

Research Article

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Genetic dissection of advanced soybean (*Glycine max* L.) germplasm for spring season cultivation in Pakistan

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Abstract

Improvement in genetic gains of crops could be achieved by phenomics' characterization of agronomic, physiological and stress-related traits. Molecular and strategic breeding programmes require broad range of foreground and background phenotypic information for crop improvement. The current experiment was performed on 123 advanced soybean (*Glycine max* L.) genotypes including seven local lines belongs to four different maturity groups (000-IV) to estimate the endogenous potential of various yield-related traits. The experimental trial was repeated for two cropping seasons. Four traits out of six, yield per plant (YPP), number of seeds per plant, number of pods per plant and plant height (PH), showed maximum variation (CV%) that directly correlate with variability in the subjected population. PH, number of pods, 100-seed weight and YPP showed strong positive correlation in both years. Among the principal components, factors 1 and 2 showed maximum contribution in phenotypic variability ranges from 19 to 48.5% and 26 to 47.7% in the first and second years, respectively. Number of pods showed significant positive correlation with genotypes in both years. Dendrogram showed two distinct groups of soybean genotypes. Genetic variation and association among the accessions is indispensable for effective conservation and utilization of germplasm. Principal component analysis helps to identify the diverse genotypes that will be used as a parent for various breeding programmes. These phenotypic data will be used for detection of heat stress-related quantitative trait loci with genotypic data in genome-wide association studies experiments.

Introduction

Soybean (*Glycine max* L.) is one of the most important oil seed crops worldwide. It belongs to family Fabaceae and subfamily Faboide (Ali *et al.*, 2015). Soybean seeds are enriched with protein (40–42%) and oil (18–22%). Its oil has two important fatty acids, i.e. linoleic and linolenic acid (85%) that normally are not produced by human body (Antalina, 2000; Balasubramaniyan and Palaniappan, 2003). Soybean oil is also highly recommended for human diet due to its cholesterol-free composition. Owing to its multipurpose feature and excellent nutritional value, it is called by different names such as 'meat without bones', 'golden bean', 'wonder crop', 'queen of pulses', 'agriculture's Cinderella', 'meat of the field' and 'farmers friend' (Kumar and Sharma, 2018; Akram and Ahmad, 2019).

Globally, during the year of 2019–20, production of oilseeds was recorded as 554.61 million metric tons (MMT) in which 341.76 MMT was contributed by soybean. Brazil is one of the countries that leads the production followed by the United States with 126 and 96.841 MMT, respectively. After Brazil and USA, Argentina is one of the major producers of soybean with 54 MMT ranking third in the world (USDA, 2020).

Despite the favourable climatic and soil conditions in Pakistan, cultivation of soybean is limited due to lack of diverse germplasm, coherent policy, disease-resistant genotypes and lack of photo-insensitive soybean lines for different regions of Pakistan. These are the major factors that limit the popularity of this crop among farmers (Asad *et al.*, 2020). There are only 7–9 soybean cultivars that are generally cultivated in the country, and only produce economical yield under short-day conditions (autumn season). Pakistan faces severe shortage of edible oil since the local production of edible oil is less than 20% of country's requirements (GOP, 2019). In the latest scenario, import of edible oil was 2.68 million tons with a value of US\$3.56 billion that negatively affected the economy (Pakistan, 2022–2023). Now there is a need to fulfil the local demand through increasing domestic cultivation of soybean in both spring and autumn seasons.

Morphological and genetic dissection is a prerequisite of crop improvement programmes. This pre-breeding morphological characterization will provide information about endogenous



genetic diversity that will be helpful for selection of parental combinations. That ultimately leads to introgression of desirable genes or chromosome segments from diverse sources into elite germplasm (Thompson *et al.*, 1998; Das *et al.*, 2001; Iqbal *et al.*, 2008). It is crucial for plant breeders to accurately characterize genotypes at every point of the breeding process, from selecting parents to applying genotypes to breeding programmes (Hausmann *et al.*, 2004; Haider *et al.*, 2015; Mehmood *et al.*, 2016).

The estimate of endogenous genetic variation in a population can be made statistically through multivariate analysis. Multivariate analysis can be used to assess the genetic diversity of germplasm. The genotypes and data are plotted in two-dimensional graph to represent its dissociation, variability and correlation (Chakravorty *et al.*, 2013). Agro-morphological trait information speeds up the process of strategic trait-based breeding as well as increases the probability of positive allele pyramiding in contrast to uncharacterized parents (Reynolds and Langridge, 2016).

The prime focus of this current research is to detect the genetic variation in the advance soybean germplasm by dissociation of agro-morphological traits to select germplasm for future spring season cultivation in Pakistan.

Materials and methods

Germplasm collection and phenotyping

A total of 123 soybean (*G. max* L.) accessions from diverse maturity groups (000-IV) were collected from the United States Department of Agriculture (USDA), Agricultural Research Institute Mingora, Swat, Ayub Agricultural Research Institute (AARI), Faisalabad, National Agricultural Research Center (NARC), Islamabad (online Supplementary Table S1). Healthy seeds of all genotypes were grown in early and mid-March at CABB, University of Agriculture Faisalabad (31°26'N, 73°6'E) for the period of two years 2020 and 2021 on two different plantation dates each year. Each entry was planted in 1.5 m long row by keeping row-to-row distance 45 cm and plantation was done by planting one seed per hole and maintaining 8 cm plant-to-plant distance. The crop was raised by following all standard agronomics practices. The experiment was conducted following the augmented RCBD (Federer, 2002) with three replications. The whole material was divided into four blocks each comprising 29 entries. A set of seven check varieties (Ajmeri, Faisal soya, Malakand, NARCII, Rawal, Swat18 and William) were randomized in each block.

One healthy plant from each replication was randomly selected and tagged from each genotype for morphological data collection. Agro-morphological traits data were recorded for each genotype (Chen *et al.*, 2007; Dubey *et al.*, 2018a) which were plant height (PH; cm), number of pods per plant (Num), number of seeds per plant (Num), 100-seed weight (HSW; g), yield per plant (YPP; g) and days to maturity (DM; Days).

Statistical data analysis

Statistical parameters including standard deviation, minimum, maximum, mean, variance, coefficient of variation and analysis of variance (ANOVA) with significance $P < 0.001$ for agronomic traits were calculated using OriginPro (2021) statistical analysis software (Mukul and Akter, 2021). Genetic diversity of agro-morphological data was determined by using multivariate analysis

(Zafar *et al.*, 2008). The construction of dendrogram on the basis of agro-morphological data for both cropping years was done by using Ward's clustering method.

Results

Statistical summary of 123 soybean genotypes was to determine the behaviour of six significant agro-morphological traits during the years of 2020 and 2021 (online Supplementary Table S2). However, genotype A62 showed highest PH, while the genotype A88 showed minimum PH. Minimum YPP was observed in A30, while in the first year the genotype A99 had higher yield. Furthermore, the genotype A54 had maximum HSW (17.63 g) among all 123 genotypes (online Supplementary Table S2).

The range of statistical values, standard deviation (n), maximum value, mean, minimum value, variance and coefficient of variation (CV%) of six agro-morphological traits provided a precise view of the population data. The range of the morphological data for the year of 2020 was: PH (10.9–72), pods (9–119), seeds (17–249), HSW (7.5–17.6), YPP (1.6–38.7) and DM (96–133). In the second year, genotype A110 had maximum HSW, while the genotype A99 had maximum YPP (online Supplementary Table S2). In both years, ANOVA of six yield-related traits showed significance in all genotypes (Table 1).

Correlation analysis

The relationship among six agro-morphological traits was determined by using Pearson correlation coefficient method. Correlation matrix of subjected traits for two cropping seasons was given in Table 2. PH showed positive correlation with pods and seeds, but non-significant correlation with HSW and YPP. Furthermore, PH showed negative correlation with DM in both years (Table 2). Seeds showed positive correlation with YPP in both years and showed non-significant but positive correlation with HSW and DM in both years. The pods showed significant and positive correlation with number of seeds and YPP in both years. Furthermore, pods showed non-significant correlation with HSW and DM. HSW revealed positive correlation with YPP and DM in both years. The YPP showed negative correlation with DM in both years. Moreover, DM showed positive but non-significant correlation with pods and seeds as well as it showed negative non-significant correlation with PH and YPP in both cropping years (Table 2).

Multivariate analysis based on cumulative variability of soybean agronomical traits

In this study, based on eigenvalue and variability, morphological data of the two consecutive years are divided into six principal factors (Fs). In both cropping years 2020 and 2021, the first three principal factors (Fs) with eigenvalue >1 highly contributed to variability as mentioned in Table 3. For further analysis and selection of principal factors (Fs), eigenvalue 1 was mainly used as a cut-off value. In both years 2020 and 2021, first three principal factors (Fs) highly contributed to variability of 84 and 88%, respectively. The first two factors F1 (48; 20%) and F2 (47; 26%) highly contributed to variability for the years 2020 and 2021, respectively, indicating their significance in the construction of biplot (Table 3). In the year of 2021, factor F2 contributed higher to variability of 44.6% followed by F1 (34%) and F3

Table 1. ANOVA of six agro-morphological traits of 123 soybean genotypes

Source	DF	PH	Pods	Seeds	HSW	YPP	DM
2020							
Rep	2	1190.47	3100.4	15937	2.9116	270.2	798.41
SD	1	9474.69	62012.5	252988	2.5171	11208.3	1042.18
Error Rep × SD	2	330.34	306	910	2.5171	7.4	798.41
Acc	122	423.6	1369.7	5213	26.5994	117.8	333.03
SD × Acc	122	150.18	584.4	2219	1.1409	68.1	9.51
Error Rep × SD × Acc	488	31.92	233.6	834	3.1613	16.9	1.2
2021							
Rep	2	446.3	532	2492.6	13.99	53.63	798.41
SD	1	26812.2	12563.7	1886.7	4591.03	3941.92	1870.77
Error Rep × SD	2	1100.9	3063	15300.7	0.17	230.79	798.41
Acc	122	438.9	1413.9	5583.3	27.06	115.11	333.03
SD × Acc	122	151	560.7	2127.3	7.21	67.35	14.87

Table 2. Correlation matrix of six agro-morphological traits of soybean genotypes

	PH	Pods	Seeds	HSW	YPP	DM
PH	1	0.177**	0.157*	0.085 ^{NS}	0.049 ^{NS}	-0.037 ^{NS}
		0.232**	0.324**	0.443 ^{NS}	0.060 ^{NS}	-0.116 ^{NS}
Pods		1	0.958**	0.036 ^{NS}	0.908**	0.122 ^{NS}
			0.926**	-0.129*	0.879**	0.140 ^{NS}
Seeds			1	0.062 ^{NS}	0.927**	0.016 ^{NS}
				0.038 ^{NS}	0.851**	0.113 ^{NS}
HSW				1	0.188**	0.170**
					0.124**	0.211**
YPP					1	-0.127*
						-0.103*
DM						1

**Significant at 0.01; *significant at 0.05. Top value of each box from the year 2020 and bottom value from the year 2021.

Table 3. Cumulative variability percentage, eigenvalue and variability contribution by morphological traits of 123 soybean genotypes

	Year 2020				Year 2021			
	F1	F2	F3	F1 + F2	F1	F2	F3	F1 + F2
PH	1.381	0.046	83.160	1.427	3.048	37.934	9.305	40.982
Pods	32.496	1.468	0.094	33.964	33.091	0.219	0.411	33.310
Seeds	32.924	1.152	0.189	34.076	32.767	0.571	0.007	33.338
HSW	0.908	47.264	8.220	48.172	0.120	44.658	4.785	44.778
YPP	31.985	0.256	1.905	32.241	29.946	1.242	3.078	31.188
DM	0.306	49.813	6.433	50.119	1.028	15.376	82.413	16.404
Eigenvalue	2.910	1.170	1.016		2.860	1.565	0.95	
Variability (%)	48.500	19.499	16.939		47.667	26.091	14.657	
Cumulative %	48.500	67.999	84.938	67.9	47.667	73.757	88.414	73.6

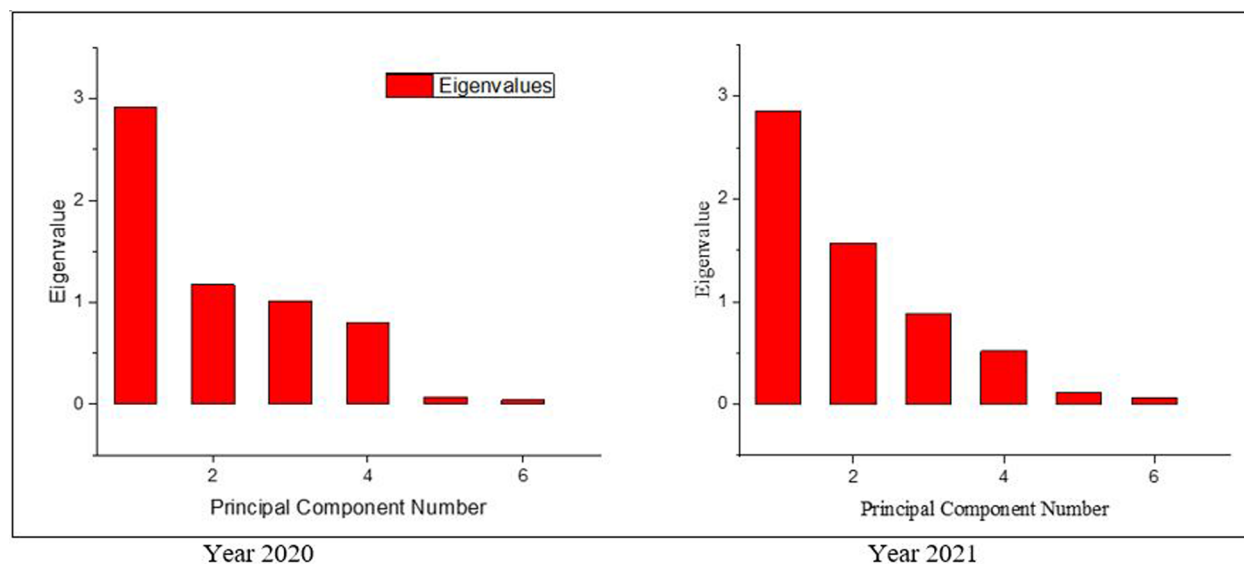


Figure 1. Scree plots representing six principal factors of agronomical traits based on variability and eigenvalues.

(10%). These three principal factors showed maximum contribution in the construction of biplot.

In the principal component analysis (PCA), scree plot is a line plot based on cumulative variability and eigenvalue showed the six principal factors and their contribution (Fig. 1). The first three principal factors in the scree plot (F1, F2, F3) distributed the genotypes based on agronomical traits. In this plot, other three factors with eigenvalue <1 played minimum role in the total variability that accounts to 15% for the year of 2020 while the last three principal factors contributed to 12% variability for the year of 2021 which is insignificant for further analysis (Fig. 1).

Pods (32%) and seeds (33%) followed by YPP (31.9%) had majorly contributed to variability of the F1 principal factor for the first year 2020 (Table 3). Although DM and PH contributed to minimum variability (0.3; 1%), in principal factor F2 DM and HSW contributed to highest variability of 49 and 47%, respectively. On the other hand, in 2021, the variables that showed maximum contribution in variability for F1 were pods (33%), seeds (32%) and YPP (30%). HSW showed minimum contribution (0.12%) in total variability (Table 3). HSW, PH and DM contributed higher to total variability of F2 factor with 44, 37.9 and 15.3%, respectively, for the year of 2021. Minimum contribution in the total variability of 0.2–1.2% by pods, seeds and YPP is observed.

Variability analysis based on correlation, principal factors and variability

The year of 2020 total variability showed by first two principal components (PCs) F1 (48%) and F2 (20%) (Table 3) was used for the construction of biplot. Variables were imposed as vectors whose length in both principal factors showed the combined variability and their major effects on the yield (Yan and Tinker, 2005). However, positive and negative factors describe the behaviour of correlation among variables. In biplot factor loading values divided the plot into four groups. The variable PH closest to the origin in biplot has less variability as compared to YPP, pods, seeds, DM and HSW for the year 2020 as shown in Table 3.

On the other hand, in the year of 2021, the factors F1 and F2 were used to construct the variable biplot with variability 47.7 and 26.1%,

respectively (Table 3). The vector including number of seeds, number of pods, PH and HSW had more contribution in variability based on their combined variability and length. On the other hand, both principal factors DM and YPP had less contribution in variability. The correlation and variability of agronomical traits revealed by multivariate analysis can be utilized for further selection of genotypes.

Variable plot

The graphical representation of diversity among two PCs (F1 and F2) disperses the 123 soybean accessions in two-dimensional scale. Biplot revealed the association of vectors of traits as well as the genotypes with one another. In the biplot graph for the year of 2020 using maximum variability from first two principal factors, the accessions were distributed into four diverse groups on the bases of x - y plane. The accessions that were present in the second group (positive axis) performed well in the year of 2020 and are more likely to be linked with HSW, DM and YPP. The genotypes that were away from the origin (negative axes) were negatively associated with that trait. The common genotypes in the second group of the year 2020 on both plantation dates were A110, A95, A109, A49, A51, A41, A67, A34 and A62 (Fig. 2). On the other hand, in the year of 2021, the common genotypes on both plantation dates in the first group were A112, A116, A89, A91 and A60 that were linked with HSW and DM. In group 2 of both years (2020 and 2021), the common genotypes were A99, A49, A95, A107, A44, A108, A97, A62, A10, A67, A104, A114, A37, A51, A54, A41, A4 and A62 (Fig. 3).

Mean comparison revealed maximum PH attained in early plantation for the years 2020 and 2021 (online Supplementary Fig. S1). The common genotypes in both years were A1, A34, A62, A97 and A123. On the other hand, maximum number of pods were attained in early plantation of first year and mid-March plantation of second year. The common genotypes in both years were A51, A52, A109, A48, A49, A60, A62, A63, A64, A65, A67, A70, A76, A79, A80, A85, A90, A99, A114, A119, A120, A121 and A123 (online Supplementary Fig. S1).

Maximum number of seeds were acquired in early plantation in 2020 and 2021 and common genotypes in both years were

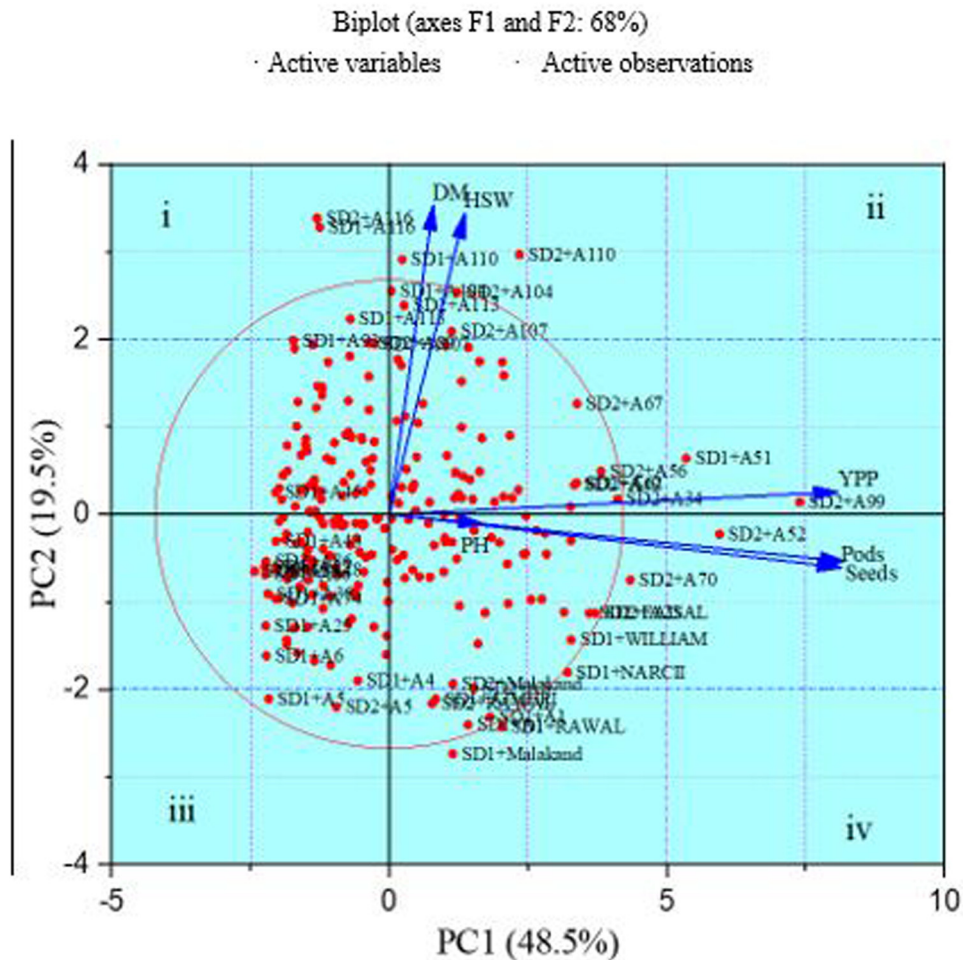


Figure 2. Biplot indicating the interrelationship of six yield-related traits with 123 soybean genotypes for the year 2020.

A4, A14, A16, A23, A27, A34, A37, A41, A46, A49, A51, A81, A85, A95, A103, A109, A114, A117, A118, A119, A120, A121, A122 and A123 (online Supplementary Fig. S2). The maximum HSW was obtained in early plantation of both years. The common genotypes for the years 2020 and 2021 were A2, A3, A13, A14, A19, A20, A21, A22, A24, A31, A34, A36, A44, A49, A51, A54, A55, A56, A57, A59, A62, A66, A67, A72, A75, A76, A77, A79, A81, A83, A89, A93, A95, A101, A102, A104, A106, A107, A108, A110, A113, A116 and A118 (online Supplementary Fig. S2).

Maximum YPP was obtained in the early plantation of first year and mid-March plantation of second year. The common genotypes for the years 2020 and 2021 were A1, A23, A34, A41, A51, A52, A62, A95, A110, A118, A120, A121 and A123 (online Supplementary Fig. S3).

Combined agro-morphological clustering of advance soybean germplasm

Ward's method was used for construction of dendrogram. All soybean accessions were divided into two major groups (C1 and C2) for the year of 2020 including 45 genotypes in C1 and 201 in C2 (online Supplementary Fig. S5). On the other hand, for the year of 2021, 83 and 163 genotypes were present in C1 and C2, respectively (online Supplementary Fig. S6).

Discussion

The present research was carried out for the genetic characterization of advanced soybean genotypes in local conditions by using PCA. However, mean value of all phenotypic traits specified the enormous variation revealed by the genotypes for PH, number of pods, number of seeds, HSW, YPP and DM. This huge variability provides scientific assistance to improve these traits with respect to diverse biotic and abiotic stresses. In this present era, statistics has become a standard for the dissection of agro-morphological traits (Yaqoob, 2016; Iqbal *et al.*, 2017; Shahid *et al.*, 2017; Din *et al.*, 2018; Pooja *et al.*, 2018; Gulnaz *et al.*, 2019).

The PCA or multivariate analysis has become an exceptional data reduction technique to identify smaller number of traits which provide maximum variability and based on PC score to prioritize the genotypes (Dubey *et al.*, 2018b).

The data showed that the first two principal factors of both years possessing eigenvalue >1 contributed maximum in biplot construction variability for both years 2020 and 2021 (Zafar *et al.*, 2008). In the first year, principal factor F1 showed 48% and F2 showed 47% variability; on the other hand, in the second year, F1 showed 20% and F2 showed 26% variability (Wang *et al.*, 2013; Hashash, 2016). PH has positive correlation with HSW and YPP. Number of pods showed positive correlation with number of

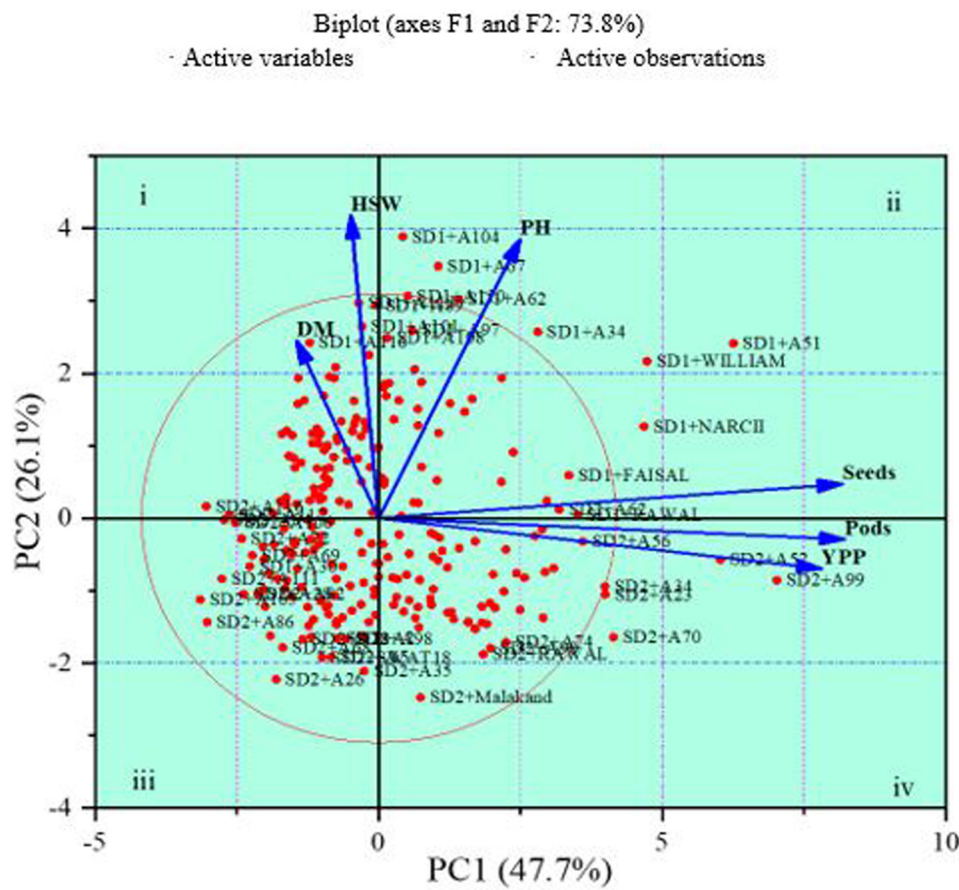


Figure 3. Biplot indicating the interrelationship of six yield-related traits with 123 soybean genotypes for the year 2021.

seeds and YPP. Number of seeds showed positive correlation with YPP. Mean comparison of both years revealed that significant yield enhancement traits of soybean advance lines showed better response in early-March plantation of both years. In combined cluster analysis of both years, the genotypes were distributed in two groups C1 and C2. Maximum number of genotypes were present in subclusters of C2 with same geographical background.

Temperature is one of the most important parameters that can adversely affect the yield and quality of soybean. Dornbos and Mullen (1991) reported that the optimum day/night temperature (26/20°C) helps to increase the soybean yield. However, a significant yield decline was observed at seed filling stage, when the temperature increases (29/20°C). In our study, the population was exposed to high temperature of >35°C at seed filling stage on both plantation dates. However, early-March plantation provides fine seed quality with better yield as compared to mid-March plantation that gives shrivelled seed due to high temperature. In the spring season under local Pakistani conditions, temperature generally remains above the optimum temperature required for soybean growth during grain filling stage as indicated (online Supplementary Fig. S4) in both growing seasons 2020 and 2021. In view of our experimental results, it is suggested that the second half of the February would be the best time for spring plantation under local conditions. Plantation after mid-February not only improves the seed germination and quality but also improves the yield due to early plantation which would save the crop from harmful effects of temperature that leads to seed shrivelling and quality deterioration.

Broschat (1979) evaluated diverse soybean panel and reported that for data reduction it is one of the most powerful techniques which wipeout interrelationships among components. Different studies reported that PCA is one of the valid systems to deal with diverse germplasm. Smith et al. (1995) characterized the soybean germplasm, conducted linkage cluster and PCA and published the value of these ramifications in the utilization and preservation of germplasm. Ghafoor et al. (2001) evaluated the morphological genetic diversity in black gram accessions using multivariate analysis and concluded that the first four PCs explained 79% of genetic variability in the data. Another study conducted by Ghafoor et al. (2003) reported that the first three PCs explained 83.3% of genetic variability in the data.

Construction of different clusters based on morphological traits in diversity panel of soybean was also published (Cui et al., 2001; Iqbal et al., 2008; Ojo et al., 2012), as well as those genotypes have similar morphological traits present in the same cluster. Similar ramifications were also published by Yu et al. (2005) and Iqbal et al. (2008) and in oil palm by Abdullah et al. (2011). Jha et al. (2016) evaluated 50 soybean advanced genotypes by using PCA to specify the ranking of genotypes on the basis of the combination of different phenotypic traits. Five principal factors with <1 eigenvalue were considered to be more significant. The traits, i.e. HSE, number of seeds per plant, number of pods per plant, PH, DM, biological YPP, are very imperative yield-contributing traits. These results indicate that genetic selection and wide hybridization among selected genotypes from the distantly related clusters could be a promising

strategy for improving soybean yield in domestic Pakistani conditions.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S1479262123001120>

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