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Genetic Diversity in Vegetable and Grain Type Soybean Genotypes Identified using Morphological Descriptor and EST-SSR Markers

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Abstract

Thirty four soybean genotypes were evaluated for morphological, quality traits and genetic parameters. Correlation and path coefficients were studied for some traits. Analysis of variance and mean performance for various traits revealed significant differences diversity between all the genotypes for traits studied. The genetic diversity among the soybean genotypes was analyzed using ten expressed sequence tag derived simple sequence repeat (EST-SSR) markers. A total of 27 alleles were detected from 334 amplicons with an average of 2.7 alleles per locus. The polymorphism information content (PIC) values of EST-SSR markers ranged from 0.334 to 0.837 with an average of 0.559. These grain and vegetable soybean genotypes could be divided into 7 subgroups based on similarity matrix and arithmetic average (UPGMA) cluster with no correlation between genetic and morphological diversity. The analysis clearly indicated that even with the EST-SSR primers, reliable estimation of genetic diversity among the population could be obtained.

Keywords

Vegetable soybean; Aroma; Oil content; Correlation; Divergence; Multivariate analysis; EST-SSR

Introduction

Soybean (*Glycine max* (L.) Merr.) is one of the legume crop species which is native to China with a history of more than 5,000 years. Since, it is one of the most important pulses cum oil seed crop and known for its useful nutrients including protein (36 to 42%) and oil content (19 to 20%), carbohydrate (35%, 17% of which dietary fiber), minerals (5%) and several other components including vitamins A, B and D and rich in lysine [1-3]. Vegetable soybean is also known by the Japanese term 'edamame', is a soybean harvested at approximately 80% maturity [4]. Soybean is popularly consumed after blanching in China, Korea, Japan, and other countries. It also has potential for cancer prevention and suppression owing to its high genistein content [4-6].

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The productivity of soybean in India is less in comparison with the average world produce. The attributes identified for such a low productivity are 1) limited genetic diversity, 2) narrow genetic base [7] of Indian soybean varieties, 3) short growing period available in Indian latitude and 4) stagnant genetic potential for yield. Further, the simulation studies indicated, increasing temperature and CO_2 levels could pose a serious threat in decreasing the growth of soybean crop and hence the yield [8]. In India, narrowing down of the genetic potential is due to the repeated use of few parents in the breeding. Global awareness of the use and benefits of vegetable soybean has been increased through the efforts of Asian Vegetable Research and Development Centre (AVRDC) the World Vegetable Center. As a result, vegetable soybeans are now produced and marketed in various part of the globe like Zimbabwe, Mauritius, Uganda, Tanzania, Zambia, Sudan, and Mozambique [9,10].

Worldwide soybean cultivation was about 51.8 Mha in 2005, 83 Mha in 2010 [11] and estimated presently on more than 92.5 million ha (about 6% of the world's arable land) to produce 217.6 million tons of production each year [12]. However, it is estimated that the current area under vegetable soybean in the USA is about 2000 ha [13]. Over the past decade improved soybean varieties bred from lines developed at AVRDC have been introduced and distributed to farmers in North-East India [10].

As per Nair et al. [14] India is a developing nation and home to almost 1.2 billion people also, India hosts a significant part of the world's poverty and health problems, providing a clear target for global initiatives against hunger. Nair et al. [14] also discussed important approach of promoting more diverse, nutritional crops to the greater Indian population to facilitate a healthier and more balanced diets. Nair et al. [14] stated vegetable soybean as rich source of protein and other nutrients also accepted as a viable and promising option to improve nutrition in India.

Young et al. [15], represent that vegetable soybean generally harvested during R6 to R7 stage of crop growth when the pods are green and seeds fill at about 80-90% of the total weight. Larger and wider green pods, more dry weight, green seed coat, higher sugar content, smooth texture and better flavors than grain soybean are product features of vegetable soybeans [16,17] for better market values. Vegetable soybean can be either sold fresh as pots, shelled beans, or sold as frozen or canned products [18]. However, in some countries like Nepal, grain soybeans are harvested at the green pod stage and marketed as vegetable soybeans and grain soybean varieties have also been used as vegetable soybean in China, Taiwan and Thailand. Such beans are unpalatable and bias consumer attitude towards using soybean as vegetable. Vegetable soybean is slightly sweeter compared with the grain type, which is oily and slightly bitter [14]. It is rich in protein (13%), cholesterol free oil (5.7%), phosphorous (150 mg/100 g), calcium (78 mg/100 g), Vitamin B_1 (0.4 mg/100 g) and, B_2 (0.17 mg/100 g). They also contain isoflavon and vitamin E [19]. The trypsin inhib itors in vegetable soybean are lower than that in grain soybean [14]. Compared to vegetable pigeon pea (*C. cajan*) and green peas (*P. sativum*) the vegetable soybean provides more protein of higher quality and is considered as an excellent and complete protein source [20]. Harvesting pods at right time just after

the R6 growth stage is critical, as loss of nutritional quality occurs when the pod turns yellow.

Assessment of genetic variability for generation of information in the existing germplasm of a particular crop is a prerequisite [21]. Also, heritability is a basic for selection which implies the extent of transmissibility of traits to next generations [22]. Similarly, high genetic advance coupled with high heritability estimate offers the most effective condition for selection for a particular trait [23]. Increased seed yield is the ultimate goal of the breeders however; seed yield itself is an outcome of direct or indirect interactions of many component traits. Therefore, understanding the relationship between yield and its component traits is of great importance to breeders. Further, this helps in selecting desirable genotypes for yield improvement programs [24]. As correlation alone cannot explicate relationships among the traits, hence the path coefficient analysis has been used in different crop species for determination of the impact of the independent variables on the dependent one and to find direct and indirect effects [25].

Genetic diversity is normally assessed by common morphological traits which are affected by different environmental conditions, development stages of the crop, also the type of plant material and need several replications to establish the genotypic contributions. In modern plant breeding, tools based on molecular markers have proved their importance and found competent. Assessment of genetic diversity with molecular markers will overcome this hurdle through excluding environmental effects and provide a true representation of the entire genome.

Various molecular markers have been used to study the genetic diversity and population structure of plants such as restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSRs), and single nucleotide polymorphisms (SNPs) etc. [26]. Among these markers, SSRs have stood out and are considered to be the most powerful tools because of its high abundance, co-dominant nature, resolving multiple alleles, reproducible behavior, wider coverage of genome, and easier detection procedure using polymerase chain reaction (PCR) [27-31]. However, due to extensive time requirement and high cost of their development, the wide use of SSRs is often limited. The recent development in studies of expressed sequence tags (ESTs) has produced a new foundation development of EST-SSRs. These EST-SSRs have some intrinsic advantages over genomic-SSR markers *viz*; (1) they are less costly (2) they are directly associated with transcribed genes; and (3) they have high transferability among related species [32]. As far as India is concerned, soybean is mainly cultivated as an oilseed crop. Considering the nutritional importance of vegetable soybean, efforts are being made to breed vegetable soybean varieties. As an initial step, present study was carried out to know variability and association among vegetable and grain type soybean genotypes for yield and quality attributing traits using morphological descriptor along with molecular markers.

Material and Methods

Experimental site

The experiment was carried out during *kharif* 2014 at the experimental field of Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra state, India located at 307.4 meters above mean sea level. The geographical situation is 20.42°N latitude and 77.02°E longitude. The soil was medium black, with clay, fairly leveled and uniform in topography with appropriate drainage.

Plant material

Thirty four soybean genotypes were used as the experimental material comprised of 7 vegetable types, 12 mutants and 15 released grain type soybean genotypes. The names of all 34 soybean genotypes included in the study are listed in Table 1 along with their source.

Experimental design and setting the experiment

The experiment was laid out in a randomized complete block design with three replicates. The seeds were sown maintaining distance of 45 × 10 cm. Seeds were sown with the help of hand drill during *Kharif* 2014. The basal fertilizer does were applied at the rate of 30 Kg N, 75 Kg P_2O_5 and 20 Kg K_2O per hectare at the time of sowing. Fertilizers were applied in the form of urea, single super phosphate and murrate of potash. Since the crop was grown during *Kharif* season, the irrigation was given at critical growth stages. As the crop is for vegetable purpose, 3-4 irrigations were provided after the initiation of flowering. Harvesting was done depending upon maturity of the genotypes.

Data collection

Data on five randomly selected plants from each replicate were recorded for various traits *viz*., days to 50% flowering, days to maturity, plant height, number of branches per plant, number of green pods per plant, pod length, pod width, 100 fresh pod weight, 100 beans weight, and test weight of beans, 100 seed weight, and photosynthetic efficiency (Table 2). Qualitative characters *viz*., protein content, total sugar content and oil content were estimated from all genotypes of each replication. Observations on flower color, seed shape, helium color, seed luster, seed coat color and pod color were recorded using color chart developed by RHS (The Royal Horticultural Society), London, United Kingdom (Royal Horticultural Society, 2001).

All soybean genotypes along with two checks, each of vegetable and grain type were evaluated for texture, aroma, taste and overall acceptability through organoleptic taste and generated data as per score card. The evaluation was done by a panel of six trained judges, including faculty members and students of the Department of Agricultural Botany, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

Determination of fragrance was done using the procedure given by Sood and Sidiq [33] with desirable modifications of the KOH concentration. The leaf and seed fragrance of each genotype was determined following the optimized procedure at R6-7 stage of the crop. About 2-3 g of green leaf and beans harvested from plants, sliced and immersed for 10 min in 10 ml of 3.0% KOH at room temperature, after which the fragrance was graded independently by six operators. Out of a random five samples of each genotype, if none were fragrant, the entry was deemed to be non-fragrant; if five successive samples were fragrant, the entry was considered to be fragrant.

Estimation of protein content (%)

Protein content of seeds harvested at R6-R7 stage was determined by Bradford method and expressed on per cent basis for each genotype. The estimation method is based on the protein dye binding method. The binding of Commassie Brilliant Blue (CBB) G-250 to protein in acidic condition shift the λ_{max} of dye from 465 nm to

Table 1: List of soybean genotypes and their source.

S No.	Genotype	Type	Source	SN	Genotypes	Type	Source
1	PK 1314	Grain	Dr. PDKV Akola, MS	18	MAUS-158	Grain	VNMKV, Parbhani, MS
2	AMS-6-1	Mutant	Dr. PDKV Akola, MS	19	IC 241949	Grain	Dr. PDKV Akola, MS
3	AMS-50(B)	Mutant	Dr. PDKV Akola, MS	20	IC-16815	Grain	Dr. PDKV Akola, MS
4	AMS-73	Mutant	Dr. PDKV Akola, MS	21	IC-118400	Grain	Dr. PDKV Akola, MS
5	AMS-247	Mutant	Dr. PDKV Akola, MS	22	IC-118452	Grain	Dr. PDKV Akola, MS
6	AMS-99-24	Mutant	Dr. PDKV Akola, MS	23	AGS-450	Vegetable	AVRDC, Hyderabad, AP
7	AMS-28(A)	Mutant	Dr. PDKV Akola, MS	24	AGS-457	Vegetable	AVRDC, Hyderabad, AP
8	AMS-353	Mutant	Dr. PDKV Akola, MS	25	Swarna Vasundhara	Vegetable	AVRDC, Hyderabad, AP
9	AMS-5-18	Mutant	Dr. PDKV Akola, MS	26	AGS-459	Vegetable	AVRDC, Hyderabad, AP
10	AMS-37	Mutant	Dr. PDKV Akola, MS	27	GC-84501-32-1	Vegetable	AVRDC, Hyderabad, AP
11	AMS-90-1	Mutant	Dr. PDKV Akola, MS	28	AGS-339	Vegetable	AVRDC, Hyderabad, AP
12	AMS-93	Mutant	Dr. PDKV Akola, MS	29	MACS-450	Grain	ARI, Pune, MS
13	AMS-65	Mutant	Dr. PDKV Akola, MS	30	MACS-1508	Grain	ARI, Pune, MS
14	EC-251411	Grain	Dr. PDKV Akola, MS	31	MACS-1188	Grain	ARI, Pune, MS
15	NRC-40	Grain	Dr. PDKV Akola, MS	32	TAMS 98-21	Grain	Dr. PDKV Akola, MS
16	NRC-2	Grain	Dr. PDKV Akola, MS	33	HIMSO 1685(C)	Vegetable	RRC Amravati, MS
17	TAMS-38	Grain	RRC Amravati, MS	34	JS-335(C)	Grain	JNKV, Jabalpur, MP

Table 2: List of traits and their description of measurement.

S No.	Traits	Method of measurement
1	Days to 50% flowering	The number of days from sowing to flowering of 50% plants
2	Days to maturity	The number of days from sowing until approximately 90% pod turned into brownish color
3	Plant height (cm)	The height from the base of the plant to the tip of last leaf
4	Primary branches per plant (number)	Total number of pod bearing primary branches in a plant
5	Pods per plant (number)	Total number of pods with seed in a plant at R6
6	Seeds per pod (number)	Total number of seeds in a pod at R6
7	Pod length (cm)	Average length of five pods from each genotype was randomly selected was measured in millimeters using vernier calliper (Tricle brand-name) and average length recorded
8	Pod width (cm)	Average width of five pods from each genotype was randomly selected was measured in millimeters using vernier caliper (Tricle brand-name) and average length recorded
9	100-seed wt. (g)	One hundred beans randomly counted and then weighed at R6
10	100- fresh pod wt. (g)	One hundred pods randomly counted and then weighed at R6
11	100- mature seed wt. (g)	One hundred seeds randomly counted and then weighed at R8
12	Test weight of seeds (g)	One thousand seeds randomly counted and then weighed at R6
13	Photosynthetic efficiency	This was recorded with the help of Chlorofluro meter at R6 stage
14	Sugar content (%)	Total sugars were estimated in edible portion using the Phenol sulfuric acid method
15	Protein content (%)	Protein content of seeds harvested at R6-R7 stage was determined by Bradford method and expressed in per cent
16	Oil content (%)	Oil content was determined by using NMR (nuclear magnetic resonance) at R8
17	Seed yield per plant (g)	Weighing the total number of seeds produced in a plant
18	Flower color	Flower colour was scored using the descriptor*
19	Seed shape	General shape of seed was scored using descriptor*
20	Helium color	Hilum colour using descriptor*
21	Seed luster	Shape of seed was scored using descriptor*
22	Seed coat color	Seed coat colour was recorded using the colour using Royal Colors Chart**
23	Pod color and appearance	Pod colour was recorded at R6 and scored using Royal Colors Chart**
24	Texture	Force required compressing the grain between one's teeth
25	Taste	Organoleptic test was carried out using 0-5 scale at R6
26	Aroma	About 0.8 to 1 g of seeds at R6 were cut into pieces and placed in a 15 ml tube. Ten ml of 1.7% KOH was added, and the tube was capped and kept for 10 min at 37°C.

Note: *Descriptor for soybean, IBPGR/84/183, Rome (1984) (http://www.bioversityinternational.org/uploads/tx_news/Descriptors_for_soyabean_252.pdf)
**The Royal Horticultural Society, London, United Kingdom (2001)

595 nm. Absorption of the blue colored protein dye complex at 595 nm is directly related to concentration of protein present in sample (Sengar and Chaudhary, 2014).

Estimation of sugar content (%)

Phenol sulfuric acid method is the most widely used colorimetric method to date for determination of total sugar concentration in aqueous solutions. The basic principle of this method is that carbohydrates, when dehydrated by the reaction using concentrated sulfuric acid, produce furfural derivatives. Further, the reaction between furfural derivatives and phenol develops the detectible color [34].

Estimation of oil content (%)

The oil composition of soybean seeds was determined using the NMR spectrometry (Nuclear Magnetic Resonance) at the

Instrumental cell, Oilseed Research Unit, Dr. PDKV, Akola. For this purpose, 25-30 gm of seeds per soybean genotype was measured with two replications. The oil content of soybean seeds was determined by calibrating the NMR signal against a suitable reference using MQC Benchtop NMR Analyzer, Oxford instrument.

Statistical analysis

The data were analyzed using MSTAT program for Analysis of variance (ANOVA). Phenotypic, genotypic and error variances were estimated following the procedure described by Johnson et al. [35]. Genotypic and phenotypic variation was estimated according to Burton [36]. Broad sense heritability and genetic advance in percent of means were estimated using the formula suggested by Johnson et al. [35].

Genotypic and phenotypic correlation coefficients for different characters were calculated in all possible combinations using the formula given by Miller et al. [37]. The path coefficient analysis was made following the procedure of Dewey and Lu [38]. Mahalanobis's generalized distance (D^2) statistics was used for clustering of genotypes by estimating the divergence among genotypes for the traits measured as per the Joshi and Vashi [39].

Molecular diversity studies

DNA isolation and PCR amplification: DNA was extracted from 15-day old seedlings of each genotype. A total of 0.3 g of fresh leaves was used for each genotype and DNA was extracted using the Cetyl Trimethyl Ammonium Bromide (CTAB) method. The relative purity and concentration of the extracted DNA were estimated with spectrophotometer. The final concentration of each DNA sample was adjusted to 20 ng/µl. Ten informative EST-SSR primers based earlier reports of Zhang et al. [40] were selected, and used in this study.

The polymerase chain reaction (PCR) was performed in a final volume of 20 µl, containing 10 mM Tris-Cl, 50 mM KCl, 2 mM $MgCl_2$, 100 mM of each dNTP, 0.4 mM of each primer, 20 ng genomic DNA, and 1 U of *Taq* DNA polymerase. Each of the 40 PCR cycles consisted of 30 s at 94°C for template denaturation, 30s at 47°C for primer annealing, and 30s at 72°C for primer extension. The PCR reaction was completed with 5 min incubation at 72°C. The PCR products were separated on 2.0% Agarose and visualized under AlfaImager.

Statistical analysis: For the statistical analysis, the patterns of all SSR loci were scored for each polymorphic amplicon as '1' for presence and '0' for absence. This allowed estimating at each locus of the number of alleles present (NA) and the polymorphic information content (PIC) value. The PIC value of each primer was calculated by the formula:

$$PIC = 1 - \sum_{i=1}^{n} (Pi)^2$$

Where, Pi is the frequency of the i^{th} allele. Similarity coefficients based on EST-SSR profiles were calculated according to procedure described by Nei and Li [41], and a dendrogram based on the similarity matrix and UPGMA clustering was produced using the online software.

Results and Discussion

Mean performances

The mean performances of all soybean genotypes for different traits are shown in Figure 1. The shortest time required to flowering and maturity was observed in vegetables genotype AGS-450 (20 and 77.67 days) closely followed by AGS-457 (20.67 and 77.33 days) and AGS-339 (20 and 81.67 days). The longest duration was required in the grain type soybean genotype TAMS-98-21 (46.70 and 89.33 days) followed by MACS-1188 (42.33 and 104.67 days) and MACS-1508 (41.00 and 100.00 days). Results suggested that some of vegetable genotypes required lower flowering and maturity period than the grain type and mutant genotypes. The wide range was observed for plant height (27.88 to 76.20 cm) and number of primary branches (1.43-5.50). Most of the mutants showed significant variation in plant height but no any genotype has found significant for containing sugar, protein, oil or green pod yield.

Among seven vegetable type genotypes, four (Swarna-Vasundhara, AGS-450, AGS-457 and AGS-459) produced significantly higher green pod yield per plant than mutants and other grain type genotypes. However, most of the genotype produced significantly higher number of green pod per plant except the GC-84501-32-1, but not found superior in green pod yield per plant. Vegetable genotypes showed significantly high pod length, pod width, 100-seedweight, 100-fresh pod weight, 100-mature seed weight, test weight and photosynthetic efficiency as compared to rest of the genotypes under study. The genotype AGS-450 had significant highest level of sugar and protein content (92.01 mg/g and 45.07%, respectively) followed by AGS-459 (91.07 mg/g and 42.91%). The highest oil content was recorded in mutant genotype AMS-353 (20.84%) followed by TAMS 98-21 (20.77%) and AMS-93 (20.65%).

Genetic variability, Heritability and Genetic Advance

Analysis of variance revealed that mean square due to genotypes were highly significant ($P<0.01$) for all the 17 quantitative traits (Table 3). These results revealed highly significant genotypic variation among genotypes for all the traits. Phenotypic and genotypic coefficient of variation (PCV and GCV), broad sense heritability and genetic advance was calculated for all seventeen traits (Table 4). The estimates of PCV were higher than corresponding estimates of GCV for all characters under study. The highest PCV and GCV with low environmental variance for all the traits indicate that the expressions of genes controlling these characters are not marked by influence of the environmental conditions. The highest PCV and GCV were observed for test weight (61.17 and 60.57%, respectively) and the lowest PCV and GCV were recorded for oil content (4.12 and 2.66%, respectively). Similarly, significant variations have also been reported earlier by several researches for various traits [42-44].

The PCV and GCV of days to 50% flowering (16.19 and 15.6%), days to maturity (6.01 and 5.62%), plant height (26.04 and 24.79%), number of primary branch (38.39 and 37.26%), number of green pod per plant (16.25 and 15.06%), number of seed per plant (9.83 and 9.20%), pod length (14.20 and 13.28%), pod width (35.21 and 33.80%), 100 beans weight (49.28 and 48.63%), 100 fresh pod weight (48.89 and 48.80%), 100 mature seed weight (59.78 and 59.53%), photosynthetic efficiency (8.47 and 7.74%), sugar content (25.05 and 24.84 %), protein content (11.44 and 10.55%) and green pod yield per plant (33.50 and 32.61%) results showed narrow difference between PCV and GCV for most of traits. All the characters exhibited high heritability which varied from 41.8% in oil content to 99.6% in fresh pod weight. Among the traits, oil content had relatively low heritability. The genetic advance as present of mean (GA%) ranged from 4.54% in oil content to 158.38% test weight of beans. Among the traits, test weight of bean, 100-mature seed weight, 100-fresh

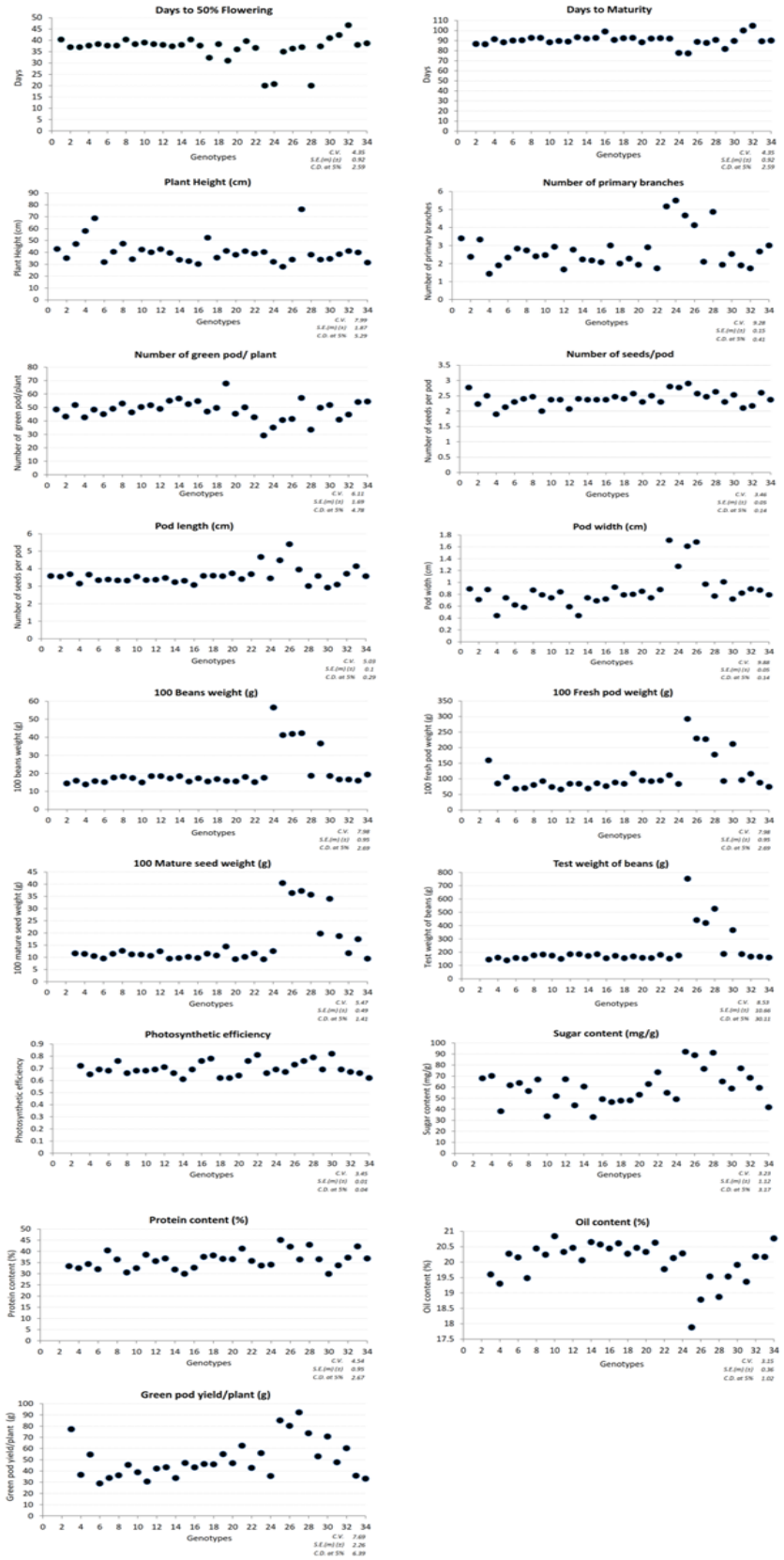


Figure 1: Mean performance of 34 genotypes for various characters in vegetable and grain type soybean.

Table 3: Analysis of variance for mean sum of squares for various characters in vegetable and grain type soybean.

Source	df	Days to 50%flowe-ring	Days to maturity	Plant height (cm)	Number of primary branches	Number of green Pod/ Plant	Number of seed /pod	Pod length (cm)	Pod width (cm)	100 Beans weight (g)	100 fresh pod weight (g)	100 Mature seed weight (g)	Test weight of beans (g)	Photosynthetic efficiency	Sugar Content (mg/g)	Protein content (%)	Oil content (%)	Green pod yield/ plant (g)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Replication	2	6.970	4.320	27.810	0.013	5.800	0.004	0.006	0.002	5.510	31.090	0.690	911.860	0.001	11.530	7.150	1.100	44.380
Genotypes	33	99.62**	81.39**	314.61**	3.19**	165.44**	0.15**	0.71**	0.26**	307.19**	8873.80**	260.89**	51946.10**	0.01**	675.64**	46.35**	1.25**	844.06**
Error	66	2.52	3.69	10.53	0.06	8.61	0.001	0.03	0.01	2.73	11.11	0.73	341.14	0.001	3.79	2.69	0.39	15.35

Note: **Significance at 1% level

Table 4: Estimation of genetic parameters in seventeen characters of 34 genotypes in vegetable and grain type soybean.

Characters	Range		Mean	Mean sum of squares	CV%	Genotypic variance	Phenotypic variance	GCV%	PCV%	Heritability (h^2) (BS) (%)	Genetic Advance (GA)
	Lowest	Highest									
Days to 50% flowering	20.00	46.70	36.47	99.62**	4.35	32.37	34.88	15.6	16.19	92.8	39.67
Days to maturity	77.33	104.67	90.41	81.39**	2.13	25.90	29.60	5.62	6.02	87.5	13.90
Plant height (cm)	27.88	76.20	40.61	314.61**	7.99	101.36	111.89	24.79	26.05	90.6	62.29
Number of primary branch	1.43	5.50	2.74	3.19**	9.28	1.04	1.11	37.26	38.40	94.2	95.46
Number of green pod/plan	29.10	67.80	47.99	165.44**	6.11	52.28	60.89	15.06	16.26	85.9	36.86
Number of seed per pod	1.90	2.90	2.40	0.15**	3.46	0.05	0.06	9.20	9.83	87.6	22.73
Pod length (cm)	2.90	5.40	3.59	0.71**	5.03	0.23	0.26	13.28	14.20	87.5	32.82
Pod width (cm)	0.40	1.70	0.86	0.26**	9.88	0.09	0.093	33.80	35.21	92.1	85.66
100 seed weight (g)	13.85	56.51	20.71	307.19**	7.98	101.49	104.22	48.63	49.28	97.4	126.71
100 fresh pod weight (g)	65.96	291.90	111.38	8873.80**	2.99	2954.26	2965.37	48.80	48.89	99.6	128.60
100 mature seed weight (g)	9.20	40.47	15.64	260.89**	5.47	86.72	87.45	59.53	59.78	99.2	156.50
Test weight of seed (g)	138.53	751.47	216.52	51946.10**	8.53	17201.65	17542.79	60.57	61.17	98.1	158.35
Photosynthesis efficiency	0.61	0.82	0.70	0.01**	3.45	0.003	0.003	7.74	8.47	83.5	18.68
Sugar content (mg/g)	32.82	92.01	60.22	675.64**	3.23	223.96	227.74	24.84	25.05	98.3	65.05
Protein content %	29.89	45.07	36.13	46.35**	4.54	14.56	17.24	10.55	11.49	84.4	25.61
Oil content %	17.88	20.84	20.03	1.25**	3.15	0.29	0.68	2.66	4.12	41.8	4.54
Green pod yield/plant (g)	28.84	92.18	50.96	844.06**	7.69	276.24	291.59	32.61	33.50	94.7	83.80

Note: **Significance at 1% level

pod weight, 100-seed weight, number of primary branch and green pod yield exhibited higher percentage of genetic advance. Narrow difference between PCV and GCV for all the characters tested indicates less influence of environmental factor on their expression and the chance of high selection gain. The heritability estimation helps breeders in selection based on the basis of phenotypic performance. Heritability and genetic advance together with high GCV could provide the best image of the amount of advancement to be expected thought phenotypic selection [35,45].

Therefore, high value of heritability and genetic advance (%) along with high GCV for the traits like green pod yield (g), 100 seed weight (g), 100 fresh pod weight (g), protein content (%) and the sugar content (mg/g) can be considered as favorable traits for improvement of vegetable soybean through effective phenotypic selection of these traits and high expected genetic gain from the selection of these traits can be achieved. This suggests that these characters are under control of additive gene action and would respond very well to continuous selection [46]. Consequently, high estimate of heritability and genetic advance (%) along with low GCV of the rest of traits like days to 50% flowering number of seed per pod, oil content (%) green pod yield per plant (g) indicated the expression of these traits are under involvement of non- additive gene action and phenotypic selection of these traits might not be effective.

Creation of new plant type with high yield is the main objective

in plant breeding. In the present investigation, it was observed that amongst 34 genotypes; five genotypes performed superiorly in respect to green pod yield per plant along with other morphological and quality like early days to 50% flowering, number of primary branches, number of seed per pod, pod length and width, 100 seed weight, 100 fresh pod weight, sugar content, oil content and green pod yield per plant. These results are in accordance of the result of Kundi et al. [47], Hussain et al. [48] and Malek et al. [49].

Character association and path coefficient analysis

Genotypic and phenotypic correlations were calculated followed by path coefficient analysis to partition the correlation coefficients of traits with yield per plant into direct and indirect effects (Table 5). The estimation of genotypic correlation coefficient was found to be higher than their respective phenotypic correlation coefficient. There are in the agreement with the result of Malek et al. [49] however, Weber and Morrthy [50] observed low phenotypic correlation due to the modifying effect of environment on the genetic association among the traits. The characters exhibited significant positive correlation with green pod yield per plant were found to be number of primary branches, number of seed/pod, pod width, pod length, 100 fresh pod weight, 100 beans weight, 100 mature seed weight, test weight of beans, photosynthetic efficiency and protein. These characters were also, positively interlinked among themselves which indicated the importance of these characters while selection.

Further the results indicated that the increase in one character will increase in the correlated character. For example, number of primary branches was positively one significantly correlated with green pod yield per plant; hence the plants having more number of primary branches are more likely to produce greater number of pods, per plant. Thus selection for higher green pod yield on the basis of above characters would be reliable.

Among the yield contributing characters themselves, number of seeds per pod was positively and significantly correlated with green pod yield per plant followed by 100 fresh pod weight, number of primary branches and while, significant and positive association with test weight of beans, photosynthetic efficiency, sugar content and protein content. This indicates the importance of the character number of seeds per pod in increasing 100 fresh pods weight, with test weight beans, photosynthetic efficiency, sugar content and protein content. Nagarjuna et al. [51] reported similar results and showed positive and significant correlation of seed yield with number of seeds per pod, number of preliminary branches, number of pod per plant and 100 seed weight. Therefore, to improve yield of soybean, emphasis should be given on the correlated traits based on strength of their correlation.

The plant height was negatively and significantly correlated with green pod yield per plant followed by number of primary branches per plant, number of seed per plant, pod length, pod width, test weight of beans, photosynthetic efficiency, sugar content, oil content and protein content. The remaining association was less important due to their positive or negative non-significant correlation coefficient values. It means that increase of plant height decreases the green pod yield per plant. Arshad et al. [52] and Rajanna et al. [53] reported similar findings for different parameters.

The genotypic correlation of green pod yield per plant (g) was positive correlated with number of primary branches, number of seed per pod, pod width (cm), 100 seed weight, 100 fresh pod weight, 100 mature seed weight, test weight of beans, photosynthetic efficiency and protein content (%). The genotypic association of 100 seed weight with 100 fresh pod weight, 100 mature seed weight, sugar content and green pod yield per plant was highly significant in positive discussion.

The significant positive correlation of number of seed per pod, 100 fresh pod weight, 100 mature seed weight, pod width, pod length, number of seed per pod, protein content and sugar content with green pod yield per plant indicated that in selecting high yielding vegetable type genotype, these characters should be more emphasis as the best detection criteria. These results are also in agreement with the results of Vijayalakshmi et al. [54].

The path coefficient analysis showed the importance of yield contributing characters *viz.* number of primary branches, number

Table 5: Estimates of Genotypic correlation coefficient (r) for different characters.

S No.	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17
X1	1	0.755**	0.072	-0.756**	0.532**	-0.536**	-0.198	-0.446**	-0.749**	-0.763**	-0.730**	-0.720**	-0.344*	-0.464**	-0.223	0.699**	-0.595**
X2		1	-0.003	-0.660**	0.486**	-0.444**	-0.371*	-0.481**	-0.651**	-0.645**	-0.591**	-0.640**	-0.208	-0.455**	-0.160	0.803**	-0.494**
X3			1	-0.271	0.169	-0.229	0.017	-0.175	-0.234	-0.229	-0.174	-0.203	-0.052	-0.120	-0.043	-0.030	-0.222
X4				1	-0.562**	0.817**	0.420**	0.701**	0.855**	0.920**	0.839**	0.811**	0.340*	0.463**	0.243	-0.705**	0.849**
X5					1	-0.169	-0.269	-0.504**	-0.703**	-0.654**	-0.687**	-0.686**	-0.117	-0.470**	-0.186	0.795**	-0.303*
X6						1	0.451**	0.649**	0.630**	0.813**	0.642**	0.592**	0.324*	0.384*	0.237	-0.516**	0.947**
X7							1	0.835**	0.628**	0.527**	0.601**	0.672**	0.261	0.549**	0.499**	-0.694**	0.543**
X8								1	0.835**	0.791**	0.840**	0.839**	0.265	0.632**	0.609**	-0.896**	0.731**
X9									1	0.919**	0.960**	0.986**	0.337*	0.647**	0.43**	-0.89**	0.739**
X10										1	0.908**	0.893**	0.337*	0.631**	0.389*	-0.893**	0.902**
X11											1	0.918**	0.402**	0.681**	0.441**	-0.920**	0.769**
X12												1	0.279	0.651**	0.484**	-0.927**	0.702**
X13													1	0.338*	0.116	-0.329*	0.384*
X14														1	0.468**	-0.959**	0.565**
X15															1	-0.552**	0.365*
X16																1	-0.681**
X17																	1

Note: * Significant at 5% probability level; ** Significant at 1% probability level

X1- Days to 50% flowering; X2- Days to maturity; X3 – Plant height (cm); X4-Number of primary branches; X5-Number of green pod /plant; X6-Number of seed per pod; X7-Pod length (cm); X8-Pod width (cm); X9-100 fresh seed weight (g); X10-100 fresh pod weight (g); X11-100 mature seed weight; X12-Test weight of beans (g); X13-Photosynthetic efficiency; X14-Sugar content (mg/g); X15-Protein content (%); X16-Oil content (%); X17-Green pod yield/plant (g)

of green pod per plant, pod width (cm), 100 fresh pod weight (g), test weight of beans, 100 mature seed weight (g), pod length (cm), number of seed per pod, protein content (%), and sugar content (mg/g) which showed high positive direct effect as the major yield contributing traits, for enhancing the yield of soybean (Figure 2).

These results are in accordance with Abady et al. [55]; Sarutayophat [56] and Malik et al. [57]. The highest direct positive effect of pod length and 100 fresh pod weight on green pod yield per plant, the residual effect is low (0.0289) which indicate selected characters are desirable to study the contribution for green pod yield per plant at R6 stage.

Both the correlation and path analysis revealed that the number of primary branches, number of seed per pod, pod length and width, 100 fresh pod weight, appeared to be the first order yield components and priority should be given to these characters during selection.

Morphological descriptor

The genetic diversity in the grain and vegetable type soybean was determined by analyzing variation in ten morphological traits. The seed coat color had the highest variation among all ten traits followed by helium color, seed luster, seed shape, flower color, texture, and aroma (Table 6).

The percentages of genotypes with white, intermediate purple and purple flower color were 35.29, 20.58 and 44.11%, respectively (Figure 3). Figure 4 showed most of the genotypes had spherical seed shape (61.76%) and few of them were oval (35.29%) and spherical flatten in shape (2.94%).

The helium color was black and brown (29.45%, respectively) and rest of the genotypes had grey (23.52%), imperfect black (14.70%) and yellow (2.94%). Four different kind of seed luster could be classified as shiny (50%), intermediate (23.52%), dull (14.70%) and shine (11.764%). Only 8.82% of genotypes were aromatic amongst 34 genotypes. The genotypes with nuttiness and beany taste were 44.11%, respectively and only 11.76% genotypes had sweet taste. Based on organoleptic test, only five genotypes viz., AGS-450 AGS-457, Swarna Vasundhara, AGS-459 and MACS-1508 have found with good acceptability.

Genetic divergence studies

Cluster analysis using twelve morphological and few quality traits grouped the 34 genotypes in to three main clusters at the genetic distance of 12230.93. It was also found that among the three clusters, cluster I was the largest consisting of 29 genotypes (all 12 mutants, 10 released varieties, 6 germplasm and 1 vegetable genotype) and the second largest group was cluster II consisted four vegetable genotypes. However, a single vegetable genotype AGS-450 was felt in cluster III (Table 7 and Figure 5). The mean values of 12 different traits for three clusters among 34 genotypes are depicted in the Table 8. Results showed that among three clusters, III had the highest average mean for all the traits except days to 50% flowering, days to maturity, plant height and number of green pod per plant. On the contrary, cluster I revealed the lowest mean for various traits like pod length, pod width, 100-seed weight, 100-fresh seed weight, sugar and protein content and green pod yield per plant.

Cluster analysis based on twelve morphological and quality traits grouped 34 soybean genotypes in to three different clusters and indicated that 34 genotypes exhibited notable genetic divergence in terms of these traits (Table 7 and Figure 5). Therefore, classifications in the study based on these twelve traits are in agreement with previous report. Formation of different number of clusters using morphological and quality traits in diverse soybean genotypes was also reported earlier [57-60]. The dendogram tends to group some of the grain type genotypes including mutant and other genotypes

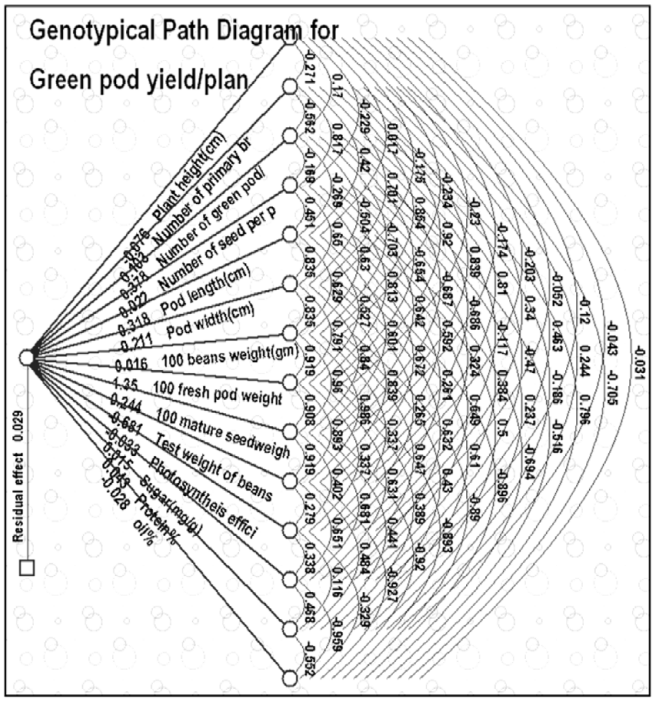


Figure 2: Genotypic path analysis diagram showing effect of different characters on green pod yield per plant.

Table 6: Qualitative characteristics of soybean genotypes.

Genotype	Flower color	Seed Shape	Helium color	Seed luster	Seed coat color at R8	Pod Color at R6	Texture	Taste	Aroma	Over all acceptability
PK-1314	Purple	Spherical	Imperfect black	Dull	Greyed-orange	Dark green	Extremely resistant	Nuttiness	Absent	Poor
AMS-6-1	Purple	Spherical	Grey	Shiny	Greyed-yellow	Dark green	Extremely resistant	Nuttiness	Absent	Poor
AMS-50	White	Spherical	Grey	Shiny	Greyed-yellow	Dark green	Extremely resistant	Nuttiness	Absent	Poor
AMS-73	Intermediate Purple	Spherical	Black	Shiny	Greyed-yellow	Dark green	Extremely resistant	Nuttiness	Absent	Poor
AMS-247	Intermediate Purple	Spherical	Imperfect black	Shiny	Greyed-yellow	Light green	Extremely resistant	Beaniness	Absent	Poor
AMS-9924	Purple	Spherical	Black	Shiny	Greyed-yellow	Light green	Extremely resistant	Beaniness	Absent	Poor
AMS-28	Purple	Oval	Grey	Shiny	Greyed-yellow	Dark green	Extremely resistant	Beaniness	Absent	Poor
AMS-353	Purple	Oval	Black	Shiny	Pale-yellow	Dark green	Extremely resistant	Beaniness	Absent	Poor
AMS-518	White	Oval	Brown	Shiny	Pale-yellow	Dark green	Extremely resistant	Beaniness	Absent	Poor
AMS-37	Purple	Spherical	Grey	Intermediate	Greyed-yellow	Dark green	Extremely resistant	Nuttiness	Absent	Poor
AMS-90-1	Purple	Spherical	Grey	Shiny	Greyed-orange	Yellow green	Extremely resistant	Beaniness	Absent	Poor
AMS-93	White	Spherical	Brown	Intermediate	Greyed-yellow	Yellow green	Extremely resistant	Beaniness	Absent	Poor
AMS-65	Purple	Spherical	Grey	Shiny	Greyed-yellow	Yellow green	Extremely resistant	Nuttiness	Absent	Poor
EC-251411	White	Oval	Brown	Intermediate	Greyed-yellow	Yellow green	Extremely resistant	Nuttiness	Absent	Poor
NRC-40	White	Oval	Black	Shiny	Greyed-orange	Yellow green	Extremely resistant	Nuttiness	Absent	Poor
NRC-2	Purple	Spherical	Black	Shiny	Pale-yellow	Dark green	Extremely resistant	Nuttiness	Absent	Poor
TAMS-38	White	Spherical	Brown	Shiny	Greyed-yellow	Yellow green	Extremely resistant	Beaniness	Absent	Poor
MAUS-158	White	Spherical	Brown	Intermediate	Greyed-orange	Yellow green	Extremely resistant	Beaniness	Absent	Poor
IC-241949	White	Oval	Imperfect black	Intermediate	Greyed-yellow	Yellow green	Extremely resistant	Nuttiness	Absent	Poor
IC-16815	Purple	Spherical	Imperfect black	Shiny	Greyed-yellow	Light green	Extremely resistant	Nuttiness	Absent	Poor
IC-118400	White	Spherical	Brown	Dull	Greyed-yellow	Light green	Extremely resistant	Nuttiness	Absent	Poor
IC-118452	Purple	Spherical	Grey	Shiny	Greyed-yellow	Light green	Extremely resistant	Beaniness	Absent	Poor
AGS-450	Intermediate Purple	Spherical flatten	Brown	Shiny	Moderate- brown	Yellow green	Not resistant	Sweetness	Absent	Good
AGS-457	White	Oval		Shiny	Moderate- brown	Light green	Not resistant	Sweetness	Present	Good
Swarna Vasundhara	Intermediate Purple	Oval	Brown	Intermediate	Greyed-yellow	Yellow green	Not resistant	Sweetness	Present	Good
AGS-459	White	Oval	Black	Shiny	Blackish	Light green	Not resistant	Sweetness	Present	Good
GC-84501-32-1	Intermediate Purple	Oval	Black	Intermediate	Light- grayish green	Dark green	Extremely resistant	Nuttiness	Absent	Moderately good
AGS-339	Intermediate Purple	Oval	Yellow	Dull	Moderate yellow	Light green	Not resistant	Beaniness	Absent	Poor
MACS-450	Intermediate Purple	Spherical	Black	Intermediate	Grayish- yellow green	Yellow green	Extremely resistant	Beaniness	Absent	Poor
MACS-1508	White	Spherical	Black	Dull	Greyed-orange	Dark green	Not resistant	Nuttiness	Absent	Good
MACS-1188	White	Spherical	Black	Shiny	Greyed-orange	Yellow green	Extremely resistant	Beaniness	Absent	Poor
TAMS-98-21	Purple	Spherical	Brown	Shiny	Greyed-yellow	Light green	Extremely resistant	Beaniness	Absent	Poor
HIMSO-1685	Purple	Oval	Brown	Dull	Grayish- yellow green	Dark green	Extremely resistant	Beaniness	Absent	Poor
JS-335	Purple	Spherical	Grey	Shiny	Greyed-yellow	Light green	Extremely resistant	Nuttiness	Absent	Poor

Note: *Descriptor for soybean, IBPGR/84/183, Rome (1984) (http://www.bioversityinternational.org/uploads/tx_news/Descriptors_for_soyabean_252.pdf)

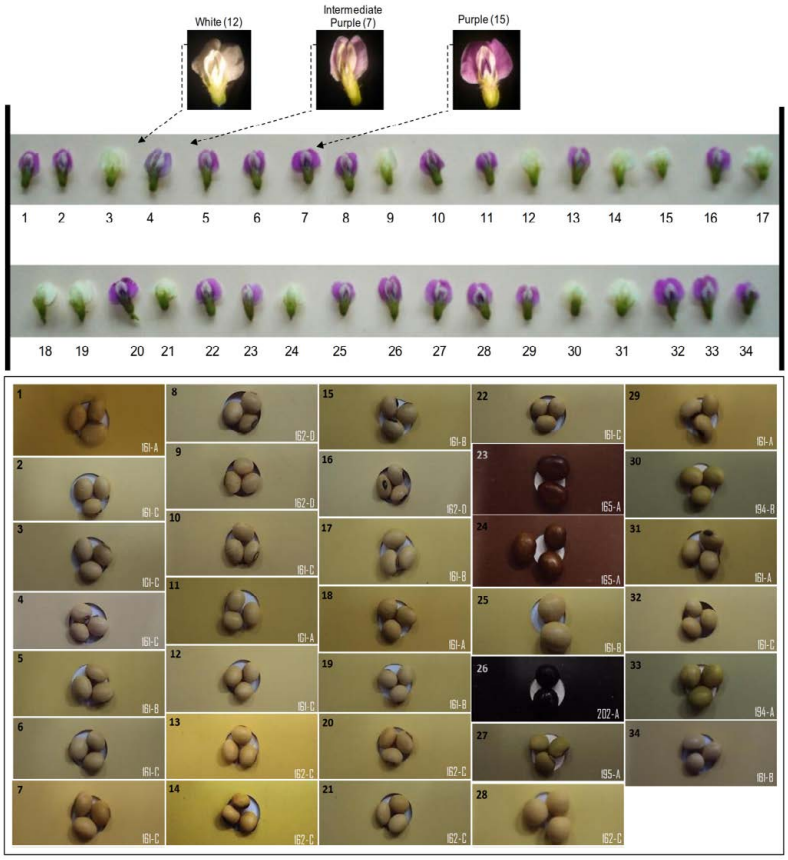
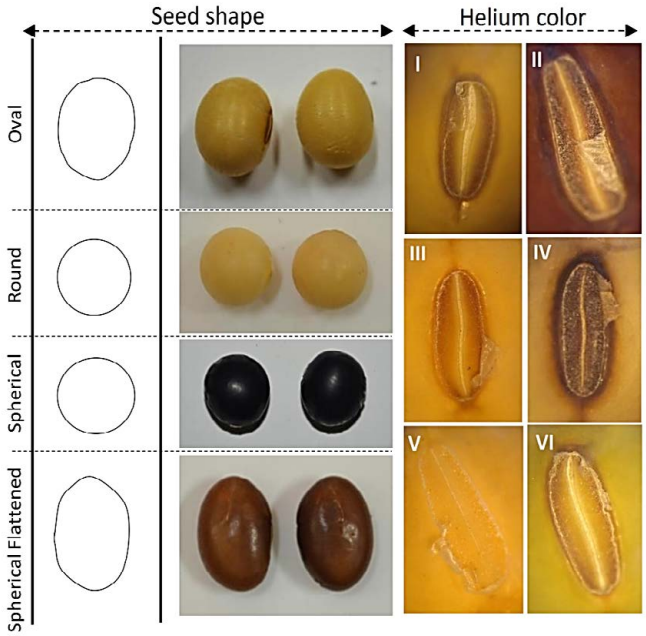


Figure 3: Variation in flower and mature seed coat color soybean genotypes under study as per The Royal Horticultural Society catalog, London.



Note: I-Grey, genotypeNo. 13; II-Dark brown, genotype no. 24; III-Medium Brown, genotype no. 25; IV-Black, genotype no. 28-Black, genotype no. 29; V-Yellow; VI-Imperfect black, genotype no. 33.

Figure 4: Variation in seed shape and helium color amongst the genotypes under study.

Table 7: Grouping of soybean genotypes in to three clusters based on various morphological traits.

Cluster	No. of genotypes	Percent of population	Name of genotypes
I	29	85.29	EC-251411, NRC-2, AMS-90-1, NRC-40, MAUS-158, AMS-99-24, IC-118452, AMS-5-18, TAMS98-21, JS-335, AMS-93, AMS-37, AMS-28(A), IC-118400, IC241949, AMS-6-1, MACS-1188, IC-16815, AMS-73, AMS-247, GC-84501-32-1, MACS-450, AMS-50(B), TAMS-38, MACS-1508, AMS-353, AMS-65, HIMSO(C), PK1314
II	4	11.76	AGS-457, Swarna Vasundhara, AGS-339, AGS-459
III	1	2.90	AGS-450

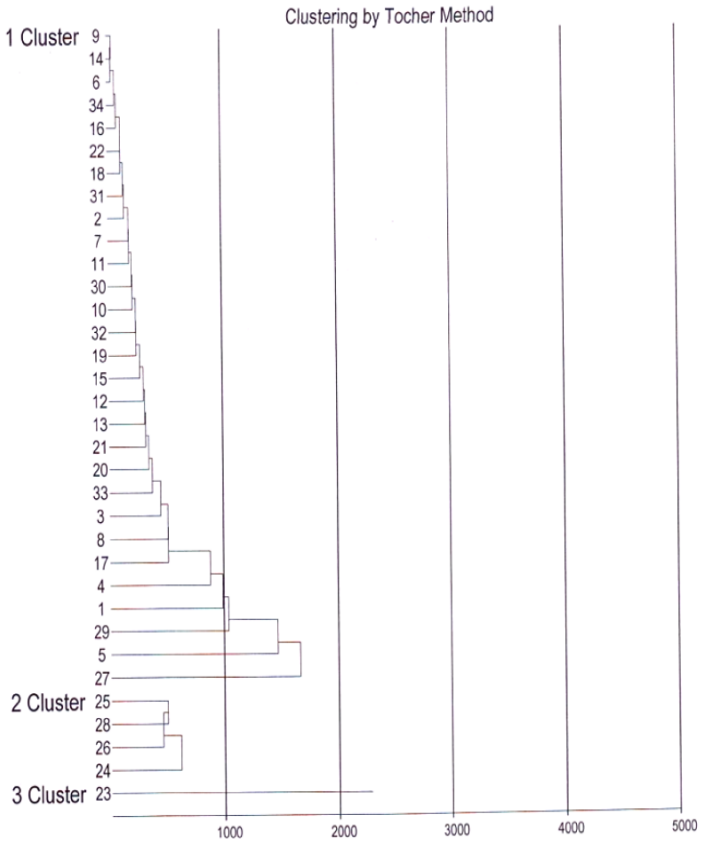


Figure 5: Dendrogram showing relationship among the soybean genotypes using morphological and quality traits.

Table 8: Mean values of different traits for three groups revealed by cluster analysis among 34 soybean genotypes.

Character	Group1	Group2	Group3	Contribution (%)
Days to 50% flowering	38.21	28.00	20.00	1.60
Days to maturity	91.76	83.83	77.67	2.50
Plant Height (cm)	41.68	32.97	40.27	7.84
No. of green pods per plant	50.07	37.62	29.10	2.14
Pod length (cm)	3.48	4.08	4.67	0.18
Pod width (cm)	0.77	1.33	1.71	0.18
100 seed weight (g)	16.75	40.47	56.51	0.18
100 fresh pod weight (g)	91.36	211.32	291.90	53.12
Sugar (mg/g)	56.57	78.78	92.01	30.66
Protein content (%)	35.60	37.80	45.07	1.25
Oil content (%)	20.21	19.27	17.88	0.18
Grain pod yield per plant (g)	45.89	79.19	85.04	0.18

with similar morphological traits in to the same cluster. However, the vegetable type soybean genotypes comprised in the cluster II separately. Similar results were also reported in soybean and other crops by Cui et al. [61], Abdullah et al. [62] and Kumar et al. [60].

Results also revealed that, the cluster III comprised of a single genotype AGS-450 on the basis of its performance of having high 100 seed weight, fresh pod weight, mature seed weight, sugar and protein content and lowest oil content at R6 stage. Therefore, the genotype

from cluster II and III could be used for hybridization programme with these genotypes in order to develop superior vegetable type soybean varieties.

Molecular diversity

Characteristics of EST-SSR markers studied: Based on the earlier studies and their polymorphic nature, ten EST-SSR primer pairs were selected for molecular characterization of 34 soybean genotypes. Among these primers, 8 were found polymorphic and two were found monomorphic. The number of repeats ranged from 8 to 18 with an average of 11.3. All the motifs of EST-SSR were ranged from 24 to 36 bp with an average of 29.1 bp (Table 9). Among 8 polymorphic SSR loci, six (75%) were trinucleotide repeats and two (25%) were dinucleotide repeats (Figure 6).

Number of alleles: Amplification of ten EST-SSR (microsatellite) markers using 34 soybean genotypes produced 334 amplicons. A total of 30 alleles were detected and distributed in the population studied with the range from 1 (CSSR391 and CSSR400) to 5 (SSR472) for respective primers. With an average of 3.0 alleles across 10 loci, with single allele for two marker, 2 alleles for one marker, 3 alleles for three markers, 4 alleles for the three markers, and 5 alleles for one marker. The overall size of the amplified products ranged from 82 bp (CSSR472) to 520 bp (GMES0709). The number of alleles, range of allele size and the PIC values of different soybean genotypes for ten EST-SSR markers are depicted in Table 9.

Monomorphic, frequent, and rare alleles: Very frequent alleles were considered to be those occurring in more than 10% of the varieties in the collection, whereas those occurring between 2% and 10% of the varieties in the collection were classified as rare alleles [63]. In this experiment amongst total of 30 alleles, 11 were monomorphic, 18 were frequent and one rare allele was indentified at 10 microsatellite loci with an average of 3.0 alleles per locus. The frequency of most common allele at each locus ranged from 36.2 (GMES4774) to 54.0 (GMES0709). Since, eighteen frequent alleles were found across the population with an average of 0.6 alleles per locus and 1 rare allele was found across the population with an average of 0.03 alleles per locus (Table 9). The rare allele was found in the genotype AMS-5-18, NRC-40 and Himso(C). There was no specific allele amplified discriminating vegetable type genotype(s).

Polymorphism in EST-SSR: All the ten EST-SSR markers used in this study generated polymorphic bands among the soybean genotypes. Similar analysis were reported by Dong (2014); Zhang [40]. The PIC values of EST-SSR loci were ranged from 0.38 (CSSR472) to 0.66 (GMES4774) with an average of 0.43. The highest PIC value (0.66) was obtained for GMES4774, followed by GMSE0709 (0.60), CSSR405 (0.58), CSSR385 (0.56), CG819919.1 (0.54), GMES0644 (0.50), and CSSR540 (0.48) (Table 4). The lowest PIC value (0.38) was obtained for CSSR472.

Genetic distance-based analysis

An un-rooted neighbor-joining tree (Figure 7) showed the genetic relationships among the soybean varieties. There were four clusters and one out-group member (NRC2) observed in NJ-Tree, interestingly; three varieties (TAMS38, IC241949, and IC118400) were grouped far from other three clusters containing remaining varieties. Vegetable type genotypes (AGS-457, Swarna Vasundhara, AGS-459, GC-84501-32-1, and AGS-339) were found grouped in a single cluster along with other genotypes however; HIMSO 1685(C) was grouped with TAMS9821. The UPGMA-based dendrogram obtained from the binary data of the samples analyzed. This pooled data analysis grouped the 34 soybean genotypes into seven clusters (Figure 8 and Table 10).

The dice similarity among the accessions ranged from 0.167 to 0.944 similarity coefficient with an average of 0.675. About 35.30% of the population among the soybean genotype showed similarity greater than 0.98 while about 64.70% showing similarity lower than

Table 9: Characteristics of EST-SSR markers used in the study.

S No.	Locus name	Corresponding ID	Forward/ Reverse	Primer sequence (5'–3')	Motif	Annealing (°C)	Range (bp)	Total alleles	Monomorphic alleles	Frequent alleles	Rare alleles	Highest freq. allele (%)	PIC values
1.	Gmp-017	CG819919.1	Forward:	ACCTCTTCCCCCATTCAGTT	$(AT)_{12}$	55	198-236	3	2	0	1	47.9	0.54
			Reverse:	ACCTCTTCCCCCATTCAGTT									
2.	Gmp-048	CSSR391	Forward:	CCGCCGAAGTACGAAGTAGA	$(GTC)_9$	54	247-263	1	1	0	0	-	0
			Reverse:	CCGCCGAAGTACGAAGTAGA									
3.	Gmp-049	CSSR400	Forward:	CTTCTCTCAGCACCCTCCAC	$(TC)_{18}$	54	250-283	1	1	0	0	-	0
			Reverse:	AACCCTTCTTCCACTTCCGT									
4.	Gmp-050	CSSR405	Forward:	AACAACAACAGCCACCACAA	$(CAA)_8$	54	197-238	4	0	4	0	47.1	0.58
			Reverse:	CTGGCATTGACACTGTTGCT									
5.	Gmp-066	CSSR472	Forward:	GGTTACGGCACTTCCTACCA	$(AAC)_9$	55	202-235	5	1	4	0	40.0	0.38
			Reverse:	AATTTTTGCGTTGTTGAGGG									
6.	Gmp-088	CSSR540	Forward:	GAGGTTGGTGCCTGGAGATA	$(GAT)_9$	56	197-235	4	1	3	0	42.0	0.48
			Reverse:	TGGCGAGTTACGAGGCTATT									
7.	Gmp-122	GMES0644	Forward:	AGATTGGAAGAGCCATCCCT	$(AGA)_{12}$	54	294-308	2	1	1	0	51.5	0.50
			Reverse:	ACTTCTCGCCCTCGTTCTTT									
8.	Gmp-133	GMES0709	Forward:	ACAGGTTGTGGGACGGTAAA	$(ACA)_9$	55	197-221	3	1	2	0	54.0	0.60
			Reverse:	ACCAAATAGCTGGAATCCCC									
9.	Gmp-197	GMES4774	Forward:	AGGATCACATACCAGGCACC	$(TA)_{18}$	56	253-285	3	1	2	0	36.2	0.66
			Reverse:	AGGATCACATACCAGGCACC									
10.	Gmp-046	CSSR385	Forward:	AACCCTTCTTCCACTTCCGT	$(CAA)_9$	55	197-211	4	2	2	0	37.8	0.56
			Reverse:	AACCCTTCTTCCACTTCCGT									
	Total							**30**	**11**	**18**	**1**	**-**	**-**
	Average							**3**	**1.1**	**1.8**	**0.1**	**35.65**	**0.43**

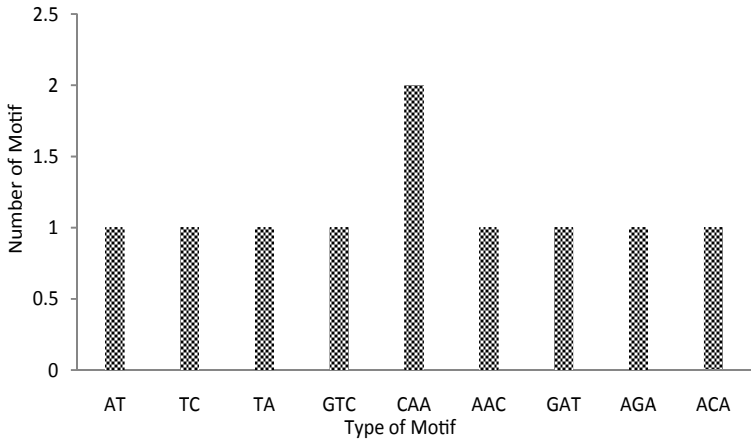


Figure 6: Figure showing distribution of different EST-SSR motifs.

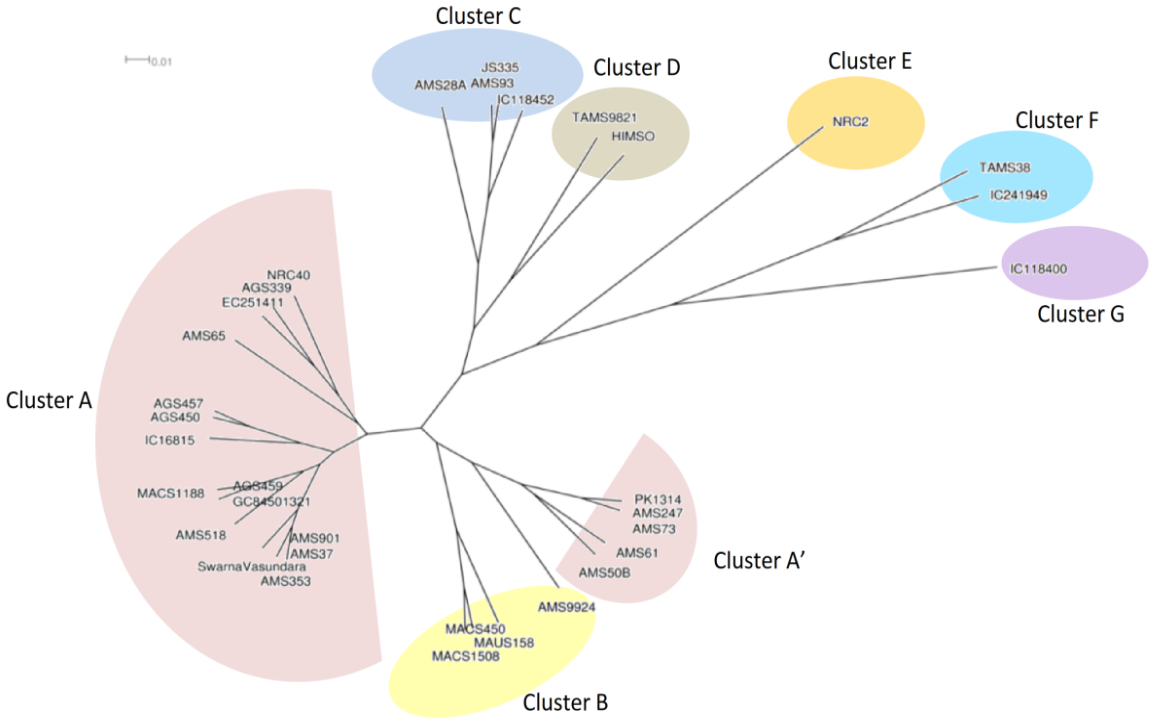


Figure 7: Neighbor joining tree showing the genetic relationship among soybean genotypes generated using molecular diversity studies.

0.675. There was strong similarity between the accessions in the clusters A, B and C. Lowest similarities were found between single individuals in the clusters E and G (Figure 8).

The results of the analysis of genetic diversity provide estimates on the level of genetic variation among the accessions that can be used in management and improvement. In this study, morphological data analysis was coupled with molecular analyses (EST-SSR markers) to investigate the genetic relationships among the soybean genotypes including vegetable-types.

The range of genetic distance based on the morphological traits was on average lower than EST-SSR markers which might be a reflection of the environmental influence on the performance of the materials. Therefore, the DNA markers and morpho-physiological traits will not necessarily gain closely matching results [64]. Mertinez et al. [65] believed that the correspondence between different methods might be improved by analyzing multiple morphological and DNA based markers. Two reasons for low or no correlations between molecular and morphological markers as well as biochemical data have been suggested by Semagn [66]. One is, DNA markers cover a larger proportion of the genome, including coding and non-coding regions, than the morphological markers and second are, DNA markers are less subjected to artificial selection compared to the morphological markers.

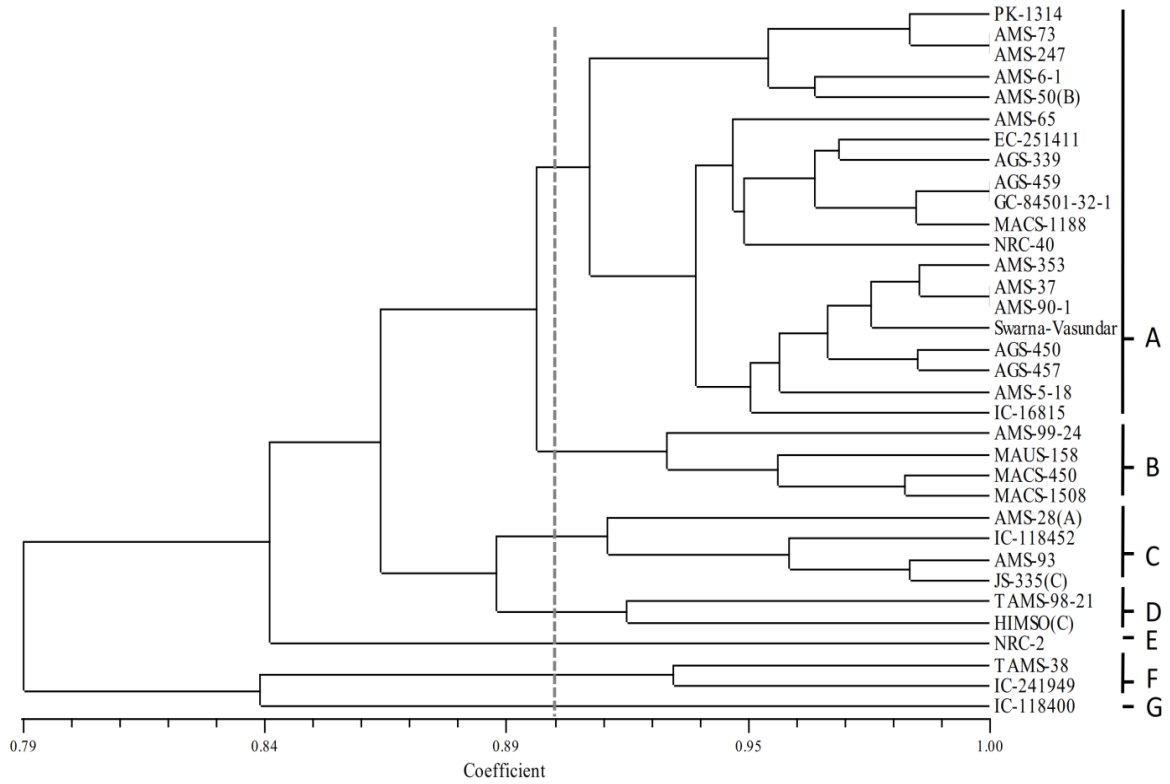


Figure 8: Dendrogram of genetic relationship among soybean genotypes based on ten EST-SSR markers. Clusters were defined at Jaccard's Coefficient. Alphabets on the right side of the dendrogram are indicating clusters which are listed in Table 10.

Table 10: Grouping of soybean genotypes in to seven clusters using SSR based molecular characterization.

Cluster	No. of genotypes	Percent of population	Name of genotypes
A	20	58.82	EC-251411, AMS-90-1, NRC-40, AMS-5-18, AMS-37, AMS-6-1, MACS-1188, IC-16815, AMS-73, AMS-247, GC-84501-32-1, AMS-50(B), AMS-353, AMS-65, PK1314, AGS-339, AGS-459, Swarna Vasundhara, AGS-457, AGS-450
B	4	11.76	MAUS-158, AMS-99-24, MACS-450, MACS-1508
C	4	11.76	IC-118452, JS-335, AMS-93, AMS-28(A)
D	2	5.88	TAMS-98-21, HIMSO(C)
E	1	2.94	NRC-2
F	2	5.88	TAMS-38, IC-241949
G	1	2.94	IC-118400

In review, the data shows significant variation among the soybean accessions their mutants and vegetable type genotypes. The information generated can be used in selecting diverse parents in breeding programme and in maintaining genetic variation in germplasm.

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