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Genetic Diversity Assessment and Characterization of Indian Mustard (*Brassica juncea* L.) Varieties using Agro-morphological Traits

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Indian mustard (*Brassica juncea* L. Czern & Coss.) is an important source of edible oil in Asian countries. Indian mustard suffers from the lack of distinguishable morphological markers. Effectiveness of a trait in establishing distinctness was determined following a criterion that involved statistical parameter of dispersion measured in terms of range and coefficient of variation. Simultaneously, stability of a trait was estimated as average of coefficient of variation estimates of three year mean values of each genotype. Extent of diversity was estimated and distinctness among cultivated Indian mustard varieties could be established. Varieties were grouped into five different clusters on the basis of multivariate analysis following Euclidean distance and UPGMA method and diverse varieties from different clusters were suggested for hybridization programme. Seed weight, days to maturity, plant height, siliqua angle with main raceme, petal length, siliqua length, plant height/length of main raceme, seeds per siliqua and days to flower initiation were found more effective than leaf length, leaf width and number of leaf lobes. There was no correspondence of grouping between classification based upon mean estimate of traits and clusters based upon multivariate analysis.

Key Words: Distinctness, Diversity, Efficacy, Indian mustard, Stability.

Introduction

Plant Breeders rights have been granted as intellectual property rights in many countries to promote the research in crop improvement. Such rights have been granted in India too under the 'Protection of Plant Varieties and Farmers Rights Act, 2001'. Establishing distinctness on the basis of morphological traits is the pre-requisite to assign the plant breeders rights. Oilseed Brassica crops, the second most important oilseed crop globally after soybean, generally lack distinguishable morphological markers. Indian mustard (*Brassica juncea* L. Czern & Coss.), which is predominantly grown in southern Asia as an important oilseed Brasscica crop, also suffers from the lack of distinguishable morphological markers (Singh et al., 2006). Most of the traits included in descriptor for distinctness, uniformity and stability (UPOV, 1996; PPV&FRA., 2009) are quantitative; hence show continuous variation unlike discrete classes of qualitative traits which are more efficient in establishing distinctness among varieties. Needs to devise more rapid and cost-effective testing procedures to improve the current testing system has been suggested (Cooke, 1999). Different biochemical techniques including comparison of seed oil fatty acid profile by GLC analysis (White and Law, 1991), HPLC analysis of leaf glucosinolates (Adams *et al*., 1999), starch-gel electrophoresis of cotyledon isozymes (Mundges *et al*., 1999) and molecular markers (Tommasini *et al*., 2003) have been reported useful to supplement morphological traits for DUS testing. However, variation within varieties in outbreeding species tends to be high and this lack of uniformity hampers the use of molecular markers for distinguishing between varieties. Preliminary studies have suggested that it might be difficult to identify markers that are sufficiently uniform within varieties and, at the same time, are sufficiently variable between varieties to allow for variety discrimination (Mailer *et al*., 1994; Plaschke *et al*., 1995; Charters, 1996; Robertson *et al*., 1996; Olufowote *et al*., 1997; Donini *et al*., 1998 and Roldan *et al*., 2000). UPOV and Indian test guidelines for DUS testing include only morphological traits. Present investigation aimed to assess the extent of diversity among cultivated Indian mustard varieties and to evaluate the effectiveness of different morphological traits in establishing distinctness using potential morphological traits.

Materials and Methods

Plant material in the present study comprised of 31 released varieties of Indian mustard. The detail of their developing organization and release year has been given

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in Table 1. These varieties were evaluated for three consecutive years in randomised complete block design. Row to row and plant to plant spacing was maintained at 45 and 15 cm, respectively. Recommended practices were followed to raise the good crop. Observations on 15 morphological traits *viz*., number of leaf lobes (LLB), leaf length (cm, LL), leaf width (cm, LW), petal length (cm, PL), petal width (cm, PW), siliqua length (cm, SL), beak length (cm, BL), number of siliquae on main raceme (SMR), siliqua angle with main raceme (0, SA), seeds per siliqua (SS), length of main raceme (cm, LMR), plant height (cm, PH), days to flower initiation (DFI), days to maturity (DM) and 1000-seed weight (g, SW) were recorded. In addition, six variables were computed as ratio between leaf length/leaf width (LL/LW), petal length/petal width (PL/PW), siliqua length/beak length (SL/BL), plant height/length of main raceme (PH/LMR), number of siliquae on main raceme/length of main raceme (SMR/LMR) and days to maturity/days to flower initiation (DM/DFI). Observations were recorded on 60 plants (20 plants from each replication) each year following DUS descriptor (Singh *et al*., 2006).

Mean values of all 21 variables (15 observed and 6 computed) were subjected to statistical analyses. Analysis of variance and multivariate analysis for hierarchical cluster analysis following Euclidean distance and UPGMA method was carried out using linear mixed model and pattern analysis module, respectively, of cropstat 7.2 software (CROPSTAT7.2). Effectiveness of a trait in establishing distinctness was determined following a criterion that involved statistical parameter of dispersion measured in terms of range and coefficient of variation. Simultaneously, stability of a trait in present investigation was estimated as average

Table 1. List of varieties of Indian mustard along with name of developing organization and year of release

Code	Variety name	Year of release	Developing organization	Pedigree
1	Arawali (RN 393)	2001	ARS, RAU Navgaon	Krishna × RS 50
2	CS 52	1998	CSSRI, Karnal	Selection from DIRA 343
3	Geeta (RB 9901)	2003	CCS HAU Bawal	Mutant of RH 30
4	GM 1	1990	SDAU SK Nagar	MR71-3-2 × TM 4
5	GM 2	1997	SDAU SK Nagar	Selection from local germplasm
6	Kanti	2003	CSAUA&T Kanpur	Selection from germplasm collected Kanpur Dehat
7	Kranti	1984	GBPUAT, Pantnagar	Selection from Varuna
8	Krishna	1998	GBPUAT, Pantnagar	Selection from Varuna
9	Laxmi (RH8812)	1997	CCS HAU Hisar	PR15 × RH 30A
10	Maya	2003	CSAUA&T Kanpur	Varuna × KRV 11
11	NDRE-4	2001	NDUAT, Faizabad	TM9 × Seeta
12	PBR 91	1996	PAU, RS Bathinda	(RLM 511× PR 18) × CM1
13	PBR 97	1997	PAU, RS Bathinda	(DIR 202 × PR 34 × V3) × RLM 619 × Varuna)
14	Pusa Agrani (Sej-2)	1998	IARI, New Delhi	Early maturing *B. juncea* × synthetic amphidiploids (*B. campestris* var. toria
15	Pusa Bahar	1991	IARI, New Delhi	(Pusa Rai 28 × Varuna) × (Pusa Rai 30 × T6342)
16	Pusa Bold	1985	IARI, New Delhi	Varuna × BIC 1780
17	Pusa Jai Kisan (Bio-902)	1994	IARI, New Delhi	Somaclone of Vrauna
18	Rajat (PCR 7)	1997	DRMR, Bharatpur	Selection from Katch germplasm line JMG
19	RH 819	1991	CCS HAU Hisar	Prakash × Bulk pollen
20	RH 30	1985	CCS HAU Hisar	Selection from P 26/3-1
21	RH 781	1991	CCS HAU Hisar	(RL 18 × P 26/3-1) × RL 18
22	RL 1359	1988	PAU, Ludhiana	RLM 514 × Varuna
23	RLM 619	1985	PAU, Ludhiana	Gamma ray induced mutant of RL 18
24	Rohini	1986	CSAUA&T Kanpur	Selection from natural population of Varuna
25	Sanjucta Asech	1989	PORS Berhampore	TM 4 × RK 2
26	Saurabh (RH 8113)	1987	CCS HAU Hisar	T 59 × RC 781
27	Swarn Jyoti (RH 9801)	2003	CCS HAU Hisar	Selection from germplasm line RC 1670
28	Urvashi (RK 9501)	2001	CSAUA&T Kanpur	Varuna × Kranti
29	Vardan	1985	CSAUA&T Kanpur	Derived through biparental mating involving Varuna, Keshari, CSU 10 and IB 1775, IB 1786 and IB 1866
30	Varuna (T 59)	1976	CSAUA&T Kanpur	Selection from Varanasi Local
31	Vasundhara (RH 9304)	2003	CCS HAU Hisar	RH 839 × RH 30

of coefficient of variation estimates of three year mean values of each genotype. High estimate indicated low stability and vice versa. A trait having wide range, high estimate of variability (coefficient of variation among genotypes) and low estimate of coefficient of variation among environments (years) was considered effective in establishing distinctness and accordingly relative performance of all studied traits was assessed. Distinctness among Indian mustard varieties was established on the basis of mean estimates for different morphological traits following Indian DUS guidelines for Indian mustard.

Results and Discussion

Combined analysis of variance revealed the existence of significant variability for 19 observations in 31 varieties of Indian mustard. Varieties did not differ for petal width and petal length/petal width (PL/PW). Interactions between variety and year were also significant for all traits except petal width. Ranges were wide for plant height, length of main raceme, siliqua angle, siliquae on main raceme, leaf length, days to flower initiation and days to maturity. Highest variability as depicted by coefficient of variation (Table 2) among varieties was recorded for seed weight (19.4%) followed by siliqua angle (16.9%). Beak length, siliqua length/beak length, leaf length, siliqua length, leaf width, number of leaf lobes, siliquae on main raceme, seeds per siliqua, plant height, plant height/length of main raceme, siliquae on main raceme/length of main raceme, length of main raceme and days to flower initiation expressed moderate variability. Remaining trait had low variability. Days to maturity (CV 3.2%) expressed least variability over years followed by petal length (CV 3.4%) indicating high stability. Leaf length, leaf width, siliqua length/beak length, beak length and number of leaf lobes were observed as fluctuating over years. Remaining traits plant height, main raceme length, seeds per siliqua, days to flower initiation, days to maturity/days to flower initiation, seed weight, leaf length/leaf width, plant height/length of main raceme, siliquae on main raceme/length of main raceme and siliquae on main raceme were moderately stable.

On the basis of multivariate analysis, 31 varieties were grouped into 5 clusters (Table 3). RL 1359 and Swarn Jyoti were most resembling varieties followed by Pusa Bold and PBR 97, while, Pusa Bold exhibited

Table 2. Mean, range and coefficient of variation (CV) estimates for 21 traits in Indian mustard varieties

Trait	Mean	Range	Minimum	Maximum	CV among varieties	CV over years
LLB	7.8	2.4	6.4	8.8	7.9	9.6
LL	31.6	13.0	23.3	36.3	9.1	12.8
LW	11.5	4.1	9.3	13.3	8.7	12.3
LL/LW	2.8	0.5	2.5	3.0	4.4	6.5
PL	1.1	0.1	1.0	1.1	2.3	3.4
PW	0.7	0.2	0.6	0.8	4.9	6.6
PL/PW	1.6	0.2	1.4	1.7	3.5	5.9
SL	4.4	2.0	3.7	5.7	8.8	6.2
BL	1.0	0.4	0.9	1.2	9.3	9.8
SL/BL	4.3	1.5	3.8	5.2	9.3	10.4
SMR	49.3	22.5	40.7	63.2	7.8	7.5
SA	28.4	28.1	7.0	35.0	16.9	7.2
SS	15.2	6.8	12.0	18.8	7.8	6.1
LMR	64.7	22.2	55.3	77.5	7.3	6.0
PH	172.6	65.4	128.3	193.7	8.5	5.1
PH/LMR	2.7	1.0	2.1	3.1	8.5	6.9
SMR/LMR	0.8	0.3	0.6	0.9	7.5	7.2
DFI	56.8	16.7	46.8	63.4	6.3	6.2
DM	124.7	17.1	114.4	131.6	3.1	3.2
DM/DFI	2.2	0.5	2.1	2.6	5.2	6.1
SW	4.3	3.0	2.9	5.9	19.4	6.5

(LLB: No. of leaf lobes; LL: leaf length; LW: leaf width; LL/LW: leaf length/leaf width; PL: petal length; PW: petal width; PL/PW: petal length/petal width; SL: siliqua length; BL: beak length; SL/BL: silqua length/beak length; SMR: number of siliquae on main raceme; SA: siliqua angle with main raceme; SS: seeds per siliqua; LMR: length of main raceme; PH: plant height; PH/LMR: plant height/ length of main raceme; SMR/LMR: number of siliquae on main raceme /length of main raceme ; DFI: days to flower initiation; DM: days to maturity ; DM/DFI: days to maturity/days to flower initiation; SW: 1000-seed weight)

Table 3. Grouping of varieties into different clusters on the basis of agro-morphological traits

Cluster No.	No. of varieties	Name of varieties
1	05	CS 52, Geeta (RB 9901), Laxmi (RH8812), RLM 619, Rohini
2	03	Maya, NDRE-4, PBR 91
3	02	Pusa Jai Kisan (Bio-902), Saurabh (RH 8113)
4	07	Arawali (RN 393), GM 1, GM 2, RL 1359, Swarn Jyoti (RH 9801), Urvashi (RK 9501), Vardan
5A	09	Kanti, Kranti, Krishna, Pusa Agrani (Sej-2), Pusa Bahar, RH 819, RH 30, S. Asech, Vasundhara (RH 9304)
5B	05	PBR 97, Pusa Bold, Rajat (PCR 7), RH 781, Varuna (T 59)

maximum distance from Saurabh. Fifth cluster comprising 14 varieties was biggest while cluster 3 comprising two varieties was the smallest. Cluster 5 could be divided into two subclusters; 5A and 5B. Subcluster 5A included Sanjuncta Asech, Pusa Agrani, Pusa Bahar, Krishna, RH 819, Kanti and Kranti while subcluster 5b included RH 781, Varuna, Rajat, PBR 97 and Pusa Bold.

Out of 21 variables studied in present investigation, petal width and petal length/petal width did not have significant variability in present set of varieties. Two parameters; coefficient of variation among varieties (variability parameter) and coefficient of variation over years (stability parameters) were plotted on a two dimensional scatter chart to visualize a biplot (Fig. 1). Trait occupying fourth quarter with high variability estimate and low coefficient of variation over years (stability parameter) were the most effective while traits occupying second quarter with low variability and low stability were unsuitable for establishing distinctness. Traits occupying 3rd quarter may be useful with material having large variability for these traits. Leaf characteristics including leaf length and leaf width displayed low stability; hence, their ratio LL/LW may be more suitable as it had high stability. These findings are in conformity with earlier findings of (Weerakoon and Somaratne, 2010) for length of main raceme and siliqua length in classifying mustard accessions, however, in disagreement for leaf length and leaf width which were recorded at seedling stage while Indian DUS test guidelines prescribes recording of leaf characteristics at bud stage. In general, new varieties

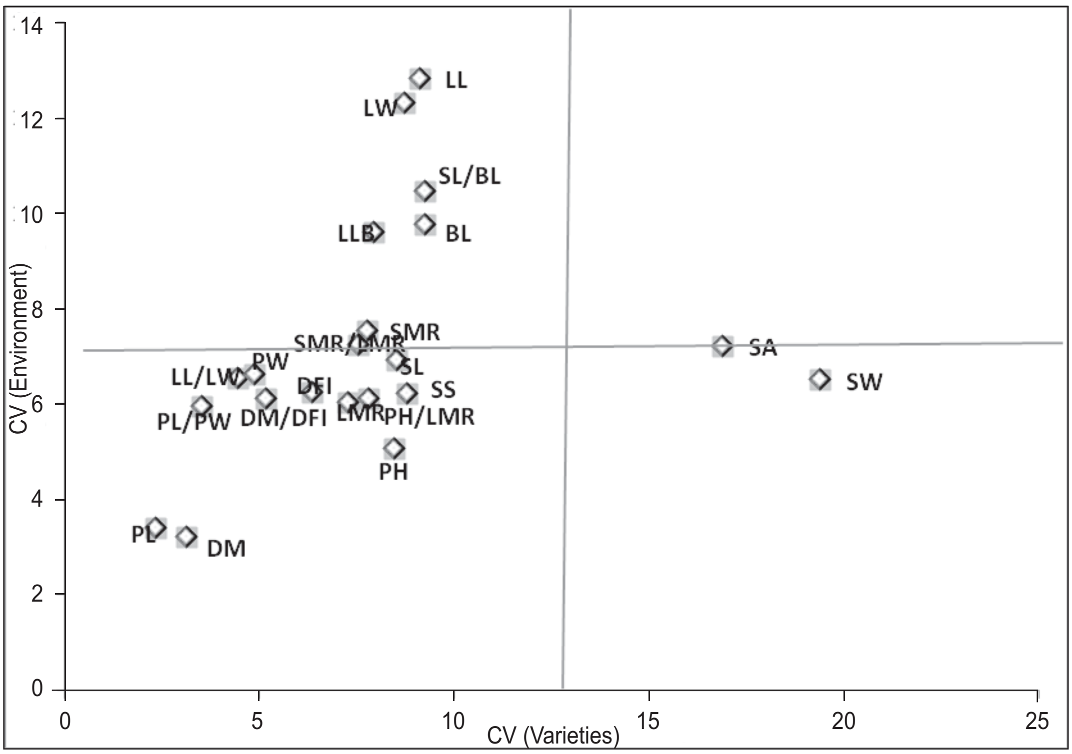

Fig. 1. Biplot of variability and stability parameters for different traits

may be considered "distinct" even in cases where they overlap with other varieties to a limited extent. However, it is necessary to quantify a threshold of the "minimum distance" between the new variety and any other variety from the reference collection, for the new variety to be considered distinct.

An analysis of pedigree of these 31 varieties revealed frequent use of single cultivar Varuna in hybridization programme. Four varieties; Kranti, Krishna, Pusa Jaikisan and Rohini have been derived through selection from natural population of Vrauna, while, seven varieties; Maya, PBR 97, Pusa Bahar, Pusa Bold, RL 1359, Saurabh and Urvashi had Varuna as one of the parentage in their pedigree. Another variety Aravali also had been derived through hybridization between Krishna and RS 50, here Krishna itself is direct selection from Varuna. Hence 12 varieties are the descendant of Varuna. Out of these four; Kranti, Krishna, Pusa Bahar and Pusa Bold have been grouped together in cluster five alongwith Varuna, indicating their resemblance. Reamaining eight varieties have migrated to other clusters which may be due to reconstellation of genes during meiosis and selection during segregating generations.

Distinctness among 31 varieties could be established using 8 morphological traits *viz*., seed weight, siliqua length, days to maturity, length of main raceme, seeds per siliqua, beak length and days to flower initiation (Table 4) proving the worth of these traits in establishing

Table 4. Distinctness based upon eight morphological traits among Indian mustard varieties

sw	sl	dm	ph	lmr	SS	BL	DFI	
small	small	early	Low					Sanjuncta Asech
			medium					Kanti
		medium	medium	medium				CS 52
	medium	early	Low	tall				NDRE-4
			medium	medium				Pusa Agrani
		medium	medium	medium	medium	low		RH 819
						medium	medium	Arawali
							late	Kranti
				small	medium			Vardan
			tall					Krishna
		late	tall					Saurabh
	long	medium	tall					RH 781
		Late	tall					RL 1359
medium	medium	medium	medium					GM 1
			tall					Geeta
		late	medium					PBR 91
			tall	medium	medium	medium	Late	PBR 97
							Medium	Swarn Jyoti
	long	medium	medium	medium	many			Pusa Bahar
			tall					Rohini
		late	tall	medium				Rajat
				tall				RLM 619
bold	medium	medium	medium	small				Urvashi
				medium	medium	medium	Medium	Pusa Jai Kishan
						medium	Late	Maya
					small			Varuna
				long				RH 30
			tall	medium	medium			GM 2
	long	medium	medium	medium	medium			Laxmi
					many			Pusa Bold

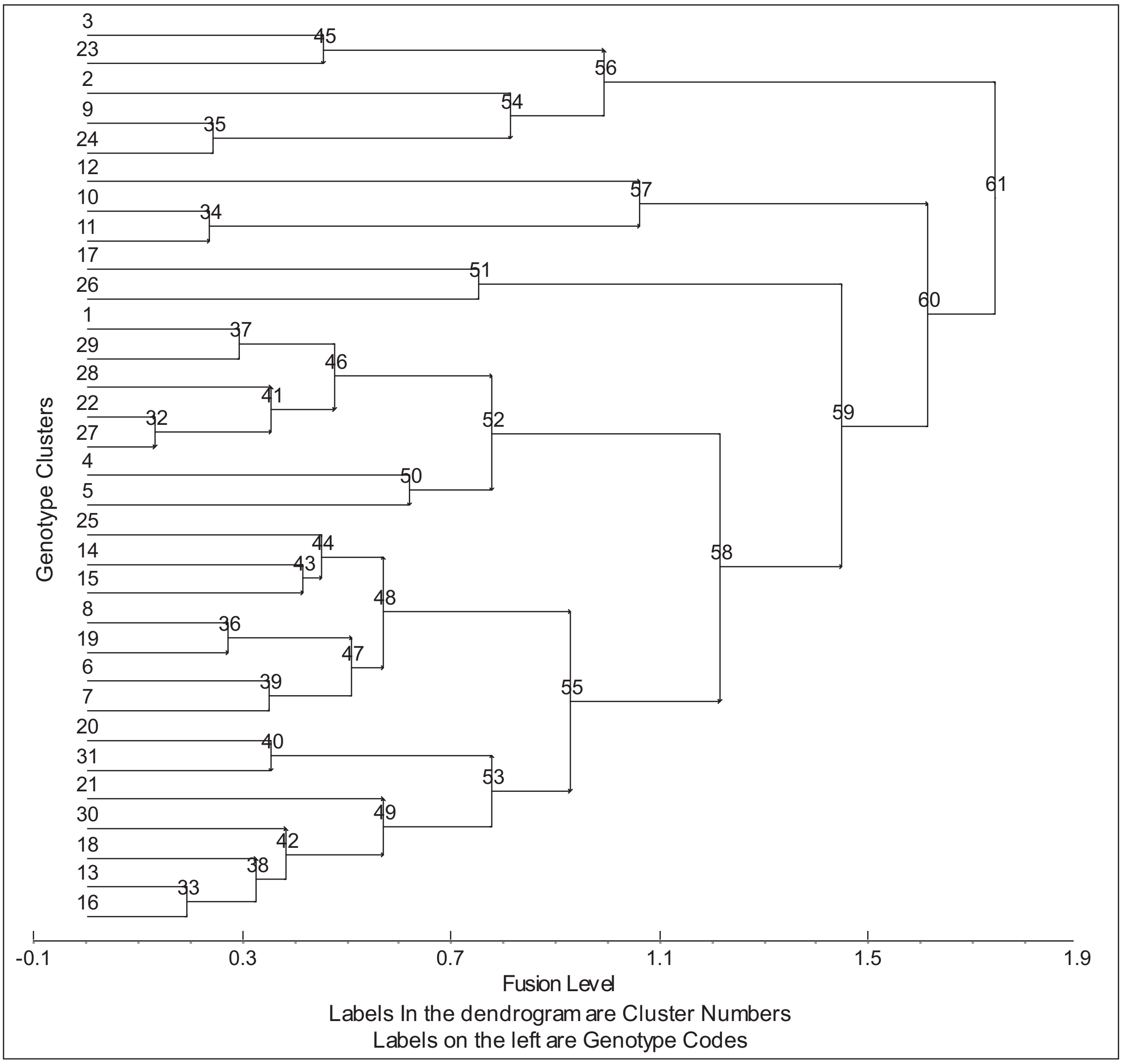

Fig. 2. Dendrogram showing different clustering pattern among 31 Indian mustard varieties on the basis of agro-morphological traits

distinctness. There was no correspondence of grouping between classification based upon mean estimate of traits and clusters based upon multivariate analysis. Though distinctiveness as defined by legislation bears no relationship to genetic distance, however, both approaches established distinctness/variability among Indian mustard varieties. High phenotypic variation in Indian mustard (Singh and Chauhan, 2010; Singh *et al*., 2006; Singh *et al*., 2013 and Singh *et al*., 2014) and canola varieties (Fahmi *et al*., 2012) have earlier been reported. On the basis of above discussion it is reported that seed weight, days to maturity, plant height, siliqua angle with main raceme, petal length, siliqua length, seeds per siliqua and days to flower initiation are more effective than leaf based traits; leaf length, leaf width and number of leaf lobes. Leaf length/leaf width estimates should be preferred over leaf based traits. Distinctness among popular 31 varieties could be established on the basis of 8 morphological traits. Varieties grouped into different clusters (CS 52, Geeta, Laxmi, RLM 619 and Rohini of cluster 1 and RH 819, RH 30, RH 781, Sanjuncta Asech, Varuna and Vasundhara of cluster 5) may be used as parents for hybridization and are likely to through wide spectrum of variability in segregating generations.

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