002. Phenotypic diversity for morphological and agronomic characteristics in chickpea core collection. Euphytica 123, 333–342.

Vieira, R.F., Carneiro, J.E.S., Lynch, J.P., 2008. Root traits of common bean genotypes used in breeding programs for disease resistance. Pesqui. Agropecuā¿â½ria Bras. 43, 707–712.

Wang, L., Liao, H., Yan, X., Zhuang, B., Dong, Y., 2004. Genetic variability for root hair traits as related to phosphorus status in soybean. Plant Soil 261, 77–84.

Wei, T., Simko, V., 2017. R Package "corrplot": Visualization of a Correlation Matrix (Version 0.84). Available from. https://github.com/taiyun/corrplot.

Wishart, J., George, T.S., Brown, L.K., Ramsay, G., Bradshaw, J.E., White, P.J., Gregory, P.J., 2013. Measuring variation in potato roots in both field and glasshouse: the search for useful yield predictors and a simple screen for root traits. Plant Soil 368, 231–249.

Yirzagla, J., Atokple, I.D.K., Haruna, M., Kusi, F., Suguri, I., Muntari, A., 2016. Scaling out cowpea production in Northern Ghana, Community seed production Scheme. Pan-African Grain Legume and World Cowpea Conference. 28 February to 4 March 2016. Livingstone, Zambia.

Zanklan, A.S., Becker, H.C., Sørensen, M., Pawelzik, E., Grüneberg, W.J., 2018. Genetic diversity in cultivated yam bean (*Pachyrhizus* spp.) evaluated through multivariate analysis of morphological and agronomic traits. Genet. Resour. Crop Evol. 65, 811–843.

Zhu, J., Kaeppler, S.M., Lynch, J.P., 2005. Mapping of QTL controlling root hair length in maize (*Zea mays* L.) under phosphorus deficiency. Plant Soil 270, 299–310.