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GENETIC DIVERSITY AND PATTERNS OF VARIATION AMONG INDIAN MUSTARD (*Brassica juncea* (L.) Czernj. & Cosson) VARIETIES

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SUMMARY

Oleiferous Brassica species are economically important agricultural commodities. India is one of the largest rapeseed-mustard growing countries. Indian mustard (*Brassica juncea*) is predominantly grown oilseed crop of India, where it is widely cultivated under diverse agro-climatic situations. As a consequence of breeding research over a century in this crop, many varieties have been bred, however little information is available about extent of variability and diversity among these varieties. Variability for 18 agro-morphological traits and for 6 computed variables was estimated in 62 extant varieties. Multivariate analyses based upon principal components and hierarchical cluster revealed 4 distinct groups of varieties based upon geographical region of their originating centers. Much of the variability could be accounted for leaf and stem characteristics and phenological stages. Closely resembling as well as distinct varieties were identified. Varieties from different clusters based upon genetic distance were suggested for utilization in hybridization program.

Keywords: cluster analysis, diversity, extant varieties, Indian mustard, pattern of variation, principal component, variability

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INTRODUCTION

The oleiferous Brassica species, commonly known as rapeseed-mustard, are one of the economically important agricultural commodities grown in more than 50 countries in Asia, Europe, America and Australia (Woods *et al.*, 1991; Kjellstrom, 1993; Gomez and Prakash, 1999). India is one of the largest rapeseed-mustard growing countries in the world, occupying the first position in area (20.2%) and second in production (10.7%) after China. Rapeseed–mustard crops in India are grown in diverse agro-climatic conditions ranging from north-eastern/north-western hills to down south under irrigated/rainfed, timely/late sown, saline soils and mixed cropping. Indian mustard (*Brassica juncea*) accounted for about 80% of the 6.3 million hectare (mha) under these crops in the country during 2012-13 (Anonymous, 2013). In India, the research work on the improvement of rapeseed-mustard started by the turn of the last century at Pusa (Bihar), the then Bengal Presidency through the collection of land races and their purification (Chauhan *et al.*, 2011). Morphological traits have been studied for their taxonomic and evolutionary importance (Gomez-Campo and Tortosa, 1974). Distinctness, uniformity and stability (DUS) tests are generally conducted using morphological descriptors (IBPGR, 1990; MAFF, 1993; UPOV, 2002; PPV&FRA, 2009).

Earlier studies on diversity analysis in Indian mustard have been with limited number of genotypes based upon few morphological traits hence, the information about pattern of variation among cultivated varieties of different regions is meagre. The present investigation aimed to assess the pattern of variation among Indian mustard varieties for morphological traits, extent of variability, relationship between different traits and delineation of varieties into different clusters for developing further breeding strategy.

MATERIALS AND METHODS

Seeds of 62 varieties bred over the years at different research centers under the All India Coordinated Research Project on Rapeseed-Mustard, formed the basis of present study (Table 1). These were grown in randomized block design with 3 replications in plot of 5 rows of 5 m length for 2 consecutive years during rabi (winter) season of 2008-2010 at Directorate of Rapeseed-Mustard Research, Bharatpur (77.30 °E longitude, 27.15 °N latitude, 178.37 m above mean sea level), India. The row to row and plant to plant spacing were maintained at 45 and 10 cm, respectively. Standard package of practices were followed to raise a good crop. The crop was irrigated twice, first at 35-40 days and second at 60-70 days after sowing. Observations were recorded on 18 agro-morphological traits (including 3 leaf, 4 flower, 5 siliqua, 2 stem, 2 seed characteristics and 2 phenological stages) following DUS test guidelines (Singh *et al.*, 2006) except for siliqua angle which was measured with the help of a compass (Table 2). In addition 6 variables were derived as ratio of different measured traits. Leaf characteristics were recorded on biggest leaf of the 60 plants (20 plants per replication) of each variety during bud formation to flower initiation stage. Flower characteristics were recorded upon freshly opened flower for which buds were bagged before flower opening. All siliquae observations were recorded upon fully developed siliquae before maturity for which, 5 siliquae from lower half of main raceme were plucked from each of 60 plants. Oil content was estimated by using pre-caliberated near infrared reflectance spectroscopy (NIR, Dickey John Instalab 600) on random bulk of seed from each variety. Days to flower initiation and maturity were recorded on plot basis. The statistical parameters for dispersion and analysis of variance were estimated by using Distribution and Fit Model module, respectively of SAS JMP 9 software. Multivariate analyses were performed by principal component following correlation matrix and hierarchical cluster analyses based upon average linkage (Sokal and Michener, 1958).

RESULTS

Analysis of variance revealed the presence of significant variation among varieties for all the characteristics except petal width, stamen and style length. The extent of variability was estimated in terms of coefficient of variation. The maximum variability (21.1%) was recorded for angle between siliqua and main raceme followed by 1000-seed weight (18.3%), and siliqua beak length (13.0%). The minimum variability (1.9%) was recorded for oil content followed by stamen length (2.6%). The distribution pattern of varieties for each trait was also studied and depicted by outlier box plots. The varieties exhibited normal distribution for seeds per siliqua, days to maturity, 1000-seed weight, oil content, stamen length, style length and for ratio between plant height and length of main raceme. Moderate deviations from normality were observed for remaining traits. RCC 4 was observed as an outlier with 39.8 cm leaf length and 14.5 cm wide leaves. Petal length had very narrow range, however, 4 varieties namely, Ashirwad (0.8 cm), Bhagirathi (0.9 cm), Sarama (0.9 cm) and Seeta (0.9 cm) were found outliers with small petals. Similarly, stamen and Style length had very narrow range (0.8-0.9 cm). Vaibhav had minimum siliqua length (2.9 cm), while RL 1359 and Basanti had longest siliquae (> 5.0 cm). Siliqua beak length also exhibited broad dispersion ranging from 0.7-1.4 cm, maximum number of varieties, however, were between 0.9-1.1 cm. There were discrete categories of 5-10°, 20-25° and > 25° on the basis of siliqua angle. Three varieties Bhagirathi, Sanjucta Asech and TM 2 were quite distinct forming an angle of < 10°. Seeds per siliqua ranged from 11.7 to 17.7 with 14 to 15.5 in the interquartile range. The varieties in present investigation had 52 – 78 cm long main raceme. Maximum varieties were grouped on the basis of plant height (between 170 to 190 cm). The 1000-seed weight ranged from 3.0 – 6.4 g. Interquartile range which comprises maximum number of varieties was 3.6 to 5.0 g. There was a continuous distribution in all categories and no outliers were observed. Oil content varied from 38.5 to 42.1 %. Maximum varieties fell between 40 - 40.5%. Distribution of variation for days to flower initiation was skewed towards earliness ranging from 40-64 days with mean value of 56 days, though maximum varieties were grouped between 55-60 days. Days to maturity ranged from 110 - 141 days with 122 -132 interquartile range. Density of siliquae bearing on main raceme was estimated as ratio between numbers of siliquae born on main raceme to length of main raceme. In all varieties it was less than 1.0 ranging from 0.6-0.98. Among the computed ratios, leaf length was 2.4 to 3.2 times higher than corresponding leaf width; petal length 1.3 to 1.8 times of petal width; plant height 2.3 to 3.3 times of main raceme length; siliqua length 3.2 to 5.6 times of beak length, while stamen and style length were in general of equal length.

Principal Component Analysis

The purpose of principal component analysis is to derive a small number of independent linear combinations (principal components) of a set of variables that capture as much of the variability in the original variables as possible. First 7 principal components accounted for 72.6% of the total variation (Table 3). The first principal component accounted for 18.4% of the variation for which leaf length, leaf width, number of leaf lobes, plant height, days to flower initiation, days to maturity and PH/LMR ratio contributed positively (Figure 1). All these traits had almost equal weights, conversely, oil content, petal width and LL/LW ratio had slightly negative weight. Principal component 2 accounted for 14.6% of variation, primarily represented by density of siliquae bearing, PL/PW and SL/BL ratios, while, beak length, siliqua length, petal width, length of main raceme, siliqua angle and 1000-seed weight had negative weights. The third principal component emphasized length of main raceme, siliquae on main raceme, petal length, plant height, number of seeds per siliqua and PL/PW ratio which increased at the expense of leaf characteristics, LL/LW and PH/LMR ratios. Principal component 4 accounted for 8.4% of variation and highlighted the variation in style length which increased with oil content, siliqua length, STL/STYL and SL/BL ratios. Score plot to study the pattern for divergence among 62 varieties was drawn on the basis of first 3 principal components. In all 3 score plots, maximum diversity was observed between RCC 4 and NDRE 4 though their relative positions have changed from one to other quarter (Figure 2). In PC 1 vs PC 2 score plot, varieties in quarter 1 had high variance for traits contributing positively to PC1 and PC 2, while varieties of quarter 2 reflected much variance for traits contributing positively to PC 2 and traits having negative weights for PC 1. Varieties of quarter 3 exhibited much variance for traits contributing negatively for PC1 and for PC 2. Likewise, varieties in quarter 4 had much variance for traits contributing positively to PC 1 and for traits with negative weights for PC 2. Varieties placed at distant points of a quarter, represented diversity from the other varieties of the same quarter and simultaneously, with other distant located varieties. Hence, varieties RCC 4, TM 2, TM 4, Bhagirathi, Sanjucta Asech, NDRE 4, Seeta and NavGold were more diverse varieties than others. Loading plots were drawn to illustrate the extent of variance and relationship among different variables. Strong association were depicted between leaf length and leaf width; days to maturity with days to flower initiation and PH/LMR ratio, density of siliquae bearing with PL/PW and SL/BL ratios while, oil content had negative association with these traits.

Cluster analysis

Sixty-two varieties were grouped into 8 clusters on the basis of 18 morphological and 6 computed variables. Cluster 1 comprising 50 varieties was the biggest (Table 4) and cluster 3, 5, 6, 7 and 8 were monogenotypic comprising TM 4, Seeta, TM 2, RCC 4 and NDRE 4, respectively. Varieties bred in eastern India particularly at Berhampore (West Bengal) share a distinct gene pool as the varieties, Bhagirathi, Sanjucta Asech and Sarama were grouped together in cluster 4 with Kanti a variety from Kanpur. Variety Seeta which displayed diversity from other varieties of Berhampore centre and belonged to separate cluster further endorsed the diversity of eastern Indian gene pool. Likewise, variety RCC 4 bred at Kangra located in Himalayan region was also quite distinct from other varieties. Similarly 2 varieties bred at Bhabha Atomic Research Centre located at Mumbai in western region of India were grouped into separate clusters, endorsing the diversity among varieties bred in different regions of country. Only 6 varieties namely, Nav Gold, Ashirwad, NDYR 8, Kanti and NDRE 4 developed in northern region fell into different clusters representing their diversity from other varieties of cluster 1. Varieties which expressed close resemblances were ACN Satabdi with Pusa Agrani; Laxmi with Krishna; RRN 505 with RGN 48 and Swarna Jyoti with Kranti. On the other hand, highest distance was recorded between RCC 4 and NDRE 4 and between TM 2 and NDRE 4.

Table 1. Developing organization and year of release of extant varieties of Indian mustard.

Name of the Variety	Developing Centre	Year of release/Identification
Durgamani	ARS Durgapura, Rajasthan	1974
JM 1	ARS Morena, MP	1999
JM 2	ARS Morena, MP	2005
JM 3	ARS Morena, MP	2005
Arawali (RN 393)	ARS, RAU Navgaon, Rajasthan	2001
Nav Gold (YRN 6)	ARS, RAU Navgaon, Rajasthan	2006
RRN 505	ARS, RAU Navgaon, Rajasthan	2006
TM 2	BARC Mumbai, Maharashtra	1993
TM 4	BARC Mumbai, Maharashtra	1993
Shivani	BAU Ranchi, Jharkhand	2005
Geeta (RB9901)	CCS HAU Bawal, Haryana	2003
Laxmi (RH8812)	CCS HAU Hisar, Haryana	1997
RH 819	CCS HAU Hisar, Haryana	1991
RH 30	CCS HAU Hisar, Haryana	1985
RH 781	CCS HAU Hisar, Haryana	1991
Saurabh (RH8113)	CCS HAU Hisar, Haryana	1987
Swarn Jyoti (RH9801)	CCS HAU Hisar, Haryana	2003
Vasundhara (RH9304)	CCS HAU Hisar, Haryana	2003
ACN Satabdi	College of Agriculture, Nagpur, Maharashtra	2007
Ashirwad	CSAUA&T Kanpur, Uttar Pradesh	2005
Basanti (RK8901)	CSAUA&T Kanpur, Uttar Pradesh	2001
Kanti	CSAUA&T Kanpur, Uttar Pradesh	2003
Maya	CSAUA&T Kanpur, Uttar Pradesh	2003
Rohini	CSAUA&T Kanpur, Uttar Pradesh	1986
Urvashi (RK9501)	CSAUA&T Kanpur, Uttar Pradesh	2001
Vaibhav	CSAUA&T Kanpur, Uttar Pradesh	1985

Vardan	CSAUA&T Kanpur, Uttar Pradesh	1985
Varuna (T59)	CSAUA&T Kanpur, Uttar Pradesh	1976
CS 52	CSSRI, Karnal, Haryana	1998
CS 54	CSSRI, Karnal, Haryana	2005
Rajat (PCR7)	DRMR, Bharatpur, Rajasthan	1997
Kranti	GBPUAT, Pantnagar, Uttarakhand	1984
Krishna	GBPUAT, Pantnagar, Uttarakhand	1998
Patan Mustard (PM67)	Govt. Of Gujarat	1984
BR-40	Govt. of Bihar	1960
Jagannath	IARI RS PUSA, Bihar	1999
Pusa Agrani (Sej-2)	IARI, New Delhi	1998
Pusa Bahar	IARI, New Delhi	1991
Pusa Bold	IARI, New Delhi	1985
Pusa Jai Kishan (Bio-902)	IARI, New Delhi	1994
Pusa Karishma (LES39)	IARI, New Delhi	2005
Pusa Mahak (JD6)	IARI, New Delhi	2005
Raya (RCC-4)	Kangra, Himachal Pradesh	2001
Shivalik (Mahon8)	Mahyco, Jalana, Maharashtra	2002
Narendra rai (NDR8501)	NDUAT, Faizabad, Uttar Pradesh	1990
NDRE-4	NDUAT, Faizabad, Uttar Pradesh	2001
NDYR8	NDUAT, Faizabad, Uttar Pradesh	2005
RL1359	PAU, Ludhiana, Punjab	1988
RLM619	PAU, Ludhiana, Punjab	1985
PBR210	PAU, RS Bathinda, Punjab	2007
PBR91	PAU, RS Bathinda, Punjab	1996
PBR97	PAU, RS Bathinda, Punjab	1997
Bhagirathi	PORS Berhampore, West Bengal	1987
Sanjucta Asech	PORS Berhampore, West Bengal	1989
Sarama	PORS Berhampore, West Bengal	1986
Seeta	PORS Berhampore, West Bengal	1982
RGN13	RAU, Sriganganagar, Rajasthan	2003
RGN48	RAU, Sriganganagar, Rajasthan	2006
RGN73	RAU, Sriganganagar, Rajasthan	2007
GM1	SDAU SK Nagar, Gujarat	1990
GM2	SDAU SK Nagar, Gujarat	1997
GM3	SDAU SK Nagar, Gujarat	2006

Table 2. Observations recorded in Indian mustard varieties.

Trait	Code	Description of the trait
Leaf characteristics		
Leaf lobes	LLB	Parts of leaf blade were counted as lobes if their length is more than the half of the width of leaf
Leaf length (cm)	LL	Length of leaf blade including petiole from stem to tip of the blade
Leaf width (cm)	LW	Width of the leaf blade at the widest point
Flower characteristics		
Petal length (cm)	PL	Length of lamina including claw
Petal width (cm)	PW	Width of lamina at widest point
Stamen length (cm)	STL	Length of tall stamens
Style length (cm)	STYL	Length of style
Siliqua characteristics		
Siliqua length (cm)	SL	Siliqua length was measured between pedicel and beak
Siliqua beak length (cm)	BL	Length of the empty tip of siliqua
Number of siliquae on main raceme	SMR	Number of siliquae on main inflorescence
Siliqua angle with main raceme (°)	SA	Angle between pedicel and main raceme
Number of seeds per siliqua	S/S	Counted total number of seeds per siiqua plucked from main raceme
Stem characteristics		
Length of main raceme (cm)	LMR	Length of upper most inflorescence
Plant height (cm)	PH	Height of the stem from ground to top of plant including branches
Seed characteristics		
1000-seed weight (g)	SW	1000-seeds were counted from random bulk of seed yield of each variety and weighed to record the 1000-seed weight in g
Oil content (%)	OC	Oil content was estimated by NIR on random bulk of seed from each variety
Phenological stages		
Days to flower initiation	DFI	Days from date of sowing to when more than 50% plants of plot bear at least 1 open flower
Days to maturity	DM	Days from date of sowing to when more than 75% siliquae in a plot turn yellow
Computed variables		
Density of siliquae bearing	DSB	Ratio between number of siliquae born on main raceme and length of main raceme (SMR/LMR)
Leaf length/width	LL/LW	Ratio of leaf length to leaf width derived from LL/LW

Petal length/petal width	PL/PW	Ratio of petal length to petal width derived from PL/PW
Plant height/length of main raceme	PH/LMR	Ratio of plant height to main raceme length derived from PH/ LMR
Siliqua length/beak length	SL/BL	Ratio of siliqua length to beak length derived from SL/BL
Stamen length/Style length	STL/STYL	Ratio of stamen length to style length derived from STL/STYL

Table 3. Eigenvalue, proportion of variance and traits that contributed to first 10 principal components of Indian mustard varieties.

Principal components	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
Eigenvalue	4.4	3.5	3.3	2.0	1.6	1.4	1.3
Proportion of variance	18.4	14.6	13.7	8.4	6.6	5.7	5.3
Cumulative variance	18.4	32.9	46.6	55.0	61.6	67.3	72.6
Trait	Eigenvector						
Llobes	0.30	-0.12	-0.21	-0.04	-0.07	0.12	0.19
LL	0.34	-0.01	-0.29	0.10	-0.03	0.15	0.13
LW	0.39	0.00	-0.17	0.14	-0.10	0.03	0.16
PL	0.06	0.12	0.31	-0.13	-0.05	0.05	0.57
PW	-0.05	-0.29	0.01	0.01	-0.26	-0.45	0.40
STL	0.07	0.09	0.08	0.54	-0.04	0.25	0.12
STYL	-0.01	-0.08	0.08	0.16	-0.61	0.40	-0.11
SL	0.20	-0.14	0.07	0.28	0.15	-0.12	-0.33
BL	0.11	-0.39	-0.01	-0.05	0.31	0.14	-0.03
SMR	0.04	0.18	0.37	-0.15	0.03	0.13	-0.07
SA	0.19	-0.26	0.20	0.13	-0.04	-0.05	0.15
S/S	0.02	0.10	0.23	0.13	-0.04	-0.33	-0.11
LMR	-0.02	-0.23	0.43	0.00	0.05	0.15	-0.12
PH	0.35	-0.12	0.24	-0.09	-0.01	0.05	-0.18
SW	0.17	-0.25	0.06	-0.24	0.11	0.00	-0.11
OC	-0.12	-0.08	0.04	0.40	0.11	0.18	0.14
DFI	0.33	0.17	0.08	-0.11	-0.01	-0.01	-0.18
DM	0.35	0.11	0.19	-0.04	0.01	-0.12	0.05
DSB	0.04	0.43	-0.13	-0.13	0.01	-0.03	0.07
LL/LW	-0.08	-0.01	-0.29	-0.04	0.18	0.30	-0.09
PL/PW	0.04	0.32	0.24	-0.12	0.16	0.35	0.17
PH/LMR	0.37	0.13	-0.20	-0.08	-0.06	-0.09	-0.06
SL/BL	0.03	0.30	0.06	0.30	-0.22	-0.24	-0.27
STL/STYL	0.07	0.13	0.03	0.35	0.53	-0.15	0.17

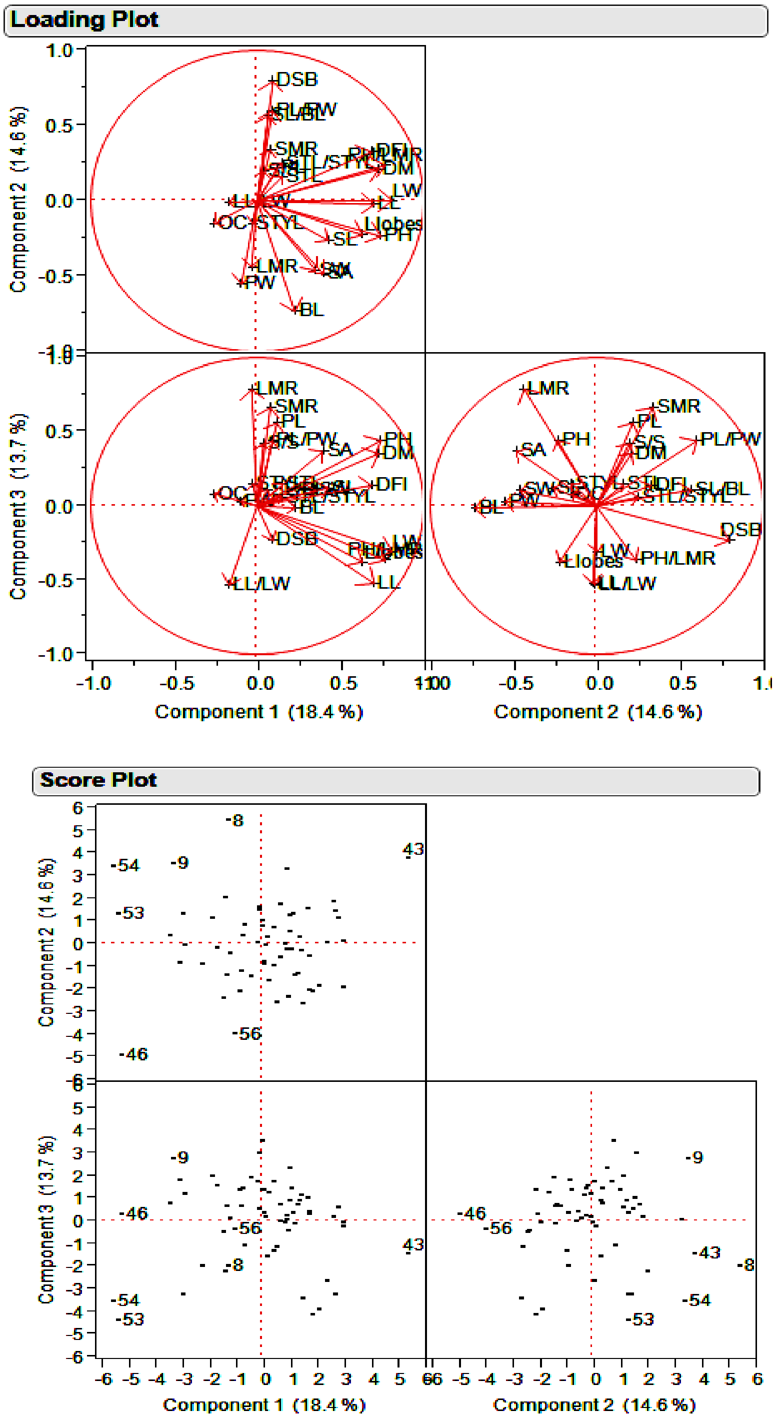

Figure 1. Loading and score plots of first 3 principal components in Indian mustard varieties.

Table 4. Grouping of varieties into different clusters.

Cluster	Variety name	Number of varieties
1	Durgamani, JM 1, JM 2, JM 3, Arawali, RRN 505, Shivani, Geeta, Laxmi, RH 819, RH 30, RH 781, Saurabh, Swarn Jyoti, Vasundhara, ACN Satabdi, Basanti, Maya, Rohini, Urvashi, Vaibhav, Vardan, Varuna, CS 52, CS 54, Rajat, Kranti, Krishna, Patan Mustard, BR-40, Jagannath, Pusa Agrani, Pusa Bahar, Pusa Bold, Pusa Jai Kishan, Pusa Karishma, Pusa Mahak , Shivalik, Narendra rai, RL 1359, RLM 619, PBR 210, PBR 91, PBR 97, RGN 13, RGN 48, RGN 73, GM 1, GM 2, GM 3	50
2	Nav Gold, Ashirwad, NDYR 8	03
3	TM 4	01
4	Kanti, Bhagirathi, Sanjucta Asech, Sarama	04
5	Seeta	01
6	TM 2	01
7	RCC-4	01
8	NDRE-4	01

DISCUSSION

In this investigation, significant variability for all the traits except petal width, stamen length and style length was observed. Normal distribution for seeds per siliqua, days to maturity, 1000-seed weight, oil content and ratio between plant height and length of main raceme indicated wide dispersion for these traits among the varieties, studied. The high variability for morphological traits has earlier been reported (Ghosh, 2002; Singh *et al.*, 2006). Cluster and principal component analyses are frequently used to study the genetic diversity and have been performed in various species of Brassica (Alemayehu and Becker, 2002; Singh *et al.*, 2013). Pattern of variation as revealed by principal component analysis indicated large variability for leaf and phenological traits. Wide range of dispersion for maturity duration may be explained in accordance to diverse climate of growing regions starting from Jammu & Kashmir and Himachal Pradesh which lies in Himalayan region representing sub temperate climate during growing period that provides longer maturity duration.

The plains of northern states, comprising Punjab, Haryana, Delhi, eastern and northern Rajasthan, Uttar Pradesh and Madhya Pradesh occupy large cultivated area of the crop and varieties bred at centers located in these states were grouped together in a single cluster representing medium maturity, plant height and leaf characteristics. The narrow genetic base of these varieties might have arisen due to common parents in their ancestry (Chauhan *et al.,* 2011; Singh and Chauhan, 2010). Western regions comprising states of Gujarat, Maharashtra and western Rajasthan and eastern region comprising Bihar, Odisha, Jharkhand, West Bengal, Asom and north eastern hill states represent shorter growing duration due to prevailing relatively high temperature at sowing time (October-November) and moderate temperature during growing season varying from October to March. Varieties, therefore, bred in these regions possess short maturity duration coupled with short plant stature (plant height and leaf size). Relationship among variables revealed by loading plots indicated close association between leaf characteristics. Traits like oil content, petal size, stamen and style length exhibited low variability. Though petal size and other floral traits have never been explored as breeding objectives, however, low variability for oil content needs to be addressed and warrants utilization of diverse exotic source for enhancing the range. 1000-seed weight and number of seeds per siliqua are important yield components for which moderate variability was observed, hence it would be desirable to focus on enhancing the extent of variability for these traits by introgressing traits from related species like yellow sarson (*B. rapa* var yellow sarson) having higher seeds per siliqua (Kumar and Chauhan, 2005). Much of the variability could be accounted for leaf, stem characteristics and phenological stages while, flower and seed characteristics had low magnitude. Leaf, stem and phenological stages were positively correlated among themselves that would have brought simultaneous improvement for these traits as a result of correlated response. Similarly, siliqua characteristics also had positive association with plant height and maturity duration that suggests varieties with shorter height and small leaves are likely to mature early and *vice versa* is true for late maturing genotypes. Considering the fact of varying growing periods of different regions, varieties of medium maturity duration, plant height and leaf size should be bred for high yield. Varieties exhibiting diversity from each of 4 regions, *viz*., RCC 4 (northern hills); Navgold, NDRE 4, NDYR 8 and Kanti (northern plains); TM 2, TM 4 (western region) and Bhagirathi, Sanjucta Asech and Seeta (eastern region) should be used as parents in hybridization program. These varieties need to be tested for general and specific combining ability for their utilization in heterosis breeding program.

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