Journal of Oilseed Brassica, 5 (2): 141-148, July 2014

Development and evaluation of Alternaria blight tolerant lines in Indian mustard (*Brassica juncea*)

Praval Pratap, PD Meena*, BK Singh, HS Meena, SS Meena, Pankaj Sharma, Rita Majumdar[1] and Dhiraj Singh

Directorate of Rapeseed-Mustard Research, Sewar, Bharatpur - 321303, Rajasthan, India;
[1]Sharda University, Noida (UP), India
**Corresponding author: pdmeena@gmail.com*
(Received: 10 March 2014; Revised: 28 April 2014; Accepted: 24 June 2014)

Abstract

The present study was aimed to evaluate and develop Alternaria blight tolerant lines in Indian mustard [*Brassica juncea* (L.) Czern & Coss.]. A total of 14 Alternaria blight tolerant lines were selected from 214 lines in F_8 generation. Amongst the selected lines, DRMR-2803, DRMR-2805 and DRMR-2806 showed the least per cent mean disease severity values of 11.4, 10.4 and 9.4, respectively. However, only DRMR-2805 exhibited seed yield/plant higher than the check variety Varuna. *Per se* performance of selected lines, evaluated for three consecutive years (2010-13), varied considerably for most yield parameters. The most pronounced range was obtained for plant height (203.7 - 264.5 cm), siliquae on main shoot (36.4 - 61.7), main shoot length (30.6 - 81.3 cm), and seed yield per plant (5.4 - 39.7g); number of primary branches/plant (7.1 -12.2) siliqua length (3.0 - 4.5 cm), number of seeds/siliqua (12.4 - 15.6), 1000 seed weight (2.3 - 5.4g), and % oil content (39.6 - 41.6) exhibited narrow range of variation. RAPD analysis of selected Alternaria blight tolerant lines, along with tolerant, (PHR-2 and PAB- 9511), and susceptible (Varuna) checks, clearly distinguished the individual lines to their available pedigree data. Highly promising tolerant lines identified in the present study may be used as potential donors for transferring Alternaria blight tolerance in high yielding mustard varieties. The advanced Alternaria blight tolerant breeding line DRMR-2805 showing seed yield/plant almost at par with check variety Varuna must be released as a variety after multi-location testing.

Keywords: *Alternaria blight tolerance, Brassica juncea, RAPD analysis, yield performance*

Introduction

Rapeseed-mustard (*Brassica* spp.) is the second most important edible oil crops after groundnut, and accounts for 22.6% of the total edible oil production in India (Anonymous, 2013). The major rapeseed crops cultivated in India are, Brown Sarson [*Brassica rapa* (L.) ssp. Brown Sarson), Yellow Sarson [*Brassica rapa* (L.) ssp. Yellow Sarson), Toria [*B. rapa* (L.) ssp. *Toria*), Gobhi *Sarson* [*B. napus* (L.) ssp. *napus*] and Taramira (*Eruca sativa* Mill.). The important mustard crops are Indian mustard [*B. juncea* (L.) Czern. & Coss.], karan rai (*B. carinata* A. Braun) and black mustard [*B. nigra* (L.) Koch]. *B. juncea* or Indian mustard is the principal crop and covers 85-90% of the total area under cultivation of rapeseed-mustard in India (Anonymous, 2013).

Although, Indian mustard is more adaptable and has higher comparative tolerance to biotic and abiotic stresses than other rapeseed-mustard crops, Alternaria blight caused by [*Alternaria brassicae* (Berk.) Sacc.] remains a major biotic stress behind the yield fluctuations in India. Alternaria blight disease has been reported from all the continents of the world and is the most devastating disease (Kolte, 1985) causing upto 35.4% yield reduction in Indian mustard (Kolte *et al*., 1987). The pathogens are greatly influenced by weather with the highest disease incidence reported in wet seasons (Humpherson-Jones and Phelps, 1989). This disease affects the crop plants at all stages of growth. Initially, symptoms appear as black points, which later enlarge to develop into prominent, round, concentric spots of various sizes. Lower leaves are affected first, and consequent to their defoliation,

middle and upper leaves are also affected. Finally, round black conspicuous spots appear on siliquae and stem which may coalesce to form elongated lesions, leading to complete blackening of siliquae or weakening of the stem. Finally, it may cause premature pod ripening and shedding of the seeds, leading to a drastic reduction in yield and oil content of the seeds (Meena *et al*., 2010).

In India, Alternaria blight causes yield losses ranging from 10-70%, but till date no perceptible breeding progress towards tolerance could be made, primarily due to the non-availability of transferable resistance in any of its hosts (Meena *et al*., 2010). Donors for Alternaria blight are almost non-existent in the present germplasm collection, which is the main deterrent to the development of resistant/ tolerant varieties for this pathogen. Under such circumstances, population improvement programme using the selected lines as base population is very likely to enhance the genetic level of tolerance. The present investigation, therefore, was initiated to evaluate and identify promising Alternaria blight tolerant lines from amongst the advanced breeding lines and develop higher tolerant lines through inter-crossing with some indigenous and exotic genotypes of Indian mustard.

Materials and Methods

Plant material

The material for present investigation was selected from 214 single plants of F_4 generation derived from 11 crosses involving Alternaria blight tolerant genotypes (Table 1). These 214 plants were screened for leaf blight disease severity under artificially created epiphytotic conditions in the field during the normal crop season. Besides, *per se* performance plant in terms of yield and oil content, superior plants were advanced to next generation through selfing. Finally, 14 lines (Table 1), consistently found to be better performing in terms of leaf blight tolerance, yield and oil content, were selected from F_8 generation, and evaluated for three consecutive crop seasons (2010-13) in three replications in augmented block design during the fourth week of October using cv. Varuna and PHR-2 as susceptible and tolerant checks, respectively. Each progeny and check variety was sown in two row of 5m length with plant to plant and row to row spacing of 30cm and 10cm respectively adjusted at 28 days after sowing. All experiments received the recommended dose of N (80 kg/ha) and P (40 kg/ha) (Anonymous, 2013), and no K fertilizer was applied. Insect-pest protection comprised of spraying with oxydemeton methyl (0.025 % a.i.) at 15 days interval. Ten plants were randomly selected from each plot to record data on Alternaria leaf blight severity, plant height, number of primary branches, number of siliqua on main shoot, siliqua length, main shoot length, number of seeds/siliqua, 1000 seed weight, seed yield/plant, and % oil content.

Table 1: Advanced breeding lines used in the study

Breeding lines	Parentage
DRMR-2800	GSL 1 × Bio 902
DRMR-2801	PR 9803 × PHR 2
DRMR-2802	BEC 107 × HYOLA
DRMR-2803	EC 399299 × EC 399301
DRMR-2804	PAB 9511 × PAB 9534
DRMR-2805	PAB 9511 × EC 399313
DRMR-2806	EC 399299 × EC 399301
DRMR-2807	JMM 915 × EC 399299
DRMR-2808	EC 399299 × EC 399313
DRMR-2809	EC 399299 × EC 399313
DRMR-2810	EC 399299 × JMM 915
DRMR-2811	PAB 9534 × EC 399301
DRMR-2812	JMM 915 × EC 399313
DRMR-2813	PAB 9534 × EC 399299

Inoculum preparation and spray

Alternaria brassicae isolated from a heavily-infected leaf of Indian mustard cv. Varuna at the experimental farm of the Directorate of Rapeseed-Mustard Research (DRMR), Bharatpur, was single spored with the help of a stereo microscope under sterilized conditions and maintained on potato dextrose agar (PDA) slants at 4°C. Seven-day-old single spore culture was sub-cultured on tomato broth medium (tomato 200 g, sucrose 20 g, volume made to 1000 ml with distilled water) incubated at 25°C (Meena *et al*., 2012). A conidial suspension was prepared by scrapping the mycelia and spores from the surface of the actively growing fungal culture into autoclaved distilled water and filtered using four layered cheese cloth to remove most of the mycelia. The filtered spore suspension was

centrifuged twice at 2000 × g for 5 min (Eppendorf 5810 R), to ensure clear spore suspension free of metabolites, washed, supernatant was discarded and spores were resuspended in deionized water containing 0.05% Tween-20 as an adhesive. The concentration of the inoculum was adjusted to 2×10^4 spores/ml with the aid of a haemocytometer slide and appropriately diluted with sterilized distilled water using an automized field sprayer, plants were inoculated in the first week of January during evening hours to keep long leaf wetness period.

Disease assessment and data analysis

Per cent disease severity on leaves was recorded on ten randomly selected plants following the pictorial rating scale of Conn *et al*. (1990), and data collected as percentage were processed by an angular transformation. All data were statistically analyzed using an analysis of variance (ANOVA) to determine the least significant difference ($P < 0.05$).

RAPD analysis

A total of 17 RAPD primers (Operon Technologies Inc.) were used for PCR amplification. The nucleotide sequences of the PCR primers are presented in Table 2. RAPD analysis was performed according to the method described by Williams *et al* (1990), with slight modifications. Amplification reactions were carried out in a volume of 20µl containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 mM each dNTP, 0.4 µM 10-mer primer, 1 unit DNA polymerase (AmpliTaq Gold) and 20 ng of template DNA. Amplifications were carried out in a Veriti 96-well Fast Thermal Cycler (PE Applied Biosystems, USA). Thermal cycler was programmed to 1 cycle of 5 min at 94°C for initial strand separation. This was followed by 45 cycles of 1 min at 94°C for denaturation, 1 min of 36°C for annealing and 2 min at 72°C for primer extension. Finally, 1 cycle of 7 min at 72°C was used for the final extension, followed by soaking at 4°C. The reproducibility of the amplification products was

Table 2: RAPD primers used in the study

Primer	Sequence of primer	No. of DNA bands amplified	No. of monomorphic bands	No. of polymorphic bands	Unique s band	% polymorphic bands
OPS-1	CTACTGCGCT	8	1	7	0	87.5
OPS-2	CCTCTGACTG	13	3	10	1	76.9
OPS-3	CAGAGGTCCC	9	1	8	1	88.9
PS-4	CACCCCCTTG	8	1	7	1	87.5
OPS-5	TTTGGGGCCT	12	3	9	3	75.0
OPS-7	TCCGATGCTG	10	2	8	0	80.0
OPS-8	TTCAGGGTGG	3	1	2	0	66.7
OPS-9	TCCTGGTCCC	8	1	7	0	87.5
OPS-10	ACCGTTCCAG	6	2	4	1	66.7
OPS-14	AAAGGGGTCC	6	1	5	0	83.3
OPB-1	GTTTCGCTCC	12	2	10	1	83.3
OPB-2	TGATCCCTGG	7	2	5	2	71.4
OPB-3	CATCCCCCTG	6	1	5	1	83.3
OPB-4	GGATGGAGT	6	1	5	2	83.3
OPB-5	TGCGCCCTTC	11	3	8	2	72.7
OPT-7	GGCAGGCTGT	8	1	7	2	87.5
OPT- 8	AACGGCGACA	12	3	9	1	75.0
Total no. of DNA bands		145	29	116	17	80.0
Average of no. of bands/primer		8.53	1.71	6.82	1.06	-

checked twice for each primer. Differences in the DNA banding patterns were qualitatively scored from gel photographs for presence (1) and absence (0) of bands assuming that each band represents a unique genetic locus. Homology of bands among samples was based on the distance of migration in gel. Scoring was done for clear, unambiguous amplicons and their sizes determined by comparing with 100 bp DNA ladder. Based on the presence or absence of amplicons, a binary 1-0 data matrix was created and used to calculate Jaccard's similarity coefficient (Jaccard, 1908). Cluster analysis was carried out among the genotypes based on Jaccard's similarity coefficients using UPGMA (Sneath and Sokal, 1973) and SAHN-clustering algorithm in NTSYS-pc, version 2.02e (Applied Biostatistics) software.

Results and Discussion

A total of 214 single plants of F_4 generation derived from 11 different crosses were screened for Alternaria blight tolerance as well as for yield

Table 3: RAPD based Jaccard similarity matrix indicating pairwise similarities between every possible pairs of the studied genotypes

	Genotype	1	2	3	4	5	6	7	8	9	10	11	12
(1)	DRMR-2805	1.00											
(2)	DRMR-2803	0.86	1.00										
(3)	EC-399301	0.90	0.95	1.00									
(4)	PAB-9534	0.90	0.78	0.82	1.00								
(5)	PHR-2	0.90	0.86	0.90	0.82	1.00							
(6)	EC-399296	0.73	0.86	0.81	0.65	0.73	1.00						
(7)	VARUNA	0.85	0.81	0.85	0.76	0.85	0.67	1.00					
(8)	EC-399000	0.77	0.90	0.86	0.69	0.77	0.95	0.71	1.00				
(9)	EC-399299	0.82	0.95	0.90	0.74	0.82	0.81	0.85	0.86	1.00			
(10)	DRMR-2806	0.82	0.95	0.90	0.74	0.82	0.81	0.76	0.86	0.90	1.00		
(11)	PAB-9511	0.95	0.82	0.86	0.86	0.86	0.68	0.80	0.73	0.77	0.77	1.00	
(12)	EC-399313	0.86	0.82	0.86	0.77	0.95	0.68	0.80	0.73	0.77	0.77	0.81	1.00

contributing traits and % oil content. Thirty seven lines showing considerable degree of tolerance, compared to the checks, were advanced to F_6 generations. Finally, 14 lines derived from different crosses (Table 1), consistently found to be tolerant to Alternaria blight and better in terms of yield and oil content, were selected from F_8 generation. Alternaria blight tolerant genotypes developed earlier (Kolte *et al*., 2008) and used as parents for making crosses in the present investigation were generally taller and lower yielder than the commonly-grown Indian mustard cultivars. Under these circumstances, crossing them with high yielding varieties, and selecting lines with better yield performance in addition to disease tolerance, is a major breeding objective in Indian mustard. In the present investigation, analysis of variance of the selected lines revealed highly significant differences among the genotypes for all the traits, indicating sufficient amount of genetic variability in the material. The tolerant reaction may include some or all disease rating criteria including longer incubation and latent periods, smaller leaf spots, poor sporulation index, and smaller leaf and cotyledon damaged area.

Per se performance of all 14 lines exhibited considerable variation for all yield traits (Table 5). The most pronounced differences were observed in plant height (203.7 -264.5 cm), number of siliquae on main shoot (36.4-61.7), main shoot length (30.6 - 81.3 cm) and seed yield per plant (5-4 - 39.7g). On other hand, characters including number of primary branches/plant (7.1 -12.2), siliqua length (3.0 - 4.5 cm), number of seeds/siliqua (12.4 - 15.6), 1000 seed weight (2.3 - 5.4g) and % oil content (39.6 - 41.6) exhibited narrow range of variation. Plant height was maximum for DRMR-2805 (264.5 cm) while it was minimum for susceptible check variety Varuna

Table 4: Alternaria blight disease severity on leaves of different Indian mustard genotypes

Genotype	Alternaria leaf blight disease severity (%)			
	2010-11	2011-12	2012-13	Mean
Varuna	27.1(31.3)	33.0(29.7)	28.8(32.5)	32.3(28.5)
DRMR-2800	12.9(21.0)	13.3(21.4)	14.1(22.0)	13.4(21.5)
DRMR-2801	12.8(21.1)	13.9(21.8)	13.1(21.2)	13.3(21.4)
DRMR-2802	12.3(20.5)	13.7(21.7)	13.7(21.7)	13.2(21.3)
DRMR-2803	10.9(19.3)	11.4(19.7)	12.0(20.2)	11.4(19.7)
DRMR-2804	16.2(23.7)	17.9(25.0)	16.5(24.0)	16.8(24.2)
DRMR-2805	9.4(17.9)	10.2(18.6)	11.5(19.8)	10.4(18.8)
DRMR-2806	8.3(16.7)	9.9(18.3)	10.7(19.1)	9.6(18.1)
DRMR-2807	13.4(21.5)	15.5(23.2)	16.7(24.1)	15.2(22.9)
DRMR-2808	14.6(22.5)	15.4(23.1)	14.3(22.2)	14.8(22.6)
DRMR-2809	15.4(23.1)	15.0(22.8)	15.8(23.4)	15.4(23.1)
DRMR-2810	16.3(23.8)	14.9(22.7)	16.2(23.7)	15.8(23.4)
DRMR-2811	15.8(23.4)	16.8(24.2)	16.3(23.8)	16.3(23.8)
DRMR-2812	17.1(24.4)	18.0(25.1)	18.1(25.1)	17.7(24.9)
DRMR-2813	17.2(24.5)	16.1(23.7)	17.1(24.4)	16.8(24.2)
EC-339000	15.5(23.2)	16.8(24.2)	15.8 (23.4)	16.1(23.6)
EC-339296	16.8(24.2)	16.9(24.3)	25.0(17.8)	17.1(24.5)
EC-399313	14.9(22.7)	16.1(23.7)	14.9(22.7)	15.3(23.0)
EC-399301	13.4(21.5)	15.1(22.8)	15.7(23.3)	14.7(22.5)
EC-399299	13.3(21.4)	13.0(21.1)	13.4(21.4)	13.2(21.3)
PAB-9511	11.8(20.1)	13.3(21.4)	13.6(21.6)	12.9(21.0)
PAB-9534	12.4(20.6)	13.4(21.4)	14.1(22.1)	13.3(21.4)
PHR-2	12.6(20.7)	13.0(21.1)	13.9(21.9)	13.2(21.2)
LSD (P=0.05)	1.04	1.16	0.76	-

*Figures in parenthesis are angular transformed values

(203.7 cm). In general, all the advanced breeding lines were taller than Varuna but at par with tolerant check PHR-2. Similarly, the values for main shoot length, number of primary branches and number of siliquae on main shoot in the breeding lines were considerably higher in the breeding lines than in Varuna. Main shoot length exhibited very high degree of variation, ranging from 30.6 in Varuna to 81.3 in EC-399299; amongst the breeding lines it was maximum for DRMR-2808 (78.2 cm). Number of primary branches ranged from 7.6 (Varuna) to 11.8 (DRMR-2807), whereas number of siliquae on main shoot varied from 36.4 (Varuna) to 61.7 (EC-399299). Amongst the breeding lines, maximum number 61.6 of siliquae on main shoot was observed in DRMR-2805. Siliqua length did not show much variation, although, it was exceptionally small (3.0 cm) in DRMR-2806. Similarly, number of seeds per siliqua and % oil content did not vary significantly among the lines studied. Thousand seed weight of promising breeding lines were found at par with check variety Varuna, maximum was for DRMR-2804 (5.4g). Except in DRMR-2813 (39.7g) and DRMR-2804(38.3g), seed yield per plant was considerably lower in breeding lines than in the check variety Varuna (35.2g) (Table 5).

The main objective of the present investigation was to identify breeding lines better tolerant to Alternaria blight disease, than the check PHR-2, although, it is also desirable to have these lines perform at least at par with the check variety Varuna in terms of yield and % oil content. In terms of Alternaria blight tolerance, although, three genotypes, DRMR-2803, DRMR-2805 and DRMR-2806 exhibited lower disease severity than PHR-2 (Table 4), only DRMR-

Table 5: *Per se* performance of selected genotypes and Alternaria blight tolerant and susceptible checks of Indian mustard (three years pooled data)

Genotype	Plant height (cm)	No. of primary branches/plant	No. of siliqua on main shoot	Siliqua length (cm)	Main shoot length (cm)	No. of seeds/ siliqua	1000 seed weight (g)	Seed yield/ plant (g)	Oil content (%)
Varuna (SC)	203.7	7.6	36.4	4.3	30.6	13.0	3.6	35.2	40.2
DRMR-2800	216.1	7.7	46.1	4.2	43.2	15.4	3.6	11.2	39.6
DRMR-2801	217.6	8.4	55.2	3.9	54.5	13.0	4.7	18.0	39.7
DRMR-2802	219.8	7.1	51.9	3.9	44.4	12.5	3.5	13.2	40.9
DRMR-2803	232.2	12.2	59.8	4.2	49.2	13.0	4.7	23.3	41.0
DRMR-2804	210.2	10.8	50.6	4.2	53.9	12.8	5.4	38.3	40.2
DRMR-2805	264.5	10.3	61.6	3.9	59.9	13.6	3.6	34.7	40.7
DRMR-2806	243.0	11.1	49.6	3.0	52.4	12.8	3.3	25.6	41.2
DRMR-2807	254.0	11.8	55.4	3.5	63.1	13.7	3.2	24.5	40.6
DRMR-2808	249.2	11.2	51.3	4.0	78.2	15.6	3.5	19.1	40.8
DRMR-2809	221.0	8.5	52.9	3.5	62.5	13.1	3.5	18.6	40.1
DRMR-2810	242.6	9.3	50.9	4.1	63.9	14.2	2.8	15.2	40.3
DRMR-2811	231.9	9.9	60.1	3.9	71.5	13.3	3.5	22.1	40.5
DRMR-2812	215.8	8.8	44.0	4.2	66.7	13.4	4.2	17.9	39.9
DRMR-2813	236.7	7.7	56.4	3.7	66.5	14.7	3.6	39.7	40.3
EC=399299 (TC)	204.3	10.7	61.7	3.8	81.3	13.9	2.8	14.3	40.4
EC=399301 (TC)	205.6	9.3	59.3	4.5	43.1	13.4	3.4	14.9	38.7
EC=399296 (TC)	228.5	10.8	42.8	3.6	44.1	12.8	2.9	5.4	41.5
PHR-2 (TC)	223.3	8.7	54.0	3.7	35.7	12.4	2.4	13.7	41.2
PAB-9511 (TC)	218.0	9.2	42.9	4.0	60.1	13.1	2.3	7.4	39.6
EC-399000 (TC)	213.8	7.3	57.2	3.9	44.9	14.6	4.7	10.2	40.3
PAB-9534 (TC)	209.6	8.7	45.7	3.5	55.5	13.0	4.8	17.6	39.6
EC-399313	214.3	9.5	52.2	3.9	43.6	14.0	2.8	10.5	41.6
CD (P=0.05)	10.7	1.3	4.4	0.26	12.2	1.4	0.96	3.6	0.95

*SC=Susceptible check; TC= Tolerant check

2805 (34.7g) produced seed yield per plant almost at par to Varuna. Therefore, on the basis of leaf blight disease severity and yield potential, only DRMR-2805 should be considered for multi-location testing. The other two genotypes may be used as potential donors in more intensive breeding programmes for developing Alternaria blight tolerant mustard varieties.

RAPD analysis

In the present investigation, a total of 40 RAPD primers were tested on three Alternaria blight tolerant lines and nine check entries. Out of the 40 primers, 17 primers produced distinct, highly reproducible amplification profile for all the screened samples. A wide variation in the number of polymorphic bands, ranging from 3 to 13, was observed. The highest number of DNA bands (13) was obtained from RAPD primer OPS-2 while the lowest number (3) was observed with primer OPS-08 (Table 2). The average number of polymorphic, monomorphic, and unique bands (1.05) per primer were found to be 6.82, 1.71 and 1.05, respectively. The percentage of polymorphism ranged from 66.7 (OPS-8 and OPS-10) to 88.9 (OPS-03). Similar genetic diversity among Indian mustard **(***B. juncea* L**.)** genotypes was also investigated at the DNA level using the random amplified polymorphic DNA (RAPD) technique by Khan *et al*. (2008). The reason that we were successful in

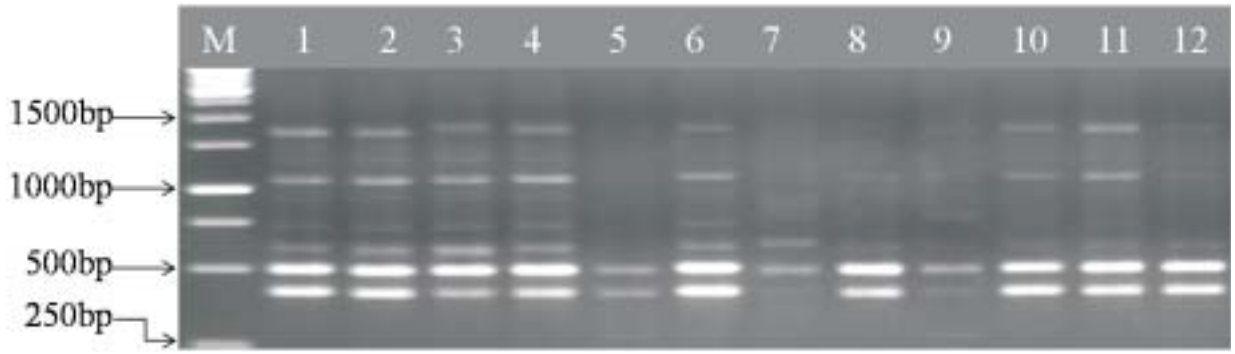

Fig. 1: Molecular profiling of advanced breeding lines, and tolerant and susceptible checks using RAPD marker OPS 07. Lane M - 250 bp DNA ladder, Lane 1- Varuna, Lane 2- DRMR 2803, Lane 3- DRMR 2805, Lane 4- DRMR 2806, Lane 5- EC339000, Lane 6- EC339296, Lane 7- EC399313, Lane 8- EC399301, Lane 9- EC399299, Lane 10- PAB9511, Lane 11- PAB-9534, Lane 12 - PHR 2

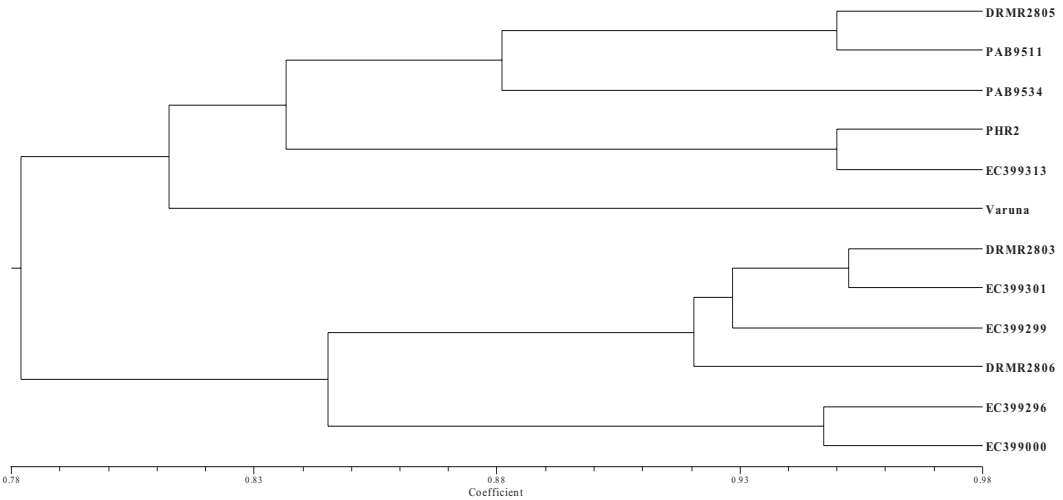

Fig. 2: Dendrogram showing genetic relationships among the advanced breeding lines, and tolerant and susceptible checks based on Jaccard's similarity coefficient using RAPD markers

identifying polymorphism in the present study was due to the use of a number of randomly selected pre-screened primers. Some researchers consider RAPD markers to represent segments of DNA with non-coding regions and to be selectively neutral (Bachmann, 1997; Landergott *et al*. 2001), while other have shown that RAPD markers are distributed throughout the genome and may be associated with functionally important loci (Penner, 1996).

The dendrogram obtained by the UPGMA analysis of polymorphic DNA bands obtained from 17 RAPD primers clearly distinguished the studied genotypes into two major clusters (Fig 2). Varuna, PHR-2 and PAB-9534 were placed in cluster I along with DRMR-2805 and EC-399313. DRMR-2805 and PAB-9511 exhibited the maximum similarity coefficient of 0.95 (Table 3). This is as expected since, PAB-9511 has been used as one of the two parents for developing DRMR-2805. All the other EC lines were placed in cluster II along with the breeding lines DRMR-2803 and DRMR-2806 (Fig 2). DRMR-2803 clustered together with EC-399301 while DRMR-2806 showed its more closeness to EC-399299. Results correspond well with their available pedigree data. The results suggest that advanced breeding lines DRMR-2803 and DRMR-2806 are more closer to their tolerant parents, and therefore, low yielding, whereas DRMR-2805 has become genotypically more similar to the high yielding cultivated varieties Varuna and PHR-2. Besides, it also possesses a high degree of Alternaria blight tolerance.

References

Anonymous. 2013. Rapeseed-mustard production technology for different agro-ecological system in India. Directorate of Rapeseed-Mustard Research, Bharatpur 321 303, India, pp. 54.

Bachmann K. 1997. Nuclear DNA markers in plant biosystematic research. *Opera Bot* **132**: 137–148.

Conn KL, Tewari JP and Awasthi RP. 1990. A disease assessment key for Alternaria black spot in rapeseed and mustard. *Can Plant Dis Surv* **70**: 19–22.

Humpherson-Jones FM and Phelps K. 1989. Climatic factors influencing spore production in *Alternaria brassicae* and *A.brassicicola*. *Annals of Appl Bot* **114**: 449-458.

Jaccard P. 1908. Nauvelles re churches sur la distribution florale. *Bull Soc Vaudoise Sci Nat* **44**: 223-270.

Khan MA, Rabbani MA, Munir M, Ajmal SK and Malik MA. 2008. Assessment of genetic variation within Indian mustard (*Brassica juncea)* germplasm using random amplified polymorphic DNA Markers. *J Integrative Plant Biol* **50**: 385-392.

Kolte SJ, Awasthi RP and Vishwanath. 1987. Assessments of yield losses due to Alternaria blight in rapeseed and mustard. *Indian Phytopath* **40**: 209-211

Kolte SJ, Nashaat NI, Kumar A, Awasthi RP and Chauhan JS. 2008. Towards improving the genetic base of rapeseed-mustard through an Indo-UK research collaboration. *J Plant Genet Resour* **21**: 132-137.

Kolte SJ. 1985. Diseases of Annual Edible Oilseed Crops, Vol.II, Rapeseed-Mustard and Sesame Diseases. CRC Press Inc., Boca Raton, Florida, pp. 135.

Landergott U, Holderegger R, Kozlowski G and Schneller JJ. 2001. Historical bottlenecks decrease genetic diversity in natural populations of *Dryopteris cristata*. *Heredity* **87**: 344–355.

Meena PD, Awasthi RP, Chattopadhyay C, Kolte SJ and Kumar A. 2010. Alternaria blight: a chronic disease in rapeseed-mustard. *J Oilseed Brassica* **1**: 1-11.

Meena PD, Rani Asha, Meena R, Sharma P, Gupta Riteka and Chowdappa P. 2012. Aggressiveness, diversity and distribution *Alternaria brassicae* isolates infecting oilseed Brassica in India. *African J Microbio Res* **6**: 5249-5258.

Penner GA. 1996. RAPD analysis of plant genomes. In: Methods of Genome Analysis in Plants. CRC Press, Boca Raton, USA, pp. 251– 268.

Sneath PHA and Sokal RR. 1973. Numerical taxonomy: the principles and practice of numerical classification. WH Freeman, San Francisco.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA and Tingey SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res* **18**: 6531-6535.