

IMAGE-BASED HIGH THROUGHPUT PHENOTYPING REVEALS VARIATION IN ROOT SYSTEM ARCHITECTURE TRAITS OF SOYBEAN

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Abstract

Soybean is a widely cultivated commercial crop mainly grown for its edible oil and protein content. Climate change and abiotic stresses are currently posing productivity and adaptation challenges to the crop. Root system architecture traits can open new avenues for ideotype development in order to reduce yield losses via efficient nutrient-water uptake. We applied semi-automated image-based root phenotyping to investigate diversity in root system architecture features among seventy soybean genotypes. Root system architecture traits such as stem diameter (Stem D), projected root area (Area), root system width (Root Sys W), number of root tips (Root Tips), number of adventitious roots (Advent Root), number of basal roots (Basal Root), tap root (TAPRD), total root length (T Length), total root volume (T volume) and number of branching points (Branching P) were evaluated after 28 DAS using a recently developed computer-based Digital Imaging of Root Traits (DIRT) and Semi-automated Root Image Analysis (saRIA) applications. The data generated by softwares were analyzed for descriptive statistics, Pearson's correlation and principal component analysis (PCA). Relatively higher variability was observed in traits i.e., Basal Root (CV= 55.82%), Branching P (CV= 44.51%), Root Tips (CV= 37.8%), Area (36.73%) and Root Sys W (35.02%). However, TAPRD and StemD exhibited low variation and had CV% of 7.64 and 18.22, respectively. All the studied traits were found to be strongly associated with each other whereas selection for one trait will lead to positive or negative selection of other trait(s). First two PCs represented 64.9% and 14.9% variability, respectively. Broadly, the classification of genotypes was manifested by root agro-morphological traits rather than geographic origin. The availability of variation in these traits provides opportunity to develop high yielding stable cultivars in response to yield limiting abiotic stresses like drought, flooding, inefficient nutrient uptake and drastic edaphic conditions.

Key words: Root system architecture; Phenotyping; Image analysis; High throughput.

Introduction

Roots are the primary plant organ exposed to environmental stimuli and stresses while acquiring nutrients and water from soil. This “hidden-half” of the plant in combination with its traits are summed as root system architecture (RSA) which determines the spatial and temporal distribution of roots in heterogenous soil matrix. The RSA augments plant potential to obtain mobile and immobile resources required for its development (Myers *et al.*, 2007). Root system architecture regulates plant strength and ability to exploit water resources under various soil conditions. Better root phenotype is considered a key to improved drought tolerance allowing better plant performance through efficient uptake of water in crops (Agbicodo *et al.*, 2009; Lopes *et al.*, 2011; Matsui & Singh, 2003). Researchers have suggested detailed morphological studies in crop root system architecture for selecting superior genotypes (Falk *et al.*, 2020; Fried *et al.*, 2018). Traditionally, breeders have favoured above-ground plant phenotype at the expense of roots for taxonomic classification and improving ideotype of crops.

In soybean, genetic variability within root architecture has been found viz-a-viz morphometric traits i.e. root length, root angle, root diameter, root surface area and depth of root (Ao *et al.*, 2010; Zhao *et al.*, 2004). Researchers have investigated soybean root architecture through manual digging and phenotyping of plant roots to examine genetic variation, response against drought, water and nutrient uptake (Boerma *et al.*, 1997; Hudak &

Patterson, 1996; Kaspar *et al.*, 1984). These studies have revealed important information regarding root dynamics under different soils and stress conditions. For example, drought stresses conditions have been found to greatly affect the root architecture and cause formation of longer lateral roots and root hairs for the purpose to encourage better water absorption by increasing the total surface area (Osmont *et al.*, 2007).

The complexity in measuring root architecture under field environment is main obstacle in the implementation of phenomics technologies for improving root related traits. Hence, the identification of crops equipped with improved root architecture remains a key bottleneck in current plant breeding. However, in recent years, the phenomenal importance of RSA studies through modern and classical phenotyping approaches has led to an emerging field of plant “shovelomics” (Trachsel *et al.*, 2011). This includes but is not limited to anatomical study of plant roots under field conditions for morphometric traits such as root depth, root angle and root branching densities, analysis of soil cores and application of standard excavation techniques (Nielsen *et al.*, 1997). Moreover, old-fashioned approaches are not enough to disclose the finer details regarding root architecture, anatomy or function (Fitter & Stickland, 1992). Besides, constraint such as higher probability of damage to root during excavation, cumbersome manual measuring of traits, time consumption, skill-dependency and labor intensiveness remain key hurdles to study plant roots.

In recent years, advancements in artificial intelligence (AI) have led to breakthrough innovations in image processing and data acquisition. The phenomenal growth of computer vision-assisted analysis tools for recognizing anatomical patterns in objects has also revolutionized traditional ways of determining plant morphology (Fahlgren *et al.*, 2015). The “High-throughput phenotyping” is increasingly replacing traditional collection of data on agromorphological traits in plants (Hartmann *et al.*, 2011). The technology has also impacted *shovelomics*, as image-based root phenotyping has enabled researchers to acquire high-throughput data on root system architecture traits from images (Wang & Zhang, 2009). Therefore, in the last few years researchers have employed various image-based root phenotyping algorithms to investigate morphological variation in root system architecture of different plants (Falk *et al.*, 2020; Fried *et al.*, 2018; Suematsu *et al.*, 2017).

There is compelling evidence in support of breeding for better root system architecture in soybean to cope with the challenges of abiotic stresses and climate adaptation. High-throughput root phenotyping methodologies have not been applied in large soybean populations which can potentially expedite selection of genotypes with superior root system architectures. Therefore, the present study aimed at utilizing imaging-based root phenotyping approach to assess variation in diverse soybean germplasm. We aimed to determine the extent of root system architecture variation in diverse soybean accessions using image-based phenotyping. Also, multivariate analyses techniques were applied to identify soybean genotypes with promising root traits.

Materials and Method

Seed of seventy geographically diverse soybean accessions and commercial varieties with origin from USDA, Pakistan, Korea and Brazil were obtained from the genebank of Bio-resources Conservation Institute, National Agricultural Research Centre (NARC), Islamabad. The experiment was conducted at Oilseeds Research Program, located at 33.6701° N, 73.1261° E NARC, Islamabad. Healthy seed of each genotypes were treated with 20% chlorox and rinsed with distilled water. Three seeds of each genotype were planted in B.N 50, 5x10 cells seedling tray whereas each cell has a volume of 73 ml. Seed was sown 1.5cm deep in trays filled with clay loam soil (pH 7.4). Trays were kept in incubator (Sanyo MIR-153) at 25°C in dark for first 72 hours and then 14-10 hrs dark and light, alternatively. Seedlings were regularly watered with spray bottle and trays were shuffled in racks to provide equal lighting opportunity to all plants. After four weeks (28 DAS), trays with seedlings were flooded with water unless soil was softend. The seedling were gently removed from soil to prevent damage to young roots. The plants were washed under tap water and soil debris was cleaned. The seedlings were dried under room conditions for an hour.

Image acquisition and analysis of root system architecture traits: For each genotypes three roots were excised from shoot part and scanned by Cannon MF-240 using a coin with 22.6mm diameter as a scale and a black

paper as background. The scanned 2D RGB images of roots were saved in .jpeg format (300 dpi) for root system architecture analysis. Root system architecture traits were measured using a recently developed computer based Digital Imaging of Root Traits (DIRT) and Semi-automated Root Image Analysis (saRIA) applications developed by (Das *et al.*, 2015) and (Narisetti *et al.*, 2019), respectively. DIRT was used to assess seven root traits i.e. stem diameter (StemD), projected root area (Area), root system width (RootSysW), number of root tips (RootTips), number of adventitious roots (AdventRoot), number of basal roots (BasalRoot) and tap root (TAPRD). The DIRT uses cloud computing platform which is publically hosted by iPlant Cyber-infrastructure which not only provides its cloud data storage but also offers the Advanced Agave API to communicate with the Texas Advanced Computing Center (TACC) for high-throughput computation of uploaded root images. The image-processing pipeline built in python runs on the TACC. The python code based algorithm starts computing traits from masked images and returns the output as pre-formatted .csv file after completion process. Likewise saRIA a matlab environment application computed three traits i.e., total root length cm (TLength), total root volume cm³, (Tvolume) and number of branching points (BranchingP). For saRIA, both the GUI and image analysis algorithms have been implemented under the MATLAB 2018b environment. The primary image analysis pipeline includes a) segmentation of root architecture b) calculation of phenotypic features of root architecture and c) image intensity such i.e., colour. In case of color images as input, the image is converted to a grescale file using the *rgb2gray* Matlab routine. The application use standard .jpeg, .bmp, .png files as input followed by preprocessing such as cropping of the region of interest (RoI), image intensity inverting, despeckling and smoothing. This is followed by adaptive thresholding for segmentation to differentiate foreground (roots) and background. Then morphological filtering is applied to remove the white noise and non-root structures. This is followed by skeletonization where additional thinning or eroding is applied on the binary images to suppress the high-frequency noise and create refined root skeleton. Finally, all the root features are computed using pixel-wise calculation whereas measurement of the traits is returned in mm after setting the pixel-to-mm conversion factor in the GUI. Whereas the pixel-to-mm conversion factor (CF) is then defined as follows:

$$CF = \frac{\text{Length of the referecne line in mm}}{\text{Length of the referecne line in pixel}}$$

Data analysis: The data on the ten RSA traits genertaed by software in a .csv output file were subjected to descriptive statistics for quantitative description of the basic data features for tendency and variation using Statistica 7.0 (Stat Soft. Inc. USA). Pearson’s correlation was computed using Microsoft Excel 2016. Interaction between various RSA traits was visualized by scatter plot through *ggplot2* package in R studio. Principal component analysis (PCA) and heatmap clustering was performed for RSA traits to determine extent of root variation in 70 soybean genotypes using packages *factoextra heatmap.plus* and *ggplot2* in R language.

Results

Morphometric variation in root traits: Analysis of the data showed that image-based semi-automated analysis of root system architecture is efficient at dissecting phenotypic variation in soybean roots (Fig. 1a, b, c). The data were subjected to descriptive statistics to quantitatively summarize the features from the data (Table 1). Relatively higher variability was observed in traits i.e. BasalRoot (CV= 55.82%), BranchingP (CV= 44.51%), RootTips (CV= 37.8%), Area (36.73%) and RootSysW (35.02%). However, TAPRD and StemD exhibited low variation and had CV% of 7.64 and 18.22, respectively. The stem diameter varied between 2.28 to 5.99 cm while average diameter was 4.34cm. Root area ranged from 4.18 to 53.46cm² with average area of 32.36 cm². Highest projected root area was observed around the roots of SPS-22 (53.46 cm²) followed by NIBGE-185 (50.9 cm²) and SA-7260 (48.20 cm²). The average RootSysW for all 70 studied soybean genotypes was 2.63 cm while it ranged from minimum of 0.47 cm to maximum of 5.69 cm. Highest RootSysW was observed in SPS-1 (5.69 cm) followed by SPS-31 (3.93 cm) and SPS-10 (3.90 cm). The number of root tips was found as a highly variable trait ranging from 6 to 56 with average of 32 in the studied genotypes. The number of adventitious roots ranged between 1 to 11 with average of 6.91. GP-18 has the highest number (11) followed by Ajmeri and E-1531 with 10 adventitious roots. The highest number of basal roots 19 were observed in E-1531 followed by 16 in SPS-23 and SPS-18. For all the studied genotypes in the experiment, the tap root diameter ranged from 0.37 to 0.53 mm with the mean diameter of 0.44 mm. Highest TAPRD of 0.53 mm was recorded in SPS-9 followed by 0.5 mm in PGRB-55 and NIBGE-115.

Significant root length variation was observed in the studied genotypes as it ranged from 3.88 cm in Black to

25.68 cm in NIBGE-185 while average value was 16.34 cm. Total root volume in the present experiment ranged between 0.61 in Black to 4.03 cm³ in NIBGE-113 with the average volume of 2.321 cm³. The number of branching points ranged between 21 in Black to 175 in SA-7260 with average number of 101.22.

Correlation among different traits of soybean root system architecture: All the studied traits were found to be strongly associated with each other (Table 2). For example, stem diameter was observed to have highly significant positive correlation with total root volume (r= 0.79**), number of adventitious roots (r=0.74**), total root length (r=0.67**), projected root area (r=0.63**) and basal root number (r=0.61**). Stem diameter also showed a positive significant correlation with number of branching point (r=0.59*) and root tips (r=0.56*). A strong positive association was found between projected root area and branching (r= 0.98**), TLength (r=0.97**), RootTips (r= 0.86**) and BasalRoot (r= 0.8**). Similarly, projected root area had strong positive correlation with AdventRoot (r= 0.66**), RootSysW (r=0.56*) and Tvolume (r=0.43*). Root system width was also observed to have significantly positive correlation with Tlength (r= 0.68**), RootTips (r= 0.68**), BasalRoot (r=0.65**) and BranchingP (r=0.59*). Number of root tips were observed to have strong positive relationship with BasalRoot (r=0.90**), Tvolume (r=0.90**), BranchingP (r=0.87**) and AdventRoot (r= 0.68**). Total root length and BasalRoot numbers had equally positive effect on AdventRoot (r= 0.69**). No negative correlation was found for the observed traits except for the very weak negative association between tap root diameter and root system width.

Scatterplots were created to depict relationship of Root system width and projected root area with other strongly correlated root system architecture traits (Fig. 2).

Table 1. Descriptive statistics for ten root system architecture traits.

Trait	Mean	Minimum	Maximum	Range	Variance	Std. Dev.	Stand. Error	CV%
Stem diameter (cm)	4.34	2.28	5.99	3.71	0.63	0.79	0.09	18.22
Projected root area	32.36	4.18	53.46	49.28	141.32	11.89	1.42	36.73
Root system width (cm)	2.63	0.47	5.69	5.21	0.85	0.92	0.11	35.02
No. of root tips	32.01	6.00	56.00	50.00	146.35	12.10	1.45	37.80
No. of adventitious roots	6.91	1.00	11.00	10.00	5.25	2.29	0.27	33.15
No. of basal roots	8.04	1.00	19.00	18.00	20.14	4.49	0.54	55.82
Tap root diameter (mm)	0.44	0.37	0.53	0.16	0.00	0.03	0.00	7.64
Total root length (cm)	16.34	3.88	25.68	21.80	32.91	5.74	0.69	35.11
Total root volume	2.32	0.61	4.03	3.42	0.39	0.63	0.07	27.12
No. of branching point	101.22	21.00	175.00	154.00	2029.46	45.05	5.38	44.51

Table 2. Pearson's correlation among various traits of soybean root architecture system.

Trait	StemD	Area	RootSysW	RootTips	AdventRoot	BasalRoot	TAPRD	TLength	Tvolume	BranchingP
StemD	1.00									
Area	0.63	1.00								
RootSysW	0.12	0.56	1.00							
RootTips	0.56	0.86	0.68	1.00						
AdventRoot	0.74	0.66	0.22	0.68	1.00					
BasalRoot	0.61	0.80	0.65	0.90	0.69	1.00				
TAPRD	0.17	0.37	-0.05	0.22	0.29	0.14	1.00			
TLength	0.67	0.97	0.68	0.90	0.69	0.83	0.28	1.00		
Tvolume	0.79	0.43	0.19	0.30	0.60	0.39	0.04	0.43	1.00	
BranchingP	0.59	0.98	0.59	0.87	0.69	0.78	0.39	0.96	0.31	1.00

StemD= stem diameter (mm), Area= projected root area (cm²), RootSysW= root system width (cm), RootTips= no. of root tips, AdventRoots=adventitious roots, BasalRoots= no. of basal roots, TAPRD= tap root diameter (mm), Tlength= total length (cm), Tvolume= Total volume (cm³), BranchingP= no. of branching points

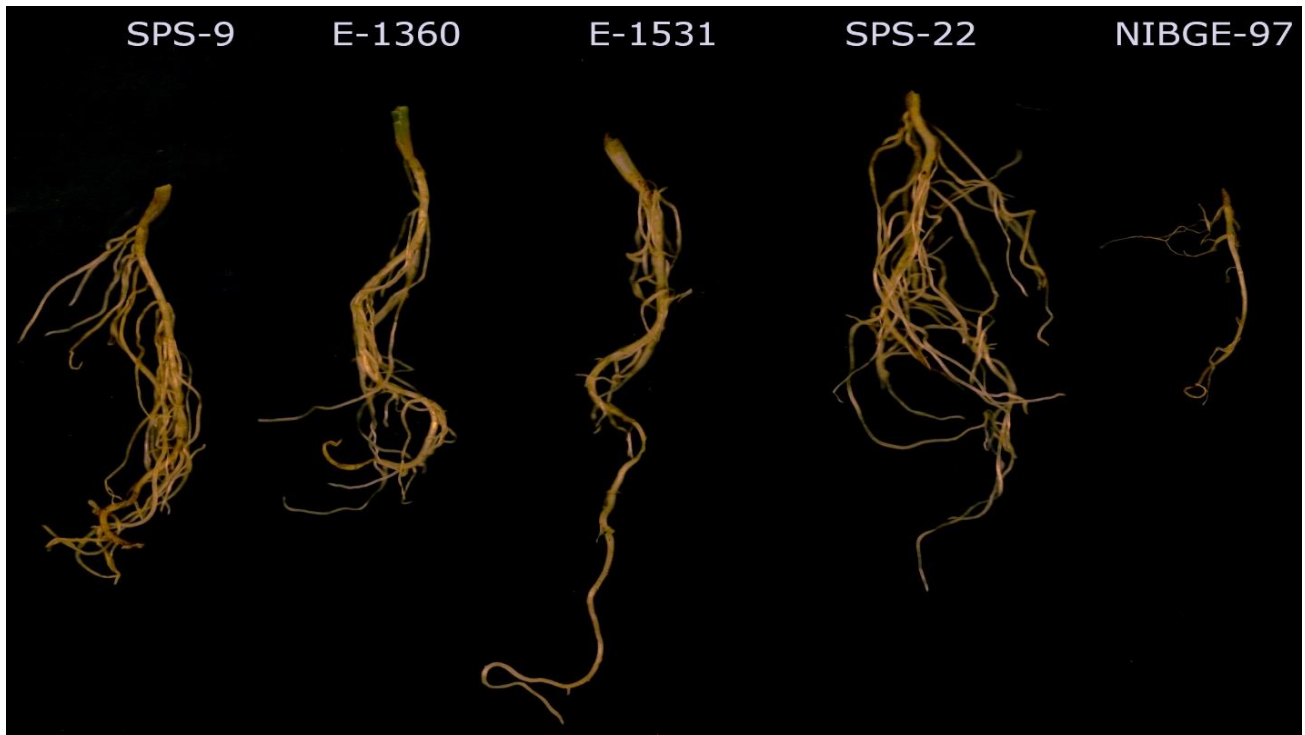


Fig. 1a. Representative picture showing phenotypic variation among root architecture system of soybean genotypes.

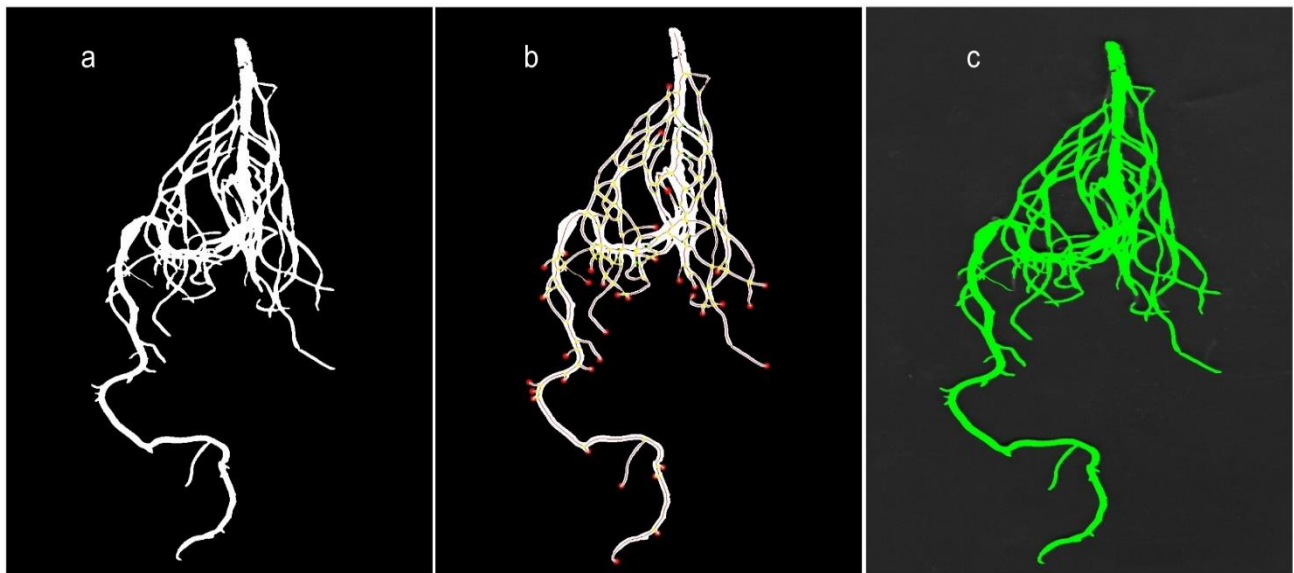


Fig. 1b. Image processing pipeline of saRIA application using algorithm to determine root traits a) binary root image b) overlay of the binary image over root skeleton c) morphometric filtering.

Multivariate analyses of root system architecture traits: Principal component analysis computed variability in ten principal components (PCs) accounting for 100% variations (Table 3). Among these PCs, the first two PCs were used to explain the results as these captured nearly 80% of the variation in RSA traits. All the studied traits contributed negatively to the phenotypic variations of PC1. The negative load of these traits on PC1 was manifested by StemD (-0.30), Area (-0.37), Root SysW (-0.24), RootTips (-0.35), AdventRoot (-0.31), BasalRoot (-0.35), TAPRD (-0.14), Tlength (-0.37), Tvolume (-0.23) and BranchingP (-0.36). With PC1 added, the principal component 2 elucidated

79.86% of the total variation. Traits such as StemD (0.46), AdventRoot (0.36), TAPRD (0.02) and Tvolume (0.54) were found having positive contribution in PC2. The rest of the studied traits contributed to negative load on PC2 i.e. Area (-0.12), RootSysW (-0.47), RootT (-0.22), BasalRoot (-0.08), Tlength (-0.15), and BranchingP (-0.15).

The biplot plot clearly divided the studied genotypes into four distinct groups based on the 10 root system architecture characters (Fig. 3a). The first group shaded with grey ellipse included 30 genotypes with almost 70% from NARC. These genotypes were characterized with shortest RootSysW (mean 1.81 cm),

medium StemD (mean 4.38 mm) and Tvolume (mean 2.29 mm). The second group (green ellipse) of PCA has only seven members all from NARC, Islamabad. These genotypes were poor performers with minimum mean values for StemD (2.73 mm), Area (13.14 cm), RootTips (10.25), AdventRoot (2.28), BasalRoot (1.51), TAPRD (0.39 mm), TLength (6.68 cm), Tvolume (1.53 cm³) and BranchingP (23.83). The third group of PCA (blue ellipse) comprised of 23 elite genotypes of which 79% were accessions from NARC while 21% were from USDA. These genotypes performed best for StemD (mean 5.29 mm), Area (mean 44.72 cm²), AdventRoots (mean 9.04), BasalRoots (mean 12.56), TotalLength (mean 22.11 cm) and Totalvolum (mean 3.07 cm³). The fourth group (pink ellipse) was also elite cluster of only 10 genotypes of which six were USDA accessions while four genotypes were from NARC. These ten genotypes had the maximum mean values for RootSysW (3.60 cm), number of root tips (47.18), TAPRD (0.46 mm), Total root length (22.34 cm) and number of branching points (151.97).

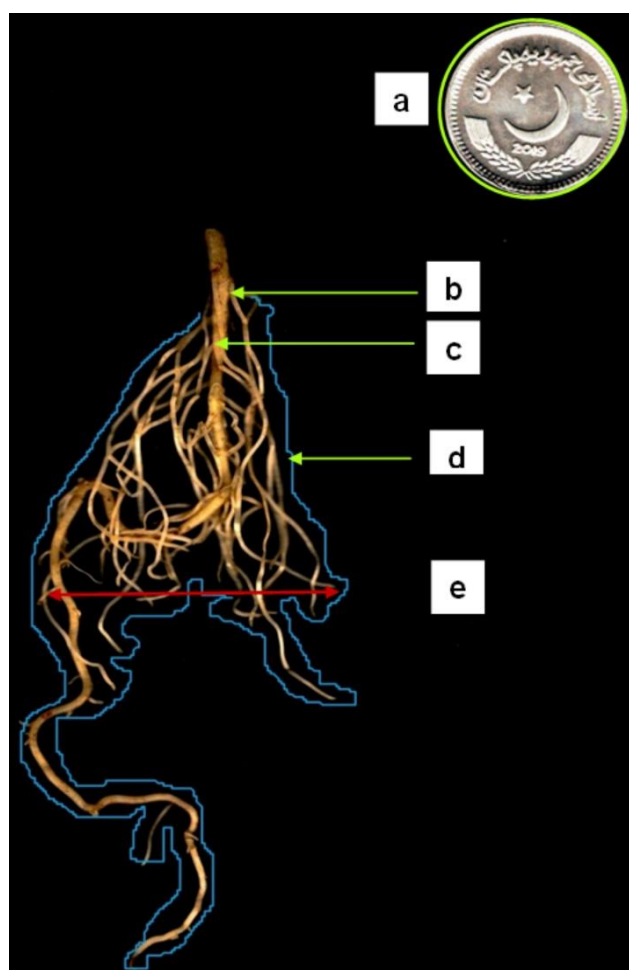


Fig. 1c. Representative picture of soybean root architecture system visualized by DIRT and saRIA. a) a coin of known diameter (22.6 mm) as a reference for size b) basal roots c) tap root d) the blue drawn polygon represents projected root area and e) the red arrow denotes root system width.

Similarly, the relationship between studied ten root traits was also visualized by constructing plot of their

interaction for first two principal components (Fig. 3b). The plot explains the relationship between these variables as well as their effect on overall phenotypic variation. The first phenomenon is construed from the angles between the eigen vectors of the traits on x-y plane while the second is depicted by the magnitude of the eigen vector i.e., its length. Hence in the present study, the vectors of variables had two distinct groups. The vectors for StemD, Tvolume and AdventRoots showed collinearity i.e. they had a positive association with each other. On the contrary, Area, BasalRoots, BranchingP, TLength, RootTips and RootSysW had parallel eigen vectors and had positive effect on each other. Moreover, the TAPRD was observed to have no significant association with any trait. Likewise, the length of the BranchingP, StemD and Area was higher which signified their role in contributing towards variability in studied genotypes.

Euclidean distances based hierarchical cluster analysis also depicted variation and grouping pattern of studied genotypes. The cultivars and improved lines i.e., Ajmeri, Rawal-I, NARC-II, Williams-82 and NARC-16 had higher root area, root length, branching points and number of root tips. Moreover, these RSA traits of the local accessions and germplasm from foreign sources scored below average (Fig. 4).

Discussion

In the current study, relatively higher variability was observed in traits i.e. basal roots, branching points, root tips, root area and root system width. However, tap root diameter and stem diameter exhibited low variation. Recently, similar observations were recorded by researchers while examining soybean root systems in the field and laboratory (Abdel-Haleem *et al.*, 2011 and Falk *et al.*, 2020). Falk *et al.*, (2020) studied genetic diversity in soybean root system architecture by evaluating 292 accessions for 32 image derived traits. Authors confirmed presence of sufficient genetic variability and high root traits values for exotic germplasm. In the current investigation, higher extent of phenotypic variation was observed for number of basal roots, number of root tips, total root length and projected root area confirmed broad genetic base of these selected 70 soybean genotypes. Similar findings were reported by Fried *et al.*, (2018) while characterizing 49 soybean genotypes for seven root traits including total root length, root area, total root volume and root diameter. They proposed presence of significant genetic variability within soybean population for root related traits which can be exploited for breeding ideotypes against drought tolerance and hard soil conditions (Sapra & Lal, 2003). The results of the current study were in agreement with the findings of the Prince *et al.*, (2015) as they studied genetic variants for root system architecture in 160 wild soybean recombinant inbred lines. They reported considerable genetic variation for traits such as total root length, surface area, total root volume and number of adventitious roots. Morphometric traits including those of root system architecture are linked directly or indirectly to each other.

In order to investigate the interrelatedness of these root traits, the data were subjected to Pearson's correlation. The resultant matrix revealed highly significant associations between important traits such as total root length, root volume, stem diameter, adventitious roots, basal root, root system width and projected root area. Prince *et al.*, (2015) also reported highly significant positive association between total root length, total volume, root area and root diameter. These findings were further supported by correlation studies of root traits in soybean (Fried *et al.*, 2018 and Falk *et al.*, 2020). In the current study, the roots phenotypic data were analyzed by principal component analysis to visualize the imminent grouping in soybean population. Clear grouping pattern was observed by principal component analysis whereas genotypes belonging from Oilseeds Research Program NARC, BCI NARC, USDA, and AVRDC. Broadly, the classification of genotypes was manifested by root agro-morphological traits rather than geographic origin. Similar pattern of root phenotype-driven classification has been reported in recent studies (Fried *et al.*, 2018 and Falk *et al.*, 2020). Genotypes such as Ajmeri, GP-18, E-1531, NIBGE-185, SA-7260, PGRA-25, SPS-1, SPS-22 and SPS-33 has higher total root length, adventitious roots, projected root area, root system width and tap root diameter. NIBGE-113, NIBGE-115 and NIBGE-314 showed potential for higher total root volume.

This trait enables them to scavenge nutrients in maximum unit per area in rhizosphere. The plasticity and genetic potential of these genotypes qualifies them for utilization in developing new varieties with improved roots to cope with challenges posed by environmental conditions. For example, waterlogging remains to be one of drastic environmental issues in soybean production particularly in rice-soybean cropping areas of southeast Asia (Suematsu *et al.*, 2017). Mainly, the flooding leads to hypoxic conditions in rhizosphere while the rate of gas diffusion is already four time slower under waterlogged conditions than open air. This inhibits nitrogen uptake and soybean leaves turn yellow and plant dies if the conditions persists indefinitely (Araki, 2006). The longer roots and ability to produce more adventitious roots enables soybean to sustain hypoxic conditions. Likewise, root surface area, total root length and root volume have been found linked to be predictor of tolerance to drought, salinity and phosphorous deficiency (Valliyodan, 2017; Shelke *et al.*, 2017 and Stangoulis., 2019). Also, these traits help soybean plant roots penetrate the soil hard pane, improve anchorage and uptake ground water and nutrients in and around rhizosphere. The availability of variation in these traits provide opportunity to develop high yielding stable cultivars in response to yield limiting abiotic stresses like drought, flooding, inefficient nutrient uptake and drastic edaphic conditions.

Table 3. Contribution of principal components (PCs) to overall variation in ten root traits.

Trait	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
StemD	-0.30	0.46	-0.07	-0.11	0.001	0.32	0.11	-0.72	0.15	-0.10
Area	-0.37	-0.12	0.06	-0.06	-0.46	-0.09	-0.16	0.05	0.55	0.51
RootSysW	-0.24	-0.47	-0.30	0.64	0.10	-0.22	0.01	-0.32	0.07	-0.15
RootTips	-0.35	-0.22	-0.01	-0.20	0.25	0.13	0.78	0.24	0.12	0.003
AdventRoot	-0.31	0.36	-0.01	-0.15	0.42	-0.74	-0.10	0.03	0.001	0.05
BasalRoot	-0.35	-0.08	-0.11	-0.005	0.52	0.49	-0.51	0.24	0.01	0.09
TAPRD	-0.14	0.02	0.90	0.37	0.12	0.04	0.03	-0.05	-0.02	0.005
TLength	-0.37	-0.15	-0.006	-0.12	-0.24	-0.01	0.003	-0.14	-0.79	0.32
Tvolume	-0.23	0.54	-0.21	0.51	-0.27	0.10	0.13	0.45	-0.10	-0.07
BranchingP	-0.36	-0.15	0.13	-0.28	-0.31	-0.07	-0.21	0.13	0.01	-0.75
Standard deviation	2.55	1.22	1.00	0.60	0.52	0.45	0.30	0.27	0.09	0.05
% Variability	0.65	0.15	0.10	0.04	0.03	0.02	0.01	0.01	0.00	0.00
Cumulative variability	0.65	0.80	0.90	0.93	0.96	0.98	0.99	1.00	1.00	1.00

StemD= stem diameter (mm), Area= projected root area (cm²), RootSysW= root system width (cm), RootTips= no. of root tips, AdventRoots=adventitious roots, BasalRoots= no. of basal roots, TAPRD= tap root diameter (mm), Tlength= total length (cm), Tvolume= Total volume (cm³), BranchingP= no. of branching point.

Conclusion

In this study, more variability was found in features such as basal roots, branching points, root tips, root area, and root system width, indicating that these parameters had higher genetic divergence. The presence of high genetic variability for these root traits within the soybean population can be used to develop ideotypes against various abiotic stresses such as drought, salinity, heat and compact soils with less aeration. Genotypes like Ajmeri, GP-18, E-1531, NIBGE-185, SA-7260, PGRA-25, SPS-1, SPS-22, and SPS-33 exhibited higher Total root length, adventitious roots, projected root area, root system width and tap root diameter. Likewise, Higher total root volume was seen in NIBGE-113,

NIBGE-115, and NIBGE-314. These characteristics allow them to absorb maximum nutrients and water from the rhizosphere. An efficient root system of these genotypes makes them ideal candidates for use in developing new varieties that can withstand harsh environmental conditions.

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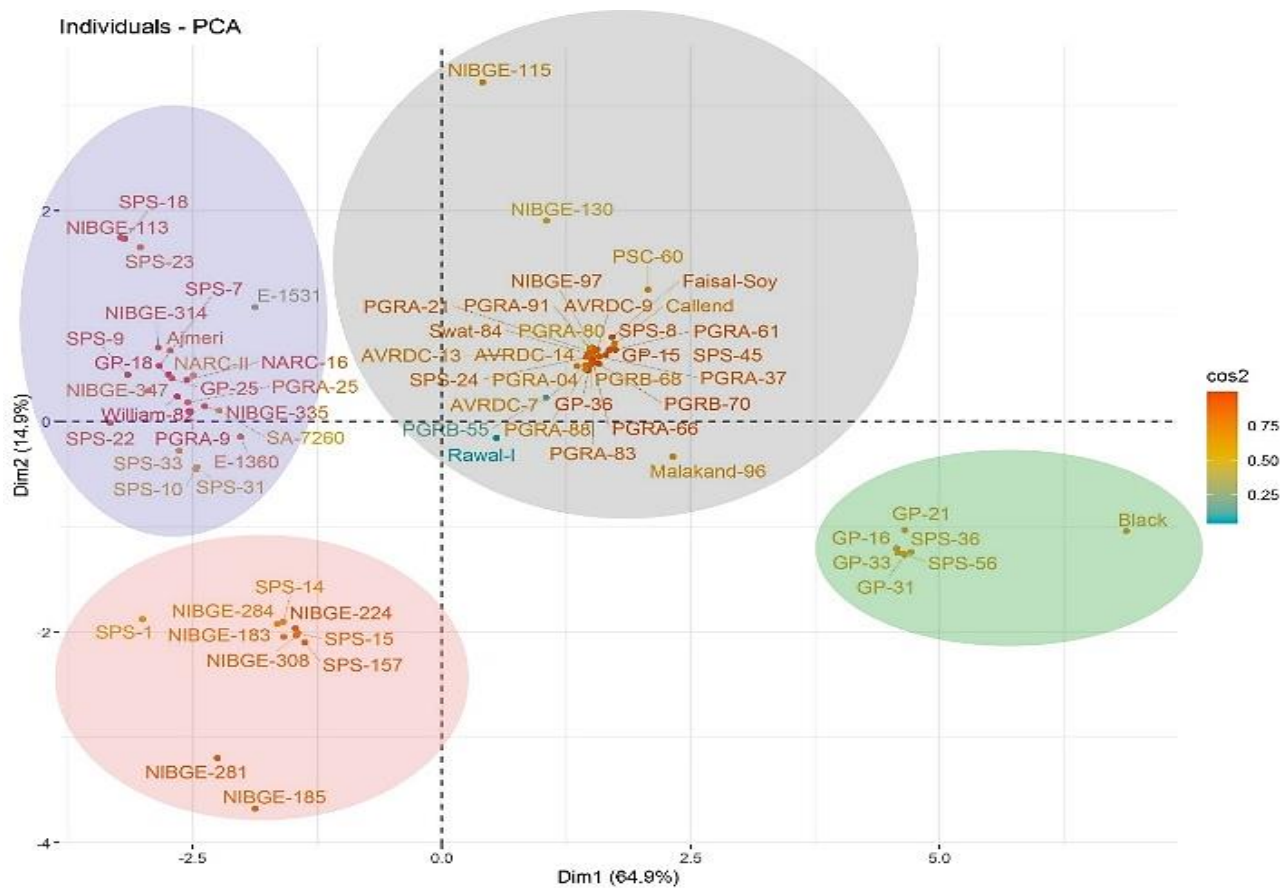


Fig. 3a. PCA scatter plot showing different groups of soybean genotypes based on root system architecture traits.

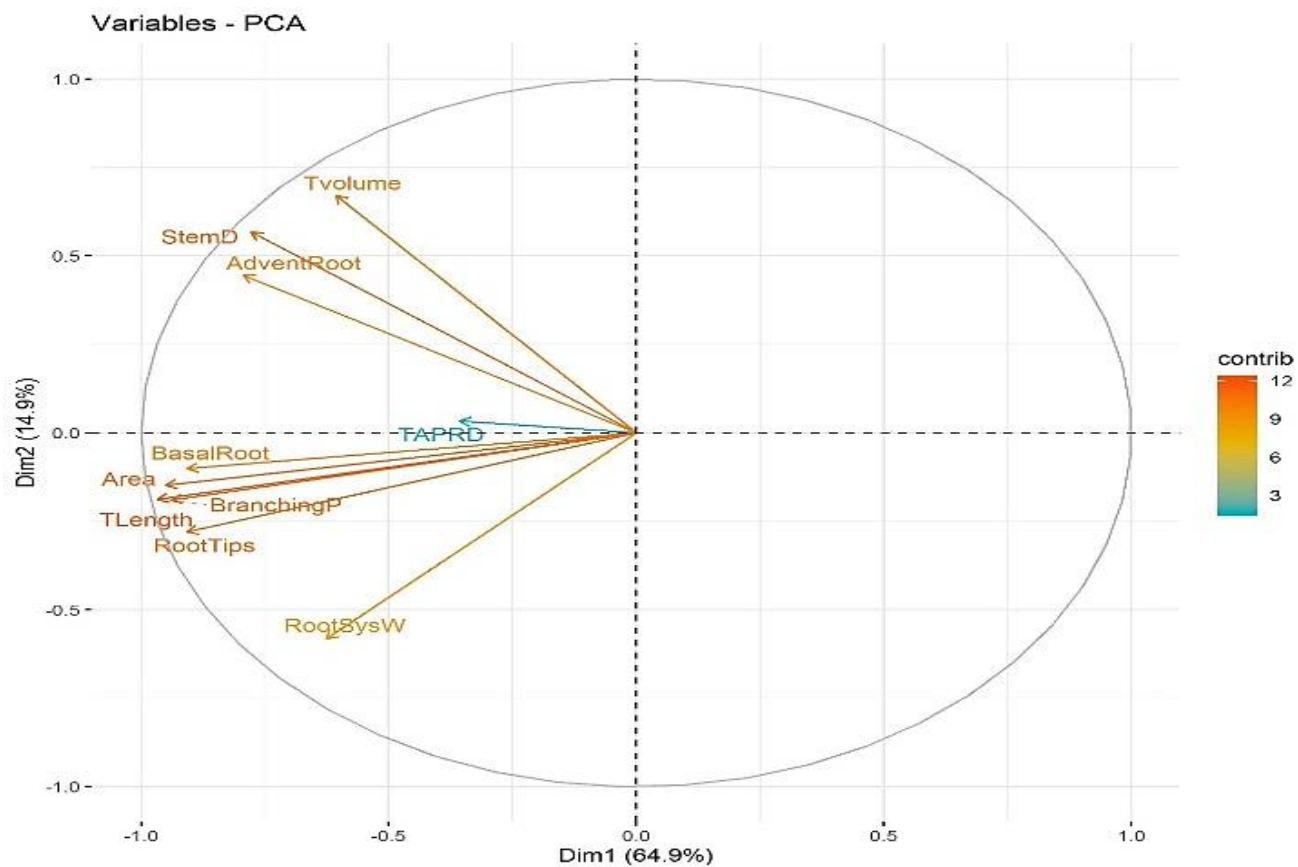


Fig. 3b. Plot showing interrelationship between different traits of root system architecture and their effect on RSA variation.

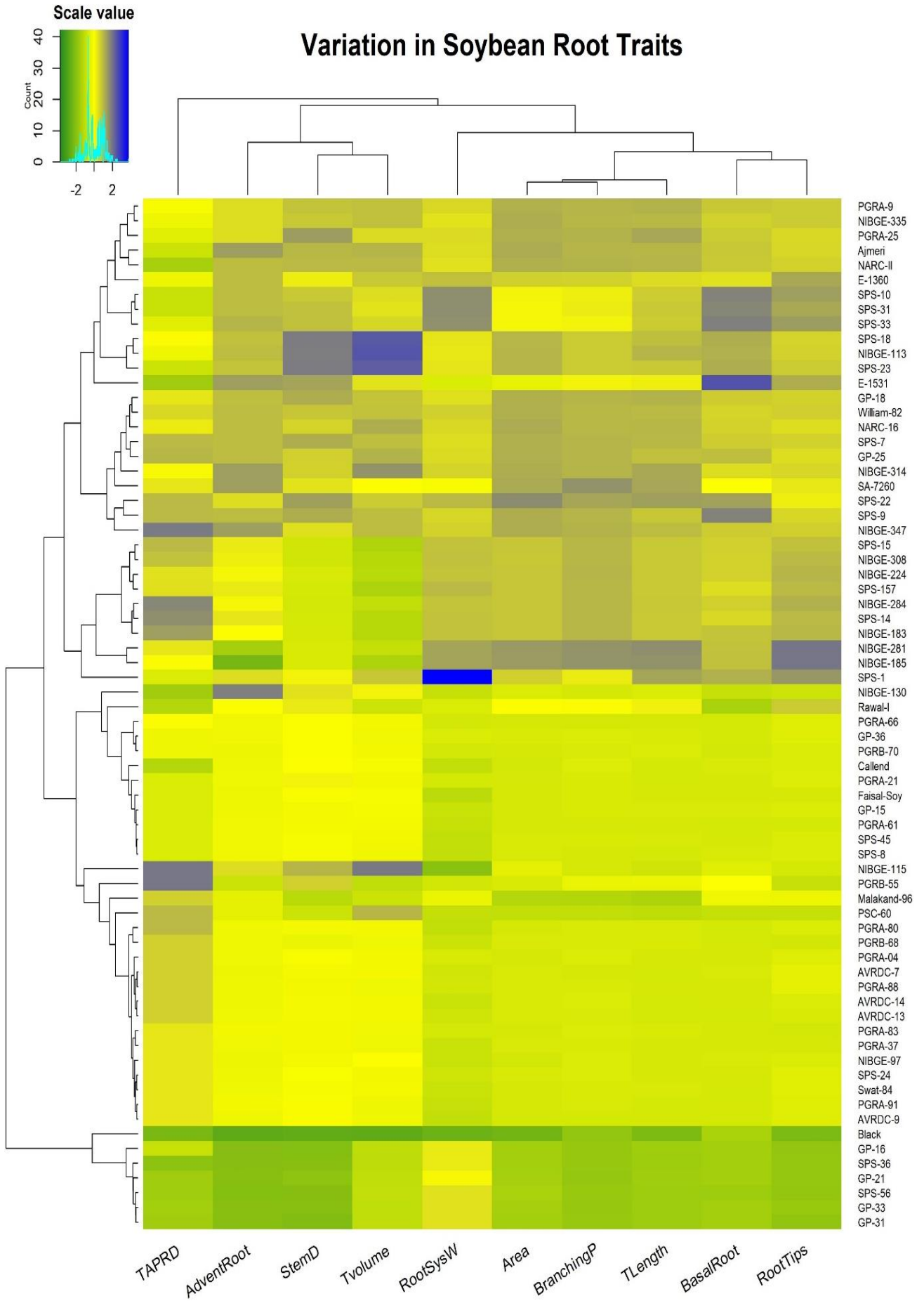


Fig. 4. Morphometric variation in root system architecture traits among 70 diverse soybean genotypes.

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