



Evaluation and clustering of soybean (*Glycine max* L.) germplasm inbred lines for root and shoot morphological traits at seedling stage

Ajmal Mandozai¹, Abdourazak Alio Moussa¹, Qi Zhang¹, Jing Qu¹, Naveed Ahmad², Yeyao Du¹, Noor al Amin¹, Rivalani Theorent¹, Gulaqa Anwari¹, Piwu Wang^{1*}

¹College of Agronomy, Plant Biotechnology Center, Jilin Agricultural University, Changchun, 130118, Jilin, China

²College of Life Sciences, Jilin Agricultural University, Changchun 130118, Jilin, China

Key words: Phenotypic diversity, Soybean germplasm, Morphological traits, Elite varieties.

<http://dx.doi.org/10.12692/ijb/15.6.152-163>

Article published on December 18, 2019

Abstract

Soybean is recognized as one of the most important economically approved beans across the world. Due to its genetic complexity of the several root traits, the study of root traits is generally ignored and not used as a selection standard. In this study, soybean germplasm consisted of 260 inbred lines was assessed for shoot and root traits at vegetative 1 stage. Analysis of variance showed highly significant differences among various varieties for different morphological shoot and root related traits. Root dry weight and total root length were positively correlated with overall core shoot and root related traits. Principal component analysis and cluster analysis structured the varieties into four groups/clusters based on their relative performances. cluster1, cluster3, and cluster4 were characterized by the varieties presenting higher, shoot length, root to shoot dry weight ratio and average root diameter, respectively. Cluster2 regrouped the varieties of the unique shoot and root characteristics. The identified genotypes including Z077, Z093, Z120, Z173, Z180, Z199, Z210, Z211, Z238 and Z248 of the above-mentioned cluster may play a crucial role in future breeding programs and could be used to improve drought tolerance for soybean elite varieties through root and shoot morphological traits specific to target environments.

* Corresponding Author: Piwu Wang ✉ peiwuw@163.com

Introduction

Soybean (*Glycine max* L.) is recognized as one of the most important economically approved beans across the world. It provides sufficient vegetable protein and various active ingredients of medicinally important chemical products for millions of people. The cultivation of soybean offers high-quality of important proteins utilized as animal and human feed (Carter *et al.*, 2004; Al Amin *et al.*, 2019). The group of proteins derived from soybean contained all essential amino acids, providing a rich source of cardio friendly vegetable oil satisfying more than 30% requirement of the prescribed oil according to the world health organization (Barbero *et al.*, 2004) along with some secondary metabolites, such as isoflavones and asponins. It also encompasses various therapeutic ingredients including lactose-free fatty acids, antioxidants, folic acid and vitamin B complex (Mathur, 2004). The global production of soybean oil shares (>61%), in comparison to rapeseed oil (12%), sunflower oil (9%), peanut oil (7%) and cottonseed oil (7%) (FAOSTAT; <http://faostat.fao.org>). The composition of soybean seed consists of 40% protein content, 20% oil content, more than 35% of carbohydrates (Liu, 1997).

Soybean roots could respond to various biotic and abiotic stresses by activating the aboveground plant tissues through multiple signaling pathways. The impact of root physiology and morphology on plant growth and development could be accelerated through modified root to shoot transport of several components, including mineral and numerous organic signaling molecules such as growth hormones, signaling proteins and RNAs (DoVale and Fritsche-Neto, 2015). With the help of complex root architecture, soybean is capable to absorb water and other growth-related nutrients essential for plants thereby providing a storage organ and facilitate the adaptation of the plants to the soil. Roots could also provide the establishment of the rhizosphere by providing the site of pathogenic as well as beneficial organisms' interactions. Recent studies have shown that the root traits of numerous crops are very complex and regulated by several genes that hold

enormous potential against various environmental stresses including wind, floods, droughts, alkaline salts and aluminum toxicity (Inoue *et al.*, 2004; Keller *et al.*, 1999; Liu *et al.*, 2005; Menchey and Aycock, 1998; Tar'an *et al.*, 2003; Zhou *et al.*, 2009). Therefore, a well-organized and sophisticated root system is one of the several advanced mechanisms of plants to protect themselves against unfavourable environments.

The area of plant genetics research continues to emphasize the traits appear above ground since the primary concern of measuring underground traits can simply limit to root studies. Many researchers have focused on the study of morphogenesis of soybean root system, physiological characteristics, and nutrient uptake (Liu *et al.*, 2005; Zwieniecki and Holbrook, 2008; King *et al.*, 2009; Manavalan *et al.*, 2009; Quan *et al.*, 2009; Zhang *et al.*, 2010). However, the study of root traits is generally ignored and not used as a selection standard. Moreover, the importance of the soybean cultivation and selection continues for decades, excluding root development, architecture, structure, and function: although it produces nearly half of the total plant biomass globally. However, to establish the root structure in-depth, it is pragmatic to explore and upgrade root traits. In the current study, we investigated the germplasm of soybean inbred lines for shoot and root related traits at seedling stage. The aims of this study were as follows: (1) to evaluate and analyze the architecture of root and shoot traits at seedling stage; (2) to analyze the phenotypic diversity among the tested soybean germplasm, and (3) to characterize and discriminate the soybean accessions according to their relative shoot and root morphological performances.

Material and methods

In this study, 260 genotypes of the Soybean natural population were tested under controlled environmental conditions in a greenhouse at the Department of Crop Genetics, Jilin Agricultural University, Changchun, China (43.8139° N, 125.4066° E). All the 260 released varieties were developed from

different breeding centers and released by varietal release committees for different agro-climatic regions of China which were coded from Z001 to Z260. Genetically pure seeds were obtained from Jilin Agricultural University experimental field in 2018. The controlled conditions of the aforesaid greenhouse for seedling were as follows: 28/23°C day/night temperature and 60/80 % day/night relative humidity, with a 12-h photoperiod. All the experiments were laid out in a complete randomized design (CRD) with three replications. Seedlings were grown in polyvinyl chloride (PVC) pipe which was sealed at the bottom with a cap and four central holes for drainage. The height of pipe was 26 cm and 9 cm inside diameter. The PVC cones filled with a mixture of sandy soil and vermiculite (2:1 ratio).

Soybean natural population seedlings were grown by constantly providing standard irrigation facilities within 5 days interval in absence of biotic or abiotic stress until the V1 vegetative stage. Then the seedling was shifted from the soil at the V1 stage nearly 14 days post-germination period (Fehr and Caviness, 1977) and three plants were selected for the analysis of root and shoot traits. The root system of each plant was subjected to washing twice using running tap water in order to remove the existing soil. The washing procedure of the roots was carefully conducted in order to avoid any supplementary root injury and losses. Shoot length was manually measured taken from the distance of the base of the plant to the tip of the top trifoliolate (Lee *et al.*, 1996) using a ruler and the measurements were expressed in centimeters (cm). After measuring the shoot length, shoots were simply cut at the base to separate them from the roots for further analysis. Using a scanner (Perfection V800 photo1A; Epson) optimized to a scanning resolution of 12800 dots per inch (dpi: 5039.37 dots per cm), with the help of DJ-GX02 software, which is an interactive scanner-based image analyser system for scanning, digitizing, and analyzing of root samples, roots were scanned and root traits were simultaneously analyzed. To operate, the collected root samples were placed on the top part of the screen (20cm×35cm) and then processed as the root system

analyzer is equipped with a light source under the scanner. A Windows-based PC, with a Pentium (R) D CPU and 4096MB RAM were used to compute the data during the experiment. A total of 10 traits including shoot length, root dry weight, shoot dry weight, root dry weight to shoot dry weight ratio, total plant biomass, total root length, surface area, average diameter, root volume, and branching number were examined initially out of which five (5) root related traits such as total root length (cm), surface area (cm²), average diameter (mm), root volume (cm³), and number of branching were electronically recorded (Table 1). Shoots and Roots samples were then separately wrapped in paper bags and subjected to oven drying until a constant weight at 72°C for 48 hours. Root and shoot dry weights were measured using an electronic weighing balance and the results expressed in milligrams (mg).

Statistical Analysis

Descriptive statistics including mean, standard deviation, coefficient of variation (CV%), distribution, and range for each measured root and shoot morphological trait were estimated using SPSS v.21- (IBM Corporation, MO, USA) software. After verification of normality and other parametric test conditions, data were subjected to analysis of variance (ANOVA) to see whether there is any significant difference among the different varieties about the various evaluated traits. As well, correlation analysis to know the relationship and magnitude of relationship existing between all traits was carried out based on the mean value. Broad sense heritability (H^2) was also calculated on the basis of entry mean (Pace, Gardner, Romay, Ganapathysubramanian, & Lübberstedt, 2015) according to the below formula

$$H^2 = \frac{\sigma_G^2}{\sigma_P^2}, \quad \sigma_G^2 = \left(\frac{MSG - MSE}{rep} \right), \quad \sigma_P^2 = \left(\frac{MSG - MSE}{rep} \right) + MSE, \quad H^2 = \frac{\left(\frac{MSG - MSE}{rep} \right)}{\left(\frac{MSG - MSE}{rep} \right) + MSE}$$

Where, σ_G^2 Genotypic σ_P^2 phenotypic variances, MSG and MSE stand for the mean square of genotype and mean square error, rep is the number of replications (rep=3). To obtain a general comprehensive characterization and classification of the samples, accession diversity was determined by Principal Component Analysis (PCA) followed by cluster

analysis in order to classify and discriminate the different varieties for the principal traits measured. Minitab 17 software was used to perform principal component analysis and cluster analysis.

Results

Descriptive statistics and analysis of variance

Descriptive statistics and Analysis of Variance (ANOVA) results related to all traits evaluated are summarized in (Table 2). The ANOVA results

indicated that the 260 natural population genotypes differed significantly ($P < 0.001$) in all seedling root and shoot morphological traits. Total root length and branching number had the largest standard deviations of 68.29 cm and 61.69, respectively. The coefficient of variation (CV) is the ratio of the standard deviation to the mean of traits where CV values ranged from 7.38% (average diameter) to 25.28% (branching number) (Table 2).

Table 1. Trait descriptions collected manually and by DJ-GX02.

Trait Name	Symbol	Measurement description
Shoot length	SL	Total length of shoot to the longest leaf tip in centimeters
Root dry weight	RDW	Total dry weight of only the plant root in gram
Shoot dry weight	SDW	Total dry weight of only the plant shoot in gram
Root dry weight/Shoot dry weight	RDW/SDW	Root dry weight to shoot dry weight ratio in gram
Total plant biomass	TPB	Root dry weight plus shoot dry weight in gram
Total Root length	TRL	Cumulative length of all the roots in centimeters
Surface area	SA	Surface area of the entire root system in cm ²
Average diameter	AD	Diameter of the primary root in millimeter
Root Volume	RV	Volume of the primary root in cm ³
Branching number	BN	Total number of branches

In order to see the inheritance about the different traits studied, heritability (H^2) for all traits was ranged from 27% (average diameter) to 85% (shoot length). Among all traits, shoot length and branching

numbers had the highest broad-sense heritability of 85% and 63%, respectively. The lowest heritability was performed by average diameter (27%) (Table 2).

Table 2. Descriptive statistics and analysis of variance results of seedling root and shoot traits.

Traits	Mean	±SD	Genotypes	Skewness	Kurtosis	Range	CV (%)	H ² (%)
SL	19.65	2.47	***	0.16	0.09	14.84	12.57	85
RDW	0.11	0.02	***	-0.40	-0.05	0.13	18.18	56
SDW	0.32	0.06	***	-0.48	0.42	0.39	18.75	56
RDW/SDW	0.35	0.06	***	0.56	0.73	0.31	17.14	47
TPB	0.43	0.08	***	-0.53	0.51	0.52	18.6	57
TRL	405.34	68.29	***	-0.41	0.09	380.43	16.85	41
SA	157.67	27.23	***	-0.22	-0.08	147.82	17.27	42
AD	1.22	0.09	***	0.02	0.06	0.54	7.38	27
RV	4.82	1.07	***	0.06	0.14	5.62	22.2	45
BN	243.99	61.69	***	0.40	0.51	408.33	25.28	63

Correlation and distribution analysis

Correlations among root and shoot traits were analyzed based on the mean values of each genotype. Globally, significantly positive correlations were observed between all traits. The highest significant

correlation was obtained between shoot dry weight and total plant biomass (0.98) and the lowest one between branching number and average diameter (0.12). The root dry weight had strong significantly ($p < 0.01$) positive correlations with total plant

biomass (0.85), surface area (0.75), shoot dry weight (0.73), root volume (0.72), total root length (0.65), branching number (0.45), root dry weight to shoot dry weight ratio (0.33) and shoot length (0.25). The

branching number and total root length had also a strong significant positive correlation with all traits except root dry weight to shoot dry weight ratio and average diameter, respectively as shown in (Table 3).

Table 3. Pearson correlation coefficients among all seedling root and shoot traits.

	SL	RDW	SDW	RDW/SDW	TPB	TRL	SA	AD	RV	BN
SL	1	0.254**	0.549**	-0.393**	0.499**	0.195**	0.178**	0.010	0.164**	0.231**
RDW		1	0.737**	0.339**	0.852**	0.650**	0.755**	0.338**	0.720**	0.450**
SDW			1	-0.352**	0.980**	0.529**	0.584**	0.229**	0.533**	0.413**
RDW/SDW				1	-0.171**	0.137*	0.202**	0.146*	0.225**	0.042
TPB					1	0.593**	0.664**	0.271**	0.615**	0.447**
TRL						1	0.845**	-0.035	0.638**	0.614**
SA							1	0.389**	0.898**	0.636**
AD								1	0.632**	0.128*
RV									1	0.552**
BN										1

SL=shoot length, RDW=root dry weight, SDW=shoot dry weight, RDW/SDW=root dry weight to shoot dry weight ratio, TPB=total plant biomass, TRL=total root length, SA=surface area, AD=average diameter, RV=root volume, BN=branching number. *Correlation is significant at the $p < 0.05$ level. **Correlation is significant at the $p < 0.01$ level.

All ten captured seedling traits have approximately followed a normal distribution, slightly skewed from right to left without any significant skewness and kurtosis (Fig. 1).

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was performed

using the means values related to each trait per variety. The correlation matrix between the principal components and the variables studied, as well as the eigenvalues, proportion and cumulative variance concentrated in each principal component are shown in (Table 4).

Table 4. Contribution of traits for principal components (PC) and, Eigenvalues, Proportion and cumulative variation.

Descriptor	PC1	PC2	PC3
SL	0.178	-0.504	-0.060
RDW	0.387	0.111	-0.016
SDW	0.362	-0.348	-0.107
RDW/SDW	0.029	0.631	0.115
TPB	0.389	-0.239	-0.088
TRL	0.345	0.079	0.475
SA	0.397	0.189	0.092
AD	0.176	0.238	-0.766
RV	0.377	0.238	-0.196
BN	0.292	0.046	0.324
Eigenvalue	5.236	1.849	1.133
Proportion	0.524	0.185	0.113
Cumulative	0.524	0.708	0.822

SL=shoot length, RDW=root dry weight, SDW=shoot dry weight, RDW/SDW=root dry weight to shoot dry weight ratio, TPB=total plant biomass, TRL=total root length, SA=surface area, AD=average diameter, RV=root volume, BN=branching number.

The first 2 components accounted for 70.9 % of the total variance that may exist in the samples. Specifically, PC1 and PC2 accounted for 52.4% and 18.5% of the total variance respectively. The

remaining components contributed 29.1% to the total variability related to the characteristics of the different soybean varieties.

Table 5. Descriptive results of morphological traits of each cluster.

Traits	Cluster 1		Cluster 2		Cluster 3		Cluster 4	
	Mean	St.dev	Mean	St.dev	Mean	St.dev	Mean	St.dev
SL	19.83	2.40	20.17	2.05	18.83	2.62	18.50	2.35
RDW	0.12	0.02	0.12	0.02	0.10	0.02	0.07	0.02
SDW	0.34	0.05	0.34	0.05	0.30	0.06	0.22	0.07
RDW/SDW	0.34	0.04	0.35	0.05	0.34	0.06	0.31	0.06
TPB	0.45	0.06	0.46	0.06	0.39	0.08	0.29	0.09
TRL	427.95	49.08	460.47	30.84	362.98	43.09	255.17	35.76
SA	166.96	20.52	179.70	16.03	142.07	19.19	102.95	12.36
RV	1.24	0.08	1.20	0.09	1.21	0.08	1.23	0.14
AD	5.13	0.97	5.26	0.83	4.31	0.85	3.05	0.79
BN	310.33	43.79	253.00	37.16	202.33	27.66	127.00	26.68

SL=shoot length, RDW=root dry weight, SDW=shoot dry weight, RDW/SDW=root dry weight to shoot dry weight ratio, TPB=total plant biomass, TRL=total root length, SA=surface area, AD=average diameter, RV=root volume, BN=branching number.

Therefore, the first two components absorbing 70.9% of the cumulative variance associated with the root and shoot traits are large enough to explain the whole variability among the traits evaluated. Nevertheless, the analysis of the correlation table of the variables with the axes shows that the average diameter is poorly represented on these first two components but well correlated with the third axis. As a result, the third principal component appears indispensable in the interpretation of the results. Thus, the first three components accounted 82.2% of all the variability related to the morphological root and shoot features.

The traits RDW, SDW, TPB, TRL, SA, and RV had major contributions towards PC1 and for PC2 the maximum variation is contributed by SL and RDW/SDW (Table 4). Other traits, BN and AD showed medium variance contributions towards PC1 and PC2 respectively (Table 4). As a result, we can say that PC1 is mainly related to the most important root traits whereas PC2 to shoot trait. Taking all together,

the results of score plot and loading plot (Figs 2 and 3), PC1 separates the group of varieties which performed better in RDW, SDW, TPB, TRL, SA, and RV contrary as for those regrouped on the negative side of the axis 1. PC2 disassociates the varieties with higher RDW/SDW ratios (thus lower SL inversely represented in the axis 2) from those with lower RDW/SDW values. Therefore, based on these results, PC1 allowed us to distinguish in one hand the group of varieties with good root characteristics from the group of varieties with other characteristics and in the other hand PC2 distinguished higher varieties with higher RDW/SDW ratios from shorter varieties with smaller RDW/SDW (Fig. 3).

Cluster analysis (CA)

The grouping of 260 soybean germplasm was analyzed according to cluster analysis to explain the similarity between different accessions, based on morphological root and shoot characters. Four (4) clusters at 51 % similarity coefficient were separated

(Fig. 4). Mean and standard deviation for each cluster are presented in (Table 5). The dendrogram shows that cluster 1 comprised 59 accessions, characterized by the relatively longest shoot length. Cluster 2, as the second-largest cluster member of 83 accessions had characteristics of a unique Root dry weight, shoot dry weight and Total plant biomass, Total root length,

Surface area, Root volume, and Branching number. Cluster 3 as the largest cluster members of 107 accessions had a high root dry weight to shoot dry weight ratio. Cluster 4 consisted of 11 accessions which were characterized by average diameter (Fig. 4).

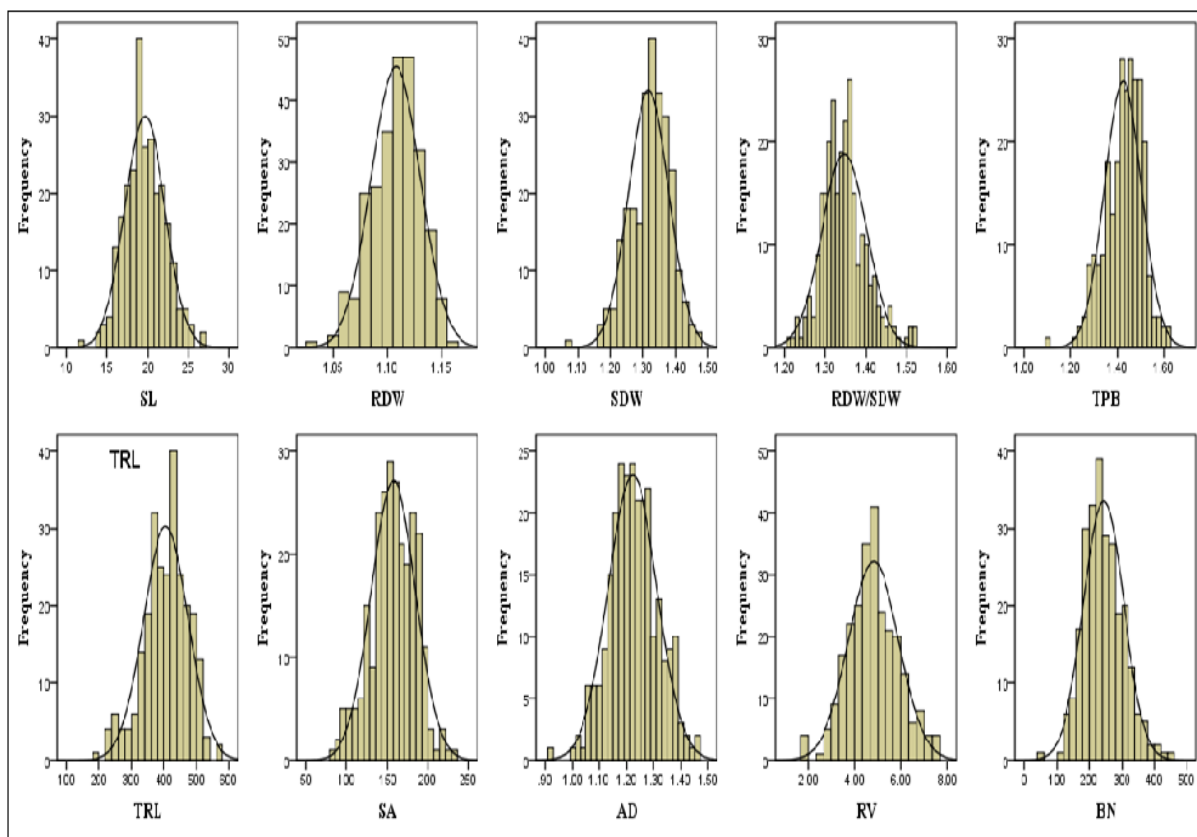


Fig. 1. Distribution of the studied root and shoot traits in 260 soybean germplasm.

Discussion

The analysis of root characteristics of soybeans and the genetic basis of these characteristics is essential to understand how soybean roots determine yield and to create more powerful and flexible varieties. All the root related morphological traits exposed extremely significant ($P < 0.001$) variations indicating the existence of enough amount of genetic variability between the genotypes for all the studied traits.

Our results are in lined with the previous findings of (Paterson *et al.*, 1991) who specified that assessment of genetic diversity would facilitate the efficient and rapid use of genetic variations related to the breeding programs. Thus, it is of great importance to study and

revisit the genetic diversity among various varieties of higher plants in order to regulate the mechanism of population, based genetic variation to explore novel morphological characters which may lead towards the development of advanced varieties. Similarly, genetic diversity and dealing between breeding materials provide key information for a plant breeder to promote an effective crop variety.

During this study, the 260 Soybean germplasm were obtained from different regions of China. The soybean natural population genotypes were grown under controlled conditions in the greenhouse until (V1) vegetative stage to investigate the variability of root and shoot related traits.

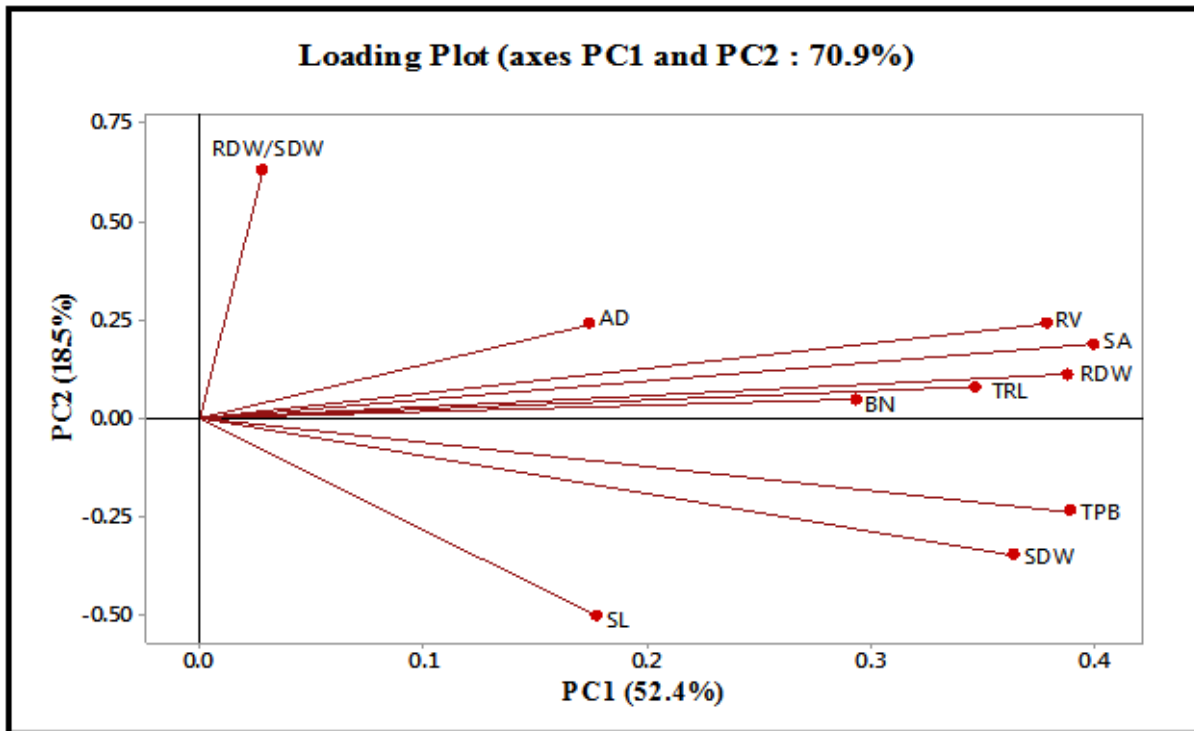


Fig. 2. Loading plot of 260 soybean germplasm describing the relationships among morphological traits SL=shoot length, RDW=root dry weight, SDW=shoot dry weight, RDW/SDW=root dry weight to shoot dry weight ratio, TPB=total plant biomass, TRL=total root length, SA=surface area, AD=average diameter, RV=root volume, BN=branching number.

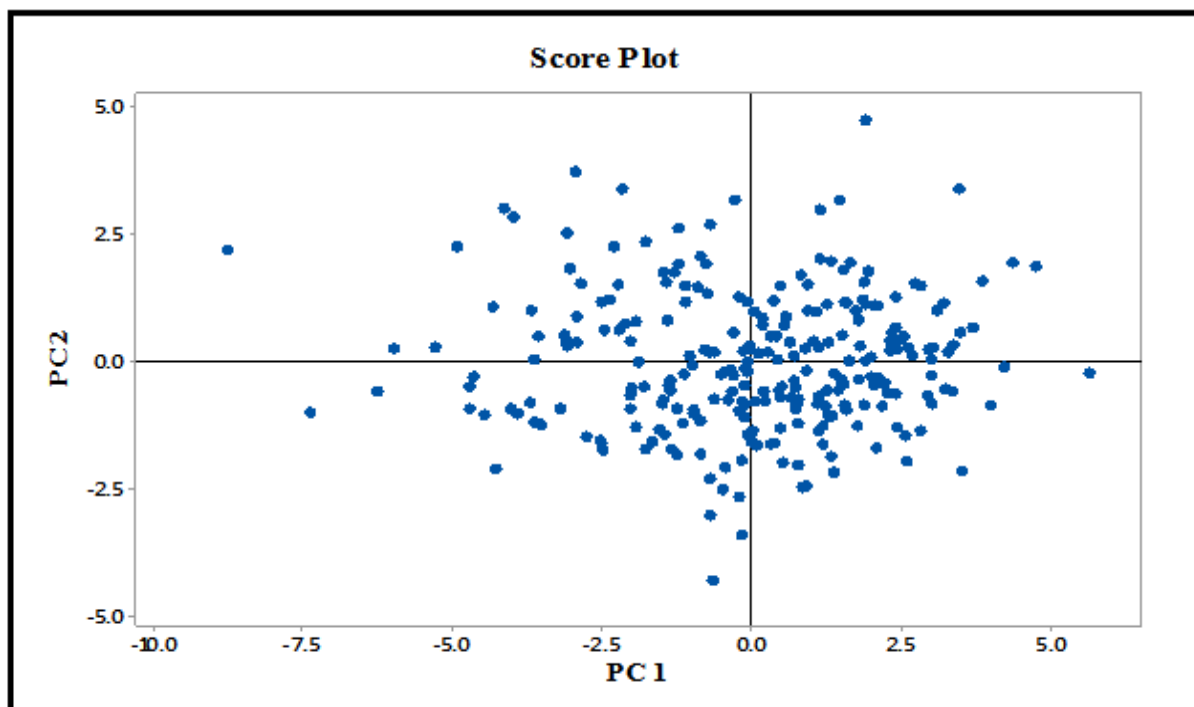


Fig. 3. Scores plot of 260 soybean germplasm lines on PC1 and PC2.

Our key findings are consistent with the confirmation of previous reports suggesting that root related traits are highly influenced by soil type and other edaphic

factors of specific growing environment conditions, especially in field situations, as it differs based on nutrients, water availability, and soil temperature and

strength (Cairns *et al.*, 2011; Whitmore and Whalley, 2009). The question of whether phenotyping system under controlled conditions can exactly represent what occurs in the field is always an issue of deliberation. Therefore, we choose to grow soybean seedling in green houses located in Jilin Agricultural

University. Keeping in view the importance of growing conditions, many studies have done under controlled environment, growing seedlings in the soil or gel media to avoid the critical process of root excavation in field studies (Gruber *et al.*, 2013; Shrestha *et al.*, 2014).

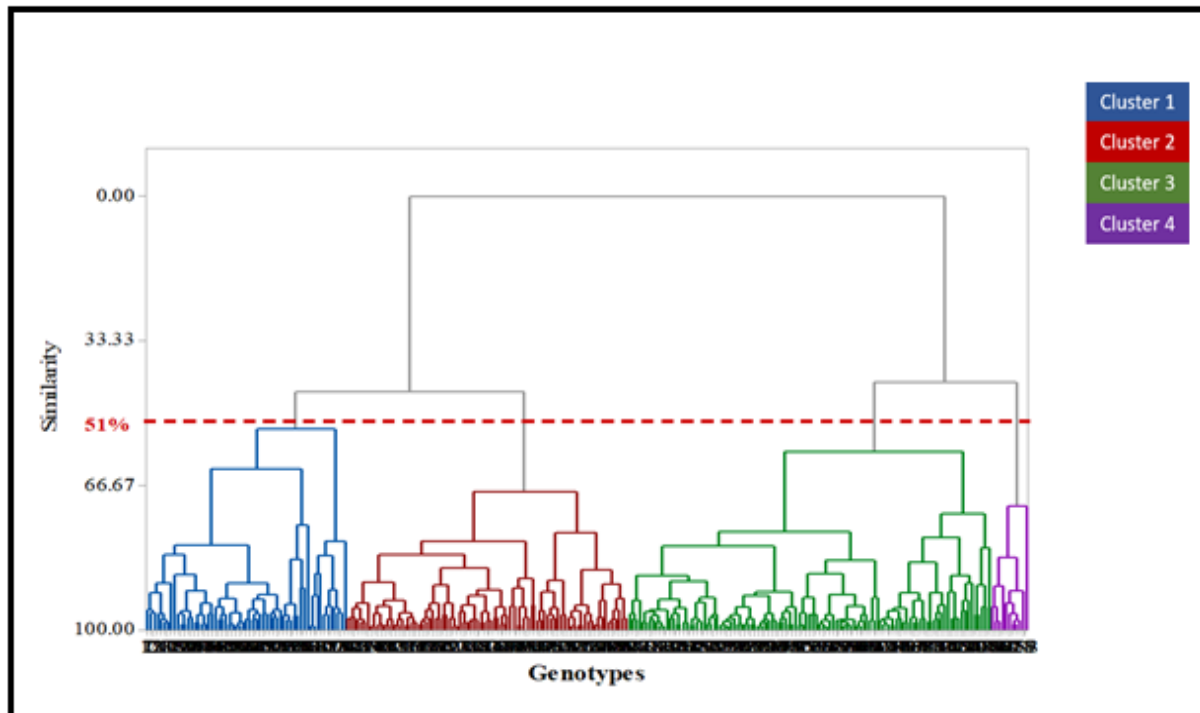


Fig. 4. Dendrogram of 260 soybean accessions based on morphological traits at a 51% similarity coefficient. four clusters were represented in different colors including cluster 1 which denotes 59 genotypes and is attributed to shoot length characters, cluster 2 denotes 83 genotypes and is attributed to root dry weight, shoot dry weight, total plant biomass, total root length, surface area, root volume, and branching number characters. cluster 3 denotes 107 genotypes and is attributed to root dry weight to shoot dry weight ratio characters, cluster 4 denotes 11 genotypes and is attributed to average diameter character.

In this study, relatively high heritability was presented for all traits in the selected soybean seedling except average diameter, suggesting that genetic effects play a principal role in the phenotype variation of these traits. We found that most of the root related traits were significantly correlated with the entire root system traits. For example, total root length and surface area were significantly positively correlated with total root volume, surface area and branching number with ranging between 0.52 and 0.89. Similar explanations have been reported by (Prince *et al.*, 2015) who revealed that fine root length, surface area of roots and root volume had a very strong and positive correlation with total root

volume. Another study of (Fried *et al.*, 2018) also described that the total root length and surface area were positively correlated with surface area, total root length, and root volume. These traits are the most energetic part of the root system in case of extracting nutrients and water described by (Hodge *et al.*, 1999; Pierret *et al.*, Moran and Doussan, 2005; Smucker, 1984). Hence, it is necessary to coordinate relations of these traits while doing selective breeding for soybean quality.

Further investigation on 260 soybean natural population genotypes showed genetically high diversity. We categorized them in different groups

using principal component analysis (PCA) followed by cluster analysis (CA). Principal component analysis (PCA) allows us to discriminate the different genotypes into four different groups by considering the two first components which accounted for 70.9% of the total phenotypic variance. The group2 is characterized by the varieties which are especially good in root characteristics as compared to the group4 which includes the varieties with otherwise characteristics. The varieties of Group 1 present higher shoot length while those of group 3 present smaller shoot length. By applying cluster analysis (CA), the 260 Soybean natural population genotypes were clustered into four clusters (as obtained from principal component analysis) at 51% similarity coefficient based on the 10 morphological root and shoot traits. Furthermore, each cluster characterized one to seven determinant characters. The morphological classification of presenting genetic variation were found in agreement with previous studies (Adie and Krisnawati, 2017) who indicated 11 agronomic characters of 150 soybean genotypes by grouping into 10 clusters at a 62% similarity coefficient. Another genetic diversity study conducted by (Dayaman, 2007) grouped 45 soybean genotypes into six(6) clusters based on 22 morphological traits (Diazcarrasco *et al.*, 1986) clustered 17 soybean accessions to ten clusters based on plant height, seed yield plant⁻¹ and days to maturity. By applying cluster analysis, (Moe, 2012) also investigated morphological traits in soybean accessions, 94 genotypes were clustered at the similarity of 52% into seven different groups by consuming Unweighted Pair Group Method with Arithmetic Mean (UPGMA) (Salimi *et al.*, 2012). Also applied cluster analysis and grouped to ten clusters to identify germplasm TNH56 and BP which are drought-tolerant, and these germplasms could be used as a source of genotypes for future breeding drought tolerance.

Conclusion

In this study, a total of 260 Soybean germplasm inbred lines were clustered and classified for the unique shoot and root characteristics including high root dry weight, high shoot dry weight, high total

plant biomass, total root length, high surface area, root volume, and high root branching number. The genotypes of cluster2 resulted in 83 genotypes of soybean inbred lines which may play a crucial role in future breeding programs and could be used as a new germplasm resource to improve drought tolerance for soybean elite varieties through root and shoot morphological traits. Thus, we selected the top ten soybean accessions from the above mentioned cluster2 such as (Z077, Z093, Z120, Z173, Z180, Z199, Z210, Z211, Z238, and Z248) which was identified as crucial candidates for at least seven root traits in soybean germplasm.

Funding

This project was funded by the National Natural Science Foundation of China under grant number (31571689).

References

Adie MM, Krisnawati A. 2017. Characterization and clustering of agronomic characters of several soybean genotypes. *Nusantara Bioscience* **9(3)**, 237-242.

<https://doi.org/10.13057/nusbiosci/n090301>

al Amin N, Ahmad N, Wu N, Pu X, Ma T, Du Y, Wang P. 2019. CRISPR-Cas9 mediated targeted disruption of FAD2-2 microsomal omega-6 desaturase in soybean (*Glycine max.* L). *BMC biotechnology* **19(1)**, 9.

<https://doi.org/10.1186/s12896-019-0501-2>

Barbero A, Johnson E, Conner TW, Paschal, EH. 2004. The challenges and potential for future agronomic traits in soybeans.

Cairns J, Impa S, O'Toole J, Jagadish S, Price A. 2011. Influence of the soil physical environment on rice (*Oryza sativa* L.) response to drought stress and its implications for drought research. *Field Crops Research* **121(3)**, 303-310.

<https://doi.org/10.1016/j.fcr.2011.01.012>

Carter T, Nelson R, Sneller C, Cui Z,

- Boerma H, Specht J.** 2004. Genetic diversity in soybean. Soybeans: Improvement, production, and uses, **3**.
- Dayaman V.** 2007. Diversity analysis in soybean (*Glycine max* L.) using morphological and simple sequence repeat (SSR) markers. Master of Science in Biotechnology, Department of Plant Molecular Biology and Biotechnology, Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, India.
- Diazcarrasco H, Abreuferer S, Velazquez O, Garcia O.** 1986. Similarities between some agronomic characters in soybean. *Ciencias de la Agricultura* **27**, 108-113.
- DoVale JC, Fritsche-Neto R.** 2015. Root phenomics. In *Phenomics* p 49-66 Springer.
https://doi.org/10.1007/978-3-319-13677-6_4
- Fehr WR, Caviness CE.** 1977. Stages of soybean development.
- Fried HG, Narayanan S, Fallen B.** 2018. Characterization of a soybean (*Glycine max* L. Merr.) germplasm collection for root traits. *PloS one* **13(7)**, e0200463.
<https://doi.org/10.1371/journal.pone.0200463>
- Gruber BD, Giehl RF, Friedel S, Von Wirén N.** 2013. Plasticity of the Arabidopsis root system under nutrient deficiencies. *Plant physiology* **163(1)**, 161-179.
<https://doi.org/10.1104/pp.113.218453>
- Hodge A, Robinson D, Griffiths B, Fitter A.** 1999. Why plants bother: root proliferation results in increased nitrogen capture from an organic patch when two grasses compete. *Plant, Cell & Environment* **22(7)**, 811-820.
<https://doi.org/10.1046/j.13653040.1999.00454.x>
- Inoue M, Gao Z, Cai H.** 2004. QTL analysis of lodging resistance and related traits in Italian ryegrass (*Lolium multiflorum* Lam.). *Theoretical and applied genetics* **109(8)**, 1576-1585.
<https://doi.org/10.1007/s00122-004-1791-9>
- Keller M, Karutz C, Schmid J, Stamp P, Winzeler M, Keller B, Messmer M.** 1999. Quantitative trait loci for lodging resistance in a segregating wheat× spelt population. *Theoretical and applied genetics* **98(6-7)**, 1171-1182.
<https://doi.org/10.1007/s001220051182>
- King CA, Purcell LC, Brye KR.** 2009. Differential wilting among soybean genotypes in response to water deficit. *Crop science* **49(1)**, 290-298.
<https://doi.org/10.2135/cropsci2008.04.0219>
- Lee S, Bailey M, Mian M, Carter T, Ashley D, Hussey R, Boerma H.** 1996. Molecular markers associated with soybean plant height, lodging, and maturity across locations. *Crop science* **36(3)**, 728-735.
<https://doi.org/10.2135/cropsci1996.0011183X003600030035X>
- Liu K.** 1997. Soybeans: Chemistry, Technology, and Utilization; Aspen Publication. Inc.: New York.
- Liu Y, Gai J, Lu H.** 2005. Identification of rhizosphere abiotic stress tolerance and related root traits in soybean [*Glycine max*(L.) Merr.]. *Zuo wu xue bao* **31(9)**, 1132-1137.
- Liu Y, Gai JY, Lu HN, Wang YJ, Chen SY.** 2005. Identification of drought tolerant germplasm and inheritance and QTL mapping of related root traits in soybean (*Glycine max* (L.) Merr.). *Acta Genetica Sinica* **32(8)**, 855-863.
- Manavalan LP, Guttikonda SK, Phan Tran LS, Nguyen HT.** 2009. Physiological and molecular approaches to improve drought resistance in soybean. *Plant and Cell Physiology* **50(7)**, 1260-1276.
<https://doi.org/10.1093/pcp/pcp082>
- Mathur S.** 2004. Soybean the wonder legume.

Beverage Food World **31(1)**, 61-62.

Menchey E, Aycock M. 1998. Anther-derived dihaploids for lodging improvement in tobacco. Crop science **38(3)**, 698-701.

<https://doi.org/10.2135/cropsci1998.0011183X003800030013X>

Moe S. 2012. Relationships of soybean (*glycine max L.*) accessions based agro-morphological, physiological traits and dna polymorphisms. School of Crop Production Technology Institute of Agricultural Technology.

Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Lincoln SE, Tanksley SD. 1991. Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. Genetics **127(1)**, 181-197.

Pierret A, Moran CJ, Doussan C. 2005. Conventional detection methodology is limiting our ability to understand the roles and functions of fine roots. New Phytologist **166(3)**, 967-980.

<https://doi.org/10.1111/j.1469-137.2005.01389.x>

Prince SJ, Song L, Qiu D, dos Santos JVM, Chai C, Joshi T, Murphy M. 2015. Genetic variants in root architecture-related genes in a Glycine soja accession, a potential resource to improve cultivated soybean. BMC genomics **16(1)**, 132.

<https://doi.org/10.1186/s12864-015-1334-6>

Quan L, Yuanping Y, Xiaolong Y. 2009. Genetic analysis of root characters in soybean using a recombinant inbred line population at two phosphorus levels. Molecular Plant Breeding.

Salimi S, Lahiji HS, Abadi GM, Salimi S, Moradi S. 2012. Genetic diversity in soybean genotypes under drought stress condition using factor

analysis and cluster analysis. World Applied Sciences Journal **16(4)**, 474-478.

Shrestha R, Al-Shugeairy Z, Al-Ogaidi F, Munasinghe M, Radermacher M, Vandenhirtz J, Price A. 2014. Comparing simple root phenotyping methods on a core set of rice genotypes. Plant Biology **16(3)**, 632-642.

<https://doi.org/10.1111/plb.12096>

Smucker A. 1984. Carbon Utilization and Losses by Plant Root Systems 1. Roots, nutrient and water influx, and plant growth (rootsnutrientan), 27-46.

Tar'an B, Warkentin T, Somers D, Miranda D, Vandenberg A, Blade S, DeKoeyer D. 2003. Quantitative trait loci for lodging resistance, plant height and partial resistance to mycosphaerella blight in field pea (*Pisum sativum L.*). Theoretical and applied genetics **107(8)**, 1482-1491.

<https://doi.org/10.1007/s00122-003-1379-9>

Whitmore AP, Whalley WR. 2009. Physical effects of soil drying on roots and crop growth. Journal of experimental botany **60(10)**, 2845-2857.

<https://doi.org/10.1093/jxb/erp200>

Zhang Y, Jia J, Zhao Y, Gu S, Xu J. 2010. Screening index for low phosphorus tolerance at seedling stage. Agricultural Science & Technology-Hunan **11(3)**, 87-97.

Zhou R, Wang X, Chen H, Zhang X, Shan Z, Wu X, Wu J. 2009. QTL analysis of lodging and related traits in soybean. Acta Agronomica Sinica, **35(1)**, 57-65.

<https://doi.org/10.3724/SP.J.1006.2009.00057>