Characterising shoot and root system trait variability and contribution to genotypic variability in juvenile cassava (*Manihot esculenta* Crantz) plants

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Abstract

The development of cassava genotypes with root system traits that increase soil resource acquisition could increase yields on infertile soils but there are relatively few work that has quantified cassava root system architecture (RSA). We used an easily adaptable and inexpensive protocol to: (i) measure genotypic variation for RSA and shoot traits of a range of cassava genotypes; and (ii) identify candidate variables that contribute the largest share of variance. Cassava genotypes were grown in soil-filled pots, maintained at 70% field capacity. Shoot and RSA traits were measured on plants grown up to 30, 45 and 60 days. Multivariate analysis was used to determine major traits contributing to variation.

The study showed that cassava roots are adventitious in origin consisting of a main root axis and orders of lateral roots, and therefore the historically used term "fibrous roots" are redundant currently not contributing to clarity. There were significant differences ($P < 0.05$) for traits evaluated. The highest relative root growth rate occurred over the first 30 days and ranged from 0.39 to 0.48 cm day^{-1}. Root fresh weight was significantly correlated with other traits, including root length ($r = 0.79$), leaf area ($r = 0.72$), number of lower nodal roots ($r = 0.60$), indicating that direct selection based on these traits might be sufficient to improve root biomass. Up to the first six principal components explained over 80% of the total variation among the genotypes for the traits measured at 30, 45 and 60 days. Leaf area, root diameter and branching density-related traits were the most important traits contributing to variation. Selection of cassava genotypes based on shoot and root biomass, root diameter and branching density at juvenile growth stage could be successful predictors of nutrient and water-use efficiency in the field. Further studies are required to relate studied juvenile cassava root traits with the performance of field-grown-mature plant with regard to drought, nutrient-use efficiency and yield.

Keyword: Agriculture

1. Introduction

Cassava (*Manihot esculenta* Crantz), is a perennial woody shrub grown as an annual crop in the humid tropics (Alves, 2002; Izumi et al., 1999). It is the sixth source of energy in the world, with over 70% global production used for human consumption in freshly cooked or processed forms, and the remaining for animal feed and/or industrial uses (El-Sharkawy, 2003; Okogbenin et al., 2013; Westby, 2002). In Africa, Asia, Latin America and the Caribbean, cassava is a major staple crop providing calories for over 500 million people daily (Okogbenin et al., 2013). In these regions, cassava is crucial to food security. In Sub-Saharan Africa, more than 90% of the 117 million tons of cassava produced is consumed as food (Okogbenin et al., 2013; Philips et al., 2006). The main economic value of the crop rests in its starchy storage root, albeit the young leaves offer a dietary protein supplement when used as vegetable (Okogbenin et al., 2013; Westby, 2002). Cassava has the ability to remain productive on marginal soils and under low input systems. It is also drought-tolerant. These attributes make cassava attractive to resource-poor farmers.

Observed yields of cassava are about 8-fold below potential yields (Okogbenin et al., 2013) due principally to the use of landraces and traditional varieties, as well as poor production conditions (El-Sharkawy, 2005; Okogbenin et al., 2013). The identification and/or breeding of nutrient and water-use efficient, other abiotic and biotic-stress tolerant varieties could be crucial in cassava yield improvement for low input

production systems. Genotypes with long nodal and basal roots and high root biomass have a good potential for high nutrient and water-use efficiency (Marschner, 1988). Descriptive root system data, which can be integrated into representative models (Kalogiros et al., 2016), are lacking for cassava, and this is limiting the dissection and genetic analysis of complex root system traits. Breeding for root system in cassava would require quantitative screening of these root features and their development in a near-natural environment with high resolution and high throughput systems (Adu et al., 2014; Downie et al., 2015) but this is as yet not very practicable and/or economical.

Cassava root system consists of adventitious roots originating from the nodes of stem cuttings (nodal roots) or from a recently formed callus at the base of cuttings (basal roots) and their attendant lateral roots of different branching orders (El-Sharkawy, 2003; Subere et al., 2009). The nodal roots are formed first from the base of more than one axillary bud 5−7 days after planting, which is then followed by the formation of basal roots from the callus formed at the base of the stem cutting (El-Sharkawy, 2003; Subere et al., 2009). Following crop establishment, cassava forms a fibrous root system, mainly in the upper 1 meter layer of soil. From 2 to 3 months after planting, the fibrous roots expand swiftly to form storage roots for starch. Starch accumulation and increase in weight continue in the storage roots during the growing season until the crop is harvested, 7−15 months after planting (El-Sharkawy and Cock, 1987).

There are relatively few prior studies that have quantified cassava root system architecture and in these few studies, diverse shoot and root variables were measured. Examples of previous research on cassava root system include the works of Banoc et al. (1999), Izumi et al. (1999), Lowe et al. (1982), Pardales and Esquibel (1996), Pardales et al. (1999) and Suber et al. (2009). In these works, various shoot and root traits were measured, ranging from shoot biomass, leaf numbers and area, development of nodal and basal adventitious roots as well as storage roots. Others also measured development of various orders of lateral roots, numbers, biomass and lengths of various root types. It is as yet to be established which different traits or variables among the measured multi-variate root system data contribute most to the observed variation or provide the most detailed differentiation among distinctive genotypes. It is possible that many of the traits that have hitherto been measured are mutually correlated and are in fact measuring the same construct (Bodner et al., 2013). This situation can further extend the long-term process of multi-trait selection in breeding for improved genotypes (Bradshaw et al., 2009; Wishart et al., 2013). The current study aims to addresses this problem by (i) using an easily adaptable and economical protocol to measure genotypic variation for shoot and root traits of a range of cassava genotypes at the juvenile stage of growth and (ii) identifying candidate variables that contribute the largest share of the variance in the original, multivariate dataset.

2. Materials and methods

2.1. Cassava genetic material

Eight cassava genotypes were used in this study. The genotypes were composed of one released variety called 'Capevars bankye' (Catalogue of Crop Varieties Released in Ghana, 2015), designated CV in this paper (Table 1). The remaining 7 genotypes are unreleased materials composed of 2 white flesh and 5 yellow flesh genotypes. These materials are designated 1A, 2B, 3C, 4D, 5E, 6F and 7G (Table 1). The first five genotypes were bred for higher carotenoid levels and the last two were bred for host plant resistant to cassava mosaic disease (CMD) through mutagenesis at Nuclear Agriculture Research Centre of the Ghana Atomic Energy Commission.

2.2. Soil and environmental conditions

This experiment was conducted at the Teaching and Research Farm of the School of Agriculture, University of Cape Coast (UCC; 5.1155°N, 1.2909°W) from April 5th to June 5th, 2017. The soil and environmental conditions for the present study has previously been described in Adu et al. (2017a). Briefly, nursery polybags (30,000 cm^3) with drainage holes underneath were filled with the air-dried soil,

Table 1. Disease, carotenoid and culinary properties of root cortex of the eight cassava genotypes.

Genotype	Disease resistance				Culinary properties				Selection
	CMD	CAD	CGM	CL	T	C	ML	S	
1A	2	3	2	7.69	1	2	1	2	Yes
2B	1	3	1	7.05	3	2	3	3	Yes
3C	1	3	1	3.93	1	2	1	1	No
4D	2	3	1	4.67	1	2	1	1	No
5E	1	3	1	7.05	1	2	1	2	Yes
6F	1	3	1	1.00	4	1	4	4	Yes
7G	1	4	1	1.00	4	1	4	4	Yes
CV	1	3	1	2.10	2	1	2	2	No
Mean	1	3	1	4	2	2	2	2	
STDEV	0.4	0.3	0.2	2.7	1.3	0.5	1.3	1.1	
Coefficient of Variation (%)	28.3	9.9	15.8	65.2	58.6	30.0	58.6	46.2	

CMD: Cassava Mosaic Disease; CAD: Cassava Anthracnose Disease; CGM: Cassava Green Mite. Disease resistances were scored from field trials and Scores 1, 2, 3, 4, and 5 indicated Resistant, Mild, Moderately Resistant, Severe and Very Severe, respectively (modified from Fukuda et al., 2010); CL: Carotenoid Level in μg g^{-1} fresh weight basis; T: Texture and Scores 1, 2, 3 and 4 indicated Hard and Glassy, Hard not Glassy, Medium Soft and Soft, respectively; C: Colour and Scores 1 and 2 indicated white and yellow, respectively; ML: Mealiness and Scores 1, 2, 3 and 4 indicated Not Good for 'fufu', Good for 'fufu', Better for 'fufu' and Best for 'fufu', respectively; S: Sweetness of root cortex after boiling for 30 minutes and Scores 1, 2, 3 and 4 indicated Very Bitter, Sour, Medium Sweet and Very Sweet, respectively (modified from Safo-Kantanka and Owusu-Nipah, 1992). Genotypes CV is not selected because it is an already released variety.

tapped very gently to achieve a bulk density of approximately 1.1 g cm^{-3}. The filled nursery bags were kept under an 8 × 12 × 2.4 m rigid rain shelter constructed with galvanized iron pipe as frames with a 0.1 mm thick ultraviolet (UV)-resistant transparent polyethylene film roof. Temperature and relative humidity at the experimental site ranged between approximately 24 °C to 32 °C and 60% to 80%, respectively. Day length ranges from approximately 11.30 to 12.40 hours while solar radiation ranges from 3151 kJ cm^{-2} day^{-1} to 3804 kJ cm^{-2} day^{-1}, respectively (Adu et al., 2017a).

2.3. Planting, watering and harvesting

Cassava cuttings of approximately 20 cm of each genotype were planted in inclined position (about 45°) into the soil at the centre of polyethylene pots, making sure that at least five nodes were within the soil. The size of polyethylene pot was 45 cm high and 30 cm wide (Fig. 1A) and this provided sufficient soil volume for root growth and distribution for the up to 60 days after planting (DAP) sampling period adopted in this study (Izumi et al., 1999). Cuttings were obtained from the middle third of stems from 12 month old plants. Diameter of the cuttings were controlled, but no
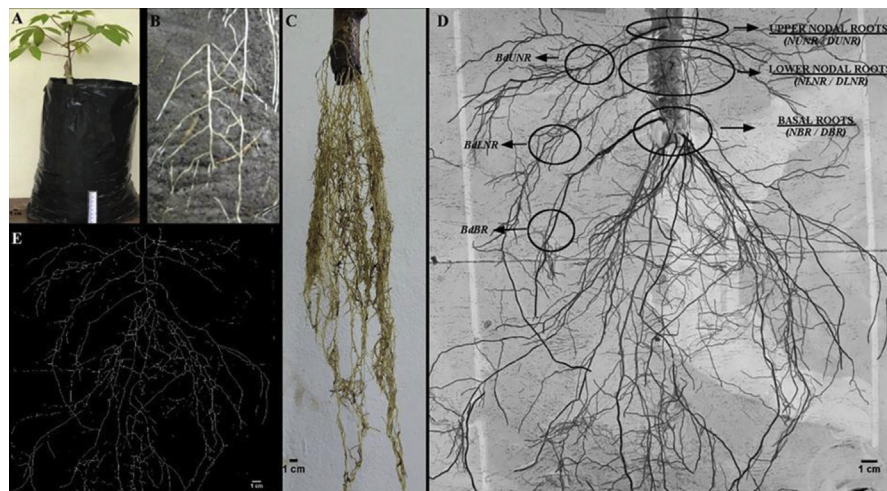

Fig. 1. A) Image of cassava plant growing in polyethylene pot; (B) Image showing a cross-section of a 30 d old cassava plant root system growing in a soil-filled polyethylene pot; (C) Image illustrating a washed root system of a juvenile (45 DAP) cassava plant; (D) Category of adventitious roots used in this study: upper nodal roots: emerged from the topmost nodes within the top 7 cm below the soil surface; lower nodal roots: emerged from the nodes on the stem cutting with 7−13 cm below the soil surface; basal roots: emerged from the callus at the base of the stem cutting. Ten traits were assessed visually on the washed root system: number of upper nodal roots (NUNR), diameter of upper nodal roots (DUNR), branching density of upper nodal roots (BdUNR), number of lower nodal roots (NLNR), diameter of lower nodal roots (DLNR), branching density of lower nodal roots (BdLNR), total number of nodal roots (TNR), number of basal roots (NBR), diameter of basal roots (DBR) and branching density of basal roots (BdBR); (E) Sample image of a cassava root system showing skeletonized root image for measuring total root length.

one specific diameter was adopted, such that the diameter of cuttings were greater than one half the diameter of the thickest part of the stem of respective genotypes (Ceballos et al., 2010). There was one cutting per pot and genotypes were arranged in a complete randomized design. Polyethylene pots were positioned side by side because we assumed that for up to 60 DAP, this spacing would not cause mutual shading. Positions of polyethylene pots were rotated under the rain shelter every 10 days to reduce effects of possible environmental gradients. A day prior to planting, the soil in each polyethylene pot was watered with tap water to 80% field capacity (FC) determined gravimetrically and allowed to drain overnight. The soils were subsequently maintained at approximately 70% FC once a week during the growth period. No other soil amendment or input was applied. There were four (4) replications per cassava genetic material for each sampling period. There were three harvesting or sampling days in this study, at 30 DAP, 45 DAP and at 60 DAP. At sampling, plants were harvested by carefully severing the polybag longitudinally at its two sides with a knife to uncover roots growing in the soil (Fig. 1B). Roots were gently washed free of soil under running tap water, using water hose. Care was taken to minimize damages to roots.

2.4. Shoot and root system measurements

To a large extent, the stem cutting were still intact up to 60 DAP (Fig. 1C). After root washing, shoots with leaves were severed from the stem cuttings and number of sprouted shoots determined (Data not shown). Leaves were subsequently cut from the shoots and number of leaves (NL) determined. Leaves were carefully placed flat and end-to-end on a sea-blue background and images of same captured with a Canon EOS 70D DSLR camera (https://www.usa.canon.com/) held stationary on tripod 50 cm above the leaves. Shoots, including the leaves, were then weighed and oven-dried at 80 °C for three days to determine shoot fresh weight (SFW) and shoot dry weight (SDW), respectively. Total leaf area (TLA) was extracted from leaf images using binarization/thresholding and feature extraction routines in *ImageJ* (US National Institutes of Health, Bethesda, MD, USA, https://imagej.nih.gov/ij/). Root-to-shoot ratio (R:S) was calculated as the quotient of root dry weight (RDW) and SDW.

Here, we adopted the classification of Izumi et al. (1999) and classified the roots into three categories, consisting of two types of nodal roots [upper nodal roots (UNRs) and lower nodal roots (LNRs)], and basal roots (BRs) as shown in Fig. 1D. Nodes on the stem cutting within the soil were divided into two; upper and lower nodal roots were roots formed from the top and lower nodes, respectively. The cuttings were buried in the soil with about one-third of it above the soil surface. Relative to the soil surface, upper nodal roots were within approximately the top 7 cm and lower nodal roots were within the 7−13 cm below the soil surface. Basal roots

were those formed from the callus at the base of the cutting. Root system traits including total root length (TRL), number of upper nodal roots (NUNR), diameter of upper nodal roots (DUNR), and branching density of upper nodal roots (BdUNR) were measured. Other root traits measured included number of lower nodal roots (NLNR), diameter of lower nodal roots (DLNR), branching density of lower nodal roots (BdLNR) and total number of nodal roots (TNR). Number of basal roots (NBR), diameter of basal roots (DBR), branching density of basal roots (BdBR) and root-to-shoot ratio were also measured (Fig. 1A). On the root systems, the number, diameter and branching density of the nodal (upper and lower) and basal roots were manually measured. Root number of each category of roots was manually counted. Subsequently, three representative roots were randomly selected from each of the three positions (Fig. 1) per plant for the measurement of root diameter and branching density. Root diameter for each root type was determined at three (3) cm from the stem cutting using digital callipers. Branching density for each category of root was determined within six (6) cm distance beginning from the cutting. It was assumed that the branching density was going to be constant for the rest of the main axis of the root and only the first order lateral root was considered. Total root length was measured by spreading and suspending the roots in water in a rectangular glass dish with black background. Care was taken to avoid roots overlying on each other. Images of the total root system were captured with a Canon EOS 70D DSLR camera (https://www.usa.canon.com/) held stationary on tripod 50 cm above roots. Images were converted to a binary image and TRLs were extracted from root images using skeletonization routines (Fig. 1E; Adu et al., 2015, 2016; 2017a) in *ImageJ* (US National Institutes of Health, Bethesda, MD, USA, https://imagej.nih.gov/ij/). The relative growth rate of the total root system (RGR) in 30-, 45- and 60-day-old plants was calculated using equation (1) (Kumar et al., 2012):

$$RGR = \frac{lnw_2 - lnw_1}{t_2 - t_1} \qquad (1)$$

where w_1 and w_2 = TRL of first and second measurement, respectively; t_1 and t_2 = time interval between two successive measurements. Genotypes were classified according to their RGR of the TRL into five groups (HLL, LHH, HHL, HLH and LLH), where the three letters indicate RGR at intervals of 0−30, 30−45 and 45−60 days, respectively. H and L indicate above-and-below average RGR, respectively (Kumar et al., 2012).

2.5. Data and statistical analysis

Descriptive statistics including mean and range for all traits were calculated for each of the three sampling dates. Estimate of the standard deviation (σ) were obtained and the coefficient of variation (CoV) was determined as the quotient of the standard deviation and the mean and expressed as a percentage. Residual

maximum likelihood (REML) procedures were used to estimate variance components for all traits and ANOVA was used to determine variation between individual genotype means. All factors were classed as random factors in REML so that the proportional contribution of genotype to overall variation in traits could be determined (Thomas et al., 2016). Both REML and ANOVA employed the following model (equation (2)):

$$y_{ij} = u + g_i + b_j + gb_{ij} + \varepsilon_{ij} \tag{2}$$

where: y_{ij} represents the observation from the ij^{th} sampling date (30, 45, or 60 DAP), u is the overall mean, g_i is the effect of the ith genotype, b_j is the effect of the jth block, gb_{ij} is the interactive effect of the ith genotypes with the jth block, and ε_{ij} is the experimental error.

Multivariate analysis of trait space was subsequently carried out. First, Pearson's correlation coefficients for pairs of shoot and/or root traits were calculated for plants sampled on 30, 45 and 60 DAP at α level of 5%. Second, roots and shoot traits were subjected to principal components analysis (PCA) to identify major traits accounting for most of the variation among the studied cassava genotypes. The PCA was based on correlation matrix and the number of significant principal components was determined based on the Kaiser criterion, retaining any component with an eigenvalue >1.00 (Kaiser, 1960; Tabachnik and Fidell, 1996). Statistical analyses were performed using GenStat (GenStat Release 14.1, VSN International, Oxford, UK) and various packages in the *R* software, the Language and Environment for Statistical Computing (R Core Team, 2013). Pearson's correlation was performed using the *r corrplot* package (Wei, 2013). Principal component analysis was performed using the functions of prcomp from the built-in *R* stats package (R Core Team, 2013) and the package *factoextra* (Kassambara, 2017) was used for the visualization of the PCA results.

3. Results

3.1. Descriptive data and variance components

For up to 45 DAP, plants grew well in the polyethylene pots and visually showed no symptoms of stress. Between this time-interval, values for all shoot and root traits increased over time (Supplementary Table 1). There were reduction in some traits, including NUNRs, DLNRs, TNRs, DBRs, NL and TLA between 45 DAP and 60 DAP (Supplementary Table 1). The root system of all tested cassava genotypes was dominated by fine (fibrous) root consisting of adventitious roots and up to 1st and 2nd order lateral roots (Fig. 1B−E). For each of the traits evaluated, descriptive statistics including the range and the means of the three sampling periods (30, 45 and 60 DAP) are summarized in Supplementary Table 1. Wide ranges of phenotypic

values were observed for all traits. Shoot dry weight ranged from 0.26−2.22, 2.1−6.5 and 2.4−7.54 mg in 30-, 45- and 60-day-old plants, respectively (Supplementary Table 1). Similarly, NBRs ranged from 0 − 20, 1−20 and 3−23 in 30- 45- and 60-day-old plants, respectively and BdLNR ranged from 0−9, 0−4.5, and 0−3.5 cm^{-1} in 30- 45- and 60-day-old plants, respectively (Supplementary Table 1).

Coefficients of variation (CoV) across the three sampling periods for the measured traits generally ranged from 4% for RDW recorded at 60 DAP to 148.6% for NUNRs recorded at 45 DAP (Supplementary Table 1). The CoVs of upper nodal root-related traits were particularly high and were 67, 148.6 and 98.44% for NUNR, 69.36, 104.2, 86.44% for DUNR and 114.5, 85.78 80.87% for BdUNR in 30-, 45- and 60-day-old plants, respectively. The CoVs recorded for some traits including SFW, DBRs NL and TRL were relatively smaller (Supplementary Table 1). Some of the variation in all the traits examined, could be attributed to vagaries in experimental conditions (i.e. block) but variation attributable to block × genotype interaction was largely small (Supplementary Table 1). The effect of genotype alone ranged from 0% for DLNRs recorded at 45 DAP to 94.35% for RDW of plants grown for 30 days. The effect of genotype alone accounted for >55% for RDW for all three sampling dates but <30% for DUNRs and NLs for all three sampling dates (Supplementary Table 1).

3.2. Genotypic variation in traits

With the exception of DUNRs and BdLNRs, a significant effect of genotype ($p < 0.05$) was observed for all traits measured, but the significant differences were not recorded for all sampling dates in some traits (Supplementary Table 1). The ranking of genotypes was however not consistent across traits. Total root length varied from 358 (7G) to 1440 cm (1A), 1346 (7G) to 2849 cm (1A) and 1557 (4D) to 3054 cm (2B) in 30-, 45- and 60-day-old plants, respectively (Fig. 2A). Total leaf area varied from 82 (7G) to 387 cm^2 (CV), 383 (7G) to 1078 cm^2 (CV) and 426 (4D) to 944 cm^2 (CV) in 30-, 45- and 60-day-old plants, respectively (Fig. 2B). At 30 DAP, the genotype CV had the highest shoot biomass with SDW of 2.1 mg, followed by genotype 7G (1.51 mg) and genotype 2B (1.4 mg). Consistent with the trend at 30 DAP, the genotype CV had the highest SDW (4.98 mg) at 45 DAP, followed by genotype 3C (4.21 mg) and genotype 2B (4.1 mg). Shoot dry biomass was not significantly different between genotypes at 60 DAP ($p = 0.56$) but root dry biomass was ($p < 0.001$). At 60 DAP, genotype CV again had the highest RDW (5.8 mg) followed by 5E and 1A (5.6 mg), and 2B (5.4 mg).

3.3. Relative growth rate of total root length

The relative growth rate (RGR) of total root length was calculated across different time intervals 0−30, 30−45, and 45−60 DAP (Fig. 3). The highest RGR occurred
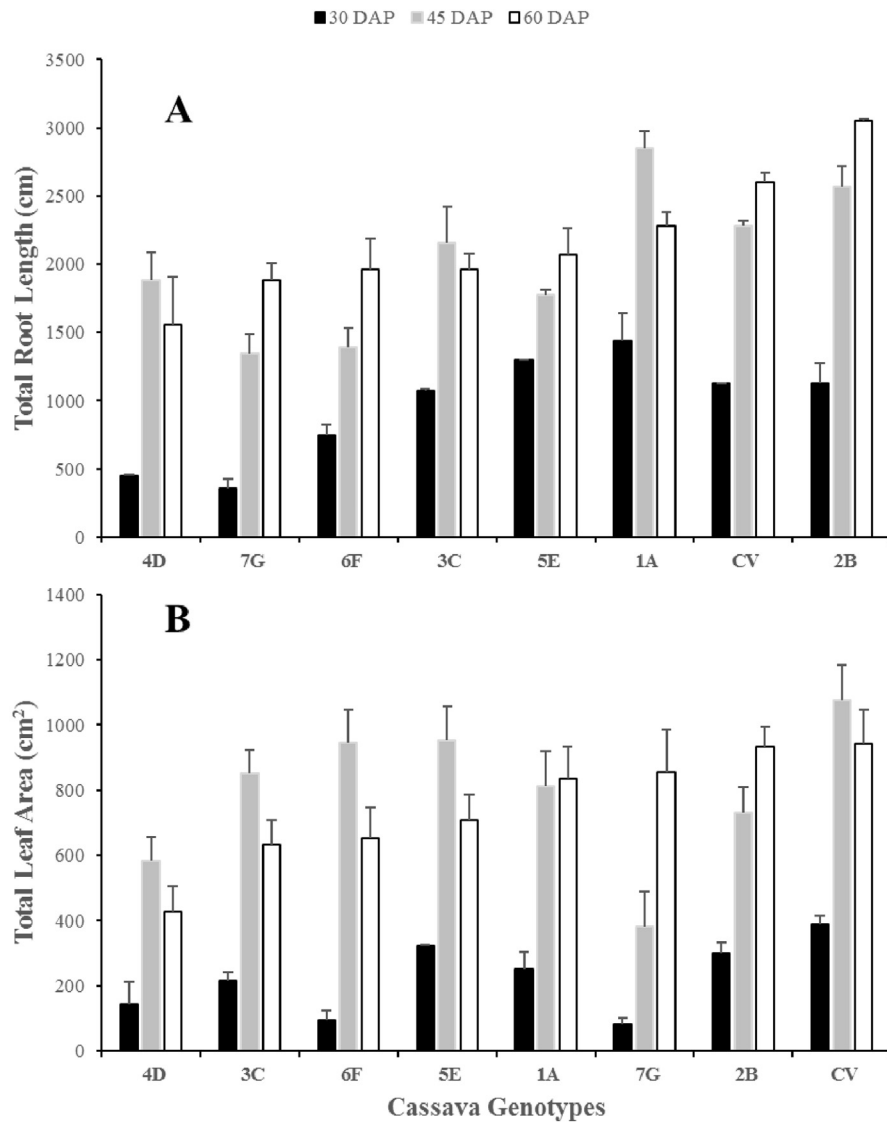
Fig. 2. Total root length (A) and Total leaf area (B) for eight cassava genotypes grown in soil-filled poly-ethylene pots for 30, 45 and 60 days after planting (DAP). (Data shows mean + SE, n = 4).

over the first 30 DAP and subsequently declined until nearly constant. Between 0–30 DAP, RGR ranged from 0.39 to 0.48 cm day^{-1}. Intermediate values were recorded for 30- to 45-day time interval ranging from 0.02 to 0.10 cm day^{-1}. The lowest RGR values were observed in the third time interval (45–60 DAP) with values ranging from 0.01 to 0.04 cm day^{-1}. No genotype showed above-average TRL RGR at all three time intervals but four genotypes recorded above average RGRs at two but different time intervals. No genotype obtained below average RGR at all three time intervals but genotypes 1A, 5E 6F and CV recorded above average RGR at only one but varying time intervals (Fig. 3). The performance of the genotypes in terms of RGR of TRL was however not completely consistent with actual TRL measurements. Out of the four genotypes that showed above-
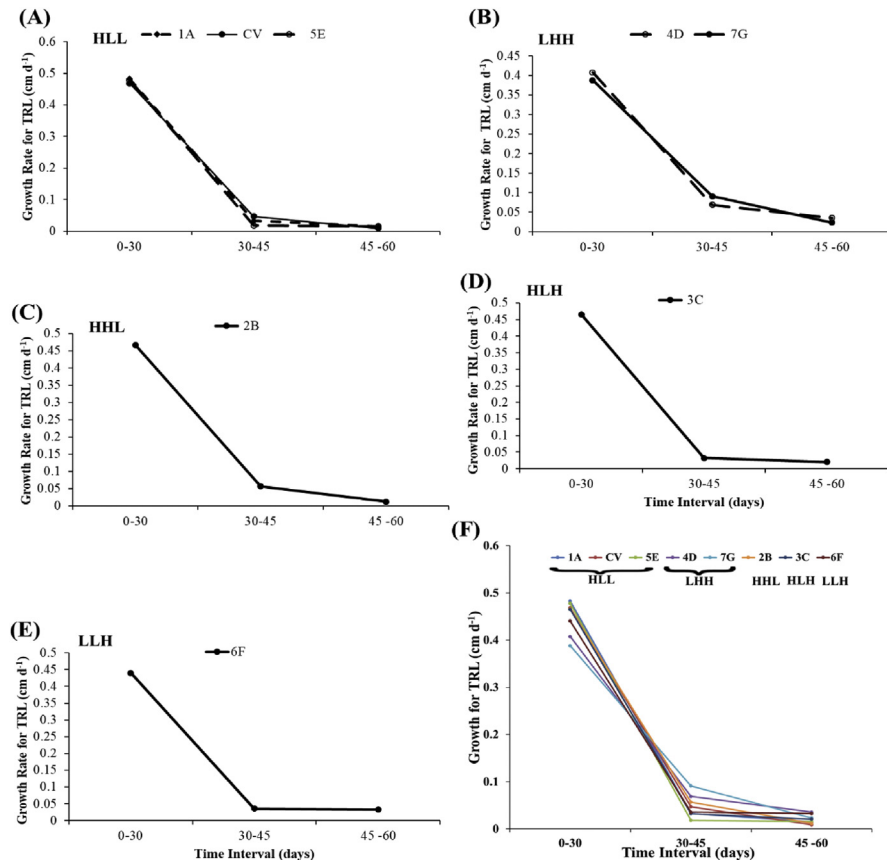
Fig. 3. Classification of 8 cassava genotypes according to their relative growth rate (RGR) of total root length at different time intervals 0−30, 30−45 and 45−60 days. (A) Genotypes CV, 1A and 5E have above average, below average and below average growth; (B) genotypes 4D and 7G have below average, above average and above average growth; (C) genotype 2B recorded above average, above average and below average growth; (D) genotype 3C recorded above average, below average and above average growth; (E) genotype 6F has below average, below average and above average growth at 0−30, 30−45 and 45−60 days interval, respectively; (F) combination of all genotypes into one figure for easy comparison. The three letters on the figures (HLL, HHL, LLH, LHH and HLH) indicate the GR at 0−30, 30−45 and 45−60 days interval, respectively. H and L indicate above and below average GR, respectively.

average RGR at more than one time interval, only 2B seem to be among the best performing genotypes in terms of TRL (Fig. 2A).

3.4. Correlations among measured shoot and root system traits

Test for the significance of relationships between all the measured variables revealed that approximately 35% (53 out of possible 153), 29% (44 of 153) and 32% (49 of 153) of all potential correlations were statistically significant ($p < 0.05$, indicated as '*' subsequently) with varying strengths and consistencies in traits measured in 30-, 45- and 60-day-old plants, respectively (Fig. 4). At 30 DAP, there were both positive and negative significant correlations. Pearson's correlation coefficient
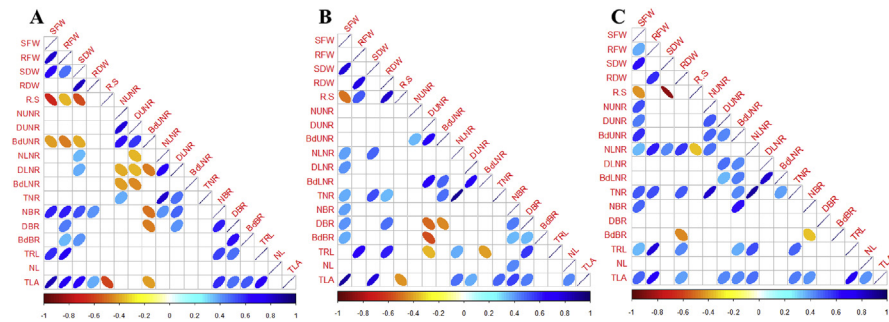
Fig. 4. Phenotypic correlations between traits observed in juvenile cassava plants grown in soil-filled polyethylene pots for 30 DAP (A), 45 DAP (B) and 60 DAP (C). Traits shown in the matrix are SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight, R: S: root-to-shoot ratio, NUNR: number of upper nodal roots, DUNR: diameter of upper nodal roots, BdUNR: branching density of upper nodal roots, NLNR: number of lower nodal roots, DLNR: diameter of lower nodal roots, BdLNR: branching density of lower nodal roots, TNR: total number of nodal roots, NBR: number of basal roots, DBR: diameter of basal roots, BdBR: branching density of basal roots, TRL: total root length, NL: number of leaves and TLA: Total leaf area. Eccentricity and colour of the ellipses represents the correlation value. The scale is indicated in the bar below the matrix. Blank boxes indicate non-significant relationships ($P < 0.05$).

analysis revealed strong positive and significant correlations between shoot and root biomass (SFW and RFW, $r = 0.81*$; SDW and RDW, $r = 0.84*$); TRL and SFW ($r = 0.84*$); TRL and RFW ($r = 0.76*$); DUNR and NUNR ($r = 0.79*$); and TNR and NLNR ($r = 0.80*$) and DBR and NBR ($r = 0.61*$) (Fig. 4A). Low to medium significant correlations were recorded between variables such as NLNR and SDW ($r = 0.36*$); NBR and SDW ($r = 0.53*$); BdBR and RFW ($r = 0.35*$); DBR and DLNR ($r = 0.42*$) and TLA and NL ($r = 0.66*$). Negative significant correlations were observed between traits such as R: S and SFW ($r = -0.68*$); TLA and R: S ($r = -0.59*$); and DLNR and BdUNR ($r = -0.47*$) (Fig. 4A).

At 45 DAP, positive significant correlations ranged from $r = 0.35*$ (BdBR and NBR; DBR) to $r = 0.88*$ (TLA and SFW) (Fig. 4B). Negative significant correlations were observed between traits such as R: S and SFW ($r = -0.40*$); TLA and R: S ($r = -0.44*$); DBR and DUNR ($r = -0.54*$); DBR and NLNR ($r = -0.45*$); BdBR and DUNR ($r = -0.57*$); TRL and DUNR ($r = -0.34*$) and TRL and BdLNR ($r = -0.40*$) (Fig. 4B). At 60 DAP, there were strong positive correlations between biomass root traits. Pearson's correlation coefficient analysis revealed strong positive and significant correlations between TLA and RFW ($r = 0.72*$); TRL and RFW ($r = 0.79*$) and NLNR and RFW ($r = 0.60*$). Similarly, there were strong positive correlations between TLA and TRL ($r = 0.75*$); BdLNR and DLNR ($r = 0.80*$); TNR and NUNR ($r = 0.89*$) and NBR and BdUNR ($r = 0.63*$) (Fig. 4C). Low to medium significant correlations were recorded between variables such as TLA and NBR ($r = 0.42*$); NLNR and SFW ($r = 0.35*$); TRL and TNR ($r = 0.50*$) and TRL and RDW ($r = 0.44*$). Negative significant correlations were observed between traits such as R: S and SFW ($r = -0.43*$); R: S and

SDW ($r = -0.87*$); BdBR and RDW ($r = -0.44*$); NLNR and R: S ($r = -0.30*$) and BdBR and NBR ($r = -0.30*$) (Fig. 4C).

3.5. Correlations between time intervals

In order to assess if measurements taken in younger juvenile plants could be used to predict performance in relatively older juvenile plants, Pearson correlation coefficients were calculated between 30 and 45-, 30 and 60-, and 45- and 60-day-old-plant-related traits (Table 2). There were positive correlations (r ranging from 0.35* to 0.77**) between 30- and both 45-, 60-day measurements but many of the correlations were intermediate, weak or not significant. Positive significant correlations were found between 30 and 45-, 30 and 60-, and 45- and 60-day measurements for RFW ($r = 0.40*$); BdBR ($r = 0.42*, 0.41*, 0.40*$), TRL ($r = 0.58**$, 0.49**, 0.41*) and NL ($r = 0.36*, 0.48**, 0.47**$) for 30 and 45-, 30 and 60-,

Table 2. Phenotypic correlation coefficients matrix between traits recorded at 30, 45, and 60 days after planting in eight cassava genotypes.

Trait*	Correlation coefficient		
	30 and 45 DAP	30 and 60 DAP	45 and 60 DAP
SFW	0.27	0.1	0.28
RFW	0.40*	0.40*	0.40*
SDW	0.13	0.1	0.77**
RDW	0.23	0.41*	0.40*
R-S	0.04	0.04	0.24
NUNR	0.32	0.2	0.17
DUNR	0.15	0.23	0.12
BdUNR	0.2	0.27	0.2
NLNR	0.31	0.26	0.36*
DLNR	0.1	0.33	0.14
BdLNR	0.29	0.29	0.22
TNR	0.23	0.41*	0.34
NBR	0.42*	0.38*	0.02
DBR	0.35*	0.1	0.01
BdBR	0.42*	0.41*	0.40*
TRL	0.58**	0.49**	0.41*
NL	0.36*	0.48**	0.47**
TLA	0.43*	0.34	0.11

Traits shown in the Table are SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight, R: S: root-to-shoot ratio, NUNR: number of upper nodal roots, DUNR: diameter of upper nodal roots, BdUNR: branching density of upper nodal roots, NLNR: number of lower nodal roots, DLNR: diameter of lower nodal roots, BdLNR: branching density of lower nodal roots, TNR: total number of nodal roots, NBR: number of basal roots, DBR: diameter of basal roots, BdBR: branching density of basal roots, TRL: total root length, NL: number of leaves and TLA: Total leaf area. *Significant at $P < 0.05$; **Significant at $P < 0.01$.

and 45- and 60-day measurements, respectively (Table 2). No significant correlation was found for SDW taken after 30 and 45 and 30 and 60 days but high significant correlation for measurements takes between 45 and 60 days ($r = 0.77**$). There was intermediate significant correlation for RDW in measurements taken between 30 and 60 days ($r = 0.41*$) and 45 and 60 days ($r = 0.40*$). Correlations for NBR was significant for measurements taken between 30 and 45- ($r = 0.42*$) and 30 and 60-day old plants ($r = 0.38*$) (Table 2). Correlations for TLA was only significant ($r = 0.43*$) for measurements taken between 30 and 45 day-old plants and that for TNR was only significant ($r = 0.41*$) for measurements taken between 30 and 60 day-old plants (Table 2).

3.6. Principal component analysis

The first six, first five and first six principal components (PCs) with an eigenvalue >1.00 explained 85.1%, 80.3% and 81.8% of the total variation among the genotypes for the 18 shoot and root system traits measured at 30, 45 and 60 DAP, respectively (Supplementary Table 2). For the 30-day time point of measurements, the relative magnitudes of eigenvectors for the first PC was 33.7%, explained mostly by biomass traits including SFW, RFW, SDW, RDW, NBR and TLA. The second to fifth PCs, contributed 16.6, 13.2, 8.5, 7.6 and 5.6% of the total variation, respectively. The most predominant traits were NUNR, DUNR, DLNR and BdLNR (PC2), NLNR (PC3), R:S ratio and BdUNR (PC4), TRL and NL (PC5) and TNR DBR and BdBR (PC6). For measurements taken after 45 and 60 days, the first PC explained 24.9 and 33.4% of total variation, respectively, with contributions from SFW, RFW, SDW, BdUNR, NLNR and TLA. For measurements taken at 45 DAP, RFW, DUNR, BdUNR, BdLNR, DBR and TRL were the highest contributors for the second PC, which explained 21.1% of total variation. Root DW and R: S resolved on PC3 which explained 16.2% of total variation. The fourth and fifth PCs had NUNR, NBR, BdBR and NL as well as NLNR, DLNR and TNR as the highest contributors and explained 10.5 and 7.5% of total variation, respectively. For measurements taken at 60 DAP, RDW, DUNR, DLNR, BdLNR and BdBR were the highest contributors for the second PC, which explained 13.9% of total variation. Shoot biomass and DBR resolved on PC3 which explained 11.5% of total variation. The major contributors to the fourth to sixth PCs were R: S, NBR, NUNR, TNR, TRL, TLA and NL and explained 9.7, 7.2 and 6.2% of total variation, respectively (Supplementary Table 2).

Fig. 5 shows the biplots obtained from the three sampling dates. Biplots in Fig. 5A1, B1 and C1 contain the traits vectors and the location of the genotypes according to their PC scores. For better visualization, trait vectors and objects, quality of variable representation and contribution of variables in explaining variability of the PCs are shown on separate graphs. A genotype by trait (GT) biplot, captured 47%−50% of the variations (Fig. 5A1, B1 and C1). For all 3 timepoints, the largest variation
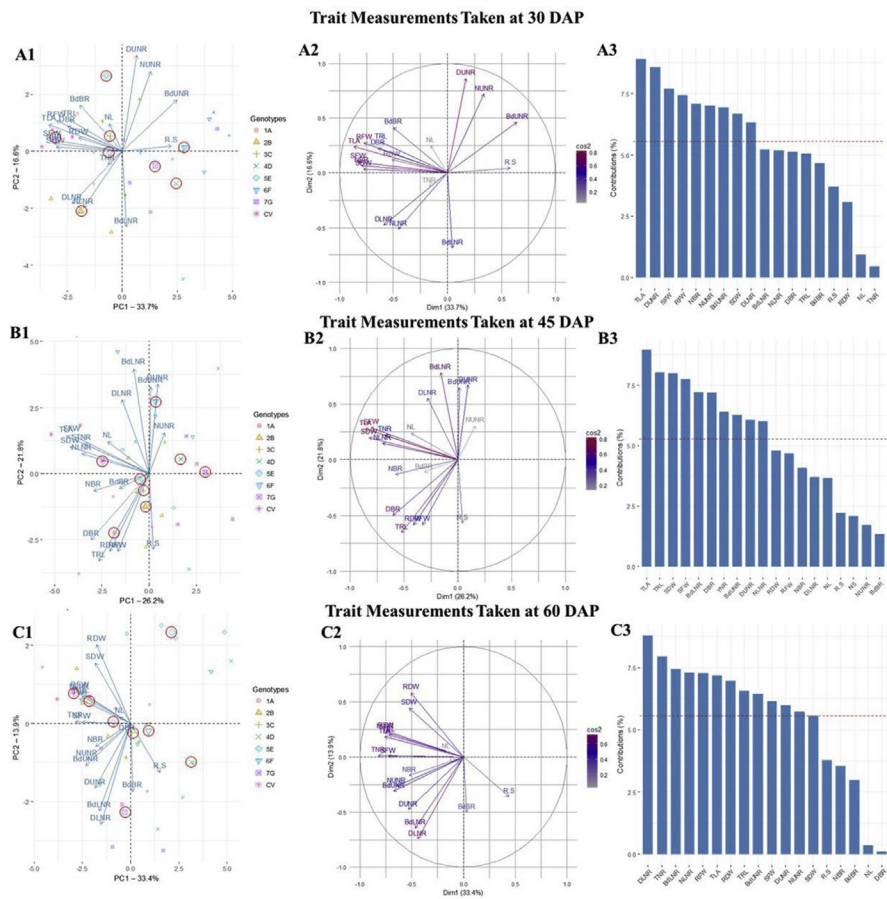
Fig. 5. Genotype by trait PCA biplot showing trait vectors and location of the single objects from (A1) measurements taken at 30 DAP; (B1) measurements taken at 45 DAP; (C1) measurements taken at 60 DAP from cassava plants grown in polyethylene pots. Arrow lines represent vectors that quantify the magnitude and direction of a trait's contribution to that axis. Trait names are shown in acronyms previously defined in this paper. The position of mean point for each genotype on the biplot is shown with enlarged markers highlighted in red circles for PC1 vs. PC2 representing 50.3% (A1), 48.0% (B1) and 47.3% (C1) of the variability in measurements taken during the 1st, 2nd and 3rd sampling dates, respectively. Plots of quality of representation of the variables (cos2) on the factor map for (A2) measurements taken at 30 DAP; (B2) measurements taken at 45 DAP; (C2) measurements taken at 60 DAP; the scale is indicated in the bar alongside the plots. Plots showing total contribution of variables in accounting for the variability in PC1 and PC2 for (A3) measurements taken at 30 DAP; (B3) measurements taken at 45 DAP and (C3) measurements taken at 60 DAP. The red dashed line on the graph indicates the expected average contribution. Traits shown in the graph are SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight, R: S: root-to-shoot ratio, NUNR: number of upper nodal roots, DUNR: diameter of upper nodal roots, BdUNR: branching density of upper nodal roots, NLNR: number of lower nodal roots, DLNR: diameter of lower nodal roots, BdLNR: branching density of lower nodal roots, TNR: total number of nodal roots, NBR: number of basal roots, DBR: diameter of basal roots, BdBR: branching density of basal roots, TRL: total root length, NL: number of leaves and TLA: Total leaf area.

explained by the biplots came from TLA and shoot/root biomass traits as indicated by the relative length of their vectors (Fig. 5A1, B1 and C1). In addition, at 30 DAP, the largest variation explained by the biplots came from DUNR, BdUNR, NUNR and DLNR. At 45 DAP, additional variables that explained the largest variation included BdLNR, TRL, and DBR. At 60 DAP, additional variables that explained the largest variation as indicated by the relative length of their vectors included DLNR, BdLNR, TRL, and TNR.

Three prominent associations were revealed by the GT biplots. First, there was a strong negative associations. These were between DUNR and BdLNR; BdUNR and DLNR or NLNR; Shoot biomass and R:S and NBR and R:S, at 30 DAP (Fig. 5A1); between DBR and NUNR, BdUNR, DUNR BdLNR, all being negatively correlated to R: S at 45 DAP (Fig. 5A2); and between shoot/root biomass traits and R:S at 60 DAP (Fig. 5C1). Second, there were also a near zero correlations. These were between R: S and both BDLN and DUNR; and between BdLNR and both shoot biomass traits and NBR, at 30 DAP; and NLNR, BdLNR and NBR, all having a near zero correlation to R:S at 45 DAP, and between BdBR and TNR or SFW, at 60 DAP (Fig. 5A1, B1 and C1). Thirdly, there was a positive associations, including between DLNR and biomass traits, NLNR and BdLNR, at 30 DAP; NLNR, TNR, shoot biomass, all being closely correlated to NBR; and BdUNR and DUNR, both being closely positively correlated to DLNR, at 45 DAP; TNR and SFW, both being closely correlated to NBR, between NUNR and BdUNR, and TLA, TRL and RFW, all being correlated to NBR, at 60 DAP (Fig. 5A1, B1 and C1).

Fig. 5A1, B1 and C1 also enable the comparison of genotypes on the basis of the measured multiple variables and to identify genotypes that are particularly superior in certain traits. There were some inconsistencies across the three sampling dates but some genotypes recorded consistent superiority in certain traits. The CV genotype had higher values of biomass, TLA and NL at all three sampling dates. At 30 DAP, the CV was grouped with genotypes 3C and 5E and had higher values also of TRL and BdBR and all the traits positively associated with root dry weight. Whilst, genotypes 1A and 2B recorded higher values for DLNR and NLNR at 30 DAP, genotypes 7G and 4D seemed to be superior in BdLNR (Fig. 5A1). At the second sampling date, CV showed additional superiority in BdLNR, NLNR and DLNR and genotypes 1A, 2B, 3C and 5E seemed to have been grouped together and obtained higher values of NBR, BdBR, TRL and all the traits positively associated with root biomass. Also Fig. 5C1 indicates that genotype CV was highest in similar traits as genotype 2B and genotypes 7G was highest in BdUNR, DLNR, NBR, BdLNR, NUNR, and DUNR and all these traits were positively associated.

Fig. 5B2−C2 are the $\cos^2$ (squared coordinates) which is the quality of representation of the variables on the factor map and shows the importance of a component for a given observation (Abdi and Williams, 2010). The value of $\cos^2$ can help find the

components that are important to interpret observations. When there is high $\cos^2$, the variable is positioned close to the circumference of the correlation circle and indicates a good representation of the variable on the PC. A low $\cos^2$ where the variable is close to the centre of the circle suggests that the variable is not perfectly represented by the PCs. For a given variable, the sum of the $\cos^2$ on the entire PCs = 1, such that if the variable is perfectly represented by only two PCs (PC1 and PC2), the sum of the $\cos^2$ on these two PCs = 1 (Kassambara, 2017). The $\cos^2$ values recorded were 0.2–0.8 for both 1st and 2nd sampling dates and 0.2–0.6 for the 3rd sampling (Fig. 5B1, B2 and C2). Total leaf area was well represented on the factor map at all sampling dates but some traits, including shoot and root biomass and TRL were well represented on two occasions (Fig. 5B1 – B3). For measurements taking at 30 DAP, DUNR and DLNR were among the variables that are well represented on the factor map and thus warrant interpretation (Fig. 5B1). At measurements for 45 DAP, BdLNR and DBR were among traits that obtained higher $\cos^2$ values (Fig. 5B2). Total number of nodal roots, NL DLNR and BDLNR were among traits that were well represented on the factor map at 60 DAP (Fig. 5B3).

Fig. 5A3, B3 and C3 present the contributions of the variables at the 3 sampling dates. In the present study, if the contribution of the variables were uniform, the expected value would be 1/length of variables = 1/18 = 5.5%. The total contribution of a given trait (contrib), on explaining the variations retained by two PCs (PC1 and PC2) is given by contrib = [(C1 × Eig1) + (C2 × 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). Here, for a given PC, a variable with a contribution larger than [(5.5 × Eig1) + (5.5 * Eig2)]/(Eig1 + Eig2) could be considered as important in contributing to PC1 and PC2. Here, five variables including TLA, shoot biomass (SFW and SDW), BdUNR and DUNR consistently contributed above average to the variability in PC1 and PC2 at all 3 sampling dates (Fig. 5A3, B3 and C3). Seven variables however contributed to the variability in PC1 and PC2 on two sampling dates. These included RFW, NUNR, TRL, BdLNR, TNR, NLNR and DLNR (Fig. 5A3, B3 and C3). Three variables, including number (at 30 DAP) and diameter (at 45 DAP) of basal roots and root dry weight (at 60 DAP) contributed to the variability in PC1 and PC2 at one sampling date (Fig. 5A3, B3 and C3).

4. Discussion

4.1. Low cost screening for genetic variation in cassava root traits

Cassava underpins livelihoods and food security for a substantial proportion of the populations in Africa and Asia. Knowledge of root system architecture in cassava is crucial for improving cassava productivity (Subere et al., 2009; Villordon et al.,

2014) as the root system is directly connected to exploitation and acquisition of soil resources (Connor et al., 1981; El-Sharkawy, 2003; El-Sharkawy and Cock, 1987; Tscherning et al., 1995). High automated throughput, high resolution, and scalable root phenotyping systems are critical to all crop breeders to aid in the selection and release of new cultivars with rooting architectures that are favourable for planting, harvesting and early bulking (Adu et al., 2014, 2015; Delgado et al., 2017; Downie at al. 2015). However, huge challenges including the high cost and complexity of current root phenotyping systems remain, especially in resource-poor jurisdictions, where root and tuber crops such as cassava are the second major source of carbohydrates (Adu et al., 2017a; Villordon et al., 2014).

In the present study, a simple, inexpensive phenotyping approach was used to characterize the RSA of selected cassava genotypes at the early stage of growth. Even though the protocol in the present study could be used to evaluate up to 60-day-old plants, measurements obtained could be used to predict lines which have better roots or even yield at later stages of development. In Fig. 2, the genotypes were ranked based on TRL and TLA and while genotypes 2B and CV were consistently among the top performing lines, genotypes 3C and 4D performed relatively poorly. It is interesting and worth noting that CV is an already released genetic material but an ongoing evaluation for the remaining genotypes has selected genotype 2B for release but is currently not considering genotypes 3C and 4D for release due low dry matter contents, low beta carotene and/or non-mealiness (Table 1). Our results thus suggest that the protocol employed in the present study could not only pick up variations in juvenile traits of cassava that could persist to maturity but also is able to detect subtle differences that occur in the growth of cassava roots at the juvenile stage. In fact, El-Sharkawy (2003) reported that genetic differences in root traits within cassava germplasm persevere throughout the production cycle. This is indicative of the possibility of screening large number of clones at the juvenile stages of growth for the evaluation and selection for better root system ideotypes in cassava. Our results however suggest that the persistence of genetic differences through the production cycle may not be uniform for all traits; while the variation or the extent of it in some traits may not persist, others show low to high persistence. This observation is supported by data in Table 2 where there were significant correlations between corresponding traits between 30- and 45-day-old seedlings, between 45- and 60-day-old seedlings, as well as between 30- and 60-day-old seedlings for some but not all traits measured. It may well be that some traits are growth-stage-specific and thus measuring them at certain growth stages will be advantageous in identifying superior genotypes in those traits.

Increase in total root length and total leaf area largely showed a similar pattern (Fig. 2) and these were reflected in the shoot and root biomass. Some genotypes however exhibited a larger total leaf area and total root length at 45 DAP than corresponding measurements at 60 DAP (Fig. 2). For leaf area, this phenomenon could

be attributed to increased leaf senescence than leaf production and/or unfolding in the genotypes whose leaf areas decreased at 60 DAP. On other hand, Izumi et al. (1999) found that decrease in total root length was associated with the onset of tuber bulking, so it is possible that genotypes that recorded reduced TRL at 60 DAP were initiating tuber bulking. Moreover, limitations in the current protocol due to root washing could be also implicated in the discrepancies observed in total root length measurements (Adu et al., 2017b). We generally did not observe the initiation of tuber bulking and this is consistent with many authors who have reported that tuber bulking normally starts from 60 DAP and rapidly advances from 100 DAP (Izumi et al., 1999; Tsay et al., 1988).

To our knowledge, field evaluation methods for root system architecture such as Shovelomics (Trachsel et al., 2011) are yet to be fully developed for cassava but in comparison with field evaluation methods, pot juvenile cassava root assays would be less laborious and time-saving. Pot juvenile cassava root assays in controlled environments would also be able to delineate confounding environmental influences among plants, which is currently one of the drawbacks to identify superior phenotypes under field conditions. However, some disadvantages could be stated for pot juvenile cassava root assays. The destructive nature of sampling, potential loss of fine root features through root washing, the difficulty or inability to measure certain root features such as lateral root insertion angle, the unnatural environment of root growth and infeasibility to grow plants until maturity are some of the limitations that need to be taken note of and possibly accounted for or circumvented. Poorter et al. (2012) has suggested that narrow pots could cause decline in root growth. This would particularly be a problem if plants are grown for extended periods, in which case, narrow pots could force downwards root growth, and might change the root traits or cause decline in root growth at later stages of the plants life. Moreover, the current study contains eight genotypes including released and unreleased varieties and may not reflect the entire variation of *Manihot esculenta*. It is also possible that the parameters measured are not the only possible sources of variation in juvenile *Manihot esculenta* (El-Sharkawy, 2003; El-Sharkway and Cock, 1987; Lowe et al., 1982). Whilst inherent genotypic variation in the capacity for adventitious root emergence is plausible, and could in turn influence other measurements taken at specific time intervals like root length and possibly root growth rate, the present study proceeded under the assumption that root emergence timing was analogous for all genotypes. Thus, data about timing of root emergence could be critical subsequently.

4.2. Quantitative variations in shoot and RSA and traits in cassava germplasm

Izumi et al. (1999) observed that differences in total number of non-storage nodal roots among genotypes could be traced to decrease in the number of lower nodal

roots from 60 DAP. There is also evidence that basal and nodal root structures originate from different regions of the stem and have divergent anatomies (Chaweewan and Taylor, 2015). In the present study therefore, we adopted the categorisation of Izumi et al. (1999) and divided the roots into three types namely UNR, LNR and BR. Significant quantitative variation for various shoot and root traits evaluated at three different stages of growth of juvenile cassava plants was observed indicating a considerable amount of morphological variability among cassava genotypes (Fig. 2 and Supplementary Table 1). Similar genetic variation was observed for juvenile traits among cassava lines at various stages of plant development by El-Sharkawy and Cock (1987) and these variations persisted in cassava's response to drought and rewatering (Subere et al., 2009).

Optimizing selection strategies for the improvement of quantitative traits requires reliable estimates of variance components (Kumar et al., 2012). In the present study, significant genetic variances were found for all root and shoot attributes except diameter of upper nodal roots and branching density of lower nodal roots (Supplementary Table 1). The presence of significant genetic variations suggests that selection for a given character would be effective (Adu et al., 2017a) and also implies good potential to select for improved root system traits of adult plants based on phenotypic selection (Tuberosa et al., 2003). Cassava genotypes with large total root system length, leaf area, root and shoot biomass, identified in this work such as genotypes 2B and CV are attractive for breeding for drought tolerance and nutrient-use efficiency, and to identify the genomic regions controlling these traits. Most of the variation in some of the traits examined, including diameter of upper nodal roots and branching density of lower nodal roots, could be attributed to vagaries in experimental conditions (i.e. block). Root systems of plants grown in soil respond dynamically to changes in their local environment (Adu et al., 2014).

4.3. Relative growth rate

All genotypes showed high RGR at 0- to 30-day interval, as compared with 30- to 45- and 45- to 60-day intervals (Fig. 3). This suggests that measurements after 30 days best display differences among genotypes (Kumar et al., 2012). Genotype 2B which has been identified as one of the outstanding lines was uniquely grouped. This genotype which showed high RGR between the first two sampling dates and possess superior root characteristics would be good candidate for drought tolerance and nutrient-use efficiency (Barber and MacKay, 1986; Marschner, 1988). Generally, it appears that, high performing genotypes are characterized mainly by high early RGR, in this case, at the 0- to 30-day and 30−45-day time intervals, but inconsistent behaviour at later growth stages. The rooting volume for the present study could be implicated in this observation, especially for the genotypes whose roots may have touched the pots by 45 DAP. It could also be that sufficient availability

of water at the upper layer caused marginal root growth after 45-days of planting. However, soils for the present study were thoroughly mixed and sieved before use. To a large extent, plants were therefore assumed to have grown in soils with homogenous distribution of water and mineral nutrients. Most of the variability observed in RGRs could therefore be intrinsic to the processes of root development. Intrinsic noise, or developmental stochasticity, is particularly significant in plant roots (Adu et al., 2014). In order to minimize such residual variations in root phenotyping, it would be critical to develop ways to characterize developmental stochasticity in root growth and other root system traits.

4.4. Multivariate analysis − correlation between traits and PCA

There were a number of significant correlations in the present study (Fig. 4) suggesting that targeting certain traits would be sufficient to explain the variation among cassava genotypes at the early growth stage and to screen large germplasm populations for superior root types in future investigations (Kumar et al., 2012). Significant positive correlations also imply that that selection for a given trait will not be detrimental to other traits (Adu et al., 2017b; Seiler, 2008) and that certain traits could be selected as proxies for others. Low correlation observed between some traits may also be beneficial in permitting independent manipulation of traits (Adu et al., 2017b; Gifford et al., 2013). For traits pairs that correlate closely and positively with each other, concentrating on the phenotyping of counterpart traits that are relatively easier to measure, highly reproducible and robust to whims of the environment, could be satisfactory and economical means to explain the variation among lines of a given population. Easy-to-measure counterparts of positivity correlated traits, such as shoot and root biomass, could also be used as indirect trait for the representation of their counterparts which are relatively more difficult to measure, such as TRL.

The results of PCA revealed that first PC generally captures shoot and root biomass related traits and TLA, while the second PC was consistently related to diameter and branching density related traits (Supplementary Table 2), indicating that these traits are explaining most of the variation present among studied cassava genotypes. The results suggests that root branching in cassava significantly contributes to genetic variability, and could be exploited in the breeding of genotypes adapted to soil moisture and soil mineral nutrient stress conditions. Our results are consistent with that of Subere et al. (2009) who observed that branching plays the key role when the entire root system shows plastic responses in development. Additionally, if the first four PCs, which accounted for 72.0%, 72.8% and 68.4% of the total variation in measurements taken after 30, 45, and 60 days, respectively, are considered then TRL, root number related traits and R: S were responsible for most of the phenotypic variation.

The GT biplot for each of the sampling times explained 47%−50% of the total variation of the standardized data (Fig. 5A1, B1 and C1). This is a relatively low

proportion of the variance that has been explained by the biplot but it reflects the complexity of the relationships among the measured traits (Yan and Rajcan, 2002). All the same, the fundamental patterns among the variable measured should be adequately captured by the biplots (Kroonenberg, 1995). For all 3 time-points, the largest variation explained by the biplots came from TLA and shoot/root biomass traits (Fig. 5A1, B1 and C1). The interrelationships among these traits would be relevant to cassava breeding. The correlation coefficients among the variables show that the GT biplot correctly captures associations among the traits that had relatively large loadings on either PC1 or PC2 in Supplementary Table 2.

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

Cos^2 values recorded were 0.2−0.8 for both 1st and 2nd sampling dates and 0.2−0.6 for the 3rd sampling (Fig. 5B1, B2 and C2). Similar to the observation in the PC loadings on PC1 and PC2 and in the GT biplots, the results of quality representation showed that TLA, shoot and root biomass traits, TRL, DUNR and DLNR were among the variables that were well represented on the factor map and thus warrant consideration in efforts to breed for improved genotypes. The contributions of variables in accounting for the variability in a given PC are expressed in percentage. Traits that are correlated with PC1 and PC2 are the most important in explaining the variability in a given dataset but variables that do not correlate with any PC have low contribution and could be classified as reductant and removed to simplify the overall analysis. Here, shoot biomass related traits including TLA, SFW and SDW, as well as BdUNR and DUNR consistently contributed above average to the variability in PC1 and PC2 at all 3 sampling dates (Fig. 5A3, B3 and C3). Other variables which contributed to the variability in PC1 and PC2 included root biomass, NUNR, TRL, BdLNR, TNR, NLNR and DLNR (Fig. 5A3, B3 and C3).

5. Conclusion

Quantifying root traits of any field crop is a challenging task and for a root and tuber crop such as cassava, the process is even more challenging. Soil-filled pots provide closest natural conditions for screening root system architectures (RSAs), albeit for cassava, it only suits screening at the juvenile stage of growth. Majority of studies on cassava roots have focused on tuber bulking but few studies have quantified cassava RSA at the early stages of growth. Accordingly, the extent of genotypic variability for root traits in cassava genotypes has not been fully established. Moreover, traits of juvenile cassava root system data that contribute most to differentiation among distinct genotypes are yet to be established. The pot-based screening of cassava RSA presented in this paper addresses these issues. The study showed that cassava roots are adventitious in origin consisting of a main root axis with 1st and 2nd order

lateral roots, and therefore the historically used term "fibrous roots" are redundant currently not contributing to clarity. The study also showed significant differences for juvenile shoot and root traits evaluated at the three different stages among the cassava genotypes used in our study, indicating a considerable amount of morphological variability among the cassava genotypes. Principal component analysis and genotype-by-trait biplots of shoot- and root biomass-related traits revealed that total leaf area, root diameter and branching density-related traits are the most important traits contributing to variation among the cassava genotypes studied, and thus, warrant consideration in efforts to breed for drought stress tolerance and efficient nutrient uptake in cassava. Selection of cassava genotypes based on shoot and root biomass, root diameter and branching density at juvenile growth stage could thus be successful predictor of nutrient and water-use efficiency in the field. Further studies are however required to relate studied juvenile cassava root traits with the performance of field-grown-mature plant with regard to drought, nutrient-use-efficiency and yield.

Declarations

Author contribution statement

Michael Osei Adu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Elvis Asare-Bediako: Contributed reagents, materials, analysis tools or data.

Paul Agu Asare, Frank Kwekucher Ackah: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Godwin Amenorpe: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mishael Nyarko Amoah: Performed the experiments.

Emmanuel Afutu, David Oscar Yawson: Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

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References

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

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

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., Wiesel, L., Bennett, M.J., Broadley, M.R., White, P.J., Dupuy, L.X., 2015. Scanner-based time-lapse root phenotyping. Bio-protocol 5 (6), e1424.

Adu, M.O., Yawson, D.O., Armah, F.A., Asare, P.A., Bennett, M.J., Broadley, M.R., White, P.J., Dupuy, L.X., 2016. Effects of rooting media on root growth and morphology of *Brassica rapa* seedlings. S. Afr. J. Plant Soil 33, 219−227.

Adu, M.O., Yawson, D.O., Bennett, M.J., Broadley, M.R., Dupuy, L.X., White, P.J., 2017b. A scanner-based rhizobox system enabling the quantification of root system development and response of *Brassica rapa* seedlings to external P availability. Plant Root 11, 16−32.

Alves, A.A.C., 2002. Cassava botany and physiology. In: Hillocks, R.J., Thresh, J.M., Bellotti, A.C. (Eds.), Cassava: Biology, Production and Utilization. Chapter: 5. CAB International, pp. 67−89.

Banoc, D.M., Yamauchi, A., Iijima, M., Kono, Y., 1999. Root system development of cassava and sweet potato during early growth stage as affected by high root zone temperature. Plant Prod. Sci. 2, 247−251.

Barber, S.A., MacKay, A.D., 1986. Root growth and phosphorus and potassium uptake by two corn genotypes in the field. Fert. Res. 10, 217−230.

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. eCollection 2013.

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, 215−227.

Catalogue of Crop Varieties Released and Registered in Ghana vol. 1, 2015.

Ceballos, H., Okogbenin, E., Pérez, J.C., López-Valle, L.A.B., Debouck, D., 2010. Cassava. In: Bradshaw, J.E. (Ed.), Root and Tuber Crops. Springer, pp. 53−96.

Chaweewan, Y., Taylor, N., 2015. Anatomical assessment of root formation and tuberization in cassava (*Manihot esculenta* Crantz). Trop. Plant Biol. 8 (1−2).

Connor, D.J., Cock, J.H., Parra, G.E., 1981. Response of cassava to water shortage. I. Growth and yield. Field Crops Res. 4, 181−200.

Delgado, A., Hays, D.B., Bruton, R.K., Ceballos, H., Novo, A., Boi, E., Selvaraj, M.G., 2017. Ground penetrating radar: a case study for estimating root bulking rate in cassava (*Manihot esculenta* Crantz). Plant Methods 13 (1), 65. eCollection 2017.

Downie, H.F., Adu, M.O., Schmidt, S., Otten, W., Dupuy, L.X., White, P.J., Valentine, T.A., 2015. Challenges and opportunities for quantifying roots and rhizosphere interactions through imaging and image analysis. Plant Cell Environ. 38, 1213−1232.

El-Sharkawy, M.A., 2005. How can calibrated research-based models be improved for use as a tool in identifying genes controlling crop tolerance to environmental stresses in the era of genomics − from an experimentalist's perspective. Photosynthetica 43, 161−176.

El-Sharkawy, M.A., 2003. Cassava biology and physiology. Plant Mol. Biol. 53, 621−641.

El-Sharkawy, M.A., Cock, J.H., 1987. Response of cassava to water stress. Plant Soil 100, 345−360.

Fukuda, W.M.G., Guevara, C.L., Kawuki, R., Ferguson, M.E., 2010. Selected Morphological and Agronomic Descriptors for the Characterization of Cassava. IITA.

Gifford, M.L., Banta, J.A., Katari, M.S., Hulsmans, J., Chen, L., Ristova, D., Tranchina, D., Purugganan, M.D., Coruzzi, G.M., Birnbaum, K.D., 2013. Plasticity regulators modulate specific root traits in discrete nitrogen environments. PLoS Genet. 9 (9), e1003760.

Izumi, Y., Yuliadi, E., Sunyoto, S., Morio Iijima, M., 1999. Root system development including root branching in cuttings of cassava with reference to shoot growth and tuber bulking. Plant Prod. Sci. 2, 267−272.

Kaiser, H.F., 1960. The application of electronic computers to factor analysis. Educ. Psychol. Meas. 20 (1), 141−151.

Kalogiros, D.I., Adu, M.O., White, P.J., Broadley, M.R., Draye, X., Ptashnyk, M., Bengough, A.G., Dupuy, L.X., 2016. Analysis of root growth from a phenotyping data set using a density-based model. J. Exp. Bot. 67, 1045−1058.

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

Kroonenberg, P.M., 1995. Introduction to Biplots for GxE Tables. Department of Mathematics, Research Report #51, University of Queensland.

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 (4), 465−478.

Lowe, S.B., Mahon, J.D., Hunt, L.A., 1982. Early development of cassava. Can. J. Bot. 60, 3040−3048.

Marschner, H., 1988. Role of root growth, arbuscular mycorrhiza, and root exudates for the efficiency in nutrient acquisition. Field Crops Res. 56, 203−207.

Okogbenin, E., Setter, T.L., Ferguson, M., Mutegi, R., Ceballos, H., Olasanmi, B., Fregene, M., 2013. Phenotypic approaches to drought in cassava. Front. Physiol. 4, 93.

Pardales Jr., J.R., Esquisel, C.B., 1996. Effect of drought during the establishment period on the root system development of cassava. Jpn. J. Crop Sci. 65, 93−97.

Pardales, J.R., Bañoc, D.M., Yamauchi, A., lijima, M., Kono, Y., 1999. Root system development of cassava and sweetpotato during early growth stage as affected by high root zone temperature. Plant Prod. Sci. 2, 247−251.

Philips, T., Taylor, D., Sanni, L., Okechukwu, R., Ezedinma, C., Akoroda, M., et al., 2006. The Nigerian Cassava Industry: Statistical Handbook. IITA, Ibadan, Nigeria, p. 30.

Poorter, H., Bühler, J., van Dusschoten, D., Climent, J., Postma, J.A., 2012. Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. Funct. Plant Biol. 39, 839−850.

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

Safo-Kantanka, O., Owusu-Nipah, J., 1992. Cassava varietal screening for cooking quality: relationship between dry matter, starch content, mealiness and certain microscopic observations of the raw and cooked tuber. J. Sci. Food Agric. 60, 99−104.

Seiler, G.J., 2008. Root growth of interspecific sunflower seedlings derived from wild perennial sunflower species. Can. J. Plant Sci. 88, 705−712.

Subere, J.O.Q., Bolatete, D., Bergantin, R., Pardales, A., Belmonte, J.J., Mariscal, A., Sebidos, R., Yamauchi, A., 2009. Genotypic variation in responses of cassava (*Manihot esculenta* Crantz) to drought and rewatering: root system development. Plant Prod. Sci. 12, 462−474.

Tabachnik, B.G., Fidell, L.S., 1996. Using Multivariate Statistics. Harper Collins, New York.

Thomas, C.L., Graham, N.S., Hayden, R., Meacham, M.C., Neugebauer, K., Nightingale, M., Dupuy, L.X., Hammond, J.P., White, P.J., Broadley, M.R., 2016. High-throughput phenotyping (HTP) identifies seedling root traits linked to variation in seed yield and nutrient capture in field-grown oilseed rape (*Brassica napus* L. Ann. Bot. 118, 655−665.

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.

Tsay, J.S., Fukai, S., Wilson, G.L., 1988. Effects of relative sowing time of soybean on growth and yield of cassava in cassava/soybean intercropping. Field Crops Res. 19, 227−239.

Tscherning, K., Leihner, D.E., Hilger, T.H., Mliller-Samann, K.M., El-Sharkawy, M.A., 1995. Grass barriers in cassava hillside cultivation: rooting patterns and root growth dynamics. Field Crops Res. 43, 131−140.

Tuberosa, R., Salvi, S., Sanguinetti, M.C., Maccaferi, M., Giuliani, S., Landi, P., 2003. Searching for quantitative trait loci controlling root traits in maize: a critical appraisal. Plant Soil 255, 35−54.

Villordon, A.Q., Ginzberg, I., Firon, N., 2014. Root architecture and root and tuber crop productivity. Trends Plant Sci. 19, 419−425.

Wei, T., 2013. Corrplot: Visualization of a Correlation Matrix. R package version 0.73. http://CRAN.R-project.org/package=corrplot.

Westby, A., 2002. Cassava utilization, storage and small-scale processing. In: Hillocks, R.J., Thresh, J.M., Bellotti, A.C. (Eds.), Cassava: Biology, Production and Utilization. Chapter: 14. CAB International, pp. 281−300.

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.

Yan, W., Rajcan, I., 2002. Biplot analysis of test sites and trait relations of soybean in Ontario. Crop Sci. 42, 11−20.