Journal of AgriSearch, 9 (1): 79-87
An Open Access International Peer Reviewed Quarterly

ISSN : 2348-8808 (Print), 2348-8867 (Online)
https://doi.org/10.21921/jas.v9i01.9899

Varietal Screening and Molecular Analysis against Alternaria Blight in Indian Mustard Genotypes

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ABSTRACT

Indian mustard [Brassica juncea (L.) Czern & Coss] is an important edible oilseed crop in India. There are 154 genotypes screened under natural condition against Alternaria blight. Disease appeared on leaves after 90 days were noted as, 9 genotypes found moderately susceptible (Rohini, PAB-14-14, PBZ-4, PRD-14-1, PAB-14-5, PRD-14-16, PAB- 14-17, PHR-2, Parvati), 29 were susceptible and 116 were highly susceptible. In case of biochemical analysis, only ten genotypes, maximum phenolic value in non-infected and infected leaves was in found same variety NDRS-2009-1 and peroxidase activity maximum values was found non-infected and infected leaves NDRE-1-11-1, Vaibhav. In catalase activity, a height value was found non-infected and infected leaves varieties NDRS-2007, NDRS-2009-1. Cluster analysis dendogram is divided in two group I and II, this similarity showing mean 0.45 and group I had only one variety namely NDRS-2009- 1. Group II had divided in to two sub groups A and B. Group A is in to two clusters A1 and A2 in this group similarly 0.60 and cluster A1 divided in to two sub cluster A1-1 and A1-2, sub cluster A1-1 had found three varieties NDRE-1-11-1, NDYR-2008 and Narendra Rai but NDRE-1-11-1 0.75 more similar both varieties NDYR-2008 and Narendra Rai. Sub cluster A1-2 had two varieties Vaibhav and Rohini both similar only 0.70. Cluster A2 had found three varieties NDRE -7, Vardan and PM-26 but both NDRE -7, Vardan dissimilar 0.81 then PM-26 and group B had found only one variety NDRS-2007.

ARTICLE INFO

Received on	:	09.01.2022
Accepted on	:	27.02.2022
Published online	:	16.03.2022

Keywords:
Brassica juncea, Catalase, Peroxidase, Phenol, Alternaria blight.

INTRODUCTION

Indian mustard [*Brassica juncea* (L.) Czern & Coss.] is an important edible oilseed crop in India, it is commonly referred to as rapeseed mustard along with four other related cultivated oilseed species viz. *B. rapa*, *B. napus*, *B. carinata* and *Eruca sativa.* Oilseed brassica shares 23.5% area and 24.2% production of total oilseeds in the country. Indian mustard is belonging to Brssicacea family. In this three diploid species, namely, *Brassica rapa* (AA, 2n=20), *Brassica nigra* (BB, 2n=16), *Brassica oleracea* (CC, 2n=18), and their three natural amphidiploid species, namely, *Brassica napus* (AACC, 2n=38), *Brassica juncea* (AABB, 2n=36) and *Brassica carinata* (BBCC, 2n=34), constitute cultivated Brassicas. These are cultivated across the globe for use as vegetables, oilseed crops and condiments (Maheshwari and Kovalchuk, 2016).

The estimated area, production and yield of rapeseed-mustard in the world was 36.59 million hectare (mha), 72.37 million tonne and 1980 kg /ha, respectively, during 2018-19. Globally, India accounts for 19.8% and 9.8% of total acreage and productivity (USDA). During 2018-19 the production was 1980 kg/ha and production 72.42 mt (DRMR, 2018-19). In Uttar Pradesh, Rapeseed – mustard was grown on 1099 ha with a production of 8041 tonnes and 1054 kg/ha productivity during 2017-18 (Anonymous, 2018).

Disease resistance depends upon the induction of defenses following exposure to organisms. Several types of biochemical changes which include the level of phenolics was found to be important in the investigation of resistance mechanism (Arora and Wagle, 1985; Meena *et al*., 2008). The aim of this research was to explore the possibility of biochemical changes for defense and symptom logical changes in the three genotypes of oilseeds *Brassica* infected by *A. brassicae* under natural conditions at different stages of Alternaria blight disease development. *Alternaria* infection causes considerable changes in the sugar and phenolic contents of the plant (Chopra and Jhooty, 1974; Nema, 1983; Chahal, 1986; Kumar and Singh, 1996; Saharan and Saharan, 2004; Joshi *et al*., 2004; Kushwaha and Narain, 2005). The biochemical and physiological changes associated with induction of resistance and plants peroxidases have been implicated in varieties of defense-related processes, including the hypersensitive response, lignifications, cross-linking of phenolics and glycoprotein, suberization and phytoalexin production. (Rakow and Raney, 2003). Catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen a water molecule thus avoiding cellular disintegration (Bolwell and Wojtaszek, 1997). Peroxidase convert H_2O_2 to water provide an efficient system to prevent oxidative damage. Induction and accumulation of POX correlated with onset of induced resistance suggest an active role for this enzyme in defense against pathogenic fungi and retard fungal growth (Jung *et al*., 2004).

MATERIALS AND METHODS

The experiment was conducted at the Student's Instruction Farm, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya (U.P.) during *Rabi* 2019-20. The planting of 154 Indian mustard genotypes were done

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under natural conditions in order to promote a severe natural epidemic of disease; the genotypes were sown in two rows each of three-meter length with spacing of 30x10 cm in augmented design with three replications. To maintain the high humidity level in microclimate of the field, time to time irrigation was applied for favoring the development of the disease. Observations were recorded on randomly selected five plants from each genotype. Numerical rating grade was given on the basis of percentage of area covered by pathogen on the leaves. On the basis of disease intensity, genotypes were classified into different groups *viz*., near immune/highly resistant, resistant, moderately resistant, moderately susceptible, susceptible, and highly susceptible. (Anonymous 2019)

Table- 1: Modified 0-9 scale for rating disease intensity of Alternaria blight in Indian mustard(Anonymous, 2019)

Rating scale	Disease Intensity (%)	Pathogen Reaction
0	0	Immune/highly resistant (HR)
1	<5	Resistant (R)
3	5-10	Moderately Resistant (MR)
5	11-25	Moderately Susceptible (MS)
7	26-50	Susceptible (S)
9	>50	Highly Susceptible (HS)

The per cent disease intensity (PDI) was calculated by following formula.

$$\text{PDI (\%)} \frac{\text{Sum of all the disease rating}}{\text{Number of plant observed} \times \text{Maximum disease grading}} \times 100$$

Area under the disease progress curve (AUDPC) was calculated on the basis of disease severity over time from 60 to 90 days after sowing using the formulae as follows (Shaner and Finney, 1977)

Area Under Disease Progress Curve (AUDPC)

$$\text{AUDPC} = \sum_{n}^{n} \ [(y_i+1 + y_i)/ 2] \ [X_i+1- x_i]$$

Where,

y_i and y_{i+1} = Disease severity in the i^{th} and $(i+1)^{th}$ observations

x_i and x_{i+1} = Time (weekly) in the i^{th} and $(i+1)^{th}$ observations

n = Total number of observations

Table 2: Sequence of SSR primer

S. No	Marker Name	Forward	Reverse
1	BR_A01	CCGTTTTTATGTCACAAATCT	AAACAAAACGAACTTTGTCAG
2	BR_A04	GACAATGTTCTTGCTATCACC	ATAGTTCCTTCGCAACCTATT
3	BR_A04	ATGGAATCTGCTCATCTCAC	TAAGCTGCAATGATCAAAGAT
4	BR_ A04	CATTTTCCTCCTTGAGATCT AT	CTGGTGGAAAACTTGATTTTA
5	BR_ A04	CATCACAAGCCAAGAAGAAT	AGAGTCTGTGGTTCATCTCCT
6	BR_A04	TTTGAACGATACACAACAACA	GTTGGTCCACGAGTAAAAGAT
7	BR_A04	AAAGAAGGGGAAAGTAAACCT	GCAACTCTCTTCATTTTCAGA
8	BR_ A05	CCTTGTGGTATCGTATTGACT	AAAGAATACAACCGCACTGTA
9	BR_A05	GTTGAGCTCTCCTTCACCTAT	CGTGCGGGTATTTATTTTTAT
10	BR_A05	ACCCAAATATAGCATCAAGGT	ATGTTTGGTATCTGGGTTTGT

Biochemical analysis

Only 10 selected genotypes were selected on the basis of total phenol content, peroxidase, catalase and true protein biochemical analysis methods employing are as follows.

Total phenol content

Total phenols were evaluated by following methods described by Bray and Thorpe (1954). A sample of 0.5 g from each replicate sample was ground in 10 times volume of 80 per cent ethanol in morter and pestle and centrifuged at 10,000 rpm for 20 min. A sample of 20 µl was taken for total phenols, estimated calorimetrically with folin-ciocalteu reagent. The absorbance was taken, and catechol was used as standard.

Peroxidase activity

Peroxidase activity was conducted by method of Plewa *et al.*, 1991. The conversion of guaiacol to tetraguaiacol was measured at 470 nm. The reaction mixture of 1 ml was contained 935 µl phosphate buffer, 25 µl of enzyme extract, 15 ml guaiacol, and 25 µl H_2O_2. The reaction was initiated by adding H_2O_2 and rate of change in absorbance were recorded at 470 nm for 1 min at an interval of 5 seconds. Peroxidase activity has been defined as the change in absorbance min1mg-protein.

Catalase activity

Catalase activity measured by the methods of (Beers and Sizer 1952). Leaf material was homogenized in 0.1M phosphate buffer, pH 6.5 (w/v, 1:1) centrifuged at 15000 RPM for 30 min at 4^0c. The reaction mixture contains 1.9 mL reagent grade water 1.0 mL of 0.059 M hydrogen peroxide which was incubated for 4-5min to achieve temperature equilibration and to establish blank rate, add 0.1 Ml of diluted enzymes and record decrease in absorbance at 240 nm for 2-3 min. the difference in absorbance was divided by the H_2O_2 molar extinction coefficient ($36M^{-1}$ cm^{-1}).

True protein

The activity of true protein was given by Lowry *et al*. (1930) Reagents percent Na_2CO_3 in 0.1 N NaOH,0.5 percent Cu SO_4 is 1 percent sodium potassium tartarate, Folin – ciocalteau (phenol reagent) 1;1, D.W., Bovine Serum Albumin (BSA) 0.2 mg /ml in 0.1 NaOH, Mix reagent 1 and 2, 50:1 before assay. Procedure- A solution of 3 ml was taken which constituted 0.2 ml of Aliquot and 2.8 ml of D.W. (1.1) A solution of Na_2CO_3 and $CuSO_4$ was prepared which was 5ml reagent (1.2). With the help of the vortex both (1.1) and (1.2) was mixed. It was kept at 25^0C for 30 minutes for colour development. It was observed that a bluish color developed. It was later read at

750nm by SP-21.

Genomic DNA Isolation

The immature leaves from two to three weeks old plant were collected for extraction of genomic DNA following the CTAB method (Murray and Thompson, 1980) with some minor modifications. The extraction buffer was prepared by using 1 M Tris-HCl (pH 8.0), 0.5 M EDTA (pH 8.0), 3.5 M NaCl, 3% (w/v) CTAB and 2% (w/v) PVP and the final concentration of the reagents in extraction buffer was 100 mMTris- HCl, 20 mM EDTA, 1.4 M NaCl, 3% (w/v) CTAB and 2% (w/v) PVP.

Primer directed amplification of genomic DNA

Using the standard protocol of polymerase chain reaction (PCR) adjusted to laboratory condition, amplification of targeted genomic regions was performed with the help of 10 SSR Primer (Table 2).

The reaction mixture (15 µl) contained 2.6 µl water, 3.0 µl 59 PCR buffer, 1.5 µl 10mM $MgCl_2$, 3.0 µl 200 µM dNTPs mixture, 1.2 µl (5 µl) primer F, 1.2 µl (5 µl) primer R, 0.5 µl Taq polymerase (1 unit) and 2.0 µl DNA template (30 ng). The amplification was carried out in a thermal cycler using initial denaturation at 94^0C for 4 min, 35 cycles of denaturation at 94^0C for 1 min, primer annealing at 50–68^0C for 1 min and extension at 72^0C for 2 min followed by final extension at 72^0C for 10 min and cooling at 4^0C till separation and resolution through electrophoresis.

RESULTS AND DISCUSSION

Screening of different genotypes of mustard under field condition against Alternaria blight

Appearance of disease

The ideal and most economical means of managing the Alternaria blight disease of Indian mustard, is the use of resistant varieties. Resistant breeding is a most suitable and economical method for the management of disease therefore, the experiments were carried out to find out the resistant genotype against Alternaria blight. One hundred fifty-four (144) genotypes were evaluated against this disease under natural field condition and the results obtained are presented in table 3. Generally, the disease symptoms firstly appeared on lower leaves. The initial symptoms of the disease were noted between to 35 to 45 DAS. The earliest appearance of the disease is noted on 35 to 37 DAS in Indian mustard. Some of these were noted on LES-54, NPJ-217, PDZ-2, PDZ-9, PDZ-3, NDRS-2008-1, NDRS-2009-1, DRMR-2035, PT-2010-5, NPJ-219, YSH-0401(NC), CS-15000-1-1-1-2, Vardan, NDRE-1-11-1, PDZ-1, EYS-2015-03, PHR-1500, RH-1573, CS-2009-154, NPJ-210, PRE-2013-3, RH-1607, RH-1599-41. The latest symptoms appeared i.e., 43-45 DAS in *brassicae juncea* namely PAB-14-14, PBZ-4, PRD-14-16, PRD-14-1, PAB-14-5, PRD-14-17, PHR-2 exhibited relatively good resistance and slow blighting against Alternaria blight. Similar studies by Bal and Kumar noted that the first appearance of Alternaria leaf spot symptoms from *A. brassicae* (RLM 619).

Per cent disease severity

In general, on average basis a progressive increase in disease severity was noted. Progress on per cent disease severity was recorded thrice, first at 60 DAS, second at 75 DAS, and third at 90 DAS. In different genotype the disease severity ranged between 12.44 to 76.64, respectively. The minimum per cent disease severity was recorded in 9 genotypes namely Rohini, PAB-14-14, PBZ-4, PRD-14-1, PAB-14-5, PRD-14-16, PAB- 14-17, PHR-2, Parvati having 12.44, 22.7, 23.28, 23.58, 24.67, 24.89, 24.98, 23.42, 24.78, respectively which were rated as moderately susceptible. On the basis of per cent disease severity and on the basis of host reaction of disease against genotype the entries were categorized into different categories given (table 3). Out of 154 genotype of Indian mustard none of the genotype was found free from disease, highly resistant and resistant (Table 3). Out of 154 genotype, nine genotype were found moderately susceptible namely Rohini, PAB-14-14, PBZ-4,PRD-14-1, PAB-14-5, PRD-14-16, PAB- 14-17, PHR-2, Parvati (11-25% leaf area covered), 29 were found susceptible against Alternaria blight some of these were DRMRSI-9-1-1, PRD-14-6, PAB-16-2, RH-1378, RMM-09-04, LES-57, DRMR-2019, DRMR-1-5, NPJ-215, RH-1585, PBR-438, RH-1650, SVJ-111, DRMRQ-4, TM-179, JMM-991, PBZ-5, RMM-09-06, NDRI-8-14-1, NDYR-2008, Vaibhav,, NDRE-7, Pusa mustard-26, NDRE-2009-1, SKM-1104, KMR(E)-16-1, PHR-126, RHH-1561, PRD-14-18 (26 to 50% leaf area covered) and 116 genotype found highly susceptible against Alternaria blight (> 50% leaf area covered) similar result reported that Kolte *et al*., (2001) genotypes PR-8988 and PR-9024 showed high degree of resistance to Alternaria blight and genotypes PR-9301 and PR-9650 showed high degree of susceptibility.

Area under disease progress curve (AUDPC)

AUDPC was ranged from 683 to 38.34 in different genotypes. Generally, on average basis AUDPC was ranged from 683 (YSH-0401 NC) to 87.8 in different genotypes. The minimum AUDPC was recorded on 87.8 (DRMR-4005). The maximum AUDPC was noted 683 (YSH-0401 NC). Kumar *et al*., (2001) also concluded that calculation for AUDPC in mustard crop sown on different dates helps in identifying the disease severity progress of *Alternaria* blight of mustard on leaves and pods.

Host reaction

Out of 154 genotypes screened, no one of the genotype was found disease free or highly resistance, 9 genotypes namely (Rohini, PAB-14-14, PBZ-4, PRD-14-1, PAB-14-5, PRD-14-16, PAB-14-17, PHR-2, Parvati), were rated as moderately resistant, 29 moderated susceptible and 116 as susceptible. (Table 3). Similarly, several research have also been reported other genotypes resistance to this time (Kumar and Singh 2012).

Table 3 Per cent disease severity and AUDPC of Alternaria blight on Indian mustard

Name of Genotypes	Appearance of Disease (DAS)	Per cent disease severity (Days after sowing)			AUDPC	Host reaction
		60	75	90		
NPJ-201	38	19.07	44.98	68.80	609.2	HS
RMT-15-29	34	17.51	42.76	62.00	575.8	HS
RRN-911	40	15.60	38.89	56.87	528.4	HS

Name of Genotypes	Appearance of	Per cent disease severity (Days after sowing)			AUDPC	Host reaction
DRMRSJ-9-1-1	41	14.00	33.20	46.08	458.9	S
PRD-14-6	39	6.700	15.89	27.29	222.6	S
NPJ-220	39	15.03	36.73	56.32	503.7	HS
RB-72	38	14.76	35.56	51.90	472.9	HS
RH-1209	42	15.65	30.56	54.98	491.6	HS
KMR(L)-15-5	36	17.03	39.06	56.80	531.5	HS
LES-54	37	16.06	34.87	55.89	418.3	HS
TS-46	35	16.89	40.34	64.98	577.8	HS
RGN-368	37	16.98	37.98	60.56	555.9	HS
YSB-9	38	19.86	53.79	68.89	682.8	HS
PAB-16-2	40	11.09	29.08	38.45	38.34	S
NPJ-217	39	17.90	39.66	58.78	540.6	HS
RH-1378	37	15.67	37.57	48.74	484.8	S
PDZ-2	40	16.10	37.81	55.89	513.9	HS
PAB-14-14	41	5.76	12.87	23.58	189.8	MS
RMM-09-04	42	8.03	21.61	31.26	287.9	S
LES-54	38	15.06	34.74	54.80	492.9	HS
PDZ-9	38	15.78	42.78	59.45	553.8	HS
TH-1603	37	20.26	48.23	65.24	640.1	HS
NPJ-216	41	14.34	34.86	54.90	475.8	HS
RMWR-09-1	32	15.76	36.45	53.89	498.7	HS
LES-57	36	14.56	36.56	47.89	476.8	S
DRMR-2019	38	13.51	30.83	46.45	436.5	S
PBZ-4	40	5.45	14.67	22.70	199.7	MS
PDZ-3	41	15.67	39.68	56.89	540.3	HS
DRMR-1-5	37	15.78	34.67	49.79	465.8	S
NDRS-2011	40	15.13	34.00	54.89	497.8	HS
NDRS-2008-1	41	15.78	39.78	54.90	538.9	HS
CS-508-1-P2	38	17.56	37.89	60.45	548.9	HS
DRMR-1153-12	37	16.89	38.65	57.80	537.3	HS
NDRS-2009-1	38	18.04	46.89	64.67	604.8	HS
EC-399299	36	11.30	29.56	49.56	424.7	HS
DRMR-2035	37	18.05	42.05	65.26	585.9	HS
CS-700-2-1-4	36	16.80	17.67	38.56	525.7	HS
PT-2010-5	35	17.65	46.89	61.45	603.7	HS
Parvati	40	7.70	17.8	24.78	234.7	MS
RHH-1561	41	10.50	24.6	34.80	343.6	S
DRMR-5206	39	15.50	39.67	58.01	535.9	HS
NPJ-219	73	15.70	33.82	52.25	474.5	HS
PBZ-5	40	6.18	19.21	28.84	257.1	S
DRMRSJ-9-1	39	18.65	42.16	64.83	584.9	SH
PRD-14-1	41	6.03	19.51	24.98	445.1	MS
PAB-14-5	40	6.34	14.97	23.28	206.5	MS
PRD-14-16	40	7.03	17.65	24.89	235.4	MS
PAB-14-17	41	6.07	16.9	24.67	218.9	MS
RMM-09-06	39	10.56	25.67	38.00	357.9	S
PRD-14-18	40	7.08	17.58	25.00	236.6	S
JMM-991	41	10.07	24.8	36.89	236.8	S
AKMS-8141	38	18.75	41.8	63.91	572.9	HS
BIOYSR	39	17.00	34.35	63.41	522.9	HS

Name of Genotypes	Appearance of	Per cent disease severity (Days after sowing)			AUDPC	Host reaction
Pusa-MH-9	98	17.63	39.82	60.02	550.4	HS
71J-0004	39	16.38	39.81	61.73	552.1	HS
PHR-2	44	5.04	13.63	23.42	195.0	MS
NPJ-203	37	17.23	46.62	63.72	609.7	HS
RH-919	40	17.08	35.16	59.43	513.9	HS
RGN-394	38	16.85	38.84	61.21	545.2	HS
PBR-422	39	16.67	36.5	52.78	495.9	HS
PRO-5222	38.	16.78	28.90	54.90	530.8	HS
RGN-761	36	18.34	47.89	65.89	634.0	HS
RLMCP-626	37	19.90	40.04	62.00	566.0	HS
SVJ-68	38.	18.81	38.10	63.32	548.0	HS
CS-13000-3-1-1-4-2	38	17.05	36.90	57.94	514.9	HS
PDZ-1	36	16.65	39.67	63.78	560.6	HS
Kranti	37	18.60	42.89	64.89	592.8	HS
EYS-2015-03	35	20.70	49.80	69.80	632.8	HS
RTM-314	35	16.78	37.89	65.80	550.5	HS
PHR-1500	39	16.89	37.00	59.56	523.6	HS
RRN-917	38	16.78	36.89	59.56	527.8	HS
RGN-761	36	17.87	48.04	65.67	636.0	HS
RH-1573	37	18.26	43.78	67.90	593.7	HS
PRE-2013-1	37	18.78	46.78	69.45	635.8	HS
CS-2009-154	38	15.76	37.89	55.89	530.6	HS
RH-1326	37	15.67	43.02	62.90	567.9	HS
NPJ-210	36	17.89	43.78	54.89	580.6	HS
PRE-2013-3	38	16.78	37.92	54.89	524.7	HS
RH-923	38	17.69	39.86	60.66	566.1	HS
Pusa MH-8	36	17.75	39.56	60.12	548.2	HS
YSH-0401(NC)	35	17.56	49.78	76.64	683.2	HS
SVJH-100	40	17.89	40.96	66.03	581.8	HS
DRMRIC-16-38	36	17.32	38.64	63.98	552.9	HS
CS-15000-1-1-1-1-2	37	16.8	32.90	59.34	495.8	HS
DRMR-4005	37	16.78	36.89	58.63	522.7	HS
Rohini	43	1.50	6.13	12.44	87.8	MS
Vardan	36	17.25	39.67	63.78	553.7	HS
NPJ-215	40	14.04	33.67	47.64	449.8	S
NDRS-2007	37	20.03	47.78	68.89	638.0	HS
RMWR-09-1	39	16.61	36.23	53.80	496.0	HS
NDRE-1-11-1	37	16.83	30.83	56.89	522.8	HS
PMH-8	38	18.56	40.56	62.78	567.8	HS
RH-1599-41	38	18.20	43.8	59.81	570.8	HS
Vaibhav	42	13.82	32.63	48.80	454.7	S
PT-2015-11	37	20.6	51,27	68.66	671.7	HS
TKM-17-2	37	16.65	37.01	56.28	521.9	HS
NDRE-7	41	14.2	36.23	49.81	571.7	S
JM-12-6	39	17.26	42.81	61.27	560.4	HS
JD-6	36	19.28	45.46	64.13	610.3	HS
RH-1699-22	40	16.34	39.35	57.64	536.2	HS
PR-2015-1	38	15.23	37.62	58.03	520.6	HS
Ashirvad	38	16.84	42.82	57.78	569.0	HS
RH-1607	38	19.23	42.91	63.81	598.0	HS

Name of Genotypes	Appearance of	Per cent disease severity (Days after sowing)			AUDPC	Host reaction
NDRS-2009-1-2	38	18.02	45.23	64.04	605.8	HS
NPJ-213	38	18.40	46.22	63.81	605.4	HS
Pusa –M-26	40	13.24	35.63	49.00	471.7	S
PHR-3278	37	17.01	42.03	64.87	584.3	HS
NDRS-2009-1	39	12.45	30.83	49.40	434.9	S
CS-2009-129	39.	17.04	41.97	64.12	574.1	HS
BAUM-08-14	42	13.89	34.67	50.10	469.4	HS
KMR(E) 17-2	37	16.72	37.34	54.78	510.5	HS
RLC-6	37	16.3	42.52	60.13	568.1	HS
Pusa Mustard-25	36	17.67	45.83	63.67	603.8	HS
TM-277	38	23.76	47.65	61.21	627.2	HS
Rohini	38	19.33	43.19	63.68	593.7	HS
NDRE-8-14-1	40	14.36	32.89	50.00	469.0	S
NDYR-2008	39	14.02	34.63	48.82	476.8	S
TH-1603	37	20.28	46.23	65.22	640.2	HS
PHR-126	39	14.26	36.64	48.98	470.8	S
Narendra Rai	39	14.45	31.12	54.23	481.9	HS
RMT-10-13	37	21.78	46.89	66.67	624.0	HS
TKM-17-2	37	15.81	30.81	54.46	534.7	HS
RH-1585	39	12.24	38.61	48.01	470.0	S
DRMRCI-98	39	14.08	36.42	58.07	514.7	HS
DRMR-2017-11	40	15.30	36.58	56.89	508.9	HS
DRMR-2017-14	39	17.34	40.56	60.46	553.1	HS
SKM-1328	38	14.44	32.12	55.28	482.7	HS
RB-94	38	16.26	38.57	58.17	530.7	HS
SVJ-111	39	14.37	32.15	47.47	428.7	S
PR-2015-5	40	15.37	37.92	55.23	512.6	HS
DRMRIJ-16-38	38	16.82	38.36	56.93	508.7	HS
DRMRQ-4	40	14.27	34.87	48.81	476.8	S
71J-0001	40	18.06	40.23	59.62	553.5	HS
TM-179	40	13.46	36.78	49.46	461.4	S
PBR-400	40	13.65	33.63	48.96	450.7	HS
SKM-1104	39	13.57	34.87	49.70	470.8	S
AKMS-9026	37	19.33	47.23	62.46	627.9	HS
KMR (E)-16-1	40	13.66	31.41	48.97	450.5	S
RH-1656	39	17.34	34.34	56.19	478.0	HS
PBR-438	40	14.58	31.62	48.98	450.7	S
RGN-419	38	17.05	41.66	59.09	654.9	HS
71J-0002	39	19.23	41.27	59.27	56.3	HS
KMR-17-4	39	14.83	30.23	50.00	438.6	HS
DRMR-2017-5	3.8	17.00	40.6	58.66	545.7	HS
DRMRCI-85	38	18.23	43.98	62.13	582.3	HS
RH-1590	39	16.10	39.25	59.61	543.8	HS
NPJ-209	38	17.00	42.20	59.40	562.4	HS
Giriraj	37	18.22	40.14	60.16	555.1	HS
PRE-2015-1	39	18.03	40.44	58.81	555.7	HS
RH-1650	38	13.23	35.61	49.22	470.6	S
DRMRIJ-16-66	37	17.22	40.84	60.22	560.2	HS
RGN-435	38	16.30	39.35	56.41	526.4	HS

Note: R-Resistant, **MR-**Moderately resistant **S-**Susceptible**, HS-**Highly susceptible

Biochemical Analysis

Biochemical analysis of only selected 10 Indian mustard genotypes based on yield and yield attribute with disease response.

Total phenol content

Total phenol content in respect of non-infected Indian mustard leaves. The range between 5.15 to14.45. It was observed highest phenol content in NDRS-2009-1 (14.45) followed by Pusa Mustard-26 (11.55), while minimum phenol content noticed in NDRS -2007 (5.15) followed by Vardan (6.35). While in case of infected leaf, maximum range was obtained in 13.15 to 5.05. It was observed that the highest phenol content in NDRS-2009-1 (13.15) followed by Pusa Mustard-26 (10.65). while minimum phenol content was noticed in NDRS-2007 (5.05) followed by Rohini (5.06) similar result was found by Neeraj *et al.*, (2010) The reduction was also recorded in total phenol contents in diseased leaf tissues**.**

Table 4: Biochemical activity in Indian mustard leaves

S No.	Name of Varieties	Catalase activity Concentration (mg/g fresh weight/minute)		Phenol content (mg/g fresh weight)		True protein content (mg/g fresh weight/minute)		Peroxidase activity (mg/g fresh weight/minute)	
		Non-Infected	Infected	Non-Infected	Infected	Non-Infected	Infected	Non-Infected	Infected
1	Rohini	34.2	30.5	5.65	5.06	36	30.5	43.5	38.2
2	NDRS -2007	60	39	5.15	5.05	44.5	39	93.4	87.3
3	NDRE-1-11-1	40.80	49.5	9.45	6.05	55	49.5	180.6	154.2
4	Vaibhav	42.20	55	8.05	6.35	62.5	55	179.3	160.6
5	NDRE-7	45.40	45.5	9.35	6.55	45.5	45.5	86.75	81.7
6	Pusa M -26	40.60	28.5	11.55	10.65	31.55	28.5	30.9	25.5
7	Vardan	38.80	99.5	6.35	5.55	100.5	99.5	80.51	75.1
8	NDRS-2009-1	54.30	51.5	14.45	13.15	89.5	51.5	120.5	108.7
9	NDYR-2008	36.20	52	7.15	5.15	99.5	52	115.30	112.3
10	Narendra -Rai	42.80	89	9.2	6.15	100.5	89	95.8	91.3
CD at 5%		2.56	2.45	1.67	2.6	1.56	2.35	2.60	2.13

Peroxidase activity

Peroxidase enzyme activity in non- infected leaves is given in regard of peroxidase activity in Indian mustard leaves. The range between 30.9 to 180.6 in (mg/g fresh weight/min). It was observed that highest peroxidase activity is found in NDRE-1-11-1 (180.6) followed by Vaibhav (179.3) and NDRS -2009-1 (120.5), while minimum peroxidase activity noticed in Pusa mustard-26 (30.9) and followed by Rohini (43.5). While in case of infected leaves it ranged between 25.5 to 160.6, maximum range obtained in Vaibhav (160.6) followed by NDRE-1-11-1 (154.2) and NDYR-2008 (112.3). While minimum peroxidase activity was noticed in Pusa Mustard-26 (25.5) followed by Rohini (38.2) similar result was shown by that Singh *et al.*, (2012) who used peoxidase as parameter to identify the resistant genotype for breeding program. Gupta *et al.* (1990) observed that specific activities of polyphenol oxidase remained higher while that of peroxidase was lower in tolerant genotypes in comparison to susceptible. In response to infection, the activity of both the enzymes increased sharply in all the genotypes. However, this increase was considerably higher in susceptible genotypes than that of tolerant.

Catalase activity

The catalase activity in non-infected leaves, ranged between 34.20 to 60.0 in (mg/g fresh weight /min.). It was observed that the highest catalase activity in non-infected leaves was in found in NDRS -2007 (60.0) followed by NDRS-2009-1(54.30) and NDRE-7 (45.40), while minimum catalase activity was noticed in Rohini (34.2). While in case of infected leaves range was obtained in 30.60 to 50. It was observed that the highest catalase activity in NDRS-2009-1 (50) followed by NDRS-2007(42.60) and Vaibhav (40.20) while minimum catalase activity was noticed in NDYR-2008(30.60) followed by Rohini (30.80). Similar result was reported by Gupta *et al.*, (1990) that catalase activity was appreciably higher at initial stage at later stages it dropped markedly. In response to infection, catalase activity decreased.

True protein content

True protein content in non-infected leaves of Indian mustard is presented in table 3. It ranged between 31.55 to100.5 (mg/g fresh weight/min). The highest true protein content in Vardan (100.5) and Narendra Rai (100.5), while minimum true protein content noticed in Pusa Mustard-26 (31.55). In case of infected leaves in table 4, maximum range was obtained in 28.5 to 99.5. It was observed that the highest phenol content is present in Vardan (99.5) followed by Narendra Rai (89). While minimum phenol content was noticed in Pusa Mustard-26 (28.5) followed by Rohini (30.5).

DNA Isolation from different varieties

DNA isolation and PCR amplification of the ten selected varieties such as varieties, **1**. Rohini, **2.** NDRS-2007, **3.** NDRE - 1-11-1, **4.** Vaibhav, **5.** NDRE-7, **6**. Pusa Mustard-26, **7.** Vardan, **8.** NDRS-2009-1, **9.** NDYR-2008, **10.** Narendra Rai.

PCR amplification using by SSR Marker

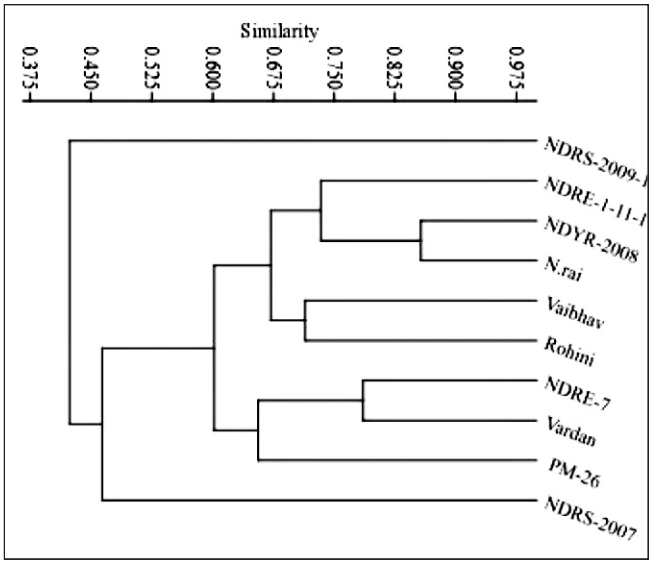

Fig-1. Cluster analysis by un-weighted pair group method (UPGMA) with model of SHAN is NTSYS package.

The cluster basis analysis on un-weighted pair group method of arithmetic mean (UPGMA) based clustering all the genotype. The dendogram is divided in two group I and II this similarity showing mean 0.45 and group I had only one variety, NDRS-2009- 1. Next II group had divided in to two sub groups A and B. group A divided is in to two cluster A1 and A2 this groups similar 0.60 and cluster A_1 divided in to two sub cluster A_{1-1} and A_{1-2}, sub cluster A_{1-1} had found three varieties NDRE-1-11-1, NDYR-2008 and Narendra Rai but NDRE-1-11-1 0.75 similar both varieties NDYR-2008 and Narendra Rai. Sub cluster A_{1-2} had two varieties Vaibhav and Rohini both similar only 0.70. Cluster A_2 had found three varieties NDRE -7, Vardan and Pusa Mustard-26 but both NDRE -7, Vardan dissimilar 0.81 then Pusa Mustard-26 and group B had found only one variety NDRS-2007. Similar result was found by Wang *et al.*, (2009) genetic diversity cultivars genetic diversity between 28 species and wild relatives of *Brassica* germplasm collection, using 60 SSR markers was studied by Redden *et al.*, (2009) who explained that this class of markers can be effectively used for the genetic analysis of a germplasm collection. In another study conducted by Chen *et al.* (2011), 15 accessions of tuber mustard were used to determine their genetic diversity employing SSR markers and these genotypes were classified into two clusters based on genetic distance.

CONCLUSION

The study revealed that Indian mustard Rohini, PAB-14-14, PBZ-4, PRD-14-1, PAB-14-5, PRD-14-16, PAB- 14-17, PHR-2, Parvati, are the potential moderate susceptible source against *Alternaria brassiceae* that was proved by physiological screening and more diversity of showing on the molecular basis only two varieties NDRE-7 and Pusa Mustard-26 out of ten varieties. These varieties may be exploited in the breeding programme to enhance the resistance against *Alternaria brassiceae*.

Table 5: Cluster analysis by un-weighted pair group method (UPGMA) with model of SHAN is NTSYS Package

Group	Sub group	Cluster	Sub Cluster	Number of genotypes	Genotypes
I				1	NDRS-2009-1
II	A	A1	A1-1	3	NDRE-1-11-1, NDYR-2008 N. Rai
			A1-2	2	Rohini, Vaibhav
		A2		3	NDRE-7 Vardan PM -26
		B		1	NDRS-2007

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