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Original Research Article

Stage Specific Seed Nutritional Quality Attributes and Genotyping of Vegetable Soybean Genotypes through DNA-SCoT Profiling

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Keywords

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A B S T R A C T

Diversity of food-grade soybeans is critical for utilization of genetic resources in cultivar development, germplasm enhancement, and end product commercialization. The experiment was carried out during *kharif*-2015 at the experimental field of Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra state, India. The objective of this study was to assess stage specific seed nutritional attributes phenotypic variability and molecular genotyping 30 vegetable and grain type cultivars. The results showed greater genetic diversity of green pod yield per plant, 100 beans weight, 100 seed weight, number of green pod per plant, plant height, days to maturity, contents of sugar, chlorophyll, protein, oil, seed hardness, taste, texture, aroma and other mineral quality traits in genotypes evaluated under the investigation. Micronutrient analysis revealed that R6 stage found to be best suited for the high level of micronutrient contents than the R8. The molecular profiling was assessed using twenty SCoT primers with 58.9% polymorphism. The Polymorphism information content (PIC) of 20 SCoT loci ranged from 0.27 to 0.70 with an average value of 0.41. The dendrogram was constructed based on DNA SCoT profiling and soybean genotypes were grouped into three clusters based on similarity matrix and arithmetic average cluster. The SCoT markers were found to be helpful in determining the genetic diversity and characterization of diverse genotypes will expedite parent selection in soybean breeding.

Introduction

Soybean is one of the protein source most used in the world. However, its organoleptic characteristics are not well accepted for human consumption (Chen and Buss, 2004). Therefore, the development of soybean cultivars more suitable for food utilization is important to attend the demand of the niche market that requires special cultivars with better quality characteristics (Pannizi and Silva, 2011).

The diet of over two-third world's population lack one or more essential mineral elements. This can be remedied through dietary diversification, mineral supplementation, food fortification, or enhancing the concentrations of mineral elements in produce (White and Broadley, 2008). As a developing nation, home to almost 1.2 billion people, India hosts a significant part of the world's poverty and

health problems, providing a clear target for global initiatives against hunger (CIA). The vastness of the country in geography and population creates difficulties in generalizing to address any one issue; however, it is clear that hunger and malnutrition are prevalent, urgent problems. The traditional Indian diet, though varied between regions, is well-balanced, rich in whole grains, vegetables, fruits and beneficial spices. However, a wholesome diet is not within easy reach of the poor, if the proper knowledge of nutritional needs is even present and around 25% of Indians live below the poverty line (CIA). Micronutrient malnutrition has many adverse effects on human health, not all of which are clinically evident (WHO, 2002). Vegetable soybean, rich in protein and other nutrients, is a viable and promising option to improve nutrition in countries under malnutrition like India.

Vegetable soybean is similar to its grain counterpart, it is the same species, but harvested earlier, when pods are bright green, yielding bigger, sweeter seeds. It is highly nutritious, rich in protein, cholesterol free fat, fiber, iron, zinc, calcium, phosphorous, folate, magnesium, potassium, isoflavones and vitamins (A,B,C,E and K) (Nair *et al.,* 2014). Compared to the grain soybean, it has a more pleasant flavor milder, sweeter, and nuttier and texture, and is easier to cook (Keatinge *et al.,* 2011; Khanande *et al.,* 2016). It has short growth duration, permitting it to fit into narrow windows in a crop rotation, and yields high values: around 40 tons/hectare, of which 10 t/ha is consumable and the rest is usable as fodder or green manure, in 65-75 days (Shanmungasundarum *et al.,* 2005). Vegetable soybean, producing among the highest yields of vegetable protein containing all essential amino acid, could help to fill this gap (Keatinge *et al.,* 2011; (Pannizi and Silva, 2011).

Japan, China, Korea and Taiwan were the major producers and consumers of vegetable soybean in the past (Shanmungasundarum and Yan, 1999). Soybean protein contains isoflavones (phytoestrogens) that reduces bad cholesterol and raises good cholesterol and functions against hypertension, osteoporosis, cancer and heart diseases (Magee *et al.,* 2012). Genetic diversity is normally assessed by common morphological traits which are affected by different environmental conditions, developmental stage of crop, also the type of plant material. In recent era molecular markers have become efficient tools for genetic diversity assessment (Wen *et al.,* 2009). The recent development is start codon targeted (SCoT) markers from the transcribed region of the genome that might be directly related to gene function. It uses 18-mer single primers with polymerase chain reaction (PCR); primers are simple to design and used to amplify the genomic region based on conservation of the ATG translation start site and flanking sequences in plant genes (Joshi *et al.,* 1997; Collard and Mackill, 2009). SCoT has some advantages over random amplified polymorphic DNA or inter-simple sequence such as being closely linked with the target gene. Keeping this view, present investigation was carried out to study stage specific seed nutritional quality and genotyping of vegetable and grain soybean through DNA-SCoT profiling.

Materials and Method

Experimental site

The experiment was carried out during *kharif* - 2015 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.

Plant material

Thirty soybean genotypes were used as experimental material comprised of 12 vegetable types, 4 mutants and 14 released grain type soybean genotypes (Table 1).

Experimental design and setting the experiment

The experiment was laid out in a randomized block design with three replicates. The spacing of row to row and plant to plant was of 45 × 10 cm. Seeds were sown using drill method during *Kharif*-2016. The fertilizers 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. As flowering initiated, 2-3 irrigations were provided as per requirement. Harvesting was done as per maturity of the genotypes.

Data collection

Five plants from each replicate were randomly selected for various traits at grain and mature stages. Qualitative characters *viz*., protein, total sugar and oil content were estimated from all genotypes of each replication. Micro-nutritional traits *viz*., calcium, iron, zinc, copper and manganese were analyzed using the atomic Absorption Spectrophotometer at R6 and R8 stages.

All soybean genotypes were evaluated for texture, aroma, taste and overall acceptability through organoleptic taste and generated data as per score card.

Determination of fragrance was done using the procedure given by Sood and Siddiq, 1978, with desirable modifications of the KOH concentration. The seed fragrance of each genotype was determined following the optimized procedure at R6 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 random five samples of each genotype, if none were fragrant, it 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 stage was determined by Bradford method and expressed in per cent basis for each genotype. The estimation method is based on the protein dye binding method. The binding of Commassie Brilliant Blue, G-250 to protein in acidic condition shift the λ_{max} of dye from 465 nm to 595 nm. Absorption of the blue colored protein dye complex at 595 nm is directly related to concentration of protein present in the sample.

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 when carbohydrates dehydrated by the reaction using concentrated sulfuric acid, produce furfural derivatives. Further, the reaction between furfural derivatives and phenol develops the detectible color (Dubois *et al.,* 1956).

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. 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

MSTAT program for Analysis of variance (ANOVA) was used for the data analysis. Phenotypic, genotypic and error variances were estimated following the procedure described by Johnson *et al.,* (1955). Genotypic and phenotypic variation was estimated according to Burton (1952). Broad sense heritability and genetic advance in percent of means were estimated using the formula suggested by Johnson *et al.,* (1955).

Molecular diversity studies

DNA was extracted using the Cetyl Trimethyl Ammonium Bromide (CTAB) method using 0.3 gm sample of 15 days old seedling and the quantification along with relative purity were estimated with nanophotometer. The final concentration of each DNA sample was adjusted to 20 ng/µl and twenty SCoT primers were used for the molecular profiling. The polymerase chain reaction (PCR) was performed in a final volume of 20 µl, containing 10X PCR buffer with 15mM $MgCl_2$, 100 mM of each dNTP, 0.4 mM of each primer, 20 ng genomic DNA, and 1 U of *Taq* DNA polymerase.

The temperature profile for DNA amplification was 30 s at 94°C for template denaturation, 30s at 47°C for primer annealing, and 30s at 72°C for primer extension for 42 cycles. The PCR reaction was completed with 5 min incubation at 72°C. Finally, the PCR products were separated on 2.0% agarose and amplicons were visualized under AlfaImager.

Statistical analysis

Polymorphic SCoT loci were scored 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 online software (PIC Calc). Dendrogram was generated by UPGMA (Un-weighted pair group method for arithmetic mean) as per the procedure given by Nei and Li, 1979 using the program XLSTAT software (www.xlstat. com).

Results and Discussion

Mean performances

The analysis of variance showed significant difference due to genotypes in all morphological character (Table 2), The CV ranges from 2.13% to 9.88%. A wide range of value was observed for 100 beans weight (g) (11.63 to 43.44), 100 mature seed weight (g) (8.36 to35.71), Green pod yield/plant (g) (31.21 to 68.17).The mean value of these traits 21.66 g, 16.28 g and 46.25 g, respectively as far as quality trait is concern mean ranges from 33.01 to 42.13%for protein content from 17.07 to 20.87 % for oil content and from 19.19 to 38.52%for sugar content. Considering the general mean value, the highest protein content was obtained with the Karune genotype (42.13%) followed by the mutant cultivar AMS-H (41.82%) and AGS-406 (41.80).the lowest value was recorded Ec-251411(33.01%).

For oil content the highest value was recorded with the mutant genotype AMS-353 (20.57%) followed by TAMS-9821 (20.74%).The vegetable genotype Karune had the lowest oil content (17.17%). The

highest level of sugar content 38.52% was recorded in vegetable genotype Swarna vasundhara followed by AGS-457(33.37) and lowest level of sugar was observed in the mutant genotype AMS 581(19.19)

The AMS-73(68.17 g) genotype found superior in green pod yield per plant, whereas, the lowest one was in MAUS-71 (Table 5).

Morphological descriptor through organoleptic evaluation

The genetic diversity in the grain and vegetable type soybean was determined by analyzing variation in four morphological traits including texture, taste, aroma and overall acceptability (Table 7).

The genotypes with nuttiness taste were 43.33% and beaniness 33.33%however only 23.33% genotypes had sweet taste. Based on the parameters like texture (good, moderately good and poor), aroma, taste (sweetness, nuttiness, beaniness) and overall acceptability (good, moderately good and poor) evaluated by score card. Based on organoleptic test, overall acceptability was observed towards only eight genotypes seven are vegetable viz., AMS-H, Karune,AGS-610, AGS-457, Swarna Vasundhara, AGS-447, AGS-459 and one grain type soybean MACS- 1508 by the panel.

Micro-nutritional traits analysis

The present study was undertaken to evaluate vegetable and grain type soybean genotypes for nutritional traits at both green (R6) and (R8) stages.

The micronutrients viz., content copper, zinc, iron, calcium, and manganese content were estimated at R6 and R8 stage in all the genotypes studied using Atomic Absorption Spectrophotometer (AAS) represented in Figure 1.

Figure 1 A elucidate the information regarding calcium content in all genotypes and R6 stage found significant for calcium content than the R8 stage. In R6 stage calcium ranged from 122.3mg/100 gm (EC-251411) to 217.3 mg/100gm (Karune). However, in R8 stage it was ranged from 69.5 mg/100gm (AMS-73) to 92.5 mg/100gm (AGS-447). Similarly, high iron containing genotype was TAMS-38 (15.72 mg/100gm) and the lowest iron content was found in EC-251411 (12.24 gm/100mg) at R8 stage (B).

As compared to R8 stage R6 stage recorded high amount of copper content in all the genotypes.

In R6 stage copper content ranged from 0.81 mg/100gm (IC-118400) to 1.91 (AMS-73). However, in R8 stage it ranged from 0.65 mg/100gm (TAMS-98-21) to 0.92mg/ 100gm (NRC-2). It is shown Figure 1 (C).

Zinc content was also found to be more in the R6 stage than in R8 stage In all the genotypes studied the range of zinc in R6 stage varied from 3.83 mg/100gm (AGS-406) to 5.00mg/100gm (AMS-73) whereas, in R8 stage the highest zinc content was recorded in the genotype AMS-73 (4.75mg/100gm) and the lowest zinc content was recorded in Karune and EC-251411 (3.56) genotype respectively. It is depicted in Figure 1 D. The obtained from micronutrient analysis were similar to the finding of Keatinge *et al.,* (2011), Dubois *et al.,* (1956).

Comparatively manganese content was found high in R6 stage than the R8 stage.

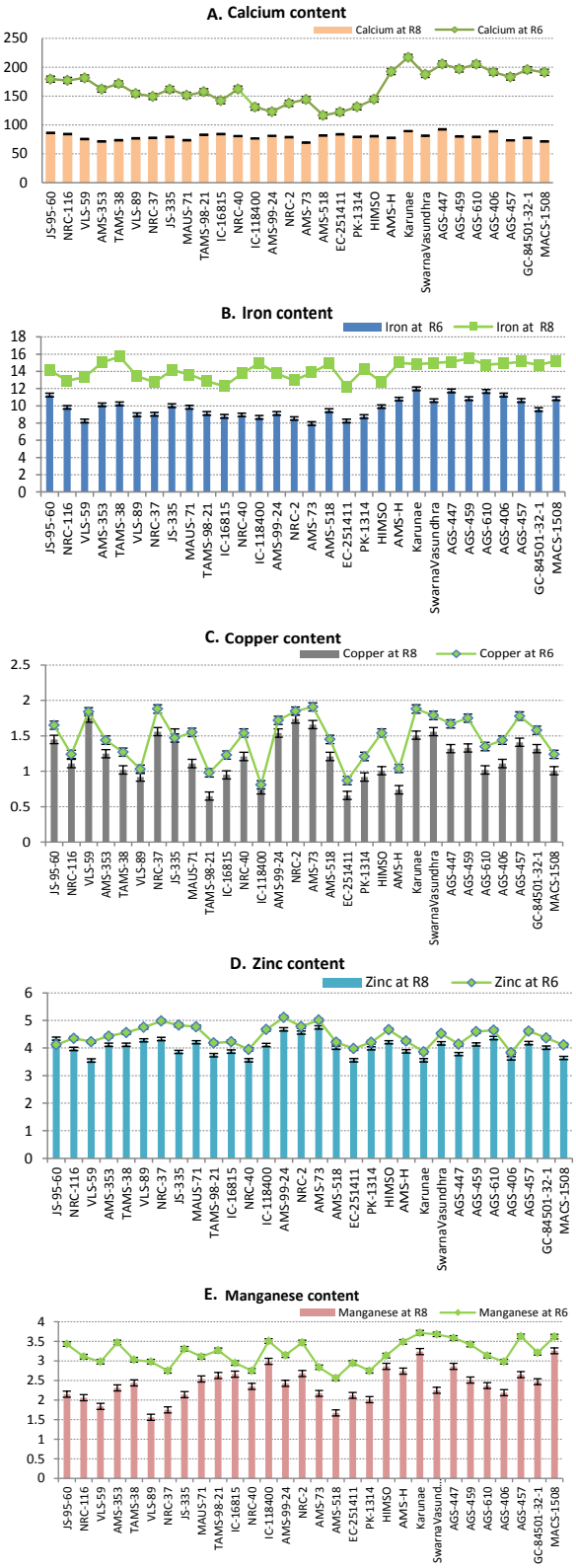

Fig. 1. Estimated micronutrients (mg/100g) from 30 soybean genotypes at green (R6) and mature (R8) stages

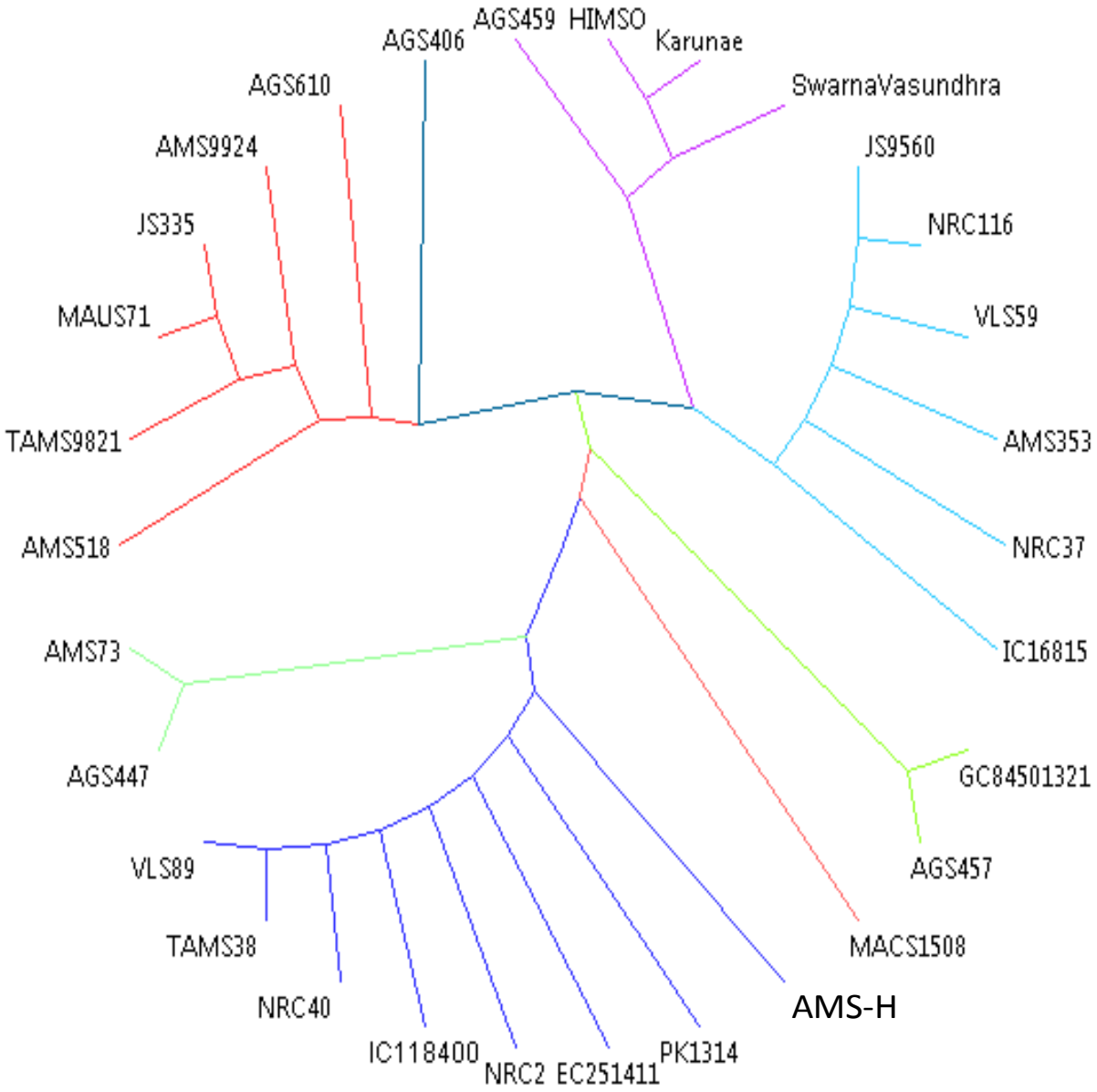

Fig. 2. UPGMA dendrogram based on Nei and Li (1979) genetic distance, summarizing the data on differentiation among 30 soybean genotypes according to DNA SCoT profiling

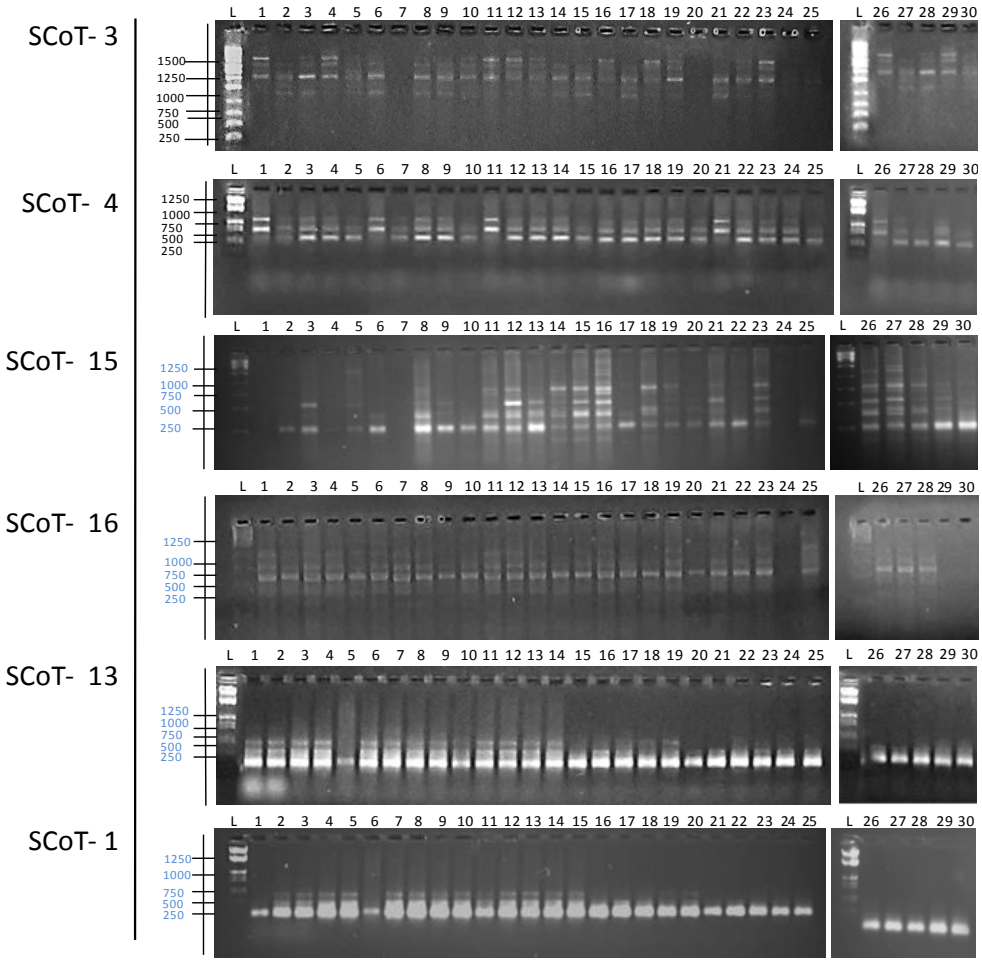

Fig. 3. Characterization of genetic diversity of soybean genotypes using SCoT primer

Table.1 List of soybean genotypes and their source

S No.	Genotype	Type	Source	SN	Genotypes	Type	Source
1	JS 95-60	Grain	Dr. PDKV Akola, MS	16	JS-335	Grain	JNKV, Jabalpur, MP
2	NRC-116	Grain	NRC Indore	17	MAUS-71	Grain	VNMKV, Parbhani, MS
3	NRC-40	Grain	Dr. PDKV Akola, MS	18	MACS-1508	Grain	ARI, Pune, MS
4	NRC-2	Grain	Dr. PDKV Akola, MS	19	AGS-457	Vegetable	AVRDC, Hyderabad, AP
5	NRC-37	Grain	Dr. PDKV Akola, MS	20	SwarnaVasundhara	Vegetable	AVRDC, Hyderabad, AP
6	AMS-99-24	Mutant	Dr. PDKV Akola, MS	21	AGS-459	Vegetable	AVRDC, Hyderabad, AP
7	AMS-353	Mutant	Dr. PDKV Akola, MS	22	GC-84501-32-1	Vegetable	AVRDC, Hyderabad, AP
8	AMS-518	Mutant	Dr. PDKV Akola, MS	23	HIMSO	Vegetable	RRC Amravati, MS
9	AMS-73	Mutant	Dr. PDKV Akola, MS	24	AGS-447	Vegetable	AVRDC, Hyderabad, AP
10	PK 1314	Grain	Dr. PDKV Akola, MS	25	AGS-610	Vegetable	AVRDC, Hyderabad, AP
11	EC-251411	Grain	Dr. PDKV Akola, MS	26	AGS-406	Vegetable	AVRDC, Hyderabad, AP
12	TAMS-38	Grain	RRC Amravati, MS	27	VLS-59	Vegetable	Dr. PDKV Akola, MS
13	TAMS 98-21	Grain	Dr. PDKV Akola, MS	28	VLS-89	Vegetable	Dr. PDKV Akola, MS
14	IC-16815	Grain	Dr. PDKV Akola, MS	29	HARA SOYA	Vegetable	
15	IC-118400	Grain	Dr. PDKV Akola, MS	30	KARUNE	Vegetable	Bangalore

Table.2 Analysis of variance for mean sum of squares for various characters in vegetable and grain type soybean genotypes

Source	Df	Days to 50%flowe-ring	Days to maturity	Number of green pod/plant	Plant height (cm)	100 beans weight (g)	100 mature seed weight (g)	Leaf area index	Photosynthetic efficiency	Chlorophyll content	Protein content (%)	Oil content (%)	Number of branches per palnt	No of seeds per pod	Sugar Content (mg/g)	Green pod yield/ plant (g)
Replication	2	0.28	2.54	3.84	8.25	0.78	242.17	6640.43	0.0011	11.98	3.46	0.16	0.14	0.43	0.24	1.81
Genotypes	29	18.59**	118.31**	182.19**	155.86**	240.18**	193.96**	21.29**	0.01**	38.56**	26.21**	3.66**	31.005**	0.76**	48.52**	340.83**
Error	58	3.05	1.13	7.78	3.40	0.75	3.70	23.10	0.0086	9.90	1.10	0.61	0.98	0.375	0.120	5.66

Note: **Significance at 1% level

Table.3 Cluster analysis of soybean genotypes derived from ScoT profiling

Group	Cluster	Genotypes
1	C1	JS-95-60, NRC-116, VLS-59, AMS-353, TAMS-38, JS-335, NRC-37, MAUS-71, TAMS-9821, IC-16815, NRC-40, IC-118400, NRC-2, AMS-99-24
2	C2	VLS-89, AMS-73, AMS-518, EC-251411, PK1314, Himso-1685, Karunae, SwarnaVasundhara, AGS-610, AGS-406, AGS-457, MACS-1508, GC-84501-32-1
3	C3	AMS-H, AGS-447, AGS-459

Table.4 Identification of promising genotypes

SN	Genotypes	Salient features														
		Number of primary branches	Oil content (%)	Chlorophyll Content	100 bean weight (gm)	Sugar content (mg/g)	Protein content (%)	Green pod yield/plant (g)	Calcium content at R6 (mg/100g)	Iron content at R6 (mg/100g)	Zinc content at R6 (mg/100g)	Copper content at R6 (mg/100g)	Manganese content at R6 (mg/100g)	Taste	Aroma	Overall acceptability
1	AGS-447	13.66	17.88	43.43	38.84	31.00	41.38	61.14	205.5	11.75	4.14	1.67	3.59	sweetness	present	good
2	Swarna Vasundhara	13.13	17.8 7	41.74	31.95	32.90	41.82	55.73	187.6	10.61	4.52	1.79	3.68	Sweetness	present	Good
3	AGS-459	16.33	18.21	49.28	30.76	31.87	41.56	54.73	197.2	10.83	4.59	1.75	3.42	Sweetness	present	Good
4	Karunae	15.33	17.17	44.73	43.44	38.52	42.13	52.98	217.3	11.98	3.86	1.88	3.72	Sweetness	absent	Good
5	HIMSO-1685(C)	14.66	19.27	37.88	24.79	28.14	41.66	48.63	144.7	9.92	4.66	1.54	3.14	beaninesss	absent	Poor
6	JS-335(C)	13.66	20.64	37.18	18.62	24.93	34.27	46.83	161.8	10.03	4.82	1.47	3.31	Nuttiness	Absent	Poor

Table.5 List of SCoT markers with their sequences

Sr. No.	Primer	Sequence (5′-3′)	%GC	Total amplicons	Monomorphic allele	Polymorphic allele	Percent polymorphism	PIC value
1	SCoT-1	CAACAATGGCTACCACCA	50	3	1	2	66	0.27
2	SCoT-2	CAACAATGGCTACCACCC	56	4	1	3	75	0.59
3	SCoT-3	CAACAATGGCTACCACCG	56	4	0	4	100	0.62
4	SCoT-4	CAACAATGGCTACCACCT	50	5	0	5	100	0.59
5	SCoT-5	CAACAATGGCTACCACGA	50	4	1	3	75	0.70
6	SCot-6	CAACAATGGCTACCACGC	56	1	1	0	0	0.00
7	SCoT-7	CAACAATGGCTACCACGG	56	2	1	1	50	0.46
8	SCoT-8	CAACAATGGCTACCACGT	50	2	1	1	50	0.37
9	SCoT-9	CAACAATGGCTACCAGCA	50	1	1	0	0	0
10	SCoT-10	CAACAATGGCTACCAGCC	56	3	1	2	66	0.44
11	SCoT-11	AAGCAATGGCTACCACCA	50	3	1	2	66	0.44
12	SCoT-12	ACGACATGGCGACCAACG	61	3	1	2	66	0.44
13	SCoT-13	ACGACATGGCGACCATCG	61	4	1	3	75	0.69
14	SCoT-14	ACGACATGGCGACCACGC	67	1	1	0	0	0
15	SCoT-15	ACGACATGGCGACCGCGA	67	3	1	2	66	0.48
16	SCoT-16	ACCATGGCTACCACCGAC	56	2	1	1	50	0.44
17	SCoT-17	ACCATGGCTACCACCGAG	61	3	1	2	66	0.44
18	SCoT-18	ACCATGGCTACCACCGCC	67	4	1	3	75	0.49
19	SCoT-19	ACCATGGCTACCACCGGC	67	3	1	2	66	0.32
20	SCoT-20	ACCATGGCTACCACCGCG	67	3	1	2	66	0.44
	Total			58	18	40	1178	8.22
	Average			2.9	0.9	2	58.9	0.41

Table.6 Mean and range values for morphological traits for each cluster

	Traits	Cluster I (14 genotype)		Cluster II (13 genotype)		Cluster III (2genotype)	
		Mean	Range	Mean	Range	Mean	Range
1.	**Days to 50% flowering**	41.66	39.66-46.33	39.862	37-44.66	38.33	34.66-42.66
2.	**Days to maturity**	93.92	89.00-103.66	89.662	81.66-99.66	80.77	77.33-83.66
3.	**Plant height (cm)**	41.39	32.60-50.45	39.65	31-53.33	34.56	25.55-39.96
4.	**Number of branches**	12.23	8.66-16.33	15.795	11.33-21.66	14.77	13.66-16.33
5.	**Number of green pod/ plant**	47.15	36.33-56.16	48.527	39.2-59.43	41.79	36.46-52.2
6.	**Number of seeds/ pod**	3.35	2.66-3.66	3.295	2.66-3.66	3.22	2.66-3.66
7.	**100 bean weight (gm)**	16.93	14.03-27.68	25.531	13.86-43.44	31.22	24.04-38.84
8.	**100 mature seed weight**	11.94	9.33-20.36	19.678	9.57-35.71	24.07	18.33-31.01
9.	**Leaf area index**	24.46	18.77-27.48	22.781	19.04-35.71	23.86	22.91-25.23
10.	**Photosynthetic efficiency**	0.69	0.61-0.74	0.721	0.62-0.81	0.73	0.69-0.8
11.	**Chlorophyll content**	41.13	34.87-47.62	42.145	38.4-44.84	44.29	40.16-49.28
12.	**Protein content%**	36.26	33.7-39.32	38.513	33.01-41.8	41.59	41.38-41.82
13.	**Oil content**	20.49	20.15-20.87	19.274	17.17-20.63	18.66	17.88-19.9
14.	**Sugar content%**	27.49	24.93-30.97	29.047	19.91-38.52	31.92	31-32.9
15.	**Green pod yield/ plant (g)**	48.60	34.49-55.16	51.016	32.6-58.63	49.85	33.68-61.14

Table.7 Qualitative characteristics of soybean genotypes

Genotype	Texture	Taste	Aroma	Over all acceptability
JS 95-60	Extremely resistant	Nuttiness	Absent	Poor
NRC-116	Extremely resistant	Nuttiness	Absent	Poor
VLS-59	Extremely resistant	Beaniness	Absent	Poor
AMS-353	Extremely resistant	Beaniness	Absent	Poor
TAMS-38	Extremely resistant	Beaniness	Absent	Poor
VLS-89	Extremely resistant	Beaniness	Absent	Poor
NRC-37	Extremely resistant	Nuttiness	Absent	Poor
JS-335	Extremely resistant	Nuttiness	Absent	Poor
MAUS-71	Extremely resistant	Beaniness	Absent	Poor
TAMS-98-21	Extremely resistant	Beaniness	Absent	Poor
IC-16815	Extremely resistant	Nuttiness	Absent	Poor
NRC-40	Extremely resistant	Nuttiness	Absent	Poor
IC-118400	Extremely resistant	Nuttiness	Absent	Poor
AMS-99-24	Extremely resistant	Beaniness	Absent	Poor
NRC-2	Extremely resistant	Nuttiness	Absent	Poor
AMS-73	Extremely resistant	Nuttiness	Absent	Poor
AMS-518	Extremely resistant	Beaniness	Absent	Poor
EC-251411	Extremely resistant	Nuttiness	Absent	Poor
PK-1314	Extremely resistant	Nuttiness	Absent	Poor
HIMSO-1685	Extremely resistant	Beaniness	Absent	Poor
AMS-H	Not resistant	Sweetness	Present	Good
Karune	Not resistant	Sweetness	Present	Good
SwarnaVasundhara	Not resistant	Sweetness	Present	Good
AGS-447	Not resistant	Sweetness	Present	Good
AGS-459	Not resistant	Sweetness	Present	Good
AGS-610	Not resistant	Sweetness	Absent	Good
AGS-406	Not resistant	Beaniness	Absent	Good
AGS-457	Not resistant	Sweetness	Present	Good
GC-84501-32-1	Extremely resistant	Nuttiness	Absent	Moderately good
MACS-1508	Not resistant	Nuttiness	Absent	Good

Note: *Descriptor for soybean, IBPGR/84/183, Rome (1984)

The highest manganese content was recorded in Karune (3.72 mg/100 gm) followed by Swarna Vasundhara (3.68 mg/100gm) and AGS-447 (3.59mg/100gm) in R6 stage. However, in R8 stage, the manganese content was ranged from 1.56 mg to 3.26mg/100gm. The highest manganese content in R8 was recorded in MACS-1508 (3.26mg/100gm) followed by Karune (3.24mg/100gm) However the genotype VLS-89 had lowest manganese content (1.56mg/100gm). It is depicted in Figure 1 (E).

Promising genotypes through present investigation

The present study illustrates a wide range of variability for all the characters. The estimates of phenotypic coefficients of variation were higher than that of genotypic coefficients of variation for all characters under study. Among thirty genotypes evaluated AMS-73, recorded highest green pod yield compared to the vegetable check Himso-1685 and grain check, JS-335.Genotype AGS-447, Swarna Vasundhara, Karune, AGS-459 recorded

significant superiority for various characters viz., number of primary branches per plant, number of pods per plant, chlorophyll content and 100 bean weight as compared to that of best check, Himso-1685 and JS-335 (Table 4). Concerned with qualitative traits, the genotype Karune exhibited highest sugar and protein content compared to check genotypes.

The micronutrients viz., calcium, iron, zinc, copper and manganese contents were significantly different in all the genotypes at R6 and R8 stage. The content of calcium, zinc, copper and manganese was higher at R6 than the R8 stage in all the genotypes. However, iron content was found high during R8 stage. The genotype, Karunae showed high content of calcium, iron, copper and manganese at R6 followed by AGS-447 for calcium and iron content.

Molecular diversity analysis

Twenty SCoT primer pairs were selected for molecular characterization of 30 soybean genotypes. Among these primers, 17 were found polymorphic and three were monomorphic (Fig. 3). Amplification of twenty SCoT markers produced 58 amplicons. A total of 40 alleles were detected polymorphic with an average of 2.0 alleles. The total amplicons including monomorphic and polymorphic, percent polymorphism and the PIC values of different soybean genotypes for twenty SCoT markers are depicted markers were enlisted in Table 5.

The molecular profiling were assessed using twenty SCoT primers with 58.9% polymorphism. The Polymorphism Information Content PIC of 20 SCoT loci ranged from 0.27 (SCoT 1) to 0.70 (SCoT 5) with an average value of 0.41. The highest PIC value was obtained for SCoT 5 (0.70) followed by SCoT 13 (0.69), SCoT 3 (0.62), SCoT 2 and SCoT 4 (0.59), SCoT 18 (0.49), SCoT 15 (0.48), SCoT 7(0.46), SCoT 10, SCoT 11, SCoT12, SCoT 16, SCoT 17, and SCoT 20 (0.44), SCoT 8 (0.37), SCoT 19 (0.32) and the least in SCoT 1 (0.27) as shown in Figure 3.

Cluster analysis

There were three clusters (Table 3), cluster I mainly represent 14 genotypes which focused the different morphological, biochemical and micronutrients attribute high oil and leaf area index. A part from this it highlighted the significant increase in photosynthesis efficiency, chlorophyll content and micronutrient; this cluster mostly involved the genotypes having no aroma and has poor acceptability and low in protein content. Cluster II represent 13 genotypes for the morphological quality base and micronutrient attributes along with same biochemical parameters. Mostly they focused maximum yield per content and presence of high content of micronutrient. This clusters involved most of the genotype having beaniness taste, presence of aroma and has overall good acceptability, whereas, cluster III included three genotypes representative for the presence of high sugar and protein content whereas, none of the genotype where found to be high in the oil content; most of the genotype has shown early flowering and maturity along with dwarf height. Micronutrient study revealed that the cluster III genotypes were mostly deficient in manganese. The UPGMA-based dendrogram obtained from the binary data of the samples analyzed (Fig. 2). This pooled data analysis grouped the 30 soybean genotypes into three clusters (s) (Table 3).

The genetic similarity were estimated by using Sneath and Sokel coefficient [20].The dice similarity among the accessions ranged from 0.772 to 0.949 similarity coefficient with an average of 0.860. There was strong similarity between the accessions in the clusters A and B. Lowest similarities were found between single individuals in the clusters C.

The range of genetic distance based on the morphological traits was on average lower

than SCoT 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 (Omondi *et al.*, 2013), 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, 2002. 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.

In review, altogether the information generated from this study shows significant variation among the soybean accessions their mutants and vegetable type genotypes which may be useful to breeders deciding breeding targets for the development of soybean and willing to use genetically diverse genotypes in soybean improvement program and also to trace the genotypes having high micro nutritional traits to overcome on the malnutrition and the disorders arises from them.

Among the genotypes studied, Karunae, Swarna Vasundhara, AGS-447 and AGS-459 are having high protein content (%) and sugar content (mg/g) thus these genotypes can be used as source for developing varieties with high protein (%), sugar, calcium, iron, zinc and manganese content. In review the morphological material and molecular related significant variation among the soybean genotypes studied at R6 and R8 stage. The information generated could be exploited in selecting diverse parents in breeding programme and in maintaining genetic variation in germplasm and development bio-fortified soybean genotype.

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Conflict of interest disclosure

There is no conflict of interest to disclose.

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