

Genome-wide association mapping and agronomic impact of cowpea root architecture

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Abstract

Key message Genetic analysis of data produced by novel root phenotyping tools was used to establish relationships between cowpea root traits and performance indicators as well between root traits and Striga tolerance.

Abstract Selection and breeding for better root phenotypes can improve acquisition of soil resources and hence crop production in marginal environments. We hypothesized that biologically relevant variation is measurable in cowpea root architecture. This study implemented manual phenotyping (shovelomics) and automated image phenotyping (DIRT) on a 189-entry diversity panel of cowpea to reveal biologically important variation and genome regions affecting root architecture phenes. Significant variation in root phenes was found and relatively high heritabilities were detected for root traits assessed manually (0.4 for nodulation and 0.8 for number of larger laterals) as well as repeatability traits phenotyped via DIRT (0.5 for a measure of root width and 0.3 for a measure of root tips). Genome-wide association

study identified 11 significant quantitative trait loci (QTL) from manually scored root architecture traits and 21 QTL from root architecture traits phenotyped by DIRT image analysis. Subsequent comparisons of results from this root study with other field studies revealed QTL co-localizations between root traits and performance indicators including seed weight per plant, pod number, and Striga (*Striga gesnerioides*) tolerance. The data suggest selection for root phenotypes could be employed by breeding programs to improve production in multiple constraint environments.

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is a primary protein source and food security crop for large portions of Africa, Asia and South America. Cowpea plays a significant role in contributing nitrogen (N) to agroecosystems and fodder to livestock especially in the low-input systems common in cowpea production zones (Ehlers and Hall 1997; Singh et al. 1997, 2003; Huynh et al. 2013). Cowpea is already cultivated in marginal environments commonly experiencing drought, low fertility and pest attack and these constraints are likely to become more severe with the effect of climate change (Yadav et al. 2015). Breeding efforts commonly target above-ground traits or incorporate studies on water balance of shoots. An underexploited breeding strategy is trait-based selection focused on linking specific root traits to efficient resource acquisition (Cattivelli et al. 2008; Lynch 2015).

Phenes are the elementary unit of the plant phenotype (Lynch 2011; York et al. 2013). The term was used as early as 1925 to describe phenotypic traits under genetic control (Serebrovsky 1925). While “trait” is often used interchangeably with phene its use is ambiguous and can cross

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several scales of biological organization. Phene is more precise and refers to an elementary unit at a given level of biological organization (Lynch and Brown 2012). The term ‘metric’ is used in this article to describe some of the mathematically derived observations generated using computer based image analysis. The term ‘trait’ is used in this article to refer to groups comprised of phenes and metrics. Agromonomically relevant traits of field grown root crowns can be quantified using shovelomics (Burridge et al. 2016) a manual phenotyping method, or DIRT (Digital Imaging of Root Traits). DIRT is an image-based phenotyping software (Bucksch et al. 2014) publically available online (Das et al. 2015). Following identification of genes controlling root phenes or metrics, marker-assisted selection (MAS) would enable trait-based selection orders of magnitude faster than field-based phenotyping of mature plants (Varshney et al. 2014). Modern breeding programs using these technologies can phenotype thousands of entries per three-month period (an average growing season) while a field-based breeder using shovelomics could phenotype only hundreds of entries in the same period. Indirect selection of root traits by MAS would also eliminate the need to phenotype roots at each generation during breeding advancement.

Cowpea production is commonly limited by multiple factors that may occur simultaneously, including biotic stress and limited access to water and nutrients. The parasitic weed *Striga gesnerioides* is a constraint to cowpea production and can be devastating in certain geographic areas (Rubiales and Fernández-Aparicio 2012; Kamara et al. 2014). Several genome regions conferring *Striga* resistance have been identified in different environments and cowpea lines but resistance is not completely conferred by a single locus, indicating resistance may be highly quantitative and may depend on a variety of resistance mechanisms (Ouedraogo et al. 2001; Ouédraogo et al. 2002; Li et al. 2009; Noubissie Tchiagam et al. 2010), of which avoidance could be one (Van Delft et al. 2000). Furthermore, some previous studies to characterize genetic control of *Striga* tolerance used pot assays (Atokple et al. 1995; Noubissie Tchiagam et al. 2010; Omoigui et al. 2011), which may negate avoidance mechanisms and amplify hypersensitive responses and chemical and physical exclusion strategies.

Soil resources are frequently stratified, with immobile resources such as phosphorus (P) and potassium (K) being more available in shallow soil strata (Lynch and Brown 2001) and mobile resources such as water being more available in deeper soil strata (Lynch and Wojciechowski 2015). Constraints to root growth and resource acquisition are often stratified, especially in oxisols, which are common in many cowpea production areas, and in which acidity and aluminum toxicity increase with depth (Lynch and Wojciechowski 2015). Biotic factors also may be stratified

such as occurs with greater concentration of *Striga* seeds in the top 10 cm of the soil profile (Van Delft et al. 2000). For these reasons root architectural phenes that affect spatial and temporal distribution of roots can have profound effects on efficient soil exploration and resource acquisition and avoidance of biotic or abiotic constraints (Lynch and Wojciechowski 2015). In maize and common bean, several root architectural phenes have been related to increased productivity under stressful conditions such as deep rooting for increased water acquisition in bean (Ho et al. 2005), maize (Zhu et al. 2010), and wheat (Kirkegaard et al. 2007). Shallow rooting has been shown to be advantageous for immobile nutrients in maize (Bayuelo-Jiménez et al. 2011) and bean (Bonser et al. 1996; Miller et al. 2003; Ho et al. 2005; Beebe et al. 2006; Miguel et al. 2015). Given the similarity between common bean and cowpea phenology and root architecture we speculate that homologous phenes may have similar benefits. The limited body of cowpea root studies indicates deep rooting may be beneficial for drought tolerance (Matsui and Singh 2003; Barros et al. 2007; Agbicodo et al. 2009) but others highlight the limited payback when investing in deeper roots when very little additional water can be acquired (Hall 2012). Research conducted in soil cylinders suggests restricting water use is important for water use efficiency (Belko et al. 2012a, b, 2014). Limited research on low P tolerance has been conducted in cowpea, but roots may be important to increase P efficiency (Kugblenu et al. 2014).

Cowpea performance in low fertility environments could be related to efficient resource acquisition and use, especially when low nutrient soils and water limitation co-occur, as is the case for many production environments. In this context, drought escape by accelerated phenology can be important, and in cowpea development of extra early maturing varieties has been used as a strategy to avoid drought effects in West Africa (Hall 2012). However, trade-offs inherent in such strategies may include decreased time to acquire immobile resources such as P and K (Bayuelo-Jiménez et al. 2011; Nord et al. 2011) and decreased yield potential and limited use of leaf fodder in cowpea (Hall 2012) and yield penalty in bean (White and Singh 1991). Plants with a strong, deep root system may be better at scavenging for available moisture and nutrients, contributing to stay-green.

The acquisition of immobile resources is important early in phenology before the topsoil becomes dry, which increases the tortuosity of the diffusive pathway and decreases root growth rates, contributing to making the acquisition of immobile soil resources more difficult (Barber 1995). In other legumes such as common bean, P acquisition is related to efficient shallow soil exploration and may enable late season root growth into deep soil where more water is available (Ho et al. 2005). As pods elongate

and seeds fill, grain sink strength and water acquisition become stronger regulators of successful maturation than new mineral resource acquisition, as significant amounts of resources are redistributed within the plant (Rao et al. 2013). We hypothesize that in a terminal drought situation the acquisition of immobile resources would be especially important in the first four vegetative stages of phenology and water acquisition is critical throughout development. A dimorphic root system possessing phenes enabling both shallow and deep exploration may be particularly well suited to environments characterized by dual limited water as well as P. Phenotypes that modulate access and rate of water extraction from a given soil domain should be considered when developing ideotypes (Vadez et al. 2012; Belko et al. 2014). Quantifying and understanding the utility of the spatiotemporal deployment of roots in heterogeneous and dynamic soil as it relates to efficiently accessing resources is important for production in environments constrained by multiple resources. Comparisons of cowpea lines with similar genetic background but varying in root architecture and water use phenotypes will be important to assess the contribution of root phenotypes to stress tolerance.

A consensus genetic map for cowpea ($2n = 2x = 22$) was constructed based on genotyping of 6 bi-parental recombinant inbred line (RIL) populations using an Illumina GoldenGate assay for 1,536 EST-derived SNP markers (Muchero et al. 2009). The map resolution was improved by genotyping seven additional RIL populations, which revealed further synteny with soybean and offers increased possibilities to identify gene function (Lucas et al. 2011). In addition, a core germplasm of traditional landraces across cowpea-growing regions in African and the world has been collected and characterized (Huynh et al. 2013). Using these markers and genetic resources, quantitative trait loci (QTL) have been discovered for key traits including tolerance to drought (Muchero et al. 2010; Muchero et al. 2013), seed quality (Lucas et al. 2013b) and resistance to root-knot nematodes (Huynh et al. 2016), root pathogens including *Macrophomina phaseolina* (Muchero et al. 2011) and Fusarium wilt (Pottorff et al. 2014; Pottorff et al. 2012), insects (Huynh et al. 2015; Lucas et al. 2013a), and the parasitic weed *Striga* (Ouedraogo et al. 2012). These performance traits could be driven by root development but until now, no research on genetic mapping has been reported for root traits in cowpea due to challenges in root phenotyping; the practical aspects of growing and excavating mature plant roots can be particularly challenging compared to above ground phenotyping. In this study, we aimed to identify genomic regions controlling root architecture in a cowpea diversity panel using state-of-the-art root phenotyping systems and established relationships between root trait QTL and yield, stress tolerance or resistance, and other agronomically important traits.

Materials and methods

Genetic materials and experimental design

A diversity panel of 189 entries including traditional cultivars (landraces) and elite breeding lines representing worldwide cowpea genetic diversity was assembled and seed-multiplied by University of California—Riverside (UCR) (Supplemental File). The panel was phenotyped in 2012 and 2013 at Ukulima Root Biology Center (URBC), Limpopo Province South Africa (24°33'00.12S, 28°07'25.84E, 1235 masl). Both experiments were designed as randomized complete blocks with four replications each year. Each line was planted in a single-row plot consisting of ten plants per plot. Row width was 76 cm and distance between plants within a row was 30 cm. URBC has a deep Clovelly loamy sand (Typic Ustipsamment). Experiments were fertilized with complete N–P–K fertilizers before planting, treated with foliar feed during the growing season and irrigated regularly with a center pivot irrigation system to ensure non-limiting growing conditions. The agrochemical NemaCur 400 EC was applied twice during the season to prevent interference by insects or nematodes.

Root phenotyping

Shovelomics was conducted 4–6 weeks after planting on two plants per plot. Following excavation of six plants per plot, the two most representative were selected for phenotyping. A complete description of the methodology is available in Bucksch et al. (2014) and BurrIDGE et al. (2016). In brief, the method involves excavation of the root crown with a shovel to 0.25 m average depth, rinsing of the root crown in a bucket of water, followed by manual measurements using a phenotyping board and digital calipers. Eleven manual root architectural parameters were collected in 2012 and 14 in 2013 (Table 1). Shoot biomass was collected both years at the same time as the root crowns.

To acquire the image needed for automated image analysis (DIRT), the washed root system was placed on a flat black background with circular scale marker, plot identifier tag and a standard color image was obtained using a standard digital camera mounted on a tripod. The color image was converted to a grayscale image, segmented into foreground and background and the foreground images were separated into root, scale marker, and tag. Then the root image was used to calculate the root-width profile and root tip paths (RTPs) and the metrics derived from the profile and RTPs. The scale marker was used to transform pixels to millimeters and to correct for imperfect lens to board alignment. A complete set of images was taken in 2013 from which 47 measures per root crown were extracted (see Bucksch et al. 2014 for a full list of traits and descriptions).

Table 1 List of root trait names with abbreviation and definition

Trait	Definition
Adventitious root growth angle (ARGA)	Average adventitious (hypocotyl) root growth angle relative to horizontal (0 is horizontal, 90 is vertical, scored in units of 10°)
Adventitious root number (ARN)	Number of 1st order lateral roots emerging from the hypocotyl
Average root density	Ratio between foreground and background pixels of the extracted root
Basal root growth angle (BRGA)	Average basal root growth angle relative to horizontal (0 is horizontal, 90 is vertical, scored in units of 10°)
Basal root number (BRN)	Number of roots in the basal region of the hypocotyl
Branching density 10 (BD10)	Number of 1st order laterals emerging from the primary root between 5 and 10 cm below soil level (tap root laterals)
CPD 25, 50, 75, 90	Diameter at 25, 50, 75, 90% length of the central path diameter
D10, D20, D50, D60	Percentage of width accumulation at $x\%$ depth
Disease score (DS)	Disease rating where 1 is severely infected with stem and root rots and 9 is extremely healthy
DS10, DS20... DS80	Slope of the line made by two sequential Dx values
Max width	Maximum width of root calculated from root image
Median width	Median width of root calculated from root image
Nodule score (NS)	Rating of nodule size and number with 1 having no nodules and 9 having many large nodules
Number of root tip paths (nr. of RTPs)	Number of unique paths from top of stem to each root apex
RTA range	Range of all calculated root tip paths
Stem diameter (SD)	Diameter of stem (mm) at soil level
Tap diameter (TD5, TD10, TD15, TD20)	Tap root diameter (mm) 5, 10, 15 and 20 cm below the soil surface
Third order branching density (3BD)	Rating of number and order of laterals with 1 being very low branching and 9 having many orders and dense spacing of laterals
1.5BD5, 1.5BD10	Number of 1st order laterals with greater than 1.5 mm diameter 2 cm from origin emerging from 0–5 cm below soil level (1.5BD5) and from 5–10 cm below soil level (1.5BD10)

Broad-sense heritability on an entry mean basis was calculated for the manually acquired root phenotypic data from the two seasons. Repeatability on an entry mean basis was calculated for the image-acquired root phenotypic data from four replications of each season (Fehr 1993).

Repeatability of DIRT traits was calculated as

$$R^2 = \frac{\sigma^2(G)}{\sigma^2(E) + \sigma^2(G)} \times 100,$$

where $\sigma^2(G)$ is the genotypic variance and $\sigma^2(E)$ is the error variance.

Heritability on an entry mean basis was calculated for the manual measurement traits with the following formula:

$$H^2 = \frac{\sigma^2(G)}{\frac{\sigma^2(E)}{ry} + \frac{\sigma^2(GY)}{r} + \sigma^2(G)} \times 100,$$

where $\sigma^2(G)$ is the genotypic variance, $\sigma^2(GY)$ is the genotype by year variance, $\sigma^2(E)$ is the error variance, r is the number of replications and y is the number of years.

Normality of residuals was checked and data were power-transformed using the lambda identified by Box-cox transformations. Spearman and Pearson correlations between years, replications, and samples suggested data could be combined using best linear unbiased predictors

(BLUPs). BLUPs were calculated and used for subsequent analysis. Statistical analyses were performed in R version 3.1.2 (R Core Team 2014).

Association mapping

Genotypic data of 1536 SNP markers for the cowpea diversity panel were derived from (Lucas et al. 2011). Version 6 of the consensus genetic map containing 1091 SNPs was retrieved from HarvEST:Cowpea (<http://harvest.ucr.edu/>). Genome-wide association study (GWAS) was performed on root phenotypes collected via DIRT image analysis in 2013 and via manual measurements in 2012 and 2013. Genotypic data for GWAS included 1091 SNP markers genotyped on 189 lines of the cowpea diversity panel. GWAS was performed using the mixed linear Q + K model (MLM) (Zhang et al. 2010) implemented in the Genomic Association and Prediction Integrated Tool (GAPIT) R package (Lipka et al. 2012) using the following model:

$$y = X\beta + Wm + Qv + Zu + e,$$

where y is a vector of phenotypic observations; β is a vector of unknown fixed effects except the SNP marker under testing, m is a vector of fixed marker effects (i.e. SNP), v is a vector of subpopulation effects, u is a vector

of unknown random effects, and e is a vector of residual effects. Q is an incidence matrix of principal component scores of marker-allele frequencies. X , W and Z are incidence matrices of ones and zeros relating y to β , m and u , respectively. The covariance of u is equal to KVA , where K is the kinship matrix that was estimated with a random set of SNPs using the VanRaden method and VA is the additive variance estimated with restricted maximum likelihood (REML). The kinship matrix estimation and the principal component analysis were performed in the GAPIT R package. The optimum number of principle components/covariates included in the model for each trait was determined by forward model selection using the Bayesian information criterion.

Additive effects were estimated relative to the minor allele. SNPs with a LOD score greater than 2.9 were considered to be significantly associated with the trait.

QTL comparison of root architecture and other traits

QTL information for yield, plant biomass, seed weight, stay-green, resistance or tolerance to biotic and abiotic stress was obtained from previous studies using GWAS and bi-parental mapping (Muchero et al. 2010, 2011, 2013; Lucas et al. 2013a, 2013b; Huynh et al. 2015, 2016; Pottorff et al. 2012, 2014; Ouedraogo et al. 2012). These QTL locations were aligned on the cowpea consensus genetic map for identification of regions that coincided with QTLs for root architecture. Root architecture SNPs with LOD score equal or greater than 2.6 were considered significant for correlations with agronomic traits.

Results

Root phenotypic assessment

Significant genotypic and phenotypic variation of root phenes is measureable using DIRT and manual shovelomics and there is some correlation between DIRT and manual measures of related traits (see Table 1 for trait definitions and Table S1 for correlations). General phenotypic results from DIRT and manual shovelomics have been discussed at length elsewhere (Bucksch et al. 2014; Burridge et al. 2016). However, several key points regarding genotypic variation and heritability are highlighted in this paragraph to prepare the reader for novel genetic and allometric analysis that follow. All manually measured traits had greater than three-fold range in values and the majority had greater than eight (Fig S1). Individual accessions are distinguishable from each other based on normalized mean values of their root phenotypic descriptor data (Bucksch et al. 2014). Heritability for root traits had a wide range.

Root traits phenotyped manually for two years at URBC with high heritability scores included 1.5BD5 (0.80), TD5 (0.27), ARN (0.27), and NS (0.45) (Burridge et al. 2016) replotted in Table S2). Repeatability of root trait values acquired by one season of image analysis highlight D20, Nr. of RTPs, and SD (DIRT) with repeatability over 0.25 (Burridge et al. 2016) (replotted in Table S3). Allometry between root phenes and shoot biomass from the 2013 South African trial was modeled as a linear regression using the \log_{10} transformed trait values to identify power-law relationships between traits and shoot biomass.

Analysis of marker-trait association

Eleven significant QTL (LOD > 2.9) were identified for manually measured phenotypes including SD (manual), BRGA, ARGA, TD10, and TD15 and 21 significant QTL were identified for DIRT measured phenotypes including SD (DIRT), CPD25, CPD75, D10,20,50,60, RTA range, max width, median width and average root density (Table 2). The most significant QTL were identified for median width (linkage group (LG) 8, 24.9 cM), SD (DIRT and manual, LG 7, 47.4 cM and LG 3, 92.9 cM), ARGA (LG 6, 48.4 cM, LOD 3.92, allelic effect -1.8) and BRGA (LG 10, 2.3 cM, LOD 3.14, allelic effect -2.6). Median and max width co-localize on LG 8, 24.9 cM with LOD of 4.46 and 3.83 and allelic effects of 12.5 and 13.6, respectively. QTL for stem diameter identified from manual measurements co-localized with QTL for stem diameter identified from DIRT measurements of stem diameter on LGs 1, 3, 6, 7 (Table 2; Fig. 1).

Six root architecture QTL identified using DIRT and manual measurements co-located with previously identified regions for agronomically relevant traits including seed weight, seed number, pod number and Striga tolerance measured in previous studies using the same genetic materials (Table 3). SNP marker 2227_693 on LG 6 affecting root system median width is co-located with QTL for seed weight per plant (Muchero et al. 2013) in which the SNP haplotype conferring high root system median width also significantly increased seed weight per plant. Markers on LG1 affecting CPD25, a measure of hypocotyl size, co-localized with QTL for pod number (Muchero et al. 2013), in which the SNP haplotype gave a negative effect on CPD25 but a slightly increased pod number effect. Markers affecting root width accumulation metrics D20, D50 and D60 were co-located on LG10, which coincided with QTL for seed number per plant (Muchero et al. 2013), however, allelic effects for D20, D50, and D60 are small and vary in the direction of their effect. When analyzed by individual trials, markers for median width and CPD 25 showed contrasting effects in different environments, suggesting their environmentally specific trait utility (Tables 5,

Table 2 Root architecture QTL with LOD >2.9

Trait	SNP marker	Linkage group	Position (cM)	Allelic ^a effect	LOD	Favorable allele	Alternative allele
Stem diameter (manual)	13772_1075	1	22.4	−0.5**	3.76	C	G
Stem diameter (DIRT)	13772_1075	1	22.4	−0.5**	3.81	C	G
Stem diameter (DIRT)	5084_519	1	23.6	−0.4**	3.26	C	G
Stem diameter (manual)	5084_519	1	23.6	−0.4**	3.25	C	G
Stem diameter (manual)	4836_807	2	6.7	0.7**	3.17	G	C
Avg root density (DIRT)	10811_937	2	71	−0.1**	3.13	A	T
RTA range (DIRT)	2326_226	3	42.7	3.0**	3.94	G	A
TD10 (manual)	12501_343	3	76.7	0.2**	3.1	G	A
Stem diameter (DIRT)	139_439	3	92.9	−0.6**	4.03	A	G
Stem diameter (manual)	139_439	3	92.9	−0.07**	2.92	A	G
TD10 (manual)	5061_428	4	21	−0.2**	3.34	A	G
CPD75 (DIRT)	7102_965	4	21.8	0.03**	4.1	G	A
Avg root density (DIRT)	1004_587	5	21.2	−0.2**	3.96	A	G
Stem diameter (DIRT)	8969_1386	6	2.2	−0.3**	3.36	A	C
Stem diameter (manual)	8969_1386	6	2.2	−0.3**	3.36	A	C
Median width (DIRT)	2227_693	6	2.9	6.5**	3.05	G	A
CPD 25 (DIRT)	9645_589	6	5.4	−0.03**	3.21	A	G
CPD 25 (DIRT)	5428_339	6	6.1	−0.03**	3.32	A	G
D10 (DIRT)	3211_511	6	21.7	0.02**	3.49	G	A
D20 (DIRT)	3211_511	6	21.7	0.02**	3.71	G	A
Adventitious root angle (manual)	4749_1972	6	48.4	−1.81**	3.92	A	G
Stem diameter (DIRT)	11138_624	7	47.4	0.5**	4.25	G	A
Stem diameter (manual)	11138_624	7	47.4	0.5**	4.26	G	A
Max width (DIRT)	14604_737	8	24.9	13.6**	3.83	G	A
Median width (DIRT)	14604_737	8	24.9	12.5**	4.46	G	A
TD15 (manual)	13848_735	9	42.1	0.1**	3.29	G	A
Basal root angle (manual)	11851_914	10	2.3	−2.6**	3.14	A	G
D20 (DIRT)	4245_136	10	50.5	0.01**	2.91	G	A
D50 (DIRT)	4245_136	10	50.5	0.01**	2.99	G	A
D60 (DIRT)	4245_136	10	50.5	−0.01**	3.01	A	G
D50 (DIRT)	2391_614	11	25.8	0.01**	3.66	G	A
D60 (DIRT)	2391_614	11	25.8	0.01**	3.65	G	A

Units for diameter measurements (SD, TD, CPD) are millimeters, Dx values are percent of total accumulated width at the specified depth meaning 0.01 corresponds to 1%, BRGA, ARG, root tissue angle (RTA) are measures in degrees from 0 to 90, max and median width are measured in millimeters, Avg root density is the ratio of background to foreground pixels in the root image and ranges from 0.2 to 6.5. See Table 1 for abbreviation definitions

^a Marker additive effect, *, ** are significance level at $P > 0.01$ and 0.001, respectively; a positive allele effect indicates the favorable allele contributing to a positive phenotypic value, while a negative allelic effect indicates the favorable allele contributing to a negative phenotypic value

6). Median width positively correlated with seed weight in the Kano field site but negatively in the Kamboinse field site (Table 6).

Markers associated with BRGA and D60 on LG10 also co-located with QTL for Striga resistance (Ouédraogo et al. 2002). Interestingly, the positive alleles for BRGA and D60 are identical to SNP haplotypes of SuVita-2, which is a donor parent for Striga resistance (Ouédraogo et al. 2002) (Table S4). Phenotypic root evaluations of SuVita-2 found few and steep basal and adventitious roots (top 10

percentile for BRGA, bottom 5 percentile for ARN), a relatively strong tap root, and low median and maximum widths.

Allometry

The slope of the regression line represents the scaling coefficient (α) (Niklas 1994) (Table 4). In our analysis, isometry is established for $\alpha = 0.33$, because traits considered have one-dimensional units whereas biomass has

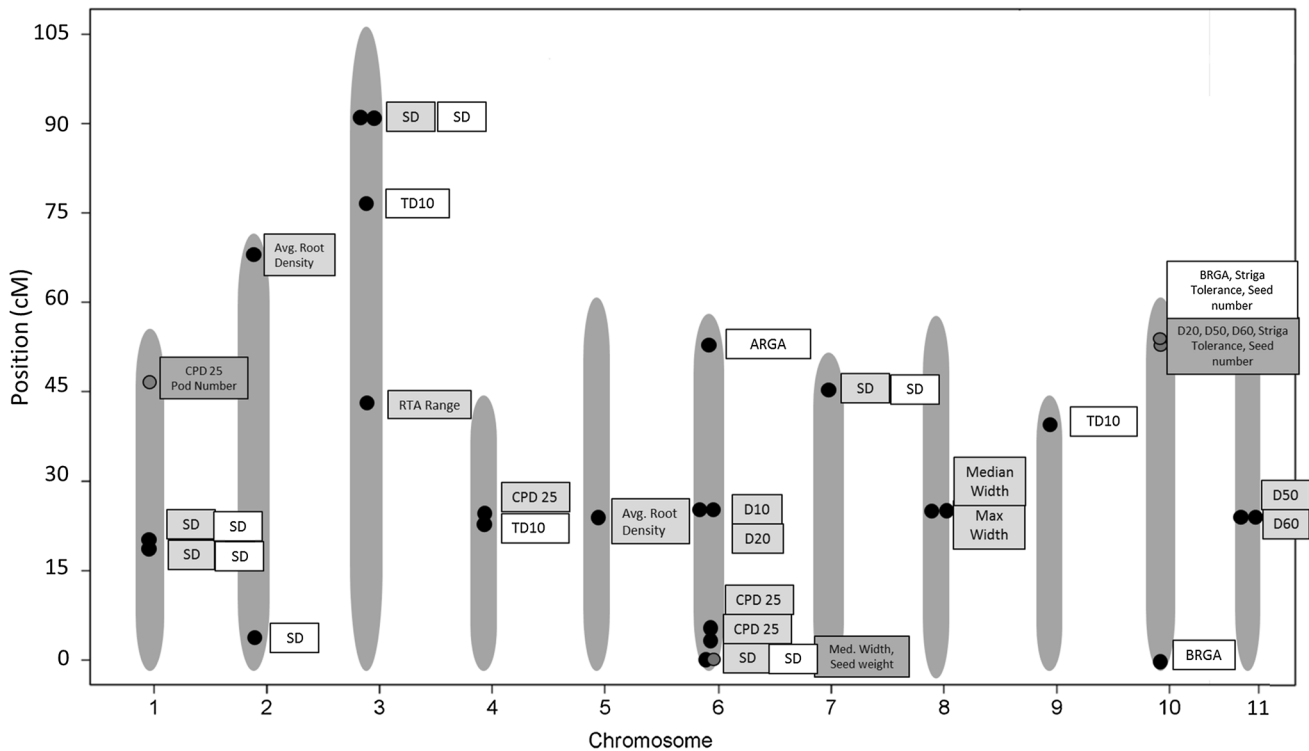


Fig. 1 Chromosome map, showing significant QTL for manually measured and DIRT root architecture traits. *Black dots* represent root architecture QTL, *grey dots* represent root architecture QTL that co-localize with agronomic traits. Traits in *light grey boxes* refer to root

trait QTL measured by DIRT. Traits in *white boxes* refer to manually measured root QTL. Trait abbreviations and descriptions can be found in Table 1

Table 3 Marker locations, allelic effects and LOD scores for root traits with annotation indicating co-localization with agronomic traits

Trait ^a	SNP marker	Linkage group	Position (cM)	Allelic effect ^b	LOD	Favorable allele	Alternative allele
Median width (DIRT) ¹	2227_693	6	2.8579	6.5**	3.05	G	A
BRGA (manual) ^{2,3}	4510_497	10	50.1197	6.0*	2.63	T	A
D20 (DIRT) ³	4245_136	10	50.5343	0.001**	2.91	G	A
D50 (DIRT) ³	4245_136	10	50.5343	0.009**	2.99	G	A
D60 (DIRT) ^{2,3}	4245_136	10	50.5343	−0.007**	3.01	A	G
CPD 25 (DIRT) ⁴	10905_418	1	45.1436	−0.029*	2.59	A	G

See Table 1 for abbreviation definitions

^a Colocation of QTL affecting root traits and agronomical traits measured in other studies: 1 = seed weight per plant (Muchero et al. 2013); 2 = Striga tolerance (Ouedraogo 2002); 3 = seed number per plant (Muchero et al. 2013); 4 = pod number (Muchero et al. 2013)

^b Marker additive effect, *, ** are significance level at $P < 0.01$ and 0.001 , respectively; a positive allelic effect indicates the favorable allele contributing to a positive phenotypic value for the root trait, while a negative allelic effect indicates the favorable allele contributing to a negative phenotypic value for the root trait

a three-dimensional unit. Traits with a scaling coefficient $\alpha > 0.33$ plus 0.15 are positively allometric and indicate that the trait scales faster than shoot biomass. Traits with $\alpha < 0.33$ are negatively allometric and demonstrate trait increases at a rate lower than expected from shoot biomass. The value 0.15 ± 0.33 was chosen to create a zone where trait scaling was roughly proportional to biomass

to increase categorization precision. We discuss here only traits with significant P values ($P < 0.001$) for the regression line. We calculated the adjusted R^2 of the relationship as a measure of the predictive power of the trait in question. Five traits had a significant adjusted R^2 and P value of the slope. Two traits, TD10, and TD15, have $\alpha > 0.33 \pm 0.15$ indicating they are positively allometric.

Table 4 Allometric comparisons of correlations between plant biomass and root traits

Trait	Adjusted R^2	Intercept	Slope (α)	P value (slope)
TD10	0.16	-0.47	0.61	<0.001***
TD15	0.34***	-0.53	0.58	<0.001***
TD	0.23***	0.17	0.37	<0.001***
SD (manual)	0.36***	0.71	0.22	<0.001***
Avg root density	0.05	-0.076	0.16	0.004**
CPD 75	0.12	-0.24	0.14	<0.001***
Median width	0.02	1.97	0.12	0.011*
Stem diameter (DIRT)	0.15***	0.98	0.1	<0.001***
CPD 25	0.07***	0.026	0.079	<0.001***
BRGA	0	1.51	0.071	0.07
Max width	0	2.35	0.037	0.16
ARGA	0	1.63	0.027	0.49
RTA range	0	1.65	0.005	0.82
D60	0.02	-0.16	-0.01	0.4
D50	0.02	-0.19	-0.02	0.17
D20	0.02	-0.33	-0.046	0.02*
D10	0.02	-0.41	-0.07	0.006**

Allometric analysis was performed by plotting a linear regression of \log_{10} of each trait against \log_{10} of total plant dry weight. The allometric scaling coefficient (α) of 0.33 with a margin of ± 0.15 was used as the threshold where trait response was proportional to a change in biomass. Traits with significant slopes greater than 0.45 exceeded proportional increase in biomass and traits with significant slopes less than 0.18 increased more slowly than if proportional to biomass. See Table 1 for abbreviation definitions

The non-significant R^2 for TD10 indicates it is not strongly controlled by shoot biomass. TD and TD15 have highly significant R^2 and P value of slope. However, the slope (α) of TD and SD are within ± 0.15 of 0.33 and are considered to be nearly isometric with shoot biomass. Highly significant R^2 for TD and SD, 0.23 and 0.36, respectively, were observed indicating greater predictive power. CPD 25 and stem diameter measured with DIRT had significant slopes close to zero and low R^2 , suggesting that the trait increases independently from shoot biomass.

Discussion

We suggest root phenotyping has utility for identifying mechanisms that drive performance. This statement is supported by moderate to high heritabilities of root traits (Tables S2 and S3) and GWAS-based co-localizations between root traits and performance indicators (Tables 2, 3; Fig. 1). Greenhouse and field based studies in other crops have identified several root phenes, particularly those that lead to more efficient deep and shallow exploration, as

being related to drought and low P tolerance, respectively. This study integrates root phenotyping over two seasons in the same environment with yield trials from multiple distinct environments. The effects of environmental variability on root architecture, shoot growth, Striga infestation, and ultimately yield introduced by these cross-location comparisons is undoubtedly large. Allelic effect and the number of co-localizations between root traits and performance indicators would likely increase if both root and performance data were collected from the same environment. Furthermore, the limited marker density available for cowpea means that the SNPs used might not be perfect markers for traits and one should not place undue importance on weak allelic effects. Nevertheless, several interesting associations were found between root traits and performance indicators that merit further investigation.

The co-localization between CPD25 and pod number with a slightly negative allelic effect, indicated a smaller hypocotyl was correlated with greater pod number. Analysis by individual trials revealed that marker effect depends on environment, suggesting the advantage of a given phenotype depends on environment. A large diameter hypocotyl could be useful as a reserve of carbohydrates for future reallocation in a perennial, fodder or multiple harvest cropping system (Gwathmey et al. 1992). However, research in common bean indicates this “insurance” strategy constrains yield in terminal drought annual cropping systems, which rewards a decisive shift to reproduction (Rao et al. 2009, 2013; Beebe 2012). Thus, a large diameter hypocotyl could constitute a misallocated reservoir of resources if those resources are not readily reallocated to reproductive processes. QTL with both positive and negative allelic effects were detected for a related measure, SD (accessed via DIRT and manual measurements), highlighting distinct loci that are related to greater and smaller SD. This suggests that selection may be possible to move towards either a smaller or larger hypocotyl.

The fact that QTL affecting root system shape (including BRGA, D20, D50 and D60) co-localized with QTL for Striga resistance also indicates a possible role of root architecture in avoiding Striga attachment and growth. An allele with a large positive effect for BRGA and an allele with a small negative effect for D60 both conferred Striga tolerance and indicated that steeper basal root growth angles are related to Striga tolerance. Since the positive allele for D20 and D50 is similar to the Striga susceptible allele while the positive allele for D60 conferred a slightly negative allelic effect on Striga tolerance, we hypothesize that D60 represents the inflection point where placing more root length above increases susceptibility and more root length below increases resistance. The implication is that less root length in the topmost layer of soil is related to Striga tolerance. The greater abundance of Striga seeds in shallow



Fig. 2 Example of *Striga* attachment on cowpea (IT86D 1010) root grown at URBC, note horizontal growth angle and relative enlargement of host lateral root

soil suggests that avoidance of the shallow soil domain may prevent triggering *Striga* germination or avoid *Striga* attachment (Van Delft et al. 2000). Based upon phenotypic data on the number and angle of adventitious and basal

roots, the donor parent of this trait, SuVita-2, deploys very few roots in shallow soil zones compared to other lines in the diversity panel (see Fig. 2 for example of *Striga* attachment). The resultant root architecture resembles a narrow but deep cone with a predominate tap root, which places few roots in shallow soil zones and may also be related to greater water extraction from deep soil (Fig. 3). Similar biotic stress avoidance mechanisms may also play a role in other parasitic plant or nematode resistance. A potential tradeoff between *Striga* avoidance and arbuscular mycorrhizal avoidance may exist as they occur in similar shallow soil and take advantage of similar chemical plant cues (Parniske 2008).

The QTL co-localization and their positive effects on both median root width and seed weight per plant (Muchero et al. 2013) suggest that a moderately large, broad cone-shaped root system is likely to be beneficial in most environments. However, marker effects for median width (marker 2227_693) and CPD 25 (marker 10905_418) on performance indicators such as seed weight and seed number varied by environment (Table 5). Correlations between median width and performance indicators also varied by



Fig. 3 From left to right SuVita-2 showing steep, narrow, tap root dominated architecture; TVu 9557 showing average root system with many adventitious and basal roots and steeper growth angle; Gorum

Local showing shallow root system with many adventitious roots and shallow adventitious and basal roots

Table 5 Marker allelic effects on median width and CPD 25 and associated agronomic traits in three environments

Trait	Marker 2227_693 (median width)			Marker 10905_418 (CPD 25)		
	AA	GG	<i>P</i> value	AA	GG	<i>P</i> value
2013_CPD25	1.3	1.3	0.267	1.4	1.3	0.002
2013_MedianWidth	119.6	139.1	0.001	132.5	129.6	0.541
2009_USA_Seed Weight	63.6	58.6	0.038	69.6	58.4	0.001
2008_Kamboinse_Seed Weight	21.8	17.9	0.020	25.4	18.1	0.001
2008_Kamboinse_SeedWtPlant	3.6	2.9	0.016	4.1	3.0	0.004
2008_Kamboinse_SeedNumberPlant	6.0	6.2	0.502	5.5	6.3	0.032
2007_Kano_Seed Weight	11.4	16.0	0.006	10.6	15.0	0.028
2009_Pobe_Seed Weight	21.1	19.3	0.089	23.4	19.4	0.002

USA, Kamboinse, Kano and Pobe refer to different locations of trials. SeedWtPlant means seed weight per plant. SeedNumberPlant means seed number per plant

Table 6 Trait correlations between median width and CPD 25 with performance indicators in three environments

Correlation coefficients	2013_MedianWidth	2013_CPD25
2013_CPD25	−0.139	
2007_Kano_Seed Weight	0.252**	0.106
2008_Kamboinse_SeedNumberPlant	0.050	0.047
2008_Kamboinse_Seed Weight	−0.250**	0.212*
2008_Kamboinse_SeedWtPlant	−0.240**	0.182*
2009_Pobe_Seed Weight	0.024	0.074
2009_USA_Seed Weight	−0.067	0.229*

*, ** are significance at $P < 0.05$ and 0.01 , respectively. USA, Kamboinse, Kano and Pobe refer to different locations of trials. SeedWT-Plant means seed weight per plant. SeedNumberPlant means seed number per plant

environment ($r = 0.25$ in Kano, $r = -0.25$ in Kamboinse) (Table 6). This suggests that median width and CPD 25 are related to enhanced performance in some environments and related to inferior performance in others. Tradeoffs between costs and benefits are in general extremely dependent upon environment, particularly when soil resources and environmental constraints occur in contrasting spatial or temporal arrangements.

A positive allelic effect on both median width and yield components suggests that a broad root system confers an advantage that leads to greater yield. We hypothesize that this root system architecture offers a balanced foraging strategy that maximizes benefits relative to costs and efficiently explores both shallow and deep soil zones. In an environment in which water is most limiting and the ideal strategy is to escape drought by rapid maturation or deep soil exploration a minimalistic or steep root system may be best. Future research needs to address the complexities of matching phenotype to environment highlighted by these environmentally dependent allelic effects.

It is a central but often overlooked task of physiologists, geneticists and breeders to distinguish independent fundamental phenes from auto-correlations and allometric relationships. This study found SD and TD to be isometrically related to plant biomass and TD15 to be positively allometrically related to plant biomass. TD10 is positively allometric but the insignificant R^2 indicates it can scale independently from shoot biomass. This suggests that it may be possible to select for a strong tap root on a relatively small plant. We speculate that TD10 affects deep soil exploration more than TD because a larger diameter root may be able to access deeper soil domains, supply greater quantities of water and contribute to drought avoidance.

The discrepancy in the allometric relationships between seemingly comparable traits such as TD and CPD 25, and stem diameter measured manually and using DIRT lies in the trait definition. TD is a diameter measurement at a defined location below the soil line. TD traits capture differential secondary growth at a given point in time independent of total plant biomass or total root system size. In contrast, CPD traits measure diameter increments relative to excavated taproot length meaning CPD 25 does not always measure the same point as TD. Because CPD traits may measure diameter at different points than TD traits they may be under different genetic control, as suggested by GWAS. Stem diameter measured manually and using DIRT differs slightly from each other. Stem diameters measured with DIRT resulted in α values and adjusted R^2 about half than achieved for SD measured manually. Manual stem diameter is taken at a point determined by subtle color variations indicating soil level. DIRT measures stem diameter at the topmost point of the excised stem, which does not always coincide with soil level. While the allometric relationships are not consistent, significant SNPs for manually and image accessed measures of stem diameter co-locate on the genome suggesting both have utility for trait-based selection. Identification of genetic control using different methods indicates that both manual and image analysis based tools have utility for breeding programs.

Our introductory study posits a root ideotype with large median width and steeper BRGA. As this root system may efficiently explore both shallow and deep zones it is termed dimorphic. This type of root system may increase tolerance to multiple edaphic stresses while avoiding *Striga* parasitism in shallow soil. The ideal stay-green phenotype for a combined stress environment may be built upon optimized root and hypocotyl phenes, including a balanced but deep root architecture and more parsimonious water uptake. Combining these phenes may permit an increased period of water extraction (Vadez et al. 2013) with a positive balance between benefit and costs of resource acquisition.

Ongoing work is investigating the mechanisms regulating soil water use and in particular the effects of root anatomical phenes such as xylem diameter on conductance and soil moisture budgets. One example of this is tap root diameter, which seems intuitively linked to greater total xylem area and to increased ability to extend into deep soil zones in a terminal drought situation. We hypothesize that a strong tap root with large number of small diameter vessels offers a balance that extracts large volumes of water in a metered fashion without depleting soil water before the growing cycle is over.

We present data indicating that both manual and image-based phenotyping of mature, field-grown cowpea root systems detected QTL describing root architectural

phenes and some of these co-located with QTL related to efficient resource acquisition and performance in sub-optimal environments. Phenotyping of mature roots under field conditions is important because it more closely approximates agricultural conditions than do greenhouse or laboratory based phenotyping platforms. Our data suggest that root architectural traits form part of the suite of traits conferring abiotic stress tolerance and *Striga* resistance. We propose that traits contributing to a cone-shaped architecture should be investigated further and potentially integrated into *Striga* management strategies such as those outlined elsewhere (Franke et al. 2006). Phenotyping root architecture may help to identify additional mechanisms related to increased productivity in sub-optimal environments and accelerate the release of climate adapted cultivars. Modeling and ideotype approaches could be useful to inform physiology studies to investigate trait integration and tradeoffs. Knowledge of allometric relationships should inform breeding targets and root ideotype development. Similarities in root architecture between cowpea, soybean, common bean, tepary bean and other legumes suggest genetic control may be homologous and agronomic impact of certain root architectures may be comparable.

Author contribution statement JB phenotyped roots and led writing, analysis, figure preparations and revisions. HS performed GWAS on root traits, correlated performance indicators to root traits, as well as contributed to writing, analysis, figure preparation, interpretation and revisions. BLH contributed expertise to genetic analysis, writing and interpretation. PR contributed interpretation, analysis and manuscript revisions. AB developed the automated image phenotyping platform and contributed to interpretation, writing and revising the manuscript. JL contributed to conceptual design, interpretation, writing and revisions of the manuscript.

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Compliance with ethical standards

The authors declare that they have no conflict of interest.

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