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Application of Morphological and Molecular Markers to Establish Distinctness in Few Genotypes of Yellow Sarson (*Brassica rapa* L. var. Yellow Sarson)

J BHAGAT[1], R PHUKAN[1], M BARUA[1], P SHARMA[1], AR BARUAH[2], RN SARMA[1] AND PK BARUA[1]*

[1]Department of Plant Breeding and Genetics, Assam Agricultural University, Jorhat, Assam-785013, India
[2]Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat, Assam-785013, India
*purna.barua@aau.ac.in

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ABSTRACT: Yellow sarson, a self-pollinated ecotype of rapeseed (*Brassica rapa* L.), is an important oilseed crop of India for use as an oilseed and as a condiment. This study was an attempt to characterize five genotypes of yellow sarson including three varieties (B9, YSH401, Pitambari) and two elite breeding lines (AAUJYS14-2 and AAUJYS15-2) by morphological characters as per DUS test guidelines and by molecular markers. Of the 24 morphological descriptors, genotypic differences were observed for 14 characters viz., leaf hairiness, leaf colour, leaf length, leaf width, plant height, main shoot length, days to flowering, days to maturity, siliqua angle, siliqua beak length, number of seeds per siliqua, siliqua density, 1000 seed weight and oil content. Mahalanobis D^2 analysis revealed that the genotypes were grouped individually into five equidistant solitary clusters. Molecular analysis was carried out by 20 random simple sequence repeats (SSR) primers, of which 12 primers turned out to be polymorphic. PIC value ranged from 0.2 to 0.96 with an average of 0.47. Broadly two clusters were formed based on Jaccard's coefficient of similarity. Cluster A was divided into two sub-clusters, AI (YSH401, AAUJYS15-2) and AII (B9). Cluster B included Pitambari and AAUJYS14-2. Thus, the use of molecular markers in conjunction with morphological descriptors could be useful for plant variety characterization and for establishing distinctness of varieties that enter the seed multiplication chain.

Keywords: Yellow sarson, distinctness, morphological descriptors, molecular markers

Oilseed brassica crops, generally referred to as rapeseed-mustard, are made up of five species of which *Brassica napus, B. rapa* and *B. juncea* are the most important [1]. Crop Brassicas are a group of six cultivated species, three diploids and three tetraploids, five are rapeseed-mustard and one is vegetable species. *Brassica rapa* has three oleifera ecotypes – Brown sarson, Yellow sarson and Toria. The two most important rapeseed crops in Assam and eastern India are Toria and Yellow sarson. Toria is the predominant oilseed crop in Assam. The major advantage of toria is its short duration, which makes it ideal to fit into the narrow sowing window in Assam. However, the plant type of toria is not favourable to high yield. Plants have weak and pithy stems, spreading branches, low siliqua density, wide siliqua angle, non-synchronous maturity, and shallow root system, which results in a low yield. Yellow sarson, on the other hand, has a deeper root system, a higher biological yield potential, more seeds per siliqua, erect branching habit.Although Yellow sarson is less grown, it is a potential oilseed crop; it fetches higher price due to its use as condiment besides for extraction of oil. Additionally, yellow sarson contains very high oil content i.e., 43-48% oil, whereas toria contains 42-44% and mustard seeds 38-40% oil only [2].

From a Seed Technological point of view, the seeds of *Brassica rapa* var. Yellow Sarson hold particular significance due to their characteristics that support various agricultural and industrial applications. The seeds of this crop have multiple uses making it highly relevant in both farming and seed industry. The high yield of oil from these seeds supports self-sufficiency in vegetable oil production in India and other South Asian regions where it is cultivated. Yellow Sarson seeds are known for their high viability and rapid germination rates, which are important for achieving a uniform crop stand. This is particularly valuable in regions that practice rainfed agriculture, where timely germination can influence the success of the crop. The seed traits of Yellow Sarson are also relevant in breeding programs since they are

correlated with traits like disease resistance, oil content, and adaptability to different climates. In nutshell, the seeds of *Brassica rapa* var. *Yellow Sarson* are significant in seed technology for their potential in oil production, breeding resilience, ensuring seed health, and enabling sustainable agriculture. These qualities make them a valuable resource in agriculture and the edible oil industry. Distinguishing varieties within the *Brassica rapa* species based solely on seed and other morphological traits is challenging due to several factors such as morphological similarities, overlapping seed size and weight, colour uniformity, influence of seed treatments, genetic overlap, need for molecular tools in addition to environmental seed traits. Therefore, distinguishing *Brassica rapa* varieties based on seed traits alone is unreliable due to these overlapping and variable characteristics. Instead, molecular markers and other advanced technologies are often needed to accurately identify and distinguish between varieties.

According to USDA Report June, 2023, [3] the global acreage of rapeseed-mustard is 41.81 million hectares with a production of 87.21 million metric tonnes. India is the third largest producer of rapeseed-mustard after Canada and China contributing around 15% of world's total vegetable oil production [4]. In India, the state of Rajasthan alone accounts for 41.44% of the total area and 45.03% of production. Assam is one of the important rapeseed-mustard producing states, which accounts for 1.81 lakh tons from 2.88 lakh hectares in 2021-22 [5]. However, there is substantial shortage of edible oilseeds; the domestic production can fulfill about 57% of demand.Consequently, huge quantities of edible oils are imported from other countries every year.

DUS (distinctness, uniformity, and stability) testing is required under the Protection of Plant Varieties and Farmers' Rights Act 2001 (PPV&FR Act, 2001) to protect plant varieties from unauthorized use. Varieties are compared typically by morphological markers. Besides plant variety protection, genotype characterization is important for germplasm documentation, conservation, quality seed production, use of genotypes in plant breeding and in variety release procedure. Yellow sarson though easily distinguishable from other rapeseed groups due to its yellow seed colour, but it is difficult to distinguish between yellow sarson varieties based on seed colour. In view of these, the present study was designed to characterize two newly developed elite genotypes and three varieties of yellow sarson by qualitative and quantitative morphological characteristics as per National DUS test guidelines and by SSR molecular markers to establish distinctness of the genotypes.

MATERIALS AND METHODS

The experiments were carried out on five genotypes of yellow sarson in the Instructional cum Research Farm of Assam Agricultural University, Jorhat during the Rabi season 2022-23. The experiment was laid out in randomized block design with 4 replications and plot size of 4 m x 2.8 m. Row to row spacing was 40 cm and plant to plant spacing within row was adjusted to about 10 cm by thinning operation at seedling stage. Fertilizers were applied @ 60:40:40 Kg/ha of N: P_2O_5: K_2O in the forms of urea, single super phosphate (SSP) and muriate of potash (MOP), respectively. Decomposed cowdung (2 t/ha), SSP, MOP and half of urea were applied as basal application. The other half of urea was applied in two top dressings at vegetative and flowering stages. Recommended package of practices was followed for raising the crop including plant protection measures (Package of Practices for Rabi Crops, Assam 2021). Observations were recorded on 10 random plants per plot for 26 characters as per the DUS test Guideline for Rapeseed (PPV&FR Authority, Govt. of India). Leaf hairiness was observed on the lower side of the leaf during bud formation to flowering stage. Leaf colour was observed by single observation of a group of plants during bud formation to flowering stage. Presence or absence of lobes on fully formed leaves was observed between bud formation and flower initiation stages in the middle portion of main shoots of sampled 10 plants. Leaf dentation of margin was observed between the bud formation and flower initiation stages on the upper one third of the leaf-blade. Colour of petals was observed by single observation in group of plants during flower initiation stage and when the few buds were open on terminal raceme. Siliqua density was observed on individual plants during maturation stage and was calculated as a ratio of the number of siliquae per cm of the main shoot. Siliqua angle was observed as the angle between the main shoot and the pedicels of siliquae at the bottom one-third of the main shoot. Siliqua texture was observed on number of individual plants during maturation stage. Seed colour was observed by a single observation of a group of plants after harvest of the crop. Number of leaf lobes was counted based on the presence or absence on individual plants during bud formation to flower initiation stages in the middle portion of main shoots

of the sampled plants. Leaf length was observed between bud formation and flower initiation stages on the sampled plants by using a centimeter scale. Petal length was observed at flower initiation stage when few buds were open on terminal raceme. Petal width was observed on sampled plants during flower initiation stage and when few buds were open on terminal raceme. Plant height was measured in cm by using a meter scale when all siliquae on terminal raceme were formed. Main shoot length (terminal raceme) was measured in cm when all siliquae on terminal raceme were formed. Siliqua length was at maturation stage and was measured from pedicel to beak at the bottom one-third of the main shoot. Siliqua beak length in cm was measured on randomly sampled 10 individual plants at maturation stage and was measured at the bottom one-third of the main shoot. While measuring the main shoot length, the number of siliquae in the main shoot of sampled 10 individual plants was counted. Average number of seeds per siliqua was counted on randomly sampled 10 individual plants from the middle portion of the main shoot at maturation stage. Time of flowering was observed by a single observation of group of plants of the plot at flower initiation stages when few buds were open on the terminal raceme. Number of days from the date of sowing to the date of 50% of the plants with at least one open flower in each plot was calculated as days to 50% flowering. Maturity period was recorded as days from date of sowing to when 75% of siliquae turned yellowish to brown, observed by a single observation of a group of plants of a plot. One thousand seeds were counted from seeds of sampled plants that were harvested, threshed, cleaned, and dried, and weighed in an electronic balance (Afcoset, model ER-200 A) in gram. Seed oil content of dry seeds of each plot was determined as percentage by nuclear magnetic resonance spectroscopy method at ICAR-Indian Institute of Oilseeds Research, Hyderabad. Seed yield per plot was recorded after threshing, cleaning, and drying and then converted to kg/ha.

The plot mean values of the sampled plots were subjected to analysis of variance by following the standard statistical procedure following Gomez and Gomez (1984) [6]. Mean values of quantitative characters were compared by critical difference at 5% and 1% levels of significance. Computations of data were done by using Microsoft Office Excel, 2007. D^2 analysis was done with the R package [8] and Bio tool [9]. The clustering of genotypes was done by Tocher's method [7]. A multivariate analysis of variance (MANOVA) was performed based on MANOVA procedure in Bio-tools package, based upon an adaptation of Wilks' statistics [10]. For molecular characterization, the five genotypes were assayed by 20 random SSR markers. For DNA extraction, young tender leaves were collected at the 3 - 4 leaf stage in the morning hours from the plants grown in pots. The extracted DNA was then purified, quantified, and used for amplification using SSR markers following published protocol [11]. Genomic DNA was extracted using SDS protocol[11] with slight modification. The DNA was loaded in 0.8% agarose gel stained with ethidium bromide and visualized under a gel documentation system to verify the quality of the DNA sample. Nanodrop readings for each DNA sample were collected to measure the quantity and quality of the genomic DNA extracted by analyzing their optical density (OD). For dilution of DNA sample, a suitable amount of sterilized distilled water was used for the final dilution of all the stock DNA solution to get 4 ng/µl of working solution and stored at -4°C until PCR amplification. For setting of PCR and SSR amplification, PCR reaction mixture of 10 µl for each sample contained 4 µl template DNA, 0.5 µl each of forward and reverse primer, 5 µl of 2X PCR master mix (Composition: 0.25U/ µl Taq DNA polymerase, 2X PCR buffer, 0.4 mM dNTPs, 3.2 Mm $MgCl_2$, 0.02% bromophenol blue) and 2.5 µl of double distilled water. To amplify the sample, the reagents were mixed thoroughly and then placed in a thermal cycler (PCR Gene AMP 2400, Applied Biosystems, USA). For agarose gel electrophoresis, agarose gel of 3.0% strength was prepared by heating 6.0 g of agarose in 200ml 1X TBE buffer and adding 10 µl of ethidium bromide (10 mg/ml), the solution was cooled down to 50°C. The solution was poured into the casting tray. The comb was removed and then mounted on a gel tank containing 1000 ml of 1X TBE after solidifying for an hour. In each well of the gel 10 µl of PCR product was loaded. The gel was run until bromophenol blue reached the end of the gel. Then the gel picture was digitally documented in Gel Documentation System (VILBER). Based on a ladder of known molecular weight, the molecular weight of PCR products was determined. During band scoring, faint bands and band with smeared backgrounds were excluded and only intense bands were scored based on their product size qualitatively and independently presence or absence across all samples. Score '0' or '1' indicated the absence or presence of band respectively. A data matrix comprising 0 and 1 were generated. SSR amplification data were analyzed by using the software package, NTYS-pc Version 2.1 [12]. Polymorphism information content (PIC) was calculated by using the

standard formula [13]. For estimation of genetic similarity analysis, using SIMQUAL module of NTSYS-pc, genetic relatedness among genotypes was computed by using the Jaccard's coefficient of similarity. The pair wise genetic similarity index was determined using Jaccard's coefficient of similarity [14]. The degree of genetic relationship among the genotypes was analyzed by cluster analysis using the algorithm of "Unweighted Pair Group Method with Arithmetic Average" (UPGMA). Graphical representation of genetic relationship among the genotypes was done in the form of dendrogram.

RESULTS AND DISCUSSION

Morphological characterization by qualitative characters

The five yellow sarson genotypes were evaluated for DUS traits and they showed broad range of variability with respect to many traits observed. Of the 24 morphological (8 qualitative and 16 quantitative) traits, considerable variation was observed for many traits. The morphological characters as presented in the Table 1 indicated that the leaves of YSH401, B9, AAUJYS14-2 and AAUJYS15-2, were sparsely hairy during bud formation to flowering stage, whereas hairiness was absent in the leaves of Pitambari. The leaf colour of YSH401, AAUJYS14-2 and Pitambari was found to be dark green. On the other hand, B9 and AAUJYS15-2 had light green leaves. Siliqua angle with main shoot of Pitambari was found to be semi-appressed, whereas the siliqua angle of the other four genotypes was open. However, the genotypes turned out to be monomorphic for leaf lobe (present), leaf dentation of margin (present), petal colour (yellow-coloured), siliqua texture (smooth), seed colour (yellow). Similarly, 10 genotypes of *Brassica juncea* were differentiated based on morphological characters [15]. Genetic diversity among yellow sarson, toria and brown sarson were estimated by using various agro-morphological traits [16]. Thus, for the qualitative traits Pitambari was the most distinct genotype from the other genotypes because of its semi-appressed siliquae and absence of leaf hairiness. AAUJYS14-2 and AAUJYS15-2 could be distinguished by leaf colour as the leaves of AAUJYS14-2 were dark green in colour and that of AAUJYS15-2 were light green. 78 genotypes of Indian mustard (*Brassica juncea*), were characterized using DUS guidelines [17]. In their study leaf hairiness was absent in three genotypes whereas, one genotype was having very sparse hairs and rest of the genotypes were having sparse hairs.

Characterization based on quantitative characters

The data recorded on quantitative traits were subjected to analysis of variance. The analysis revealed highly significant variation (Table 2) among the genotypes for all the characters except for petal width. Similarly, genetic diversity was reported among brown sarson, yellow sarson and toria for agro-morphological traits except for petal length and petal width [16]. The variation was classified into categories as per the Test Guidelines. The characteristics of the genotypes for the 16 quantitative characters are given in Table 3. Mean values of the genotypes for the 16 quantitative characters are given in Table 4. The genotypes turned out to be monomorphic for main shoot length (all short), number of leaf lobes (few), petal length (short) and width (narrow), siliquae on main shoot (very few) and siliqua length (all long siliquae). The number of lobes was low in all the genotypes (<5). Leaf length ranged from 12.05 cm to 15.22 cm with a mean of 13.63 cm. The genotype B9 (12.05 cm) was found in the category of short leaf length (<12); AAUJYS14-2 (13.89 cm), AAUJYS14-2 (13.52 cm) and

Table 1. Qualitative characters of the five yellow sarson genotypes based on DUS Test Guidelines

Characteristic	States	Notes	Frequency	Genotypes
Leaf hairiness	Absent	1	1	Pitambari
	Sparse	3	4	AAUJYS14-2, AAUJYS15-2, YSH40, B9
Leaf colour	Light green	1	2	B9, AAUJYS15-2
	Dark green	3	3	AAUJYS14-2, YSH401, Pitambari
Leaf lobes	Present	9	5	AAUJYS14-2, AAUJYS15-2, YSH401, B9, Pitambari
Leaf dentation	Dentate	2	5	AAUJYS14-2, AAUJYS15-2, YSH401, B9, Pitambari
Colour of petals	Yellow	3	5	AAUJYS14-2, AAUJYS15-2, YSH401, B9, Pitambari
Siliqua angle with main shoot	Semi-appressed	2	1	Pitambari
	Open	3	4	AAUJYS14-2, AAUJYS15-2, B9, YSH401
Siliqua texture	Smooth	1	5	AAUJYS14-2, AAUJYS15-2, B9, YSH401, Pitambari
Seed colour	Yellow	1	5	B9, AAUJYS14-2, AAUJYS15-2, YSH401, Pitambari

Table 2. Analysis of variance (mean squares) for different quantitative traits in yellow sarson

Source	df	PH	DF	DM	MSL	SMS	SD	LL	LW
Replications	3	14.49	2.53*	7.53*	6.92	0.61	0.003	0.41	0.05
Genotypes	4	94.77**	88.83**	183.57**	48.21**	8.75**	0.022**	6.40**	1.28**
Error	12	10.42	0.99	2.24	3.91	0.40	0.002	0.55	0.09
CV %		3.99	2.34	1.56	5.28	2.45	6.69	5.34	4.78
Source	df	PL	PW	LLB	SL	SBL	SS	TSW	OC
Replications	3	0.0009	0.0005	0.07	0.13	0.0022	0.20	0.03	2.24*
Genotypes	4	0.008**	0.0002	5.00**	0.55**	0.0384**	11.19**	0.18**	19.67**
Error	12	0.0019	0.0003	0.07	0.10	0.0056	0.34	0.01	0.35
CV%		4.61	3.52	10.33	5.02	6.75	3.83	2.79	1.49

*Significant at P=0.05 and **Significant at P=0.01

PH= Plant height, DF=days to 50% flowering, DM=Days to maturity, MSL= Main shoot length, SMS = Number of siliquae on main shoot, SD = Siliqua density, LL = Leaf length, LW= Leaf width, PL= Petal length, PW= Petal width, LLB=Leaf lobe, SL=Siliqua length, SBL= Siliqua beak length, SS= Number of seeds per siliquae, TSW=Thousand seed weight, OC= Oil content.

Pitambari (14.94 cm) were in the category of medium leaf length (12-15 cm), and YSH401 (15.22 cm) was in the category of long leaf length (>15). Leaf width ranged from 5.49 cm to 6.92 cm with mean of 6.2 cm. B9 (5.49 cm) and AAUJYS15-2 (5.88 cm) were found in the category of medium leaf width (4-6 cm), whereas Pitambari (6.92 cm), YSH401 (6.55 cm) and AAUJYS14-2 (6.36 cm) were found in the category of broad leaf width (>6 cm). Petal length ranged from 0.91 cm to 1.03 cm with mean of 0.97 cm and all the genotypes, were found in the category of short petal (<1.2 cm). Petal width ranged from 0.48 cm to 0.49 cm with mean of 0.48 cm and genotypes were in the category of narrow petal width (<0.6 cm). Plant height ranged from 75.18 cm to 87.73 cm with a mean of 81.45 cm. B9 (75.18 cm) and YSH401 (77.65cm) were found in the category of short plant height (<80), whereas Pitambari (87.73 cm), AAUJYS14-2 (83.08 cm) and AAUJYS15-2 (80.53 cm) were of medium plant height (81 to <90). Main shoot length ranged from 34.40 cm to 43.33 cm with mean of 38.86 cm. B9 (34.40 cm), YSH401 (35.45 cm), AAUJYS14-2 (36.90 cm) and AAUJYS15-2 (37.15 cm) were with short main shoot (<40 cm), whereas Pitambari (43.33cm) was with medium main shoot length (41 to<50). Siliqua length with mean 6.16 cm all the genotypes were long (>5.5 cm). Siliqua beak length ranged from 1.02 cm to 1.25 cm, with mean of 1.09 cm. Pitambari had long beaks (>1.2 cm) whereas AAUJYS14-2, YSH401, B9 and AAUJY15-2 had medium beaks (0.8 to<1.2 cm). Siliqua number on main shoot ranged from 27.58 to 24.20, with mean of 25.89, and thus all the genotypes belonged to category that exhibited very few numbers of siliquae on the main shoot (<40). Siliqua density of YSH401 (0.69/cm) and Pitambari (0.57/cm) was low (<0.7), whereas B9,AAUJYS14-2 and AAUJYS15-2 (0.74/cm) had medium siliqua density (0.7-0.8/cm). Number of seeds per siliqua ranged from 13.42 to 18 with mean value of 15.71. YSH401 (18) and AAUJYS14-2 (16.16) were in the category of medium number of seeds per siliqua, whereas B9 (14.65), AAUJYS15-2 (14.17) and Pitambari (13.42) were in the category of few seeds per siliqua. The time of flowering was between 40 and 51 days. B9, YSH401, AAUJYS14-2 and AAUJYS15-2 exhibited medium flowering time (36-45 days), and Pitambari exhibited late flowering (>45 days). Maturity period was between 92 and108 days. B9, YSH401, AAUJYS14-2 and AAUJYS15-2 showed medium maturity duration (80-100 days), whereas Pitambari showed late maturity duration (100-120 days). Similar findings were reported [18] to distinguish and categorize 18 varieties of mustard in the field by studying six morphological traits from the DUS protocol. They found that days to maturity, number of siliquae on main shoot and number of seeds per siliqua were important characters in classification of the varieties.Thousand seed weight varied from 3.03 g to 3.54 g with mean of 3.28 g. YSH401, Pitambari, AAUJYS14-2 and AAUJYS15-2 had small sized seed (<3.5g), but B9 (3.54 g) had medium sized seed.The seed oil content ranged from 35.39% to 41.60% with mean value of 38.49%. B9 (41.60%), YSH401 (41.58%), AAUJYS15-2 (40.16%), AAUJYS14-2 (40.16%) had medium oil content (38-<42%), whereas Pitambari (35.39%) had low oil content (<38%).It was reported that 54 *Brassica rapa* L. genotypes were evaluated for DUS characterization by 9 characters and

Table 3. Quantitative characters of five yellow sarson genotypes as per the DUS Test Guidelines

Character	States	Notes	Frequency	Genotypes
Number of Leaf lobes	Low (< 5)	3	5	AAUJYS14-2, AAUJYS15-2, YSH401, B9, Pitambari
	Medium (6 – <8)	5	0	-
	High (> 8)	7	0	-
Leaf length (cm)	Short (< 12)	3	1	B9
	Medium (12 –15)	5	3	AAUJYS14-2, AAUJYS15-2, Pitambari
	Long (>15)	7	1	YSH401
Leaf width (cm)	Narrow (< 4.0)	3	0	-
	Medium (4 –6)	5	2	AAUJYS15-2, B9
	Broad (> 6)	7	3	AAUJYS14-2, YSH401, Pitambari
Time of flowering	Early (<35 days)	3	0	
	Medium (36 - <45 days)	5	4	AAUJYS14-2, AAUJYS15-2, YSH401, B9
	Late (> 45 days)	7	1	Pitambari
Length of petals (cm)	Short (<1.2)	3	5	AAUJYS14-2, AAUJYS15-2, YSH401, B9, Pitambari
	Medium (1.2-1.5)	5	0	-
	Long (>1.5)	7	0	-
Width of petals (cm)	Narrow (<0.6)	3	5	AAUJYS14-2, AAUJYS15-2, YSH401, B9, Pitambari
	Medium (0.6-0.7)	5	0	-
	Broad (>0.7)	7	0	-
Main shoot length (cm)	Short (< 40)	3	4	AAUJYS14-2, AAUJYS15-2, B9, YSH401
	Medium (41- <50)	5	1	Pitambari
	Long (51 - < 60)	7	0	-
	Very long (> 60)	9	0	-
Plant height (cm)	Short (< 80)	3	2	B9, YSH401
	Medium (81- <90)	5	3	AAUJYS14-2, AAUJYS15-2, Pitambari
	Tall (91 - <100)	7	0	-
	Very tall (> 100)	9	0	-
Siliqua length (cm)	Short (<4.5)	3	0	-
	Medium (4.5-5.5)	5	0	-
	Long (> 5.5)	7	5	AAUJYS14-2, B9, YSH401, Pitambari, AAUJYS15-2
Siliqua beak length (cm)	Short (<0.8)	3	0	-
	Medium (0.8 – <1.2)	5	4	AAUJYS14-2, AAUJYS15-2, B9, YSH401
	Long (> 1.2)	7	1	Pitambari
Number of siliquae on main shoot	Very few (< 40)	3	5	AAUJYS14-2, AAUJYS15-2, B9, YSH401, Pitambari
	Few (41 - <50)	5	0	-
	Medium (51 - <60)	7	0	-
	Many (> 60)	9	0	-
Siliqua density on main shoot	Low (<0.7)	3	2	YSH401, Pitambari
	Medium (0.7- 0.8)	5	3	AAUJYS14-2, AAUJYS15-2, B9
	High (>0.8)	7	0	-
Number of seeds per siliqua	Very few (<12)	3	0	-
	Few (13-<16)	5	3	AAUJYS15-2, B9, Pitambari
	Medium (17-<20)	7	2	AAUJYS14-2, YSH401
	Many (> 20)	9	0	-
Maturity period	Early (<81 days)	3	0	
	Medium (82 - <100 days)	5	4	B9, AAUJYS14-2, AAUJYS15-2, YSH401
	Late (101-<120 days)	7	1	Pitambari
	Very late (> 120days)	9	0	-
Seed size: 1000 seed weight	Small (<3.5g)	3	4	AAUJYS14-2, AAUJYS15-2, YSH401, Pitambari
	Medium (3.5-4.0g)	5	1	B9
	Bold(>4.0g)	7	0	
Seed oil content	Low (<38)	3	1	Pitambari
	Medium (38-<42)	5	4	AAUJYS14-2, AAUJYS15-2, YSH401, B9
	High (42-46)	7	0	-
	Very high (>46)	9	0	-

found that all the traits were suitable for characterization [19]. 78 genotypes of Indian mustard were characterized by DUS test guidelines to establish distinctness among the genotypes based on several morphological descriptors [20]. They observed wide diversity for leaf length and leaf breadth.

Pitambari was the most diverse genotype from the other four. It was characterized by late flowering and late maturity, medium long leaves, medium wide leaves, medium wide petals, long siliqua beaks, low siliqua density, few seeds per siliqua, small seed size and low oil content. All the other four genotypes were medium in flowering time and maturity, medium oil content, medium long beaks and narrow petals. B9 was characterized with short and medium wide leaves, narrow petals, medium siliqua density, medium seed size and few seeds per siliqua. YSH401 was distinct with long and broad leaves, medium number of seeds per siliqua, low siliqua density and small seed size. AAUJYS14-2 was distinguished with medium long and broad leaves, medium siliqua density, medium number of seeds per siliqua and small seed size. On the other hand, AAUJYS15-2 was characterized with medium long and medium wide leaves, medium siliqua density, few number of seeds per siliqua and small seed size. Thus, there were subtle differences between the genotypes, by which the genotypes could be distinguished despite the fact that there were statistically significant quantitative differences among them as seen in Table 4.

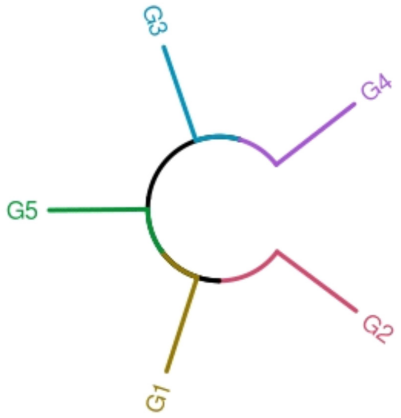

Figure 1. Dendogram based on D^2 analysis with morphological data G1: B9; G2: YSH401; G3: AAUJYS14-2; G4: AAUJYS15-2; G5: Pitambari

Diversity analysis

Mahalanobis' D^2 statistics was applied for assessing genetic divergence among the genotypes by using the morphological quantitative data. The genotypes were grouped by Tocher's method [7]. Each of the five genotypes was grouped into a separate cluster (Figure 1). The five clusters were equidistant from one another (Table 5). This indicated that the genotypes were distinct from one another. Based on D^2 analysis the five yellow sarson genotypes were grouped individually into five equidistant clusters. In similar study, some researchers reported genetic diversity by using D^2 statistics in 38 genotypes of mustard into seven different clusters [21]. Cluster I had the largest number of genotypes (22)

Table 4. Mean values of five yellow sarson genotypes for different quantitative characters

Character	B9	YSH401	AAUJYS14-2	AAUJYS15-2	Pitambari	Mean ±SE	CD5%	CD1%
Plant height (cm)	75.2a	77.6a	83.1b	80.5b	87.7c	80.8±1.61	4.97	6.97
Days to flowering	40.0a	41.0a	40.7a	40.3a	51.0b	42.6±0.50	1.53	2.15
Days to maturity	92.7a	92.7a	92.5a	93.5a	108.0b	95.9±0.7	2.3	3.2
Main shoot length (cm)	34.4a	35.5a	36.90a	37.2a	43.3b	37.5±0.99	3.05	4.27
Leaf length (cm)	12.1a	15.2c	13.9b	13.5b	14.9c	13.9±0.37	1.15	1.61
Leaf width (cm)	5.5a	6.5bc	6.4b	5.8a	6.9c	6.3±0.15	0.46	0.64
Petal length (cm)	0.96ab	0.93a	1.1b	0.94a	0.9a	0.95±0.02	0.07	0.09
Petal width (cm)	0.49	0.48	0.49	0.48	0.48	0.48±0.01	NS	NS
Number of leaf lobe	2.0a	2.0a	2.0a	2.0a	4.5b	2.5±0.13	0.40	0.56
Siliqua length (cm)	6.2a	6.4b	6.2b	5.6a	6.6b	6.2±0.16	0.48	0.67
Siliqua beak length (cm)	1.08a	1.02a	1.2b	1.1a	1.3b	1.2±0.04	0.12	0.16
Number of siliquae on main shoot	25.5b	24.2a	27.2c	27.6c	24.7ab	25.8±0.32	0. 97	1.37
Siliqua density	0.74a	0.69a	0.74a	0.74a	0.57b	0.7±0.02	0.07	0.10
Number of seeds per siliquae	14.6a	17.6b	16.2d	14.2ac	13.4c	15.2±0.29	0.90	1.26
1000 seed weight (g)	3.5b	3.2a	3.2a	3.1a	3.4b	3.3±0.05	0.14	0.20
Oil content (%)	41.6b	41.6b	40.2a	40.3a	35.4c	39.8±0.34	1.12	1.63

Table 5. Mahalanobis' Intra and inter-cluster distances

	B9	YSH401	JYS14-2	JYS15-2	Pitambari
B9	0.00	8.00	8.00	8.00	8.00
YSH401		0.00	8.00	8.00	8.00
JYS14-2			0.00	8.00	8.00
JYS15-2				0.00	8.00
Pitambari					0.00

followed by Cluster III (11). The other clusters were solitary with single genotype. 31 germplasm lines of *Brasssica rapa* var were classified [22] as yellow sarson. Yellow sarson into 8 and 12 clusters under timely sown (E_1) and late sown (E_2) conditions, respectively. In E_1, cluster I had the maximum number of 15 genotypes whereas cluster III, VII and VIII had only one genotype each. In E2, cluster III had maximum number of 15 genotypes whereas cluster IV, V, VII, VIII, X, XI, and XII had only one genotype each.Thus, in the present study B9 was characterized with short plant height, medium time of flowering, medium maturity duration, short but medium wide leaves, few leaf lobes, short and narrow petals, long siliquae with medium beaks, short main shoots with very few numbers of siliquae on main shoot, medium siliqua density, few number of seeds per siliqua, medium seed size and medium oil content.YSH 401 was characterized with short plants, medium time of flowering and maturity, few leaf lobes, long and broad leaves, short and narrow petals, long siliquae with medium long beaks, short main shoot with very few numbers of siliquae on it, low siliqua density, medium number of seeds per siliqua, small seed size and medium oil content. AAUJYS14-2 was characterized with medium in flowering and maturity, short plants, few leaf lobes, medium long and broad leaves, short and narrow petals, long siliquae with medium long beaks, short main shoot with very few numbers of siliquae, medium siliqua density, medium number of seeds per siliqua, small seed size and medium oil content. AAUJYS15-2 was characterized with medium flowering time and maturity, short plants, few leaf lobes, medium leaf length and width, short and narrow petals, long siliquae with medium long beaks, short main shoot length with very few numbers of siliquae, medium siliqua density, few seeds per siliqua, small seed size and medium oil content. Pitambari was late in flowering and maturity with short plants, few leaf lobes, medium leaf length and width, short and narrow petals, long siliquae with long beaks, short main shoots with very fewer number of siliquae and low siliqua density on main shoot, few number of seeds per siliqua, small seed size and low oil content.Seed yield was not considered as a DUS character. However, in the present study yield was recorded as a subsidiary character, not for genotype distinction. There was significant variation among the five genotypes for seed yield per hectare. AAUJYS14-2 (689 kg/ha), YSH401 (667 kg/ha) and AAUJYS15-2 (623 kg/ha) were at par and they yielded higher than B9 (593 kg/ha) and Pitambari (416 kg/ha).

Characterization by Molecular Markers

Molecular assay was carried out on the five yellow sarson genotypes by using 20 random SSR markers. Twelve of the 20 primers were found to be polymorphic that could explain the molecular diversity among the five genotypes. The polymorphic SSR markers are presented in Table 6 and Figure 2 and 3. For allelic diversity computation, the number of alleles amplified along with the PIC (Polymorphic Information Content) values were computed and are presented in Table 6. A maximum of 2 alleles

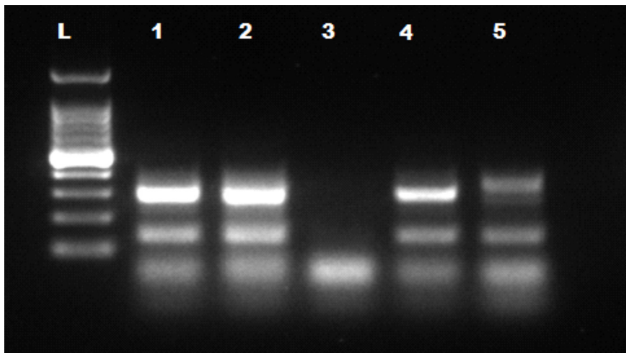

Figure 2. Amplification by using primer A03_12095
Legends: L= DNA Ladder (100 bp); 1= YSH401; 2= B9; 3=AAUJYS14-2; 4= AAUJYS15-2; 5=Pitambari

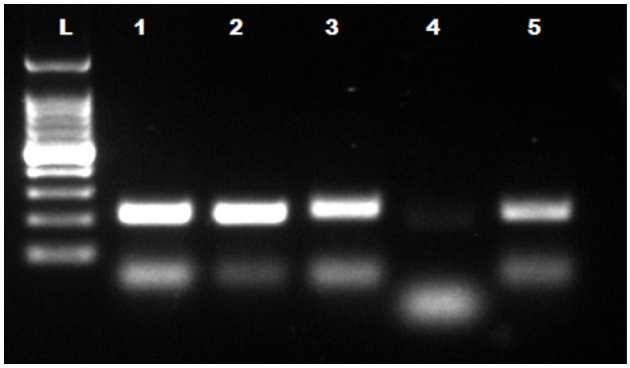

Figure 3. Amplification by using primer A03_905
Legends: L= DNA Ladder (100 bp); 1= YSH401; 2= B9; 3=AAUJYS14-2; 4= AAUJYS15-2; 5=Pitambari

Table 6. Allele amplification and PIC values of polymorphic markers

S. No.	Marker Code	Forward Primer	Reverse Primer	Reference	No. of alleles amplified	PIC value
1	A01_898	CGTCACCATCTCCTTGTCCT	CCGGGTTGAACACTCGTAAT	Hobson and Rahman (2016)	1	0.84
2	A01_8925	TCGTAGATCGGTGATGATGC	AGTCTTTTGGTTTTGGGCCT		1	0.84
3	A01_10214	TTTTGCCATTCGTGTCGTAA	AAAAGGAAGGGCAAAGTGGT		1	0.36
4	A02_497	GAGCCGCTCATATGAAGTTGA	GTTGCTTCGAAAGCGGTTAG		2	0.84
5	A02_6263	CTTGCCTATGGATCTTTCTCG	TTTTTGGGGAAGGAGCATAA		2	0.2
6	A02_8575	TGGACAGACCTGTCAGCAAC	GCAGGCTACCATCAAAGCTC		2	0.8
7	A02_10613	CATCACCGGAGCTATCGAAT	ACAATCAAAGACCTGGACCG		1	0.36
8	A03_5090	AAAGCGACAAATACATGTGACAA	CCAAAAATGTGATCCAAACTCA		1	0.96
9	A03_6890	TGTCAAGACAATTGAAGCCG	CATCTCCTTCCACGCAAAAT		1	0.64
10	A03_12095	AAAGCGGAAGAAGGAGAAGC	GACCTTGGCTTGAAGACAGC		2	0.36
11	A04_905	GAAGATCTGGTGCGGAAGAG	GCCAAAAGAGAAAGCGAATG		1	0.36
12	A04_2310	GCTAGCATGAGTCGTCCTCC	TGCTCTAAGGCGGTAGCATT		2	0.6

were amplified by five primers while seven primers amplified one allele each. The average allele per locus were 1.41. The PIC value, which is the measure of allelic diversity of SSR markers ranged from 0.2 to 0.96. The maximum PIC was exhibited by the primer A03_5090 while minimum PIC was shown by the primer A02_6263.The average PIC value was found to be 0.47. Jaccard's similarity coefficients determined the genetic relationship among the five genotypes. The coefficients ranged from 0.375 to 0.625 with an average of 0.51. The maximum similarity was observed between the genotypes YSH401 and AAUJYS14-2 and the minimum similarity was observed between the genotypes YSH401 and AAUJYS15-2.The software R version 3.6.3 was used to generate dendrogram based on Jaccard's coefficients of similarity (Figure 4). The five genotypes were divided into two clusters A and B. Cluster A comprised of two sub-clusters, namely AI and AII. Sub-cluster AI consisted of two genotypes (AAUJYS15-2, YSH401) while sub cluster AII with only one genotype (B9). Cluster B consisted of two genotypes viz., AAUJYS14-2, Pitambari (Table 7). Cluster mean values were calculated for sixteen

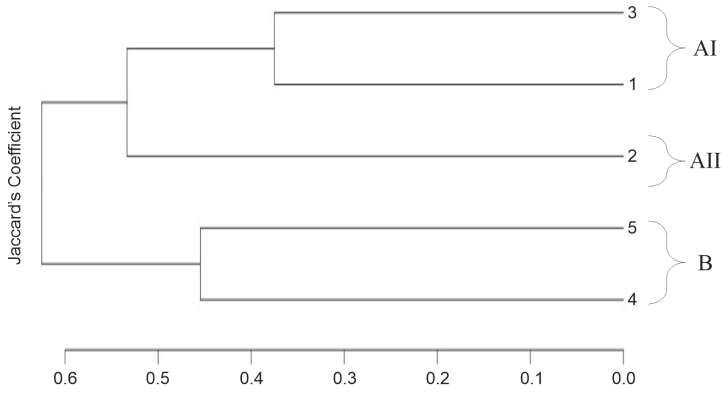

Figure 4. Clusters of genotypes based on Jaccard's coefficients of similarity

Table 7. List of genotypes according to the cluster analysis based on SSR marker data

Cluster		No. of genotypes	Genotypes
Cluster A	Sub-cluster AI	2	AAUJYS15-2, YSH401
	Sub-cluster AII	1	B9
Cluster B		2	AAUJYS14-2, Pitambari

Table 8. Cluster means for different characters

Cluster	PH (cm)	DF	DM	MSL (cm)	SMS	SD	LL (cm)	LW (cm)
Cluster AI	80.36	40.88	92.63	36.18	25.69	0.71	14.56	6.46
Cluster AII	75.18	40.00	92.75	34.40	25.55	0.74	12.05	5.49
Cluster B	84.13	45.63	100.75	40.24	26.16	0.66	14.23	6.40
Cluster	PL (cm)	PW (cm)	LLB	SL (cm)	SBL (cm)	SS	TSW (g)	OC (%)
Cluster AI	0.98	0.49	2	6.29	1.09	16.88	3.15	40.93
Cluster AII	0.96	0.49	2	6.03	1.08	14.65	3.54	41.60
Cluster B	0.92	0.48	3.25	6.16	1.14	13.79	3.23	37.78

PH= Plant height, DF=days to 50% flowering, DM=Days to maturity, MSL= Main shoot length, SMS = Number of siliquae on main shoot, SD = Siliqua density, LL = Leaf length, LW= Leaf width, PL= Petal length, PW= Petal width, LLB=Leaf lobe, SL=Siliqua length, SBL= Siliqua beak length, SS= Number of seeds per siliquae, TSW=Thousand seed weight, OC= Oil content.

quantitative characters (Table 8). The sub-cluster A-I exhibited higher mean values for leaf length, leaf width, petal length, petal width, siliqua length, number of seeds per siliqua whereas sub-cluster AII exhibited higher mean values for siliqua density, thousand seed weight and oil content. Cluster-B exhibited higher mean values for plant height, main shoot length, leaf lobe, days to flowering, days to maturity, siliquae on main shoot, and siliqua beak length. In this present investigation, a total of 20 SSR markers were assayed to assess genetic variation among the five genotypes. Twelve markers were found to be polymorphic. The primers A02_497, A02_6263, A02_8575, A03_12095 and A04_2310 amplified maximum of two alleles and the remaining 7 primers amplified only one allele. The average number of alleles per locus was 1.41. The Polymorphism Information Content (PIC), which is the measure of allelic diversity of SSR, ranged from 0.2 to 0.96 with an average value of 0.47 PIC. Similar average PIC value of 0.45 was observed [16] and average PIC value of 0.46 was observed [23] in mustard. Jaccard's coefficients of similarity indicated that the maximum similarity was observed between the genotypes YSH401 and AAUJYS14-2 and the minimum similarity was between YSH401 and AAUJYS15-2. In a similar study, genetic diversity was reported at molecular level in 24 genotypes of toria, finding two large clusters with 53% genetic similarity [24]. In their study, two genotypes (GPT-13 and GPT-59) were grouped into one cluster and remaining 22 fell into the second cluster. Thirteen out of 55 gamma-ray-induced mutants of *Brassica rapa* spp yellow sarson, along with their two parents (NDYS-2 and YST-151) were studied to characterize genetic variability using morphological and ISSR markers [22]. The five genotypes were divided into two clusters A and B. Cluster A comprised of two sub-clusters. Sub-cluster AI consisted of two genotypes (AAUJYS15-2, YSH401) while sub cluster AII with one genotype B9. Cluster B consisted of two genotypes viz., AAUJYS14-2 and Pitambari. The sub-cluster AI exhibited higher mean values for leaf length, leaf width, petal length, petal width, siliqua length, number of seeds per siliqua whereas sub-cluster AII exhibited higher mean values for siliqua density, thousand seed weight and oil content. Cluster B exhibited higher mean values for plant height, main shoot length, leaf lobes, days to flowering, days to maturity, siliquae on main shoot and siliqua beak length.

CONCLUSION

Significant variation was observed among the five genotypes for all quantitative traits except in petal width. It signified existence of good amount of genetic variation among the genotypes. Therefore, the analysis was useful for distinguishing the genotypes and for selection of genotypes for further breeding. Out of 24 qualitative characters, the genotypes were polymorphic for 14 characters including seed size, which could be used for variety distinction. The genotype Pitambari was found to be the most distinct among the all genotypes for several morphological traits. Each of the genotypes could be distinguished by some of the polymorphic traits. Diversity analysis showed that the genotypes were distinct from one another. Based on the study of 20 random SSR primers, 12 markers were polymorphic to characterize the five genotypes. Three clusters (including sub-clusters) were formed based on Jaccard's coefficients of similarity. Each cluster showed characteristic mean values for the polymorphic morphological traits. Thus, the use of molecular markers in conjunction with morphological descriptors was useful for characterization of the five

yellow sarson genotypes for management and utilization of the variation.

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REFERENCES

1. CHAUHAN JS, KH SINGH, VV SINGH AND S KUMAR (2011). Hundred years of rapeseed-mustard breeding in India: accomplishments and future strategies. *Indian Journal of Agricultural Sciences*, **81**(12):1093–1109.
2. JAT RS, VV SINGH, PSHARMA AND PK RAI (2019). Oilseed brassica in India: Demand, supply, policy perspective and future potential. *OCI,* **26**: 8.
3. USDA, (2023). https://www.usda.gov./in.
4. HANIF A, MR ASHRAF, A MASROOR, GM IQBAL AWAN, S AHMAD AND S ZAMAN (2022). Botanical description of six brassica species with relevance to their quantitative and morphological characteristics, *Journal of Xi'an Shiyou University, Natural Sciences*, **65**(6): 89-101.
5. DRMR (2022). Annual Progress Report, Directorate of Rapeseed-Mustard Research, Bharatpur. http://www.drmr.res./ /in.
6. GOMEZ KA, AND AA GOMEZ (1984). Statistical Procedures for Agricultural Research. John Wiley & Sons, New York.
7. RAO CR (1952). Advanced statistical methods in biometric research. John Wiley and Sons Inc., New York.
8. R CORE TEAM (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/
9. DA SILVA, ARG MALAFAIA, AND IPP MENEZES (2017). Biotools: An R functions to predict spatial gene diversity via an individual-based approach.*Genetics and Molecular Research*, **16**(2):1-6.
10. SILVA AR AND RP LIMA. (2014). Determination of maximum curvature point with R package soil physics. *International Journal of Current Research,* **9**(1): 45241-45245.
11. DELLAPORTA SL, J WOOD AND JB HICKS (1983). A plant DNA mini preparation: Version II. *Plant Molecular Biology Reporter,* **1**: 19-21
12. ROHLF FJ (2000). NTSYS-pc: numerical taxonomy and multivariate analysis system Version 2.1. Exeter Publishing Setauket, New York.
13. ANDERSON JA, GA CHURCHILL, JE AUTRIQUE, SD TANKSLEY, AND ME SORRELLS (1993). Optimizing parental selection for genetic linkage maps. *Genome.* **36**(1):181–186.
14. JACCARD P (1908). Nouvelles rechearches sur la distribution florale. *Bull. Soc. Vaud. Sci. Nat.,* **44**: 223-270.
15. HINGE NS, AND SN MALODE (2019). Determination of morphological variability among 10 genotypes of mustard (*Brassica napus* L.) and their application for DUS testing. *Current Updates in Life sciences*, **1**(1): 175-186.
16. THAKUR AK, KH SINGH, L SINGH, V VERMA AND D SINGH (2016). Morphological and molecular descriptors for first Indian mustard hybrid, NRCHB 506. *Indian Journal of Biotechnology*, **15**(1): 116–119.
17. YADAV AK, D SINGH AND RK ARYA (2013). Morphological characterization of Indian mustard (*Brassica juncea*) genotypes and their application for DUS testing. *Indian Journal of Agricultural Sciences*, **83**(12): 1305-1316.
18. TIWARI M, VB BABU, N KOTWALIWALE, R HAMAD AND K SINGH (2017). Identification of Indian mustard (*Brassica juncea* L.) varieties by DUS testing using morphological characters. *Journal of Oilseed Brassica*, **1**(1): 57-61.
19. BANO T, SA NAGOO, MAI QURESHI, MA WANI, S KUMAR, S MAJEED, ZI BUHROO, S ASHRAF, M, BASHIR AND ZA DAR (2021). Morphological characterization of brown sarson (*Brassica rapa* L.) genotypes for DUS traits, *Plant Archives*, **21**(1): 290-295.
20. YADAV AK, D SINGH AND RK ARYA (2013). Morphological characterization of Indian mustard (*Brassica juncea*) genotypes and their application for DUS testing. *Indian Journal of Agricultural Sciences*, **83**(12): 1305-1316.
21. SAIKRISHNA A, B ARUNKUMAR, H KHAN, PH KUCHANUR AND A PATIL (2021). Genetic diversity studies in Indian mustard [*Brassica juncea* (L.) Czern&Coss.] For yield and its component traits. *The Pharma Innovation Journal,* **10**(10): 534-538.
22. KUMAR H, MKV ANUBHA AND JP LAL (2011). Morphological and molecular characterization of *Brassica rapa*ssp yellow sarson mutants. *Journal of Oilseed Brassica*, **1**(1): 1-6.
23. TURI NA, MA FARHATULLAH, MA RABBANI AND ZK SHINWARI (2012). Genetic diversity in the locally collected Brassica species of Pakistan based on microsatellite markers. *Pak. J. Bot.,* **44**: 1029-1035.
24. SALEM JL, N MEHTA, AK SARAWAGI, RR SAXENA, S VERULKAR AND N TOMAR (2018). Genetic Diversity Analysis Based on Molecular Level in Selected *Brassica compestris* L. var. toria Genotypes. *Int. J. Curr. Microbiol. App. Sci*, **7**(7): 3604-3611.