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Estimation of Genetic Divergence and Proximate Composition in Advanced Breeding Lines of Soybean (*Glycine max* (L.) Merrill)

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ABSTRACT

The present study was carried out on fifty seven advanced breeding lines of soybean along with 3 checks viz. JS 20-98, JS 20-116 and JS 20-34 during *kharif* 2020. The objective of this study was to estimate the genetic divergence among genotypes with the help of Mahalanobis's D^2 statistics. In the present investigation eleven yield contributing traits were recorded with 3 replications in a Randomized Complete Block Design. These 57 lines alongwith three checks were grouped into 8 clusters. Cluster I was polygenotypic (53) and rest all 7 clusters were monogenotypic (1). Principal component analysis revealed six putative genotypes which were JS 20-116 (PC1, PC2), JS 22-33 (PC1, PC2, PC3) JS 22-13 (PC1, PC3), JS 22-42 (PC1, PC3, PC4), JS 22-56 (PC2, PC3, PC4), JS 22-36 (PC2, PC3). Proximate analysis reported significant differences among the genotypes with respect to moisture content, ash, proteins, fat, fiber and carbohydrate. The moisture content ranged from 4-7%. The oil content ranged from 15% (JS 23-03, JS 20-98) -23% (JS 22-46). Genotypes exhibited significant variations in protein content ranging from 42.8% (JS 22-42) to 33.6% (JS 22-25). The ash content ranged from 6% (JS 22-22 and JS 22-38) to 3% (JS 23-02, JS 22-10, JS 22-16 and JS 22-44) whereas the carbohydrate content was found in the range of 35.2% (JS 22-54) to 12.1% (JS 22-46).

Keywords Genetic divergence, Polygenotypic, Principal component analysis, Proximate, Protein.

INTRODUCTION

Soybean (*Glycine max* L. Merrill) is a leading oilseed crop in India as well as of the world, having an enriched nutrition profile that comprises 40% protein, 20% edible oil along with a wide range of minerals and vitamins. Soybean is the richest and easiest source of good quality plant protein and fats and has

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multiple roles in food and other industrial products it has been named a "wonder crop" (Gopinath and *Pavadai* 2015). In addition to this, the soybean crop also contributes to foreign exchange by the export of soy meal that is produced after the extraction of oil. (Rani *et al.* 2018). Due to this versatility of soybean, the demand for this crop in the industrial, agricultural, and medicinal sectors is increasing at a rapid rate. Being a self-pollinated crop, it has a narrow genetic base, it is highly susceptible to biotic and abiotic stresses as well as improper exploitation of heterotic potential are a few reasons for the lower yield of soybean, (Swar *et al.* 2021). Therefore, there is a need to develop new varieties to meet the requirements of human as well as livestock populations and also be suitable for climate-smart agriculture. The development of new varieties is solely dependent on the magnitude and availability of genetic variability in the parent material. Genetic variability along with genetic divergence are the main interests of any plant breeder to develop desirable varieties. The varieties that are developed from diverse parents are likely to show high heterotic effects as well as a large range of segregation, (Baraskar *et al.* 2014). The information about the genetic diversity present in a population helps in identifying superior donors from the germplasm for hybridization, (Mishra *et al.* 2018). The patterns of variation existing in germplasm are estimated with the help of multivariate analysis such as Principal Component Analysis (PCA) and cluster analysis to ease the process of data collection from such large variable data. These cluster patterns formed can be related to geographical origin with the help of the Mahalanobis D_2 statistic tool to assess the genetic divergence between the genotypes.

Soybean is considered to be the cheapest source of protein as compared to foods like meat, fish, and eggs. According to Sathe *et al*. (2009), Soybean seeds have been described as the "protein hype" of the future. Despite being a rich protein source soybean also contains unsaturated, cholesterol-free fatty acids, minerals, carbohydrates, fiber, and vitamins A, B, C, and D which are essential parts of the human diet, (Nwosu *et al.* 2019). The proximate composition of foods includes 6 components viz. moisture, ash, lipid, fiber, protein, and carbohydrate contents (Anonymous 2016). These components are necessary for the food industry for product development, quality control, and other purposes. While selecting desirable cultivars, evaluating these collections for their nutrition profile also becomes a necessary stage in any breeding program. Therefore, this present study is an attempt to unravel the proximate composition of soybean seeds to provide depth knowledge of nutrients in each studied genotype. This information will also help plant breeders to select varieties with an enriched nutrition profile to fulfill their nutritional needs. Considering the importance of genetic divergence and proximate composition, this experiment was designed with the sole purpose to estimate genetic diversity between genotypes, selecting superior genotypes, and also conclude proximate composition in seeds of these advanced genotypes.

MATERIALS AND METHODS

Experimental material and design

The experiment was carried out on fifty-seven advanced genotypes along with three best check varieties (JS 20-98, JS 20-116, and JS 20-34) of soybean at the research field of AICRP Soybean, Seed Breeding Farm, Department of Plant Breeding and Genetics, J.N.K.V.V., Jabalpur during *kharif* 2020. All these 57 genotypes evolved from the crosses of recently developed/identified superior genotypes at JNKVV, Jabalpur. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The entries were sown in three rows each of 3 m in length with a plant-to-plant spacing of 7 cm and row-to-row spacing of 30cm. The experimental site had uniform topography, medium black soil with 7.5 pH, and was free from water-logged conditions. All the recommended packages and practices were followed for caring for and nourishing plants throughout the growth period.

Climate and weather

The total rainfall received during the cropping season of *kharif* 2020 was1213.1mm. year. The average temperature was maximum in the second fortnight of July which created an adverse condition for the initial growth of the crop. The rainfall was also recorded low during this week. The maximum rainfall was received

in August (641.4 mm) with 25 rainy days.

Observations and statistical analysis

The observations for yield-associated traits were recorded from five randomly selected competitive plants from each replication for each genotype. The observations were taken from 11 yield attributing traits viz. number of primary branches per plant, number of pods per plant, number of seeds per plant, 100 seed weight (g), biological yield per plant (g), harvest index (%) seed yield per plant (g) and 3 phenological traits like days to flowering, days to 50% flowering and days to maturity. The data collected on different characters were analyzed through generalized distance given by Mahalanobis (1936). The populations were grouped into various clusters by using Tocher's method as described by Rao (1952). After the formation of the clusters, the average inter and intra-cluster distances were calculated. The average intra and inter-cluster D^2 values were estimated as per the procedure given by Singh and Choudhary (1979). The square root of the D^2 value obtained from the above represents the (D^2) between and within clusters. Principal Component Analysis based on Pearson's correlation matrix cluster analysis and a heat map was performed using a demo version of XLSTAT–Pro.

Proximate composition analysis

Proximate analysis is a chemical analysis method to identify the food content of any ingredient (Eden and Rumambarsari 2020). The proximate analysis is composed of six components viz. moisture, ash, protein, fiber, fat, and carbohydrate these components correlate with the nutritional value of food. Soybean is a widely grown crop in the USA, Argentina, Brazil, and the rest of the world it is used as a raw material for processed food such as tofu, soya chunks, soya curd, soymilk, and bakery items. Therefore, it becomes necessary to analyze the proximate content of soybean genotypes and classify them based on their nutritive value.

The analysis was carried out to estimate fat, protein, fiber, ash, and carbohydrate contents in soybean seeds.

***Fat content* **

The fat content in each sample was estimated by PELICAN EQUIPMENT SOCS PLUS based on the principle of Soxhlet's extraction method as described in AOAC (2002). 1g of sample was weighed accurately into a thimble and plugged with fat-free cotton. The extraction flask was named (A). Fat content was determined by extracting the sample with solvent, petroleum ether AR grade 60-80°fraction, for 6 hrs by Soxhlet's extraction procedure. After extraction, the excess solvent was distilled off and the residual solvent was removed by heating at 80ºC in the oven for 4-6 hr. The flask was weighted (B) and the fat content was determined as below :

$$\text{Crude fat (\%)} = \frac{\text{Weight of flask (B)} - \text{Weight of flask (A)}}{\text{Weight of sample}} \times 100$$

Fiber content

After the extraction of fat with petroleum either, 2 g of dried sample was boiled with 200 ml of H_2SO_4 solution for 30 min, filtered through muslin cloth, and washed with boiling water until washings were no longer acidic. After the acid, the same residue was boiled with 200 ml of NaOH solution for 30 min. The filtering was the same as for acid. The residue was then transferred to an ashing dish (preweighed dish W_1) where the residue was dried for 2 hr at 130±2°C, cooled, and ignited for 30 min at 600±15°C. Again, cooled in a desiccator and reweigh (W_2). The fiber content was determined using this formula :

$$\text{Crude fiber \%} = \frac{W_2 — W_1}{\text{Weight of sample}} \times 100$$

Ash content

The ash content in the seed sample was estimated as AOAC (2002). The weighed sample was kept in a Proclaim crucible (which was precisely been heated to about 600°C and cooled). The crucible was first heated over a low flame till all the material became completely charged, after that it was heated in a muffle furnace for about 6 hrs. It was then cooled in a des-

iccator and weighed to ensure the completion of the ashing. The crucible was again heated in the muffle furnace for about 1-2 hrs accompanied by cooling and again weighed. This was repeated till two concurrent readings of weights were the same and then the ash was almost white or grayish-white in color.

$$\text{Ash \%} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Protein content

The protein content in the sample was determined by using conventional Micro-Kjeldahl digestion and distillation procedure as given in AOAC (1984).

The sample (0.2 g) was weighed accurately and transferred to a Kjeldahl flask the catalyst mixture (100g K_2SO_4, 20 g of $CuSO_4$ and 2.5 g of SiO_2) of about 1g and concentrated sulphuric acid (10 ml) were added. Then the flask was heated in an inclined position in the digestion chamber for about 4-6 hrs till the liquid became clear (green-blue color). The content in the flask was allowed to cool and the digested material was transferred quantitatively to a vacuum-jacketed flask of micro Kjeldahl distillation apparatus and the ammonia liberated by the addition of 25 ml of 40% NaOH on heating was absorbed in 25 ml of boric acid containing 2-3 drops of mixed indicator in 100 ml conical flask. The distilled ammonia was titrated against 0.1 N sulphuric acid. The blank was also run similarly.

$$\text{N (\%)} = \frac{\text{Normality of } H_2SO_4 \times \text{Volume of 0.1 N } H_2SO_4 \times 14}{\text{Weight of sample} \times 1000} \times 100$$

$$\text{Crude protein (\%)} = \text{N \%} \times 6.25$$

Carbohydrate content

Total carbohydrates in the samples were estimated by the hydrolysis method as described in AOAC (2002). Weigh 100 mg of the sample into a boiling tube. Hydrolyze by keeping it in a boiling water bath for 3hr with 5 ml of 2.5 N HCl and cool to room temperature. Neutralize it with solid sodium carbonate until the effervescence ceases. Make up the volume up to 100 ml and centrifuge. Pipette out 0.2,0.6, 0.8, and 1ml of working standard into a series of test tubes. Make up the volume in each test tube to 1ml of water. Add 1 ml of phenol solution to each tube. Add 5 ml of 96% of sulphuric acid to each tube and shake. After shaking place, the tubes in the water bath for 20 min. Read the color at 490 nm.

$$\text{Total carbohydrate} = \frac{\text{Sugar value from graph (µg)}}{\text{Aliquot sample used (0.1 or 0.2)}} \times \frac{\text{Total volume of extract (100 ml)}}{\text{Weight of sample (100 mg)}} \times 100$$

RESULTS AND DISCUSSION

Contribution of characters towards genetic divergence

The trait number of primary branches per plant (19.77%) contributed most towards genetic divergence followed by biological yield per plant (18.53%), 100 seed weight (16.16%), no. of pods per plant (12.26%), days to flower initiation (9.32%), days to maturity (9.27%), no. of seeds per plant (7.97%), days to 50% flowering (3.11%) and plant height (2.32%) whereas, the magnitude of genetic divergence was less than one percent for harvest index (0.73%), seed yield per plant (0.56%). The above results indicate that the maximum divergence was contributed by major yield-attributing traits. Similar results were reported by Dubey *et al.* (2018), Adsul and Monpara (2014) for pods per plant, Mishra *et al.* (2018) for no. of pods per plant and also Jadeja (2015) for days to 50% flowering.

Grouping of genotypes into clusters

Based on D^2 values, all the 60 genotypes were classified into 8 clusters using Tocher's method (Fig. 1). The cluster I was polygenotypic that consisted 53 genotypes the reason behind this can be common ancestry and similar geographical conditions. The rest of the genotypes were grouped in seven clusters each as described in Table 1. Similar results were obtained by Thakur *et al.* (2015) where 40 genotypes were grouped into six clusters, Kumar *et al.* (2018) grouped 31 genotypes into 10 clusters. Mishra *et al.* (2018) grouped 60 genotypes into 16 clusters,

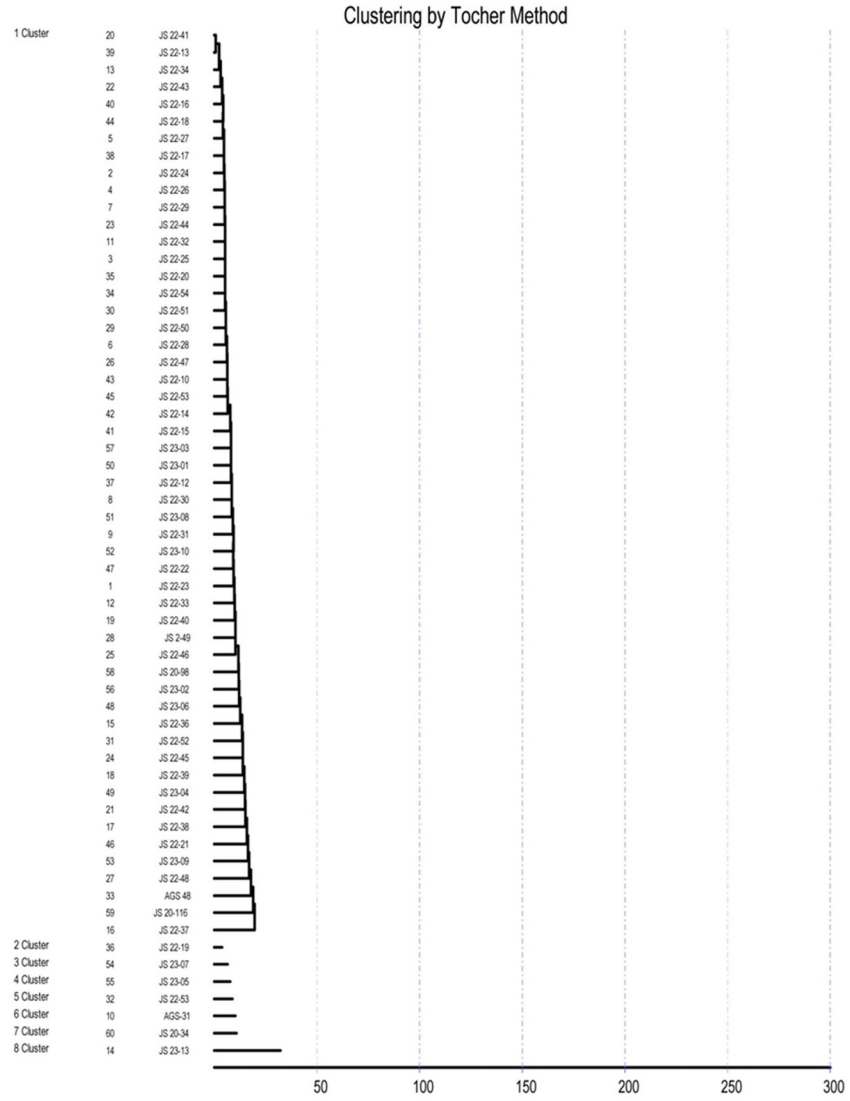

Fig 1. Clustering of Genotypes using Tocher's method.

Soniasabanam *et al.* (2018) found 5 clusters for 20 genotypes, Bijarania. 2020 found 9 clusters for 30 genotypes.

Intra and Inter cluster distances

The average intra and inter-cluster D^2 values are presented in Table 2. Cluster I showed maximum intra-cluster D^2 value (31.70), while other clusters were mono-genotypic with no intra-cluster divergence. The highest inter-cluster divergence was observed between genotypes of cluster II and VIII (322.82), followed by cluster III and VIII (321.84), cluster IV and VIII (276.84), VII and VIII (255.59), VI and VIII (177.42) and I and VIII (175.08) whereas least inter-cluster divergence was observed between genotypes of cluster III and IV (20.48). This suggests that the crosses made between the genotypes of the clusters having large inter-cluster distance result in high heterotic effects. The genotypes of clusters III

Table 1. Grouping of genotypes into clusters.

Clusters	No. of genotypes	Genotypes
1	53	JS 22-41, JS 22-13, JS 22-34, JS 22-43, JS 22-16, JS 22-18, JS 22-27, JS 22-17, JS 22-24, JS 22-26, JS 22-29, JS 22-44, JS 22-32, JS 22-25, JS 22-20, JS 22-54, JS 22-51, JS 22-50, JS 22-28, JS 22-47, JS 22-10, JS 22-55, JS 22-14, JS 22-15, JS 23-03, JS 23-01, JS 22-12, JS 22-30, JS 23-08, JS 22-31, JS 23-10, JS 22-22, JS 22-23, JS 22-33, JS 22-40, JS 22-49, JS 22-46, JS 20-98, JS 23-02, JS 23-06, JS 22-36, JS 22-52, JS 22-45, JS 22-39, JS 23-04, JS 22-42, JS 22-38, JS 22-21, JS 23-09, JS 22-48, JS 22-57, JS 20-116, JS 22-37
II	1	JS 22-19
III	1	JS 23-07
IV	1	JS 23-05
V	1	JS 22-53
VI	1	JS 22-56
VII	1	JS 20-34
VIII	1	JS 23-13

and IV have a close relationship as indicated by their inter-cluster distance. Similar results were reported by Kumari *et al*. (2019), Mishra *et al.* (2018), Bijarania (2020). None of the characters had recorded the highest and the lowest cluster mean value in cluster I. However, the genotypes of cluster II comprised traits like a smaller number of seeds per plant (39.10) and less plant height (56.01) whereas the genotypes of cluster III exhibited traits of highest plant height (81.53) and high harvest index (60.01) whereas number of pods per plant (14.13), Number of primary branches per plant (2.87) and biological yield per plant (9.57) were lowest in cluster III. The highest cluster mean values were recorded for days to maturity (105.00) in cluster IV. Cluster V recorded the highest cluster mean value of 100 seed weight (18.21) whereas none of the characters showed the lowest mean values. In cluster VI the highest cluster mean value was recorded for days to flower initiation (46.33) and days to 50% flowering (51.67) whereas the lowest cluster mean value was noted for 100 seed weight (5.30), harvest index (27.61) and seed yield per plant (3.70). Cluster VII portrayed no character which recorded the highest cluster mean values however characters like days to flower initiation (35.33) and days to 50% flowering (41.0) recorded the lowest cluster mean values. Cluster VIII recorded the highest cluster mean value for number of pods per plant (78.10), no. of seeds per plant (139.50), no. of primary branches per plant (7.93), biological yield per plant (36.10), and seed yield per plant (20.20) however none of the characters recorded the lowest cluster mean values.

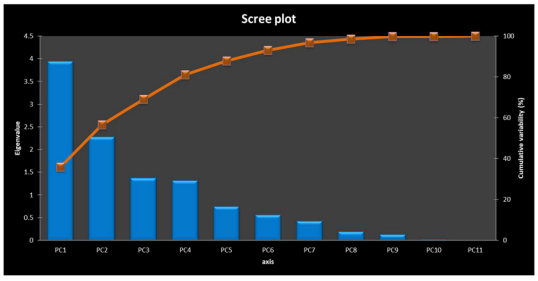

Fig 2. Scree plot depicting Eigen value and cumulative variability for 11 principal components.

Principal component analysis

In the present investigation, eigenvalues, % variance, and cumulative % are mentioned in Table 3. Out of eleven characters, only 4 principal components (PCs) showed more than a 1.00 eigenvalue and exhibited about 81.03% variability among the traits studied, further explanation. PC1 exhibited the highest variability (35.874%) followed by PC2 (20.708%), PC3 (12.514%), and PC4 (11.936 %) for traits under study. The scree plot laid out between eigen value and Principal Components reflected total variation in PC1 (35.87%) and PC2 (20.70%), followed by PC3 (12.51%) and PC4 (11.93%.). 81.03%. The semi-curve line after the fourth PC exhibiting very little variation in each PC indicated that maximum

Table 2. Intra and Inter cluster distances.

Cluster no.	I	II	III	IV	V	VI	VII	VIII
I	31.70	55.82	57.99	54.82	55.90	66.75	64.54	175.08
II		0.00	26.59	23.45	101.93	135.11	69.44	322.82
III			0.00	20.48	112.47	109.49	57.39	321.84
IV				0.00	83.44	113.94	57.80	276.84
V					0.00	104.84	145.37	100.10
VI						0.00	110.41	177.42
VII							0.00	255.59
VIII								0.00

variation was found in PC1; therefore, the selection of genotypes for characters under PC1 will prove to be desirable as seen in Fig. 2.

The above results revealed that 6 genotypes JS 20-116 (PC1, PC2), JS 22-33 (PC1, PC2, PC3), JS 22-13 (PC1, PC3), JS 22-42 (PC1, PC3, PC4), JS 22-56 (PC2, PC3, PC4), JS 22-36 (PC2, PC3) were found promising as they fall in more than one PC.

Similar results were found by Dubey *et al.* (2018) for no. of pods per plant, no. of seeds per plant, number of branches per plant, biological yield per plant, Jha *et al.* (2016) for no. of pods per plant, biological yield per plant also by Mahbub *et al.* (2016) and El-Hashash (2016).

Table 3. Eigen values, percentage variation and cumulative percentage of eleven traits of soybean.

Characters	Principal components (PC)	Eigenvalue	Variability (%)	Cumulative %
Days to flower initiation	PC1	3.946	35.874	35.874
Days to 50(%) flowering	PC2	2.278	20.708	56.583
Days to maturity	PC3	1.377	12.514	69.096
Plant height	PC4	1.313	11.936	81.032
No. of primary branches/plant	PC5	0.748	6.804	87.836
No. of pods/ plant	PC6	0.563	5.121	92.957
No. of seeds/ plant	PC7	0.428	3.891	96.847
Biological yield/plant	PC8	0.193	1.752	98.600
100 seed weight	PC9	0.127	1.156	99.755
Harvest index	PC10	0.017	0.154	99.910
Seed yield/ plant	PC11	0.010	0.090	100.000

Each genotype was assigned a PC score in each PC and based on that JS 23-13 possessed the highest PC score in PC1 followed by JS 22-53, JS 22-48, JS 22-38, JS 22-15, JS 20-116, JS 22-33, JS 22-32, JS 22-17, JS 22-13, JS 22-41, JS 22-49, JS 22-42 and JS 22-31 this shows that these genotypes possess high values for traits like no. of pods per plant, number of seeds per plant, primary branches per plant, biological yield per plant, seed yield per plant.

JS 22-56 possessed the highest score in PC2 followed by JS 22-57, JS 22-40, JS 22-46, JS 22-23, JS 20-116, JS 20-98, JS 22-36, JS 22-33, JS 22-20, JS 22-44, JS 22-39 and JS 23-09 this suggests that these genotypes possessed high values of traits like days to flower initiation and days to 50% flowering.

JS 22-43 had the highest PC score in PC3 followed by JS 22-52, JS 22-37, JS 22-19, JS 22-33, JS 22-36, JS 22-41, JS 22-12, JS 22-16, JS 22-56, JS 22-13, JS 22-42 these genotypes possess high values for trait days to 50% flowering.

JS 23-07 revealed the highest scores in JS 23-07 followed by JS 23-05, JS 22-42, JS 22-56, JS 23-04, JS 22-22, JS 22-52, JS 23-10, JS 22-32, JS 22-47, JS 22-45 and JS 23-08 these genotypes show high values for traits like days to maturity and harvest index.

Similar findings were reported by Jha *et al. (*2016) for no. of pods per plant, no. of seeds per plant, biological yield per plant., Berhanu *et al.* (2019) for

primary branches per plant, and for seeds per plant. Baria (2019) for seed yield per plant and biological yield per plant, and no. of pods per plant.

Proximate composition

The soybean seeds exhibited moisture in the range of 4-7%. This can be attributed because of the conditions of drying of the grains after harvest, the storage period, and the ability of the grains to lose moisture. The average oil content found in these advanced breeding lines of soybean was 18.9% out of which the maximum was found in JS 22-46 (23%) and the least was found in genotypes JS 22-38, JS 22-57, JS 22-54, JS 22-19 all of them has 15% oil content. It is reported that yield is negatively correlated with oil content Malik *et al*. (2007). Hence increase in oil content will lead to a decline in yield. It is similar in the case of protein content as well. In the present study, all 60 genotypes exhibited the average protein content in the range of 33-42% in which the highest protein content was exhibited in genotypes JS 22-37 (42.8%) and the lowest protein content was around 33.6% which was present in JS 22-25. The genotypes that are poor in protein content can be used in oil production and channeling them toward animal feedstock. The average ash content evaluated in the 60 genotypes came out to be 4.5% where the highest was observed in the genotypes JS 22-22, and JS 22-38 both having 6% ash content whereas the lowest was observed in JS 23-02, JS 22-10, JS 22-16, JS 22-44 all being at amount of crude fiber which was in the range of 6-14% with the highest content present in JS 23-02 (14.4%) and the lowest content present in JS 22-32 (6.8%). The total carbohydrate content showed significant variations among all genotypes studied where the average content was found to be 22.2% with the highest amount attributed to JS 22-54 (35.2%) and the lowest in JS 22-46 (12.1%). Some genotypes had carbohydrates close to the highest which was JS 22-33 (30.1%). This is due to the active respiration process during the germination of seeds. The presence of high fiber content in soya seeds is considered beneficial and has some physiological effects on the gastrointestinal tract. The high carbohydrate genotypes could be used in managing protein—energy malnutrition since there will be a sufficient amount of carbohydrates to derive energy from and spare protein for its primary function which is building the body for wear and tear. Similar results were reported by Kaur *et al.* (2014) for moisture, carbohydrate, and protein content, Ciabotti *et al.* (2019), Abd alla *et al.* (2019) for moisture and ash content, Nwosu *et al.* (2019) for fiber, moisture and oil content.

CONCLUSION

The above results revealed that 6 genotypes JS 20-116 (PC1, PC2), JS 22-33 (PC1, PC2, PC3) JS 22-13 (PC1, PC3), JS 22- 42 (PC1, PC3, PC4) JS 22- 56 (PC2, PC3, PC4), JS 22-36 (PC2, PC3) were found promising as they fall in more than one PC. The most important characters that were identified were number of pods per plant, number of seeds per plant, primary branches per plant, biological yield per plant, seed yield per plant, days to flower initiation, days to 50% flowering, days to maturity and harvest index. In future more consideration can be given to these genotypes and traits for improving genotypes. Biochemical analysis reported significant differences among the genotypes with respect to moisture content, ash, proteins, fat, fiber and carbohydrate. The chemical composition of the seed may vary due to climatic aberrations, soil fertility, disease infestation and erratic factors also.

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