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PHENOTYPIC CHARACTERIZATION OF VEGETABLE SOYBEAN ((*GLYCINE MAX* (L.)) GERMPLASM ACCESSIONS

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ABSTRACT

Vegetable soybean, commonly known as edamame, is a special type of soybean. While fresh pods are economic harvestable products, fresh immature beans are consumed as vegetables. Breeding for vegetable soybean is still in its infancy. Exploiting natural genetic variability is a short-term strategy to meet the immediate cultivar needs of farmers. Based on this premise, 116 vegetable soybean germplasm accessions, along with two check varieties, were evaluated for fresh pod and seed yield and their component traits during the 2019 *kharif* season. Based on fresh pod yield and days to 50 % flowering a total of 18 high-yielding genotypes selected from these 116 germplasm accessions were evaluated for both fresh bean and dry seed yield in replicated trials during the 2019-20 rabi season. The results indicated substantial variability, as evidenced by the standardized range and phenotypic coefficient of variance (PCV). Fresh bean yield of the genotypes was higher during the rainy season than during the post-rainy season. Most of the traits under study performed similarly across the two seasons, as indicated by the significant correlation coefficients among them. These results are discussed in relation to strategies for breeding vegetable soybeans.

Keywords: Vegetable soybean (*Glycine max* (L.) Merrill), Phenotypic Coefficient of Variance (PCV), Germplasm accessions

Introduction

Vegetable soybean (*Glycine max* (L.) Merrill), commonly known as "edamame" in Japan, "mao dou" in China, and "Poot kong" in Korea, is a highly nutritious leguminous crop. Rich in protein (13% at the R6 stage), iron, and calcium, this crop plays a crucial role in combating chronic malnutrition worldwide (Nair *et al*., 2023). Although it is a minor crop globally, edamame is widely popular in East Asia, where it is consumed as a snack, vegetable, and in soups and sweets. Beyond East Asia, the crop is gaining attention in other regions due to its high nutritional value and appealing taste.

Vegetable soybeans are characterized by large seed size (weighing between 60-75 g per 100 seeds fresh weight), a sweeter taste, and a lack of the beany flavor typical of grain soybeans. These traits distinguish them from other soybean varieties. Harvesting time is main critical factor in determining consumer acceptability and marketability. Marketable and consumable part in vegetable soybean is pods and fresh seeds, respectively. The optimum time to harvest vegetable soybean is when the pods are still green, immature and tight i.e., when 85 per cent of pod filling is attained. At this stage, the pods are marketable as fresh, frozen, or canned products.

One of the crop's notable advantages is its low input requirement and short growing period, typically 65-75 days to harvest. Its ability to fix atmospheric nitrogen in association with Rhizobia makes it a valuable addition to various cropping systems, enhancing agricultural sustainability. After harvesting the pods, the remaining green plant stalks and leaves can be utilized as livestock feed or incorporated into the soil as green manure, further enriching soil health.

Given the nutritional benefits, increasing the production of vegetable soybeans is essential, especially in regions facing malnutrition. Breeding programs aimed at improving yield and disease resistance could benefit from the assessment and exploitation of existing genetic variability within the crop. Germplasm resources, which contain natural variability, are invaluable for selecting superior genotypes and developing varieties with improved traits through hybridization. Hence, the present study framed to assess the variability in the vegetable germplasm cultivars and to check their performance across two seasons.

Material and Methods

The material for the present study comprises of 116 vegetable type soybean germplasm accessions procured from the Indian Institute of Soybean Research, Indore, Madhya Pradesh and four checks (two vegetable type check *i.e.,* Karune and Hara soya and two grain type checks *i.e.,* JS-335 and KBS-23). The experiment was conducted in two seasons *i.e.* during *kharif* and *rabi*, 2019-20. During *kharif* one set of 116 vegetable type soybean germplasm accessions were sown in augmented design in two rows with row length of 4m and a spacing of 40cm × 10 cm. Based on fresh pod yield and days to 50% flowering top 18 high yielding vegetable soybean germplasm accessions were identified. During *rabi* 2019-20, the experiment was conducted using randomized complete block design (RCBD) with three replications to assess the grain and vegetable yield of high yielding vegetable type soybean germplasm accessions.

Record of observation: Observation on quantitative traits and qualitative traits (Table 1) were recorded on five randomly picked plant. During *kharif* 2019-20, observation on nine quantitative traits viz., plant height, days to 50% flowering, primary branches plant^{-1}, number of seeds pod^{-1}, days to R6 stage or physiological maturity, number of fresh pods plant^{-1}, fresh pod yield plant^{-1}, fresh seed yield plant^{-1} and 100 fresh seed weight. During *rabi* 2019-20, observation on eleven quantitative traits viz., plant height, days to 50% flowering, primary branches plant^{-1}, number of seeds pod^{-1}, days to R6 stage or physiological maturity, number of fresh pods plant^{-1}, fresh pod yield plant^{-1}, fresh seed yield plant^{-1}, 100 fresh seed weight, dry grain yield plant^{-1} and 100 dry seed yield.

Table 1: Protocol followed to record data on qualitative traits in vegetable soybean accessions

Sl. No.	Characteristics	Stage of observation	Note	Phenotype
1	Flower color	At Flowering	1	White
			2	Purple
2	Hilum color	After harvesting	1	Yellow
			2	Grey
			3	Brown
			4	Black
			5	variegated
3	Pubescence color	At maturity	1	Yellow
			2	Grey
			3	Brown
			4	Black
			5	Variegated
4	Fresh pod color*	At R6 stage	1	Light green
			2	Green
5	Fresh seed : Size *	After harvest	1	Large
			2	Medium
			3	Small
6	Fresh seed : Color*	After harvest	1	Green
			2	Light green
			3	Brown
			4	Greenish brown
7	Dry seed: Color	At maturity	2	Yellow
			4	Yellow green
			6	Green
			8	Black
8	Seed: Shape	After harvest	1	Spherical
			2	Elliptical
9	Seed: Luster	After harvest	1	Shiny
			9	Dull

Statistical analysis

The mean of five plants calculated was used for statistical analysis and the following descriptive statistics and genetic parameters were estimated using R software *Ver*., 4.00, 4.04. The analysis of variance was carried by following the augmented design suggested by Federer (1956) and RCBD design suggested by Panse and Sukhatme (1967).

Estimation of genetic parameters

Genetic parameters like GCV (Genotypic Coefficient of Variance), PCV (Phenotypic Coefficient of Variance), h^2_{bs} (Broad sense heritability) was estimated for all the 11 quantitative traits under consideration.

Estimation of correlation coefficient

Phenotypic correlation coefficients were worked out for all possible combinations of characters using the following formula given by Al-Jibouri *et al.* (1958).

Phenotypic correlation coefficient

$$r_{xy} = \frac{CoV_{xy}}{(\sqrt{Var_x} \times \sqrt{Var_y}}$$

Where,

r_{xy} - Phenotypic correlation coefficient between the characters x and y

CoV_{xy} - Phenotypic covariance between characters x and y

Var_x - Phenotypic variance of the character x

Var_y - Phenotypic variance of the character y

Results and Discussion

Genetic variability for fresh pod and seed yield

ANOVA mainly divides total variability into components and also it acts as a pre-requisite step for detecting the presence of genetic variability in the experimental material. ANOVA was performed for yield and its attributing traits in vegetable type soybean germplasm accessions. The mean sum of squares of 116 germplasm accessions were found significant for all the traits under study indicated the presence of variability in the germplasm accessions (Table-2). Further significant difference was observed for sum of squares due to due to checks *vs* germplasm accessions in all 116 vegetable type germplasm accessions for all the traits. Which indicated the presence of large amount of genetic variability for the traits studied and hence further analysis of experimental material can be carried out. Similarly, there was substantial amount of variation was observed in 18 top high yielding individuals as indicated by the significant mean sum of squares for all the traits (Table-3&-4). The above results were in agreement with the findings of Satpute et. al. (2016) Qureshi *et al.* (2006), Mebrahtu *et al.* (2006) and Iqbal *et al.* (2010 & 2017).

Table 2: Analysis of variance of vegetable soybean germplasm accessions for quantitative traits

Sources of variation	Degrees of freedom	Days to 50 % flowering	Days to physiological maturity	Fresh pods plant^{-1}	Plant height (cm)	Fresh pod weight plant^{-1} (g)
Checks + germplasm accessions	117	26.81 **	211.88 **	303.33**	64.6 **	1063.37 **
Check	01	16.96 **	7.54 **	1447.54**	11.91	22857.18 **
Germplasm accessions vs. Check	01	20.65 **	5547.37 **	748.58 **	521.58 **	12954.17 **
Germplasm accessions	115	26.95 **	167.26 **	289.5 **	61.09 **	770.46 **
Blocks	12	0.43	33.04 **	4.41	8.74	147.06
Error	12	0.38	0.29	3.14	3.55	99.8

* P <= 0.05; ** P <= 0.01

Sources of variation	Degrees of freedom	Primary branches plant^{-1}	seeds pod^{-1}	Fresh seed weight plant^{-1} (g)	Fresh 100 seed weight (g)
Checks + germplasm accessions	117	1.42 **	0.24	287 **	397.37 **
Check	01	4.4 **	2.22 **	5588.98 **	11327.82 **
Germplasm accessions vs. Check	01	9.27 **	5.51 **	2783.86 **	63.93 **
Germplasm accessions	115	1.33 **	0.17	219.18 **	305.23 **
Blocks	12	0.08	0.18	51.11	0.98 *
Error	12	0.15	0.16	25.66	0.26

* P <= 0.05; ** P <= 0.01

Table 3: Analysis of variance of selected vegetable soybean germplasm accessions evaluated for dry seed yield and yield related traits

Sources of variation	Degrees of freedom	Fresh pods plant^{-1}	Fresh seeds plant^{-1}	Plant height (cm)	Primary branches plant^{-1}	Dry seed weight plant^{-1} (g)	100 dry seed weight (g)	Days to 50 % flowering
Replication	2	22.74	0.06	1.33	0.37	0.75	0.07	0.11
Genotypes	21	479.62**	0.24**	173.69**	1.14**	20.33**	85.09**	94.03**
Error	42	10.76	0.04	3.01	0.15	1.61	0.75	0.04

* P <= 0.05; ** P <= 0.01

Table 4: Analysis of variance of selected vegetable soybean germplasm accessions evaluated for vegetable yield and yield related traits.

Sources of variation	Degrees of freedom	Days to R6 stage	Fresh pod weight plant^{-1} (g)	Fresh seed weight plant^{-1} (g)	100 fresh seed weight (g)
Replication	2	0.017	33.24	8.2	6.08
Genotypes	19	421.45**	785.16**	175.83**	603.15**
Error	38	0.02	40.23	13.5	8.62

* P <= 0.05; ** P <= 0.01

Qualitative traits

As qualitative traits have simple inheritance (oligogenic), stable expression, selectively neutral, can be easily scored, they serve as diagnostic descriptors for germplasm accessions (Smith and Smith, 1992). Therefore, they are very useful for identification, to avoid mistakes in labeling and to minimize duplication in the germplasm. A high level of polymorphism was observed and observation was recorded among the accessions for the qualitative traits.

One hundred and sixteen vegetable type germplasm accessions along with two checks were characterized in to different status or groups with respect to flower color, presence or absence of pubescence on pod and leaf, pubescence color, fresh pod color, fresh seed color, fresh seed size during *kharif* 2019. Trait-wise groups of accessions and their *per cent* distribution are presented in Figure-1. Eighteen vegetable type germplasm accessions along with four checks for dry seed yield related traits were characterized in to different status or groups with respect to hilum color, dry seed color, dry seed shape, dry seed lusture during *rabi* 2019. Trait-wise groups of accessions and their *per cent* distribution are presented in Figure-2.

Floral, pubescence traits: In accessions the *per cent* distribution of purple flowers (62.71%) was more compared to white flowers (37.28%). About 99.15 *per cent* accessions possessed the pubescence on plant parts like leaves, pods, stem, where only one (0.84%) *i.e.* check karune did not possess pubescence on these parts. Grey pubescence (55.93%) was prominent followed by brown (35.59%), black (6.78%) and variegated (1.69%) among the accessions.

Fresh pod color: The accessions with light green pods (94.07%) were more frequent compared to those with green (5.08%) and dark green (0.85%) fresh pods.

Fresh seed traits: Like fresh pod color, light green fresh seed (86.44%) was also dominated the accessions followed by green (8.7%), brown (0.84%) and variegated (4.24%) fresh seeds. With respect to fresh seed size both large (46.61%) and medium (44.07%) sized seed accessions were prominent than smaller (9.32%) seed size.

The results observed in the present findings are in comparison with the reports made by Zeffa *et al.* (2019), Kandwal (2018) for traits like fresh pod color, flower color, pubescence color, seed coat color and hilum color.

Dry seed related traits: Brown hilum was mostly seen in most of germplasm accessions followed by yellow and black. Seeds of Most accessions were yellow and trace of accessions had green and black seeds, some had variegated seeds like blackish green. Most of the accession had elliptical seed shape with shiny lusture compare to spherical with dull lusture (Table 20). These results are in comparison with results of Kandwal (2018) for traits like fresh pod color, flower color, pubescence color, seed coat color and hilum color.

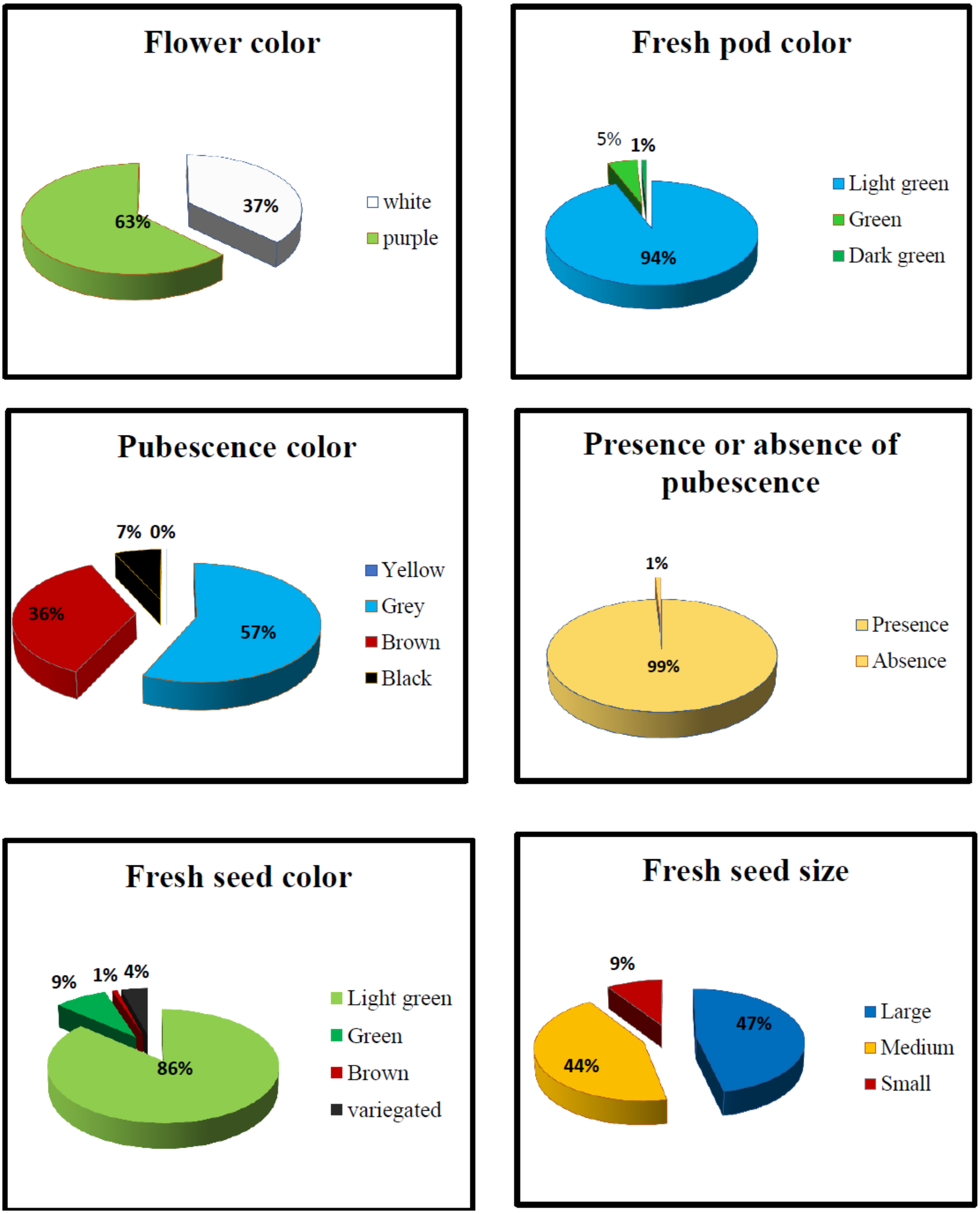

Fig. 1 : Variability for qualitative traits, their frequency and *per cent* distribution among vegetable type soybean germplasm accessions evaluated during 2019 rainy season.

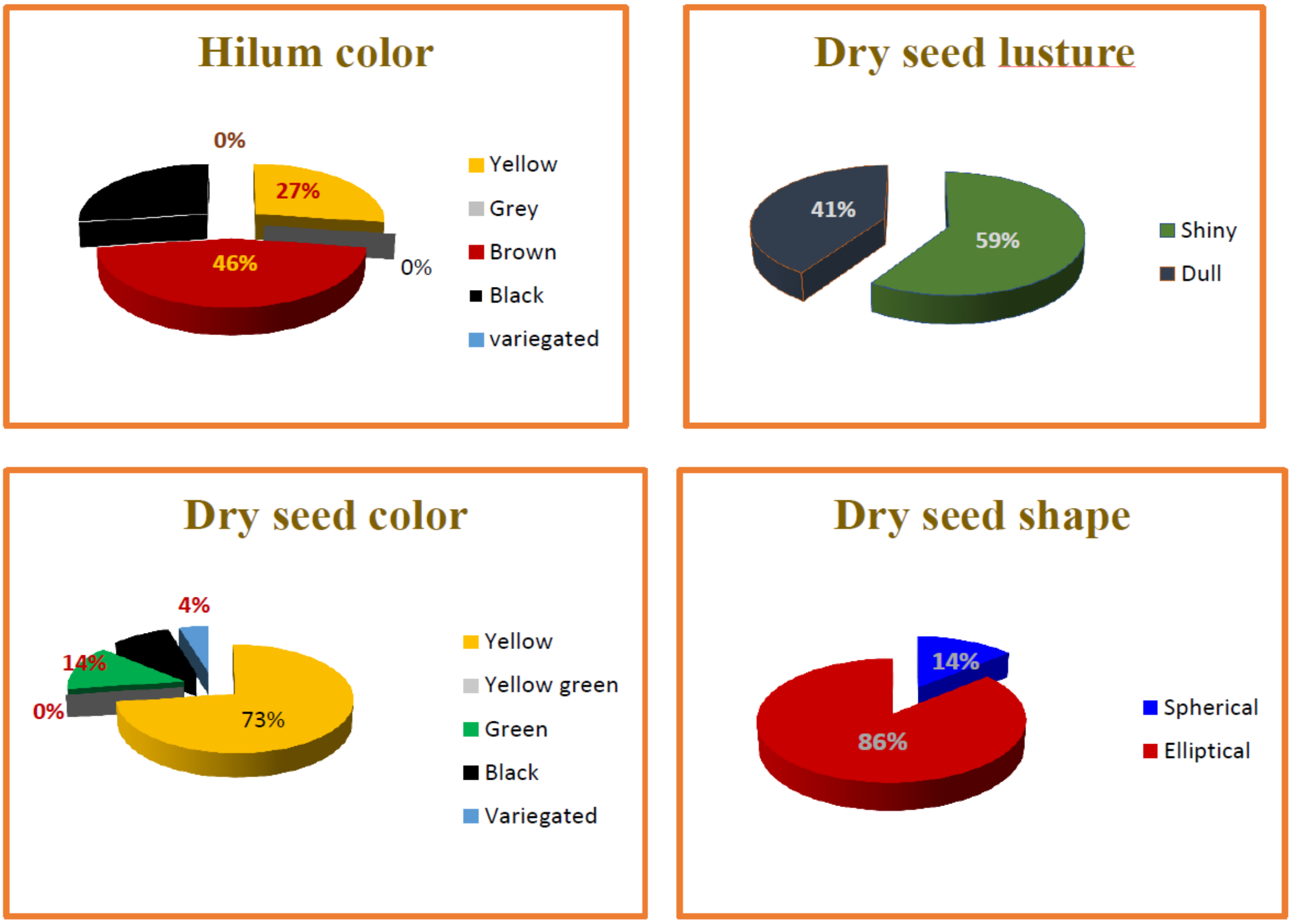

Fig. 2 : Variability for qualitative traits, their frequency and *per cent* distribution among selected vegetable type soybean germplasm accessions evaluated for dry seed yield during rabi 2019.

Variability study for quantitative traits

For any plant breeding programme, genetic variability is a prerequisite, hence selection of the desired genotype depends upon the amount of genetic variability available in the experimental material. Knowledge of genetic variability, heritability, helps to utilize the variability present in the population. The present investigation was carried out by utilizing the genetic variability in relation to breeding for high yield and its component traits in vegetable soybean.

The total variability present in the study is characterized into phenotypic and genotypic variance for all the traits under study. As each trait has different units of measurement, the degree of variability cannot be compared. Hence, estimates of standardized range which provides information about the presence of accessions with variable expression of the trait. However, standardized range as such does not reflect variability for the expression of traits in all the accessions. The estimate of co-efficient of variation is a measure that uses *per cent* of mean to express the variance is being used and the results obtained are expressed as PCV (phenotypic coefficient of variability), GCV (genotypic coefficient of variability) and heritability (h^2_{bs}).

The estimates of mean, range and standardized range for yield and its attributing traits in vegetable type soybean germplasm accessions are presented in Table-5 and Table-6.

The accessions were highly variable for most of the traits under study as stipulated by estimates of PCV (>20%) and the accessions were moderately variable (10.1% $\leq$ PCV $\geq$ 19.9%) for days to 50 *per cent* flowering, days to R6 stage or physiological maturity. Among all traits mentioned in Table-5, a wide difference between GCV and PCV was only observed for seeds pod^{-1} it indirectly indicates the higher influence of environment on the genes governing this character. Similarly, the top 18 high yielding individuals were highly variable for most of the traits under study except fresh seeds pod^{-1} which showed moderate level of variability (Table-6).

Table 5: Estimates of descriptive statistics and genetic variability parameters among vegetable type soybean germplasm accessions.

Traits	Mean ± SEm	Range		Standardized range	PCV (%)	GCV (%)	h^2_{bs} (%)
		Lowest	highest				
Plant height (cm)	28.6±0.69	72.20	14.6	1.93	27.33	26.52	94.19
Primary branches plant^{-1}	3.38±0.11	7.00	1.00	1.80	34.13	32.09	88.39
Fresh seeds pod^{-1}	2.19±0.05	3.90	1.40	1.16	19.09	6.31	10.94
Fresh pod weight plant^{-1} (g)	44.34±2.66	206.08	5.24	4.55	62.61	58.41	87.05
Fresh pods plant^{-1}	22.12±1.53	144.00	3.80	6.20	76.92	76.5	98.92
Fresh seed weight plant^{-1} (g)	22.68±1.44	105.06	4.48	4.36	65.28	61.34	88.29
Fresh 100 seed weight (g)	58.01±1.63	96.40	22.1	1.30	30.12	30.11	99.92
Days to 50 % flowering	35.15±0.47	60.00	23.00	1.02	14.77	14.66	98.6
Days to physiological maturity	75.46±1.20	142.00	65.00	1.05	17.15	17.13	99.83

Table 6: Estimates of descriptive statistics and genetic variability parameters for vegetable and dry seed yield and its component traits among vegetable type soybean germplasm accessions.

Traits	Mean ± SEm	Range		Standardized range	GCV (%)	PCV (%)	h^2_{bs} (%)
		Lowest	Highest				
Fresh pods plant^{-1}	32.10 ± 1.89	66.66	18.73	1.49	38.94	40.25	93.55
Fresh seeds pod^{-1}	2.27 ± 0.12	2.73	1.73	0.44	11.45	14.49	62.44
Plant height (cm)	27.99 ± 1.00	46.07	17.00	1.04	26.95	27.65	94.96
Primary branches plant^{-1}	3.37 ± 0.22	5.10	2.67	0.72	17.10	20.53	69.11
Dry seed weight (g)	7.34 ± 0.73	13.09	4.17	1.21	34.01	38.16	79.48
100 dry seed weight (g)	24.01 ± 0.50	33.20	14.70	0.77	22.08	22.37	97.40
Days to 50 % flowering	37.33 ± 0.35	48.00	31.00	0.46	14.10	15.10	99.86
Fresh pod weight plant^{-1} (g)	52.08 ± 3.66	82.83	28.72	1.04	30.25	14.61	99.99
Fresh seed weight plant^{-1} (g)	26.86 ± 2.12	43.73	18.17	0.95	27.38	32.61	86.06
100 fresh seed weight (g)	32.10 ± 1.89	66.66	18.73	1.49	38.94	30.60	80.04
Days to physiological maturity	81.12 ± 0.07	102.00	67.00	0.43	14.61	29.45	95.83

All the traits possessed higher broad sense heritability (> 60%) except fresh seeds pod^{-1} (10.94%). The estimates of GCV and broad sense heritability were higher for most of the traits under study and were moderate for days 50 *per cent* flowering, days to R6 stage. Similarly in top 18 high yielding accessions all the traits possessed higher broad sense heritability (> 60%). The estimates of GCV and broad sense heritability were higher for all traits except for fresh seeds plant^{-1}, days to 50 *per cent* flowering, primary branches plant^{-1} and days to physiological maturity where they possessed the moderate GCV. The higher GCV and broad sense heritability indirectly indicates the less influence of environment on the genes governing these characters. The above results are in comparable to the results obtained by Zeffa *et al.* (2019), Van der and Smit (2016), Vaijayanthi *et al.* (2016), Ramyashree *et al.* (2016), Mahbub *et al.* (2015), Reni *et al.* (2013), Rao *et al.* (2002), Shanmugasundaram *et al.* (1991), and Mbuvi (1995).

Comparative performance and association study of vegetable soybean germplasm accessions during *kharif* and *rabi* 2019.

The mean fresh pod weight plant^{-1} of all selected accessions was on par over two seasons except the accessions like EC 915900, EC 916016, and EC 892809, where the difference was observed for fresh pod weight plant^{-1} over two seasons. With respect to 100 fresh seed weight also, during both season the 100 fresh seed weight was on par except some accessions like EC 916019, EC 916004, EC 916013, EC 916016 and EC 916017, where there was huge difference was observed (Table-7).

The results of association studies between mean values of the selected vegetable lines for vegetable yield for nine traits over two seasons are described below and values of phenotypic correlation coefficient values are presented in Table-8. There was positive significant association between fresh number of pods plant^{-1} (0.92), plant height (0.66), days to 50 *per cent* flowering (0.58), fresh pod weight plant^{-1} (0.82), fresh seed weight plant^{-1} (0.7), primary branches plant^{-1} (0.55), 100 fresh seed weight (0.8) and positive non-significant association between days to first picking or R6 stage (0.45) in *kharif* with that of *rabi* season. Whereas traits, number of seeds pod^{-1} (-0.03) in *kharif* exhibited negative correlation with number of seeds pod^{-1} in *rabi*. This result is presented in the form of heat map (Figure-3).

Table 7: Comparative performance of vegetable soybean germplasm accessions during *kharif* and *rabi* 2019

Accessions	**Fresh pod weight plant^{-1} (g)**		**Fresh 100 seed weight (g)**	
	***Kharif* 2019**	***Rabi* 2019**	***Kharif* 2019**	***Rabi* 2019**
EC 916003	47.34	58.74	60.00	57.96
EC 916010	36.20	35.81	43.00	42.80
EC 916019	63.88	55.60	63.00	39.47
EC 916020	41.74	47.08	52.00	50.73
EC 916038	82.96	82.81	94.00	94.13
EC 916018	98.02	74.29	50.00	44.50
EC 915900	55.24	35.68	46.50	45.20
EC 916004	46.24	36.07	57.60	32.70
EC 916013	40.00	41.48	92.60	40.73
EC 915937	39.04	46.62	56.70	56.17
EC 916016	125.76	72.53	92.60	40.20
EC 916001	45.72	66.92	56.70	60.53
EC 915918	43.24	47.46	60.70	46.18
EC 915906	36.64	28.72	54.30	56.10
EC 915903	47.88	50.39	66.50	46.17
EC 915927	36.22	36.27	61.30	49.89
EC 916017	100.92	77.04	63.50	37.70
EC 892809	27.14	44.33	53.80	32.87
	Check			
Karune	107.21	65.70	78.10	66.63
Hara soy	38.90	38.17	35.42	35.80
SEm ±	2.66	3.66	1.63	1.70
CD @5%	12.7	10.48	0.65	4.85

Table 8: Estimates of phenotypic correlation coefficients between for vegetable yield and yield related traits of vegetable type soybean germplasm evaluated during rainy season and post rainy season.

	Association between rainy and post rainy season
Fresh pods plant^{-1}	0.92**
Fresh seeds pod^{-1}	-0.03
Plant height (cm)	0.66**
Primary branches plant^{-1}	0.55*
Fresh pod weight plant^{-1} (g)	0.82**
Fresh seed weight plant^{-1} (g)	0.70**
100 fresh seed weight (g)	0.80**
Days to 50 % flowering	0.58*
Days to physiological maturity	0.45

Fig. 3: Heat map depicting association between vegetable yield and yield related traits A. Fresh pods plant^{-1}, B. Fresh seeds pod^{-1}, C. Plant height D. Primary branches plant^{-1}, E. Fresh pod weight plant^{-1}, F. Fresh seed weight plant^{-1}, G. 100 fresh seed weight, H. Days to 50 *per cent* flowering, I. Days to physiological maturity in *kharif* and *rabi* of 2019

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