



Identification and evaluation of Indian mustard genotypes for white rust resistance and agronomic performance

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Abstract

The screening of 2000 Indian mustard accessions against white rust and phenotypic evaluation of resistant accessions to assess the nature and magnitude of genetic diversity including their agronomic potential was carried out at three locations during *rabi* of 2014-2016. Out of 2000 germplasm accessions, 168, 46 and 185 accessions were found resistant at Ludhiana, Pantnagar, and Hissar, respectively but only 27 were identified as resistant across the locations indicating the pathogenic variability at different locations. These 27 accessions were further validated under artificial inoculation and eight of them, namely, IC265495, IC313380, EC766091, EC766133, EC766134, EC766192, EC766230 and EC766272 were identified as highly resistant with disease severity reaction (PDI = 0) to *A. candida* at both cotyledonary and true leaf stages. Agronomic evaluation of these 27 accessions facilitated the identification of superior genotypes with high magnitude of genetic diversity for their use in breeding. As compared to check varieties, elite genotype(s) such as IC313380 was found promising for days to flowering (40), silique on main stem (52.6), silique length (4.2 cm), seeds per silique (17.2) and oil content (41.4%); EC766230 for no. of primary branches (8), seeds/ silique (16.8), seed yield/plant (16.0g), oil content (38.7%) while EC766272 for silique on main stem (55.50), seed yield/plant (18g) and oil content (40.5%). The resistant accessions coupled with agronomic superiority may be useful genetic resources for improvement of Indian mustard. In addition, the resistant accessions can serve as a rich gene pool for breeding programmes.

Key words: Indian mustard, *Albugo candida*, white rust, germplasm

Introduction

India is the fifth largest vegetable oil economy in the world next to USA, China, Brazil and Argentina accounting for 5.8% vegetable oil production; 11.2% of world oil import and 9.3% of the world edible oil consumption (Anon 2015). Among rapeseed and mustard, which is grown in about 6.7 m ha in the Indian subcontinent, Indian mustard (*Brassica juncea* (L.) Czern. & Coss.) accounts for about 75-80% of the 5.47 m ha area with average productivity of 1183 kg/ha in the country during 2015-16 (MOA&FW 2016). Indian mustard is an agriculturally important oilseed crop with a long history of cultivation in India, China and increasingly in Australia. In India, it is predominantly cultivated in Rajasthan, Uttar Pradesh, Haryana, Madhya Pradesh and Gujarat, which contribute 81.5% area and 87.5% production during 2013-14.

Despite having large area under oilseeds, India is the major importer of edible oil. The low productivity is attributed to yield stagnation in new varieties and increasing pressure of biotic and abiotic stresses. Rapeseed and mustard crops have been known to be affected by diseases such as downy mildew, white rust, *Alternaria* blight and *Sclerotinia* stem rot (Bisht et al. 2015). White rust and Downy mildew can occur separately or in association with each other and can cause 37-47 per cent loss in pod formation and 17-

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54.5 per cent reduction in grain yield (Mukherjee et al. 2001). The germplasm has been screened against white rust and varying levels of host resistance have been identified (Ahmad et al. 2014; Dharavath et al. 2017). However, the information on the sources of resistant donors from germplasm conserved in the National Gene Bank at NBPGR and their genetic diversity status based on agronomic traits is limited. The analysis of the nature and magnitude of genetic diversity is important for plant breeding because progenies derived from diverse origin parents may show better heterosis than those between closely related strains (Chauhan et al. 2008; Zahan et al. 2010). Therefore, a large number of accessions were screened at different hot spots under field and artificially inoculated conditions in laboratory with the objectives, (i) to identify germplasm accessions resistant to white rust and (ii) to assess the agronomic performance along with nature and magnitude of genetic diversity in the resistant accessions.

Materials and methods

The material was comprised of 2000 germplasm accessions of Indian mustard procured from 21 countries including India. Out of 2000 accessions, 1350 were collected from different parts of Indian states majoring Andhra Pradesh (91), Bihar (63), Haryana (166), Himachal Pradesh (59), Madhya Pradesh (24), Jharkhand (47), Karnataka (92), Rajasthan (24), Uttar Pradesh (114), Uttarakhand (21), Delhi (16), Arunachal Pradesh (11), Assam (9), Mizoram (9), Odisha (9) and others (595), while 650 exotic accessions were introduced primarily from United Kingdom (506), Canada (57), Sweden (18), France (16), USA (13) and Australia (11) and others (29).

All the accessions were grown for screening against white rust resistance at three experimental hot spot locations viz., Punjab Agricultural University, Ludhiana, Chaudhary Charan Singh Haryana Agricultural University, Hissar and Govind Ballabh Pant University of Agriculture and Technology, Pantnagar in augmented block design during *rabi* crop season of 2014-15 and 2015-16 along with resistant and susceptible check varieties viz., RLC 3 as resistant, RGN 73 and Varuna as susceptible and Kranti as National Check, using standard agronomic practices. Germplasm accessions scored as resistant (PDI = 0) across the location and years were further validated under artificially inoculated conditions at Division of Plant Pathology, ICAR-IARI, New Delhi during 2016-17 as per the method described by Fox and Williams (1984). Observations for per cent incidence (PDI) of white rust on leaves were recorded 20 days before the maturity of crop as per disease scoring scale adopted by Conn et al. (1990) and Annon. (2011) (Table 1). The disease severity under natural epiphytotic conditions was calculated using the formula given below:

$$\frac{(N-1 \times 0) + (N-2 \times 1) + (N-3 \times 3) + (N-4 \times 5) + (N-5 \times 7) + (N-6 \times 9)}{\text{No. of leaf samples}}$$

Average severity score =

Screening of germplasm under artificial conditions

For artificial screening of 27 accessions which showed 0-PDI under natural epiphytotic conditions were sown in plastic pots (15cm dia.) with sterilized soil in glass house. Plant stand was maintained uniformly as 10

Table 1. Disease score, its severity percentage and corresponding reaction for white rust in Indian mustard

Disease score	Disease severity (%) and symptoms	Disease reaction
0	No infection on either leaf surface	Immune
1-2	Up to 5% leaf area covered with small pinpoint to larger brown necrotic flecks under inoculation point	Highly resistant (HR)
3-4	> 5%-10% leaf area covered with very sparse sporulation, one to few pustules on lower surface and no of pustules on upper surface	Resistant (R)
5-6	11%-25% leaf area covered with few to many scattered pustules with good sporulation on lower surface and none to few pustules on upper surface	Moderately resistant (MR)
7-8	26%-50% leaf area covered with many pustules with abundant sporulation on lower surface with none to few pustules on upper surface	Susceptible (S)
9	> 50% leaf area covered with many large coalescing pustules on lower surface with few to many pustules on upper surface of the cotyledon	Highly susceptible (HS)

plants in each pot. The inoculum of *Delhi isolate* was prepared from fresh sporangia by scraping from a single pustule with a sterile scalpel and collected in 1.5 ml Eppendorf tube. The sporangial suspension was kept at 4°C for 4 h for zoospore release. The concentration of zoospore was adjusted to a concentration of 8×10^4 zoospores/ml by using haemocytometer (Sachan et al. 2004). The seedlings were drop inoculated with 5 μ l each onto adaxial surface of every lobe of each cotyledon 8 days after sowing with zoosporangial suspension with the help of micropipette. To promote infection, the inoculated plants were subjected to enhanced humidity (>98%) for 72 h in a high humidity chamber sealed with plastic sheets and free water up to 2 cm height. After three days the plants were taken out from the chamber and kept outside at temperature 18°C and 13°C, day and night, respectively under 70-120 μ mol/m/s irradiations for 12 days to reach peak sporulation.

At seedling stage infection, phenotypes were recorded after 12 days of incubation. The selected resistant plants were further inoculated at juvenile stage between 25-30 days after seedlings and at flowering stage (40 to 45 days after sowing). The germplasm accessions found resistant 20 days before maturity were selected. On the cotyledonary leaves, disease reaction assessment was done after 12 days of inoculation (DAI) and scored as per 0-9 scale, while on the true leaves, assessment was done on a 0-5 scale at 14 DAI (Fig. 1).

Statistical analysis

The data on morphological traits viz., no. of primary branches/plant (PB), days to 50% flowering (DF), plant height (PH), days to 80% maturity (DM), silique on main stem (SMS), silique length (SL), seeds/silique (SS), yield/plant (YP), 1000-seed weight (SWT) and oil content (OC) were determined for two years in 27 accessions found resistant under natural field

screening. The pooled data were analysed for mean, variances, correlations, genetic diversity to find out genetic similarity/dissimilarity and principal component analysis (PCA) using the statistical software SYSTAT-12. Phenotypic and genotypic coefficients of variation (PCV and GCV) were computed as per Burton (1952). Heritability was estimated (Lush 1940) and further classified into low, medium and high magnitude (Robinson 1966) while genetic advance estimated as per Johnson et al. (1955). The significance was assessed at the 5% probability level.

Results and discussion

Screening of germplasm against white rust

White rust is a highly destructive disease of oilseed Brassicas and therefore, it is essential to identify useful sources of host resistance in *B. juncea* as breeding and/or selection for resistance is the most cost-effective method of control. In this study, we screened 2000 accessions of *B. juncea* germplasm at three locations viz., Ludhiana, Pantnagar and Hissar during 2014-15 and 2015-16 crop seasons. Over two years, 168, 46 and 185 germplasm accessions showed 0% disease severity with no infection (rated as immune) at Ludhiana, Pantnagar, and Hissar, respectively (Table 2). However, over the locations only 27 accessions have shown 0% disease severity with no infection indicating that these accessions are genetically more diverse and stronger than others. The differential reaction of germplasm accessions at different locations showed that there is a pathogenic variability for white rust. Pandey et al. (2013) have reported morphological and pathogenic variability of six *Albugo candida* isolates collected from Pantnagar, Ludhiana, Hissar, Bharatpur, Delhi, Kangra and Jammu. *A. candida* isolates from different *Brassica* species/cultivars or from different geographical regions have shown differential response in their incubation period, latent period and production of sporangia and

Table 2. Disease reaction to white rust of Indian mustard accessions at different locations

Disease Score	No. of acc. identified at			Promising accessions over the locations	Resistant acc. under artificially inoculated conditions
	Ludhiana	Pantnagar	Hissar		
0	168	46	185	IC597932, IC267703, IC313380, IC20167,	IC 265495, IC313380,
1-2	90	-	30	IC265495, EC657030, EC699003, EC766091,	EC766091, EC766133,
3-4	110	101	17	EC766133, EC766134, EC766136, EC766144,	EC766134, EC766192,
5-6	714	1037	336	EC766145, EC766148, EC766152, EC766164,	EC766230, EC766272
7-8	801	626	426	EC766191, EC766192, EC766193, EC766230,	
9	-	86	2	EC766232, EC766272, EC766311, EC766313,	
				EC766315, EC766316, EC766402	

acc. = Accession

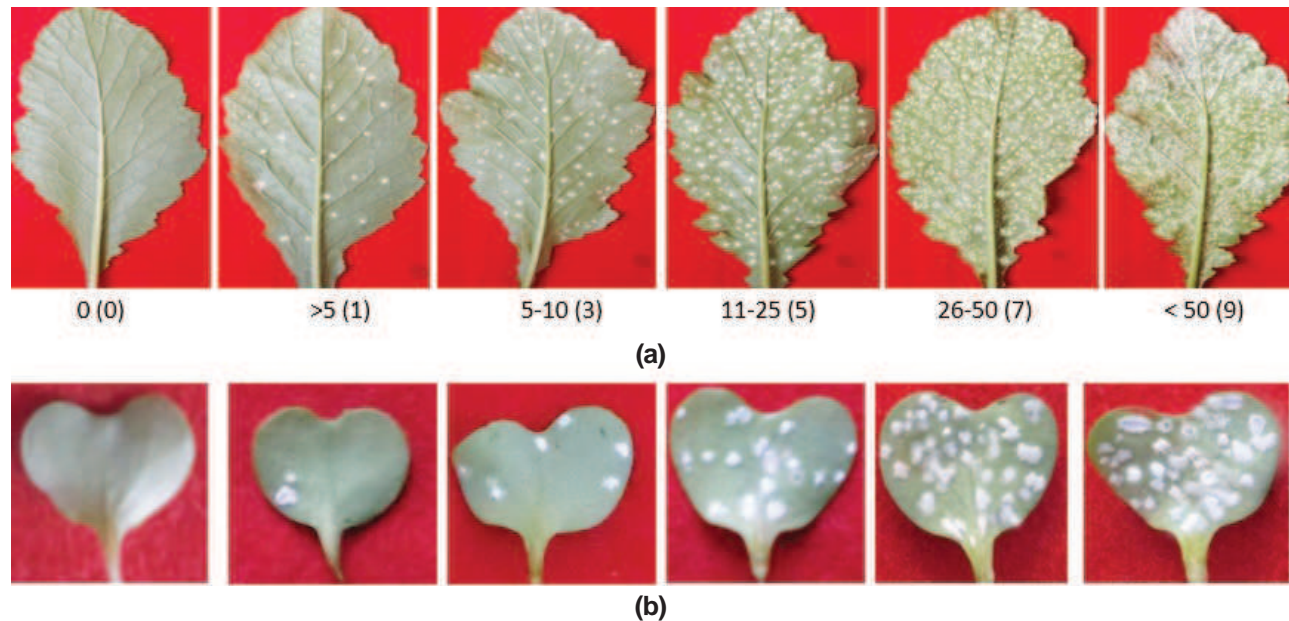


Fig. 1. Photographic scale for the screening of germplasm against white rust (0-9 scale) at true leaf (a) and cotyledonary stage (b). Source: Oilseed Seed Lab., Plant Pathology, GBPUAT, Pantnagar

zoospores, pustule size, shape and texture and aggressiveness (Gupta and Saharan 2002; Patni et al. 2005; Mishra et al. 2009).

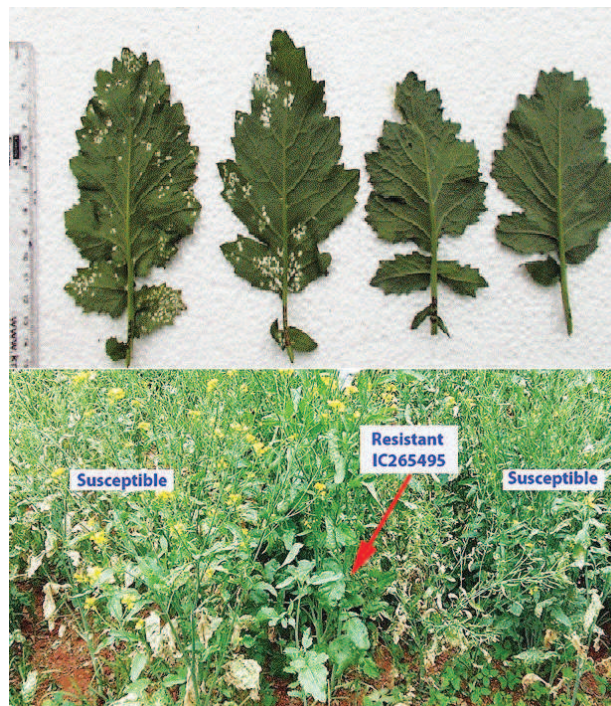


Fig. 2. Resistant and susceptible disease reaction to white rust under laboratory inoculation (above) and resistant accession IC264595 under field screening (below)

The white rust resistant, 27 accessions were further tested artificially in the pathological laboratory for assistance against Delhi isolate of *A. candida*. Out of 27 accessions, only 8 namely, IC 265495, IC313380, EC766091, EC766133, EC766134, EC766192, EC766230 and EC766272 displayed resistant reaction (PDI = 0) to *A. candida*. While analysing the geographical affinity of 8 accessions found resistant to white rust it was found that they primarily belong to cold regions. Indigenous accessions IC265495 (Mizoram), IC313380 (Himachal Pradesh) and exotic accessions viz., EC766091, EC766133, EC766134, EC766192, EC766230, EC766272 are from United Kingdom suggesting the possibility of augmenting more germplasm from cold regions. Further, less number of accessions found resistant under artificial inoculation indicates that for identifying resistant sources in the field evaluation is not enough as sometimes disease escape happens and hence they exhibit resistant reactions. Therefore, resistance must be confirmed under artificial inoculation in controlled environmental conditions (Gairola and Tiwari 2017).

Donors for white rust resistance have been identified by various workers, for instance Bisht et al. (2015) while screening 240 germplasm accessions of Indian mustard found IC296685, IC399678 and IC401570 as immune having 0% disease severity and IC326253 and IC417020 resistant with 5-10% disease

severity. Meena et al. (2011) found PBC 9221, and EC 414299 as resistant to white rust. A number of germplasm lines, namely, EC414291, EC 414293, MCB1, DRMR 243, DRMR 261, DRMR 270, NRCDR 705, JMWR 945-2-2-75 Kr, EC 399313, JYM 11 and NDWR 5-1 were identified as resistant (Annon. 2011). Pandey et al. (2013) also found that GSL-1, PBC-9221, NDCDR- 515 were highly resistant. Awasthi et al. (2012) demonstrated that almost all the important varieties of *B. juncea* being grown in India are susceptible and Yadav et al. (1999) while screening 74 Indian mustard germplasm lines found that none of the genotype showed resistance to white rust.

Study on genetic parameter

Evaluation of germplasm for its agronomic performance is the first requirement to ameliorate any crop therefore, apart from identifying donors for resistance to white rust, the resistant germplasm were also assessed for genetic diversity and agronomic superiority. It is perceived that all these accessions have some better underlying genetic mechanism due to which they showed white rust resistance across the locations. The mean performance of 27 resistant accessions as given in Table 3 showed significant variability as many accessions displayed significantly high values for different traits in comparison to standard

Table 3. Mean performance of 27 resistant accessions for various agronomic traits

Accessions	BP	DF	PH (cm)	DM	SMS	SL (cm)	SS	YP(g)	SWT(g)	OC(%)
IC597932	7.6	40.0	195.3	137.0	33.3	3.8	11.6	20.0	5.2	41.0
IC267703	4.7	53.7	177.3	125.0	36.6	4.0	12.5	5.7	3.2	39.9
IC313380	4.6	40.0	181.0	136.0	52.6	4.2	17.2	12.0	2.1	41.5
IC20167	7.0	30.0	124.6	120.0	31.3	3.3	14.1	23.2	2.9	42.1
IC265495	4.9	42.5	180.3	112.0	31.8	4.4	15.2	14.6	4.7	34.6
EC657030	5.3	49.5	196.0	137.0	41.3	3.6	11.2	24.8	4.3	40.5
EC699003	5.3	68.7	155.7	115.0	39.9	4.0	13.3	6.7	1.3	39.9
EC766091	8.6	67.0	186.0	156.0	35.6	3.4	12.0	12.3	1.6	42.5
EC766133	4.3	36.0	121.3	136.0	22.0	3.6	12.2	10.0	2.3	40.3
EC766134	4.6	33.0	109.0	133.0	24.3	3.2	12.2	7.7	1.4	40.7
EC766136	6.3	37.0	126.3	136.0	26.0	4.0	13.1	15.3	2.1	39.8
EC766144	5.0	37.0	109.3	133.0	20.0	3.5	9.6	10.7	2.4	42.8
EC766145	7.0	38.0	155.6	136.0	32.0	2.8	12.4	11.7	1.7	41.7
EC766148	7.0	35.0	119.0	133.0	36.0	3.7	10.9	12.0	1.9	42.2
EC766152	5.0	38.0	107.6	133.0	25.0	3.4	12.2	10.0	1.5	41.0
EC766164	7.2	56.0	194.7	142.0	45.1	3.8	12.6	20.2	3.6	40.6
EC766191	7.0	75.0	189.0	150.0	33.3	3.2	13.7	20.0	1.4	41.2
EC766192	7.6	50.0	144.0	146.0	33.0	3.3	15.2	18.3	2.7	39.3
EC766193	8.0	50.0	202.6	142.0	44.6	3.1	13.8	7.7	1.2	40.4
EC766230	8.0	48.0	174.0	145.0	37.3	4.2	16.8	16.0	2.3	38.7
EC766232	8.7	74.0	220.3	146.0	38.0	4.1	13.8	18.8	3.3	40.7
EC766272	6.5	55.5	208.2	143.5	55.5	3.3	13.7	18.3	3.5	40.6
EC766311	6.5	43.5	176.2	129.0	34.8	4.2	16.1	14.8	3.2	42.3
EC766313	7.0	35.0	158.0	130.0	32.6	4.0	14.3	7.7	2.5	43.1
EC766315	7.6	36.0	129.3	130.0	24.6	3.5	10.2	8.3	2.1	42.4
EC766316	4.6	38.0	139.6	130.0	34.0	3.6	9.6	9.3	2.3	42.5
EC766402	6.2	51.0	182.0	140.0	44.8	4.1	14.4	10.7	3.2	40.4
CD	1.5	4.8	11.2	5.00	3.4	0.3	1.7	1.9	0.4	1.6
Kranti [@]	6.3	46.0	176.4	143.6	48.9	3.6	12.7	20.7	3.5	41.7

[@]National Check

check variety Kranti. For instance, IC313380 showed agronomic superiority for traits like days to flowering (40), number of silique on main stem (52.6), silique length (4.2 cm), no. of seeds per silique (17.2) and oil content (41.4%). EC766230 was good for no. of primary branches (8), seeds/silique (16.8), seed yield/plant (16g), oil content (38.7%) while EC766272 was superior for silique on main stem (55.5), seed yield/plant (18.0g) and oil content (40.5%). Similarly, EC766091 produced high oil content (42.5%) and more no. of primary branches (8.6). It is assumed that accessions having high level of genetic diversity and superior agronomic background may yield better progenies than those having poor agronomic performance while using them in breeding programmes.

The analysis of data showed wide range of genetic variability for all the traits in 27 accessions (Table 4). Phenotypic and genotypic coefficient of

determining the magnitude of heritable variation. High GCV coupled with high heritability and genetic gain is useful for predicting the result of selection based on phenotypic performance. High heritability for all the traits ranging from 77.67 % for oil content to 93.68% for yield/plant was observed but genetic advance was high only for yield/plant, seed weight, days to flowering and silique on main stem indicating the presence of additive gene action and efficacy of simple selection methods for improving these traits. The low genetic advance for all other traits revealed the presence of more complex gene action such as dominant and epistasis, thus improvement needs to be done through hybridization. Moderate to high magnitude of heritability coupled with high genetic advance, and high level of phenotypic and genotypic coefficients for number of branches, main shoot length, main shoot height, length of siliqua, number of seeds per siliqua and yield per plant have been reported in other similar studies

Table 4. Statistical parameters of genetic variability in 27 accessions of *B. juncea*

Traits	Range	Mean \pm SE	PCV (%)	GCV (%)	Heritability (%)	Genetic advance (%)
PB	4.30 - 8.65	6.37 \pm 0.86	21.20	16.6	78.30	34.21
DF	30.00 - 82.50	48.05 \pm 2.78	26.10	24.36	81.20	50.27
PH	107.60 - 220.30	161.56 \pm 6.56	21.10	17.63	83.55	36.31
DM	112.00 - 156.00	135.24 \pm 2.92	7.04	5.69	80.82	12.30
SMS	20.00 - 55.50	35.01 \pm 1.97	24.72	21.23	85.88	43.76
SL	2.80 - 4.40	3.68 \pm 0.15	11.25	9.63	85.60	19.67
SS	9.60 - 17.20	13.10 \pm 0.98	15.42	13.89	90.08	28.18
YP	4.60 - 24.77	13.22 \pm 1.08	27.50	35.47	83.66	63.15
SWT	1.21 - 5.18	2.59 \pm 0.20	30.04	37.8	93.68	68.33
OC	34.62 - 43.10	40.84 \pm 0.92	4.12	3.2	77.67	6.54

variance were highest for seed weight followed by seed yield/plant, oil content, days to flowering, silique on main stem and primary branches. The differences between the values of PCV and CGV were not too wide thus indicating lesser influence of environment on the expression of traits. A wide range of genetic diversity among Indian mustard germplasm has been reported for traits like number of primary branches/plant, number of secondary branches/plant, siliqua angle, siliquae on biological yield, seeds/silique, main shoot, primary branch angle, 1000-seed weight, and main shoot length (Lodhi et al. 2013; Singh et al. 2013; Bind et al. 2015; Rameeh 2015). However, these parameters of variability alone are not sufficient for

(Kumar et al. 2013; Bind et al. 2015). Correlation coefficient was also calculated to measure the degree of association (genetic and non-genetic) between two or more traits. The correlation coefficients among various traits showed significant positive association of seed yield/plant with primary branches, days to flowering, days to maturity and seed weight. It is interesting to note that oil content does not show significant positive correlation with any of the traits rather it was negatively correlated with days to flowering, silique length, and seeds/silique. This shows that improvement for oil content in Indian mustard need hybridization followed by use of rigorous selection procedures.

Principal component analysis (PCA)

The PCA used to eliminate the redundancy in data set revealed that nine quantitatively measured traits have been loaded on first four components contributing 77.1 % of the total variability. Among four components, PCI accounted for 33.1 % of variation through days to flowering, plant height, silique on main stem, seeds per silique and yield/plant; PCII accounted for 23.5% of variation loaded on no. of primary branches, days to maturity, seed weight and oil content; PCIII accounted for 11.6% variation through seeds per silique and seed weight, while PCIV accounted for 9.1% variation though seeds per silique and days to flowering

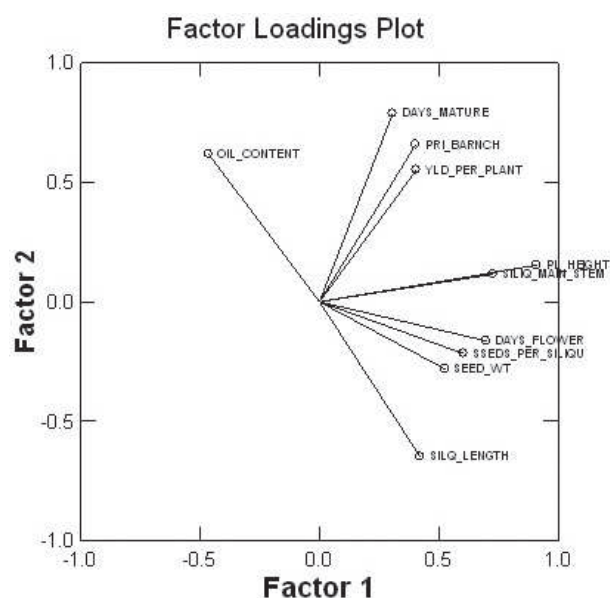


Fig. 3. Biplot of different variables loaded on PC1 and PC2

(Fig. 3). In similar experiments, Verma et al. (2016) while performing PCA on 60 accessions observed that the first seven PCs explained about 74% of the total variation and indicated leaf width, leaf length, days to maturity, days to 50% flowering, no. of siliques on main shoot, silique density, seed yield/plant, oil content, main shoot length, 1000-seed weight and silique length as more useful traits. The importance of these traits in creating genetic variability and in selecting the genotypes for higher seed yield in mustard has been highlighted at various other levels (Iqbal et al. 2014; Noor et al. 2017).

The dendrogram constructed to explain the genetic distance among 27 germplasm accessions grouped them into four clusters and each cluster have 7, 7, 8

and 5 accessions, respectively. Based on cluster (C) means, it was observed that CI had accessions with high oil content, low seed weight and shorter plant height; CII had accession with early flowering and maturity; CIII with tall types, high silique on main stem and high yield/plant and CIV had accessions with high seed weight, low oil content and late flowering. The grouping of accessions based on traits help in selection of parents from different clusters for further use in breeding. Grouping of the accession does not show any geographical affinity, except for cluster I where all the accessions were from UK. Several workers have used cluster analysis based on agronomic traits to discriminate genotypes into different clusters and observed high genetic diversity and absence of relationship between place of origin and clustering of genotypes as observed in the present study also (Gupta et al. 2014; Mohan et al. 2017). The occurrence of resistance in temperate origin germplasm may also help in augmenting the germplasm from temperate regions with desired levels of resistance to white rust.

Authors' contribution

Conceptualization of research (RY, SK, JCR); Designing of the experiments (RY, LP, JCR); Contribution of experimental materials (RY, JR); Execution of field/lab experiments and data collection (RY, LP, JN, AKT, PS, PSS, UP, RA, MR); Analysis of data and interpretation (RY, JCR, LP); Preparation of manuscript (RY, LP).

Declaration

The authors declare no conflict of interest.

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