Journal of Advances in Biology & Biotechnology

Volume 27, Issue 5, Page 13-31, 2024; Article no.JABB.114560
ISSN: 2394-1081

Biochemical Changes: Their Potential Role against Fungal Disease Resistance Development in Mustard

Aditi Shrivastav [a], Manoj Kumar Tripathi [a,b*], Prakash N. Tiwari [b] and Purnima Singh [c]

[a] Department of Genetics and Plant Breeding, College of Agriculture, Rajmata Vijayaraje Scindia Agricultural University, Gwalior, 474002, India.
[b] Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Rajmata Vijayaraje Scindia Agricultural University, Gwalior, 474002, India.
[c] Department of Plant Pathology, College of Agriculture, Rajmata Vijayaraje Scindia Agricultural University, Gwalior, 474002, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2024/v27i5758

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/114560

Original Research Article

Received: 15/01/2024
Accepted: 21/03/2024
Published: 26/03/2024

ABSTRACT

Rape-seed mustard is an imperative edible oilseed crop which is adversely affected by different biotic stresses causing import of edible oils from abroad. The experiment was carried out for screening mustard genotypes based on disease indexing and putative role of diverse biochemical parameters in development of resistant against three major fungal diseases. A modified 0-9 scale was used for rating of disease indicators to calculate disease incident (DI). In disease indexing, mustard genotypes L-4, GSC-7 and PC-6 were considered as immune against *Alternaria brassicae,* Maya, L-4, China, GSL-1, GSC-7, PC-5, PC-6 and RP-9 were classified as immune against *Albugo candida* while L-4 and PC-5 were found immune against *Erysiphe cruciferarum*.

**Corresponding author: E-mail: drmanojtripathi64@gmail.com;*

The study of biochemical constituents demonstrated that these immune genotypes accumulated higher osmolytes content including free proline content and total phenol under diseased condition showing their tolerance ability against these pathogens. The tolerant genotypes also had lesser lipid peroxidation rate as indicated by malondialdehyde content analysis to induce lesser effect on total chlorophyll, amino acids, proteins and soluble sugars including reducing and non-reducing sugars. These identified genotypes have wider scope for further phyto-pathological studies and utilization in development of fungal resistance breeding.

Keywords: Biochemical parameters; disease indexing; fungal diseases; mustard.

1. INTRODUCTION

Mustard is one of the leading oilseed crops by sharing approximately 24% area and production of entire oilseeds in the nation [1-2]. It is cultivated all around the world for its ideality as agricultural crops as well as potential source of biodiesel production [3-7]. Natural compounds present in mustard crop make it imperative crop used as industrial purposes, with the residual cake used for animal feed [8-11, 3]. The protein content in mustard is 25-30%, making it exceptional source of food used as oil in commercial and industrial purposes [12-15]. Mustard plant can be used as natural booster of immunity and removes the heavy metals in the central system of the biological pollution [16-22].

India is the second-largest producer of rapeseed-mustard, but Nation is unable to supply the demand and a sizable portion of national budget is used to import edible oil from oversees due to the prevalence of disease and insect pests [23-26]. “White rust caused by *Albugo candida,* Alternaria blight by *Alternaria brassicae* and powdery mildew instigated by *Erysiphe cruciferarum* were accounted annual yield losses up to 20–60%, 15–71% and 17 %, respectively in mustard crop in India” [27-28,4].

“At both cellular and molecular levels, plants are accounted to demonstrate a broad range of responses during exposure to different biotic stresses such as fungal infection. Against various diseases, the resistance character of plants has been found correlated with different biochemical parameters. These may be pre-existed in plants or are freshly provoked by the disease. Therefore, the accumulation or enhancement in pre-existing concentrations or activities of these biochemical ingredients under stress conditions are concluding factors of stress brutality and plant resistance against stresses” [29].

The control of the disease by applying fungicides is not recommended due to complexity in obtaining whole foliage coverage through aerial application and environmental concerns. Therefore, management of the disease by breeding for resistance is extremely advantageous. However, the genetic foundation of cultivated *Brassica* is constricted and resistance genes are scarce. In absence of resistance sources against these diseases (*Alternaria brassicae, Albugo candida*) the repetitive cultivation of susceptible varieties tends to attract oomycetes causing serious yield losses [30, 5]. In this context, mustard genotype (s) resistant to Alternaria blight, white rust and powdery mildew needs to be screened and introduced to minimize the yield loss and improve productivity without affecting quantity and quality of oil. Therefore, the present study was undertaken to screen resistant mustard genotype (s) against these three destructive diseases based on disease indexing and putative impact of biochemical responses to these diseases’ incidences.

2. MATERIALS AND METHODS

The study was conducted at Research Farm, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, Madhya Pradesh, India at 26.22°N, 77.45°E during *Rabi* 2020–2021. Biochemical work was conducted at Biochemical Analysis Laboratory, Department of Plant Molecular Biology & Biotechnology, College of Agriculture, RVSKVV, Gwalior.

2.1 Plant Material

The experimental material consisted 75 mustard genotypes obtained from All India Coordinated Research Project on Rapeseed and Mustard, Zonal Agricultural Research Station, Morena, RVSKVV, Gwalior, M.P., India (Table 1).

2.2 Crop Raising

The mustard genotypes were sown in randomized block design with three replications

under controlled and diseased conditions. Every genotype was planted in a plot with a 2 meters long row, with spacing of 30 cm apart and the plants themselves being spaced 15 cm apart. From each treatment, five plants were selected at random and labelled so that observations could be recorded.

2.3 Field Screening of Genotypes for Disease Assessment

The 0-9 disease rating scale was used for calculating the percent disease intensity of the all three fungal diseases *viz*., Alternaria blight, white rust and powdery mildew. The incidence for disease was monitored regularly. The scale was as follows:

Average severity score={(N-1×0) + (N-2×1) + (N-3×3) + (N-4×5) + (N-5×7) + (N-6×9)}/ No. of leaf sample

Per cent Disease Intensity (PDI) = [{(N-1 x 0) + (N- 2 x 1) + (N-3 x 3) + (N-4 x 5) + (N-5 x 7) + (N-6 x 9)} x 100]/ No. of leaf samples x 9

Where N-1 to N-6 represents frequency of leaves in each score

2.4 Biochemical Analysis of Defense Related Compounds

To know the putative role of different biochemical parameters in development of fungal diseases resistance in mustard, magnitude of different biochemical parameters was estimated in healthy as well as diseased leaves twice; first on 35 days and second on 70 days after sowing.

2.5 Determination of Lipid Peroxidation Rate

Lipid peroxidation was assessed by computing the MDA content according to method of Naserwafaei *et al*.[31]. Hundred mg leaf samples were homogenized with 1.0 ml of 20% w/v trichloroacetic acid and centrifuged at 15,000× g for 10 min at 4°C. An equivalent volume of supernatant and 5% w/v TBA were supplemented to the TCA. The blend was warmed at 96°C for 30 min and placed in an ice bath for 5 min. The absorbance at 532 nm and 600 nm wave lengths were recorded.

2.6 Determination of Fungal Effect by Measuring Various Biochemical Parameters

Amount of chlorophyll content was estimated as per method suggested by Arnon [32]. Hundred mg fresh leaf sample was taken, finely crushed in 10 ml of 80% Acetone and transferred into a 15 ml falcon tube. Falcon tube was centrifuged for 15 minutes at 10000 rpm and transferred the green supernatant into a fresh falcon tube. Readings were recorded in a UV–Vis spectrophotometer at 643 nm, 663 nm and 470nm wavelengths.

Total amino acid was estimated by the method given by Moore and Stein [33]. Fresh leaf sample was crushed in 5.0 ml of 80% ethanol in a mortar pestle until the leaf completely disappears and a fine liquid solution was made. The solution was then transferred in the 15 ml falcon tube and centrifuged at 1000rpm for 10 minutes. The 10ml supernatant was transferred in a fresh falcon tube and dried in glass bottle in oven at 65ºC until it gets dried then 1.0 ml distilled water was added and left it until it gets dissolved. Then, 50 microliters solution was taken from glass bottle and 100 microliter distilled water was added. Afterward 100 microliters of Ninhydrin (0.10 ninhydrin in 100 ml of methanol/ethanol) are added to the sample and boiled for 20 minutes. After cooling down, 1.0 ml of propanol (1: 1: propanol: distil water) was added and absorption was taken at 570 nm wavelength in the UV–Vis spectrophotometer.

List 1. Disease rating scale (0-9)

Rating	Percent Disease Severity	Reaction
0	0	Immune/ highly resistant
1	≤5	Resistant
3	5-10	Moderately resistant
5	11-25	Moderately susceptible
7	26-50	Susceptible
9	≥50	Highly susceptible

Table 1. List of mustard genotypes with their parentage/ source

S. No.	Genotypes	Parentage/ Sources
1	RB-50	Laxmi X RH-9617
2	Pusa Bold	Varuna x BIC1780
3	Varuna	Selection from Varansi Local
4	Rohini	Selection from natural population of Varuna
5	Kranti	Selection for Varuna
6	RH- 725	CCSHAU Hisar
7	Maya	Varuna x KRV 11
8	Vardan	Derived through biparental mating involving Varuna, Keshari, CSU 10 and IB 1775, IB 1786, IB 1866
9	Vasundhara	RH 839 x RH 30
10	Swarn Jyoti	Selection from genotypes line RC 1670
11	Pusa Jagannath	Varuna x Synthetic juncea
12	Pusa JaiKisan	Somaclone of Varuna
13	Albeli	ZARS, Morena, RVSKVV, Gwalior
14	Sej-2	Derived from a cross of B. juncea to a amphidiploid
15	Shraddha	ZARS, Morena, RVSKVV, Gwalior
16	DMH 1	CMS based hybrid
17	L-4	Canada
18	JMWR-908-1	ZARS, Morena, RVSKVV, Gwalior
19	RGN-73	RGN 8 x Pusa Bold
20	NRC-HB-101	BL 4 x Pusa Bold
21	NRC-HB-506	(MJA 05 x MJR 1)
22	RVM-3	ZARS, Morena
23	RH-749	RH-781 xRH-9617
24	NRC DR-2	MDOC 43 x NBPGR 36
25	DRMR IJ-31	HB 9908 x HB 9916
26	China	ZARS, Morena, RVSKVV, Gwalior
27	GSL-1	Punjab agricultural university, Ludhiana
28	GSC-7	Punjab agricultural university, Ludhiana
29	PC-5	ZARS, Morena, RVSKVV, Gwalior
30	PC-6	ZARS, Morena, RVSKVV, Gwalior
31	RP-9	ZARS, Morena, RVSKVV, Gwalior
32	Kiran	ZARS, Morena, RVSKVV, Gwalior
33	JTC-1	ZARS, Morena, RVSKVV, Gwalior
34	JM-1	Pusa Bold x L 6
35	JM-2	MutantofRL9
36	JM-3	Varuna x YRT 3
37	RVM-1	ZARS, Morena
38	RVM-2	Selection from Chambal growing region
39	PM-25	Sej-8 x Pusa Jagannath
40	PM-26	VEJ Open x PusaAgrani
41	PM-27	Derived from the cross [(Divya x Pusa Bold) x (PR 666EPS) x PR 704EPS-2 x B85)]
42	PM-28	SEJ8 x PUSA JAGANNATH
43	PM-30	Bio 902 x ZEM 1
44	Pusa Vijay	Synthetic *Brassica juncea* x VSL 5
45	JMM-927	ZARS, Morena, RVSKVV, Gwalior
46	JMM-991	ZARS, Morena, RVSKVV, Gwalior
47	RMM-10-01-01	ZARS, Morena, RVSKVV, Gwalior
48	RMM-12-01-18	ZARS, Morena, RVSKVV, Gwalior
49	RMM-12-03-18	ZARS, Morena, RVSKVV, Gwalior
50	WRR-5	White rust resistant advance breeding lines, DRMR, Bharatpur
51	WRR-6	White rust resistant advance breeding lines, DRMR, Bharatpur
52	WRR-7	White rust resistant advance breeding lines, DRMR, Bharatpur

S. No.	Genotypes	Parentage/ Sources
53	WRR-8	White rust resistant advance breeding lines, DRMR, Bharatpur
54	WRR-9	White rust resistant advance breeding lines, DRMR, Bharatpur
55	WRR-10	White rust resistant advance breeding lines, DRMR, Bharatpur
56	WRR-11	White rust resistant advance breeding lines, DRMR, Bharatpur
57	WRR-12	White rust resistant advance breeding lines, DRMR, Bharatpur
58	WRR-13	White rust resistant advance breeding lines, DRMR, Bharatpur
59	WRR-14	White rust resistant advance breeding lines, DRMR, Bharatpur
60	WRR-15	White rust resistant advance breeding lines, DRMR, Bharatpur
61	WRR-16	White rust resistant advance breeding lines, DRMR, Bharatpur
62	WRR-17	White rust resistant advance breeding lines, DRMR, Bharatpur
63	WRR-18	White rust resistant advance breeding lines, Bharatpur
64	WRR-19	White rust resistant advance breeding lines, DRMR, Bharatpur
65	WRR-20	White rust resistant advance breeding lines, DRMR, Bharatpur
66	WRR-21	White rust resistant advance breeding lines, DRMR, Bharatpur
67	WRR-22	White rust resistant advance breeding lines, DRMR, Bharatpur
68	WRR-25	White rust resistant advance breeding lines, DRMR, Bharatpur
69	WRR-26	White rust resistant advance breeding lines, DRMR, Bharatpur
70	WRR-27	White rust resistant advance breeding lines, DRMR, Bharatpur
71	WRR-28	White rust resistant advance breeding lines, DRMR, Bharatpur
72	WRR-29	White rust resistant advance breeding lines, DRMR, Bharatpur
73	WRR-30	White rust resistant advance breeding lines, DRMR, Bharatpur
74	WRR-31	White rust resistant advance breeding lines, DRMR, Bharatpur
75	WRR-32	White rust resistant advance breeding lines, DRMR, Bharatpur

Protein estimation was carried out by employing method of Lowery et al. [34]. The 0.2 ml working standard was taken in 5 test tubes and volume was made up to 1.0 ml using distilled water. The test tube with 1.0 ml distilled water served as blank. Then, 4.5 ml of reagent I was added and incubated for 10 minutes. After incubation, 0.5 ml of reagent II was added and incubated for 30 minutes. The absorbance was measured at 660 nm wave length.

Total sugar was estimated as per protocol described by DuBois *et al*. [35]. Hundred mg fresh leaf sample was crushed in 5 ml 80% ethanol using a mortar pestle to makes a fine liquid solution. The solution was poured in the 15 ml falcon tube and centrifuged at 1000rpm for 10 minutes. The supernatant was transferred in glass bottle and dried in oven at 65ºC. After drying, 1.0 ml distilled water was added and left to get dissolved. Now, 100 microliter solution was taken in a falcon tube then anthrone reagent was added. The falcon tube was heated at 100ºC for 30 minutes and cooled to room temperature. The absorption was taken at 630 nm wave length in the UV–Vis spectrophotometer.

Reducing and non-reducing sugar was estimated by dinitro salicylic acid (DNSA) method as proposed by Miller [36]. The DNSA reagent contained a 1:1:1 volumetric mixture of 1% 3, 5-dinitrosalicylic acid, 40% rochelle salt, 0.2% phenol and 0.5% potassium disulphide, all in 1.5% sodium hydroxide. Typically, 100µl DNS reagent was added to 100µL sample mixture. After 10 min of incubation at 50°C, 0.9 ml of the DNS reagent was added to the test tube and the mixture was incubated in a boiling water bath for 5 min. After cooling to room temperature, the absorbance of the supernatant at 540 nm wave length was measured to estimate the magnitude of reducing sugar. Amount of non-reducing sugar was estimated by subtracting the amount of reducing sugar from the value of total sugar.

2.7 Determination of Antioxidant Accumulation by Measuring Osmolytes (free proline, sugar and phenol contents)

Proline concentration was estimated as per method suggested by Bates *et al*. [37]. A 0.25 mg fresh leaf sample was crushed very finely in 3ml solution of 3% homogenize sulpho-salicylic acid using mortar pestle. Homogenized solution was centrifuged at 1000 rpm for 15 minutes and 2 ml supernatant was taken in a 15 ml falcon tube. Then, 2.0 ml Ninhydrin acid (ninhydrin + glacial acetic acid) was added and heated at 100ºC for 60 minutes in water bath. The heated supernatant was cooled using ice bath until temperature comes and 4 ml toluene was added.

Upper layer (a pink layer) was used for taking absorbance at 520 nm wave length in UV–Vis spectrophotometer.

Phenol concentration was estimated according to method suggested by Swain and Hills [38]. Hundred mg fresh leaf sample was crushed in 5ml 80% ethanol in a mortar pestle until the leaf completely disappears and made a fine liquid solution. Then solution was transferred in the 15ml falcon tube and centrifuged at 1000 rpm for 10 minutes. The 10ml supernatant was transferred in a fresh falcon tube and dried in glass bottle in oven at 65ºC until it gets dried. After drying 1.0 ml distilled water was added and left until it gets dissolved. Then, 50 microliters were taken in an Eppendorf tube and 50 microliters FC reagent was added. Then, 200 microliters of 20% sodium carbonate were added in it and the volume was made up to 1.0 ml with distilled water. Solution was boiled for 1 min and kept at room temperature for 2 hours. Finally, the absorption was taken at 650 nm wave length in a UV–Vis spectrophotometer.

2.8 Statistical Analysis

Biochemical responses from both healthy and diseased plants were subjected to diversity assessment among different mustard genotypes. Principal component analysis, dendrogram, and heatmap were generated to identify the significance of different biochemical responses towards the development of fungal disease.

3. RESULTS AND DISCUSSION

3.1 Field Screening of Brassica Genotypes Against three Detrimental Fungal Diseases

The field screening of three fungal diseases was performed based on disease intensity in the scale of 0-9. Since the growth of *E. cruciferarum* is superficial, disease symptoms can be observed easily by visual inspection. Powdery mildew first appeared on the upper surface in the lower most (oldest) leaves as small (1 to 2 cm diameter), scattered, white almost circular colonies which coalesced as the colonies grew further, eventually covering the entire leaf surface, stem, primary and secondary branches, and siliquae, progressively. “On the contrary in case of white rust, in the vegetative phase, the fungal pathogen infects leaves and cotyledons resulting in the appearance of white to creamy yellow pustules on abaxial (lower) surfaces. At the flowering stage, the fungus causes systemic infection leading to extensive distortion, hypertrophy, hyperplasia and sterility resulting in severe inflorescence malformation known as stag head” [39]. In Alternaria blight infection, leaf symptoms include round, brown spots with concentric rings. Spots often have a yellow halo, and can crack through the middle. Spots often occur first on older leaves. As the disease spreads, leaves can develop enough spots that they begin to meld together to create large necrotic areas on leaves.

The Brassica genotypes were classified based on categorizations of reactions against three detrimental fungal diseases. For white rust invasion, genotypes such as Maya, L-4, China, GSL-1, GSC-7, PC-5, PC-6, RP-9 were found to be immune, genotype JMWR-908-1 was found to be highly resistant, while WRR-15 and WRR-25 were found to be resistant, Vasundhara, Pusa Jagannath, Kiran, PM-27, JMM-991, WRR-5, WRR-7, WRR-11, WRR-12, WRR-14, WRR-16, WRR-17, WRR-19, WRR-26, WRR-27, WRR-29, WRR-31 and WRR-32 were found to be moderately resistant whereas rest of the genotypes were exhibited their susceptibility to disease (Fig. 1; Table 2). For powdery mildew infestation, genotypes *i.e*., L-4 and PC-5 were found to be immune, China and RP-9 found to be highly resistant, GSC-7, PC-6 found to be resistant, RB-50, Pusa Bold, WRR-10 and GSL-1 were found to be moderately resistant whilst rest of the genotypes were exhibited their susceptibility for disease (Fig. 2; Table 3). For Alternaria infestation, genotypes such as L-4, GSC-7 and PC-6 were found to be immune, China, GSL-1, RP-9 were found to be highly resistant, Pusa Bold, Kranti, Maya, Kiran and JM-2 were found to be resistant, RB-50, Varuna, Rohini, Vardan, Vasundhara, Swarn Jyoti, Pusa Jagannath, Shraddha, DMH1, JMWR-908-1, NRC-HB-101, NRC-HB-506, RVM-3, RH-749, NRCDR-2, DRMRIJ-31, PC-5, JM-1, JM-3, RMM-10-01-01, RMM-12-01-18 and WRR-5 were found to be moderately resistant whereas rest of the genotypes were exhibited their susceptibility to disease (Fig. 3; Table 4).

“The mustard varieties have the potential source of genes for enhancing Alternaria blight resistance in the mustard gene pool [40]. Total 1,020 Indian mustard accessions were evaluated against *E. cruciferarum* PMN isolate, at Wellington, The Nilgiris, Tamil Nadu, India under natural hot spot conditions. The study identified accession RDV29 with complete resistance against *E. cruciferarum* PMN isolate for the first

time, which was found consistent in five independent evaluations" [41]. "Eighteen different Brassica genotypes were evaluated against twelve *A*. *candida* isolates accumulated from the different geographical locations in Indian. DLSC-1 showed complete resistance against all WR isolates. Two DH *B. juncea* mutant lines C66 and C69 exhibited a promising level of resistance against *A. candida* where C66 was shown completely resistant against white rust isolates (WRI) except susceptible to WRI-K isolate" [42].

Table 2. Categorizations of reactions of *Brassica* genotypes against white rust

Severity (%)	Disease reaction	Number of genotypes	Name of genotypes
0	Immune	8	Maya, L-4, CHINA, GSL-1, GSC-7, PC-5, PC-6, RP-9
<5	Highly Resistant	1	JMWR-908-1
5.0-10	Resistant	2	WRR-15, WRR-25
10.1-25	Moderately Resistant	18	Vasundhara, Pusa Jagannath, KIRAN, PM-27, JMM-991, WRR-5, WRR-7, WRR-11, WRR-12, WRR-14, WRR-16, WRR-17, WRR-19, WRR-26, WRR-27, WRR-29, WRR-31, WRR-32
25.1-50	Susceptible	36	RB-50, Pusa Bold, Rohini, RH- 725, Vardan, Swarn Jyoti, Pusa JaiKisan, Albeli, Sej-2, Shraddha, DMH 1, RGN-73, NRC-HB-101, RVM-3, RH-749, NRC DR-2, JTC-1, JM-1, JM-2, JM-3, RVM-2, P M -26, PM-30, RMM-10-01-01, RMM-12-01-18, WRR-6, WRR-8, WRR-9, WRR-10, WRR-13, WRR-18,WRR-20, WRR-21, WRR-22, WRR-28, WRR-30
>50.1	Highly Susceptible	10	Varuna, Kranti, PM-25, DRMR IJ-31, RVM-1, PM-28, Pusa Vijay, JMM-927,RMM-12-03-18

Table 3. Categorizations of reactions of *Brassica* genotypes against powdery mildew

Severity (%)	Disease reaction	Number of genotypes	Name of genotypes
0	Immune	2	L-4, PC-5
<5	Highly Resistant	2	CHINA, RP-9
5.0-10	Resistant	2	GSC-7, PC-6
10.1-25	Moderately Resistant	4	RB-50, Pusa Bold, WRR-10, GSL-1
25.1-50	Susceptible	47	Varuna, Kranti, Maya, Albeli, Shraddha, DMH 1, JMWR-908-1, RGN-73, NRC-HB-101, NRC DR-2, DRMR IJ-31, KIRAN, JTC-1, JM-2, RVM-1, PM-25, PM-26, PM-27, PM-30, RMM-12-01-18, R M M -12-03-18, WRR-5, WRR-6, WRR-7, WRR-8, WRR-9, WRR-11, WRR-12, WRR-13, WRR-14, WRR-15, WRR-16, WRR-17, WRR-18, WRR-19, WRR-20, WRR-21, WRR-22, WRR-25, WRR-26, WRR-27, WRR-28, WRR-29, WRR-30, WRR-31, WRR-32, Shraddha
>50.1	Highly Susceptible	18	Rohini, RH- 725, Vardan, Vasundhara, Swarn Jyoti, Pusa Jagannath, Pusa JaiKisan, Sej-2, , NRC-HB-506, RVM-3, RH-749, JM-3, PM-28, Pusa Vijay, JMM-927, JMM-991, RMM-10-01-01

Table 4. Categorizations of reactions of *Brassica* genotypes against *Alternaria*

Severity (%)	Disease reaction	Number of genotypes	Name of genotypes
0	Immune	3	L-4, GSC-7, PC-6
<5	Highly Resistant	3	CHINA, GSL-1, RP-9
5.0-10	Resistant	5	Pusa Bold, Kranti, Maya, KIRAN, JM-2
10.1-25	Moderately Resistant	22	RB-50, Varuna, Rohini, Vardan, Vasundhara, Swarn Jyoti, Pusa Jagannath, Shraddha, DMH 1, JMWR-908-1, NRC-HB-101, NRC-HB-506, RVM-3, RH-749, NRC DR-2, DRMR IJ-31, PC-5, JM-1, JM-3, RMM-10-01-01, RMM-12-01-18, WRR-5
25.1-50	Susceptible	39	RH-725, Pusa JaiKisan, Albeli, Sej-2, RGN-73, JTC-1, RVM-1, RVM-2, PM-26, PM-27, PM-28, Pusa Vijay, JMM- 927, RMM-12-03-18, WRR-6, WRR-7, WRR-8, WRR-9, WRR-10, WRR-11, WRR-12, WRR-13, WRR-14, WRR- 15, WRR-16, WRR-17, WRR-18, WRR-19, WRR-20, WRR-21, WRR-22, WRR-25, WRR-26, WRR-27, WRR- 28, WRR-29, WRR-30, WRR-31, WRR-32
>50.1	Highly Susceptible	3	PM-25, PM-30, JMM-991

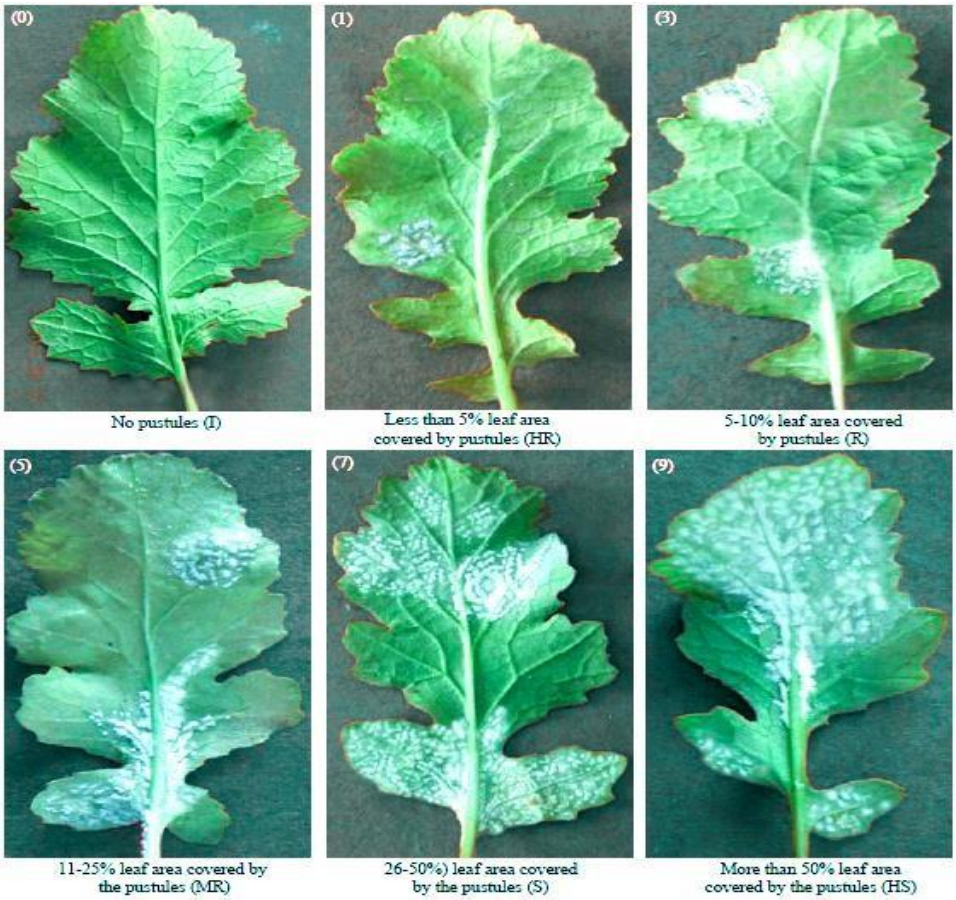

Fig. 1. Categorizations of reactions of Brassica genotypes against White Rust (*Albugo candida*)

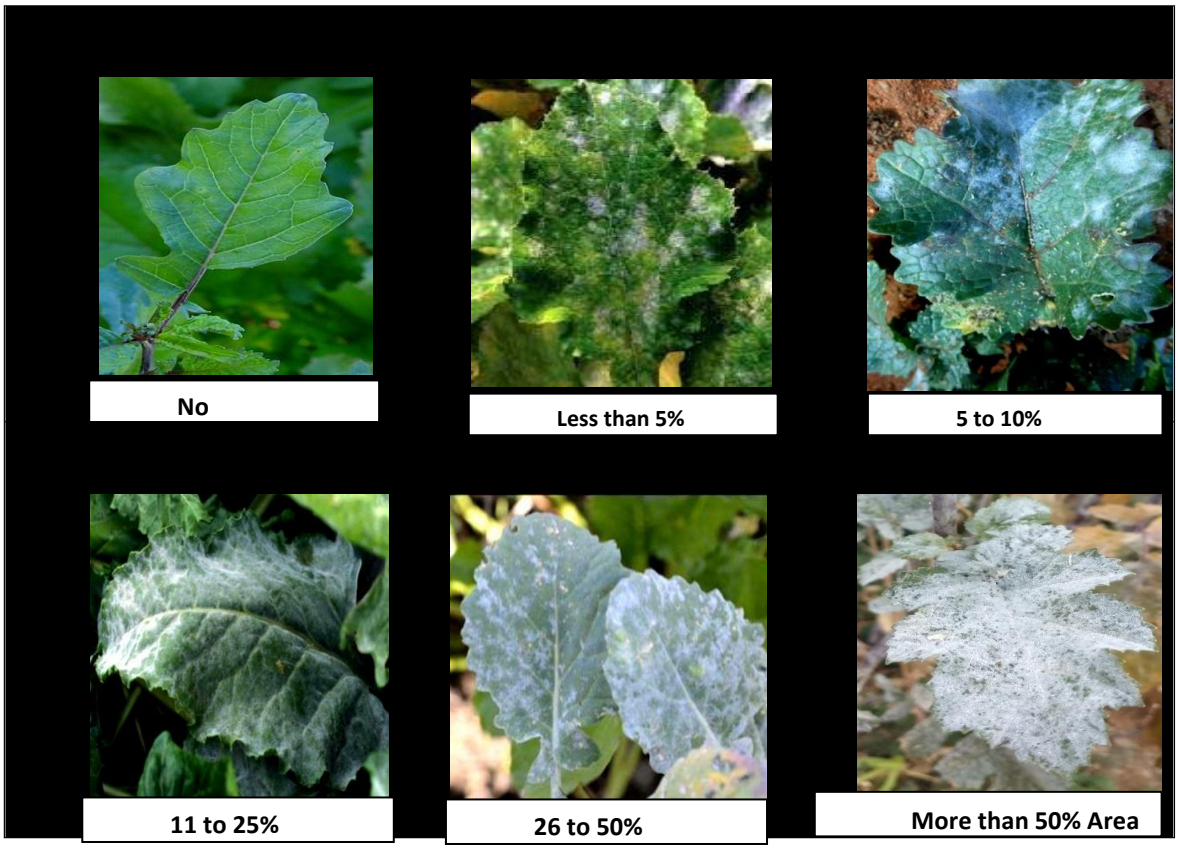

Fig. 2. Categorizations of reactions of Brassica genotypes against powdery Mildew (*Erysiphe cruciferarum*)

3.2 Biochemical Alteration in Mustard Leaves due to Fungal Infection

The total chlorophyll, total amino acid, total protein, reducing and non-reducing sugar, total soluble sugar, phenol, proline and malondialdehyde (MDA) contents in the healthy and diseased leaves of *Brassica* genotypes were estimated to know the biochemical changes and find out the putative roles of these parameters in inducing resistance in the experimented genotypes (Table 4; Fig.4; Fig.5; Fig.6).

MDA is an extensively used marker of membrane lipid peroxidation caused by biotic stress [43]. "Inhibition of mycelial growth in the fungi varied considerably and revealed sensitivity groups within the tested genotypes (Table 5; Fig. 4). In the present investigation, in general, MDA content was increased in diseased leaves. MDA, a by-product of lipid peroxidation, was quantified to ascertain the implication of lipid peroxidation. Pathogens were used as living model membranes to elucidate the role of membrane lipids and lipid peroxidation in the relative sensitivity of microorganisms to shed light on the possible mode of action of these genotypes on pathogen membranes. There was an optimistic association between MDA content and lipid peroxidation and it can weaken the integrity of cell wall" [44].

Chlorophyll content was documented minimum in diseased leaves and maximum in healthy leaves. Total chlorophyll levels in the leaf tissues of the resistant (DRMRIJ12-48) and susceptible (RH30) genotypes showed that the resistant (DRMRIJ12-48) genotype had somewhat greater total chlorophyll in healthy leaves than the susceptible (RH30) genotype. There was a gradual decrease in total chlorophyll as the disease increased. After infection, amount of total chlorophyll reduced in both genotypes. Furthermore, it was observed that there was more total chlorophyll content in the resistant genotype compared to the susceptible genotype. As a necrotrophic fungus, induces host cell death to enable rapid colonization of plants and derive nutrients from sacrificed cells, a 50 to 80% amino acid decrease was observed in the current study. Main pathogenicity factors, toxin and lytic enzyme secretion act synergistically to kill,

degrade and macerate host plant tissues. The amino acid concentration could be modified by host metabolism changes, induced by the necrotrophic pathogen. The findings of this study demonstrated that the protein content was higher in genotypes that were vulnerable to white rust than in genotypes that were resistant. Additionally, protein content reduced as white rust severity increased; however, the initial infection up to the severity level of 4.6% did not modify the content; instead, the protein content declined by 0.2% with each additional 1% increment in disease severity (Fig. 5). The protein content in the leaf of mustard genotypes increased with crop age and dropped owing to fungal infection vulnerable genotypes contents higher. This explains the role of protein in plant defense through its impact on pathogens that invade the cell wall.

Under diseased conditions, free amino acid levels increased indicating fungal infectivity causes proteolysis and denaturation of membrane proteins, resulting in augmentation of free amino acid content in host tissues [25]. Under fungal infection stress, the levels of proline, total amino acid and total soluble protein amplified with concurrent reduction in chlorophyll content and total sugar in all mustard genotypes. Alternaria infection reduced chlorophyll content causing the decline in photosynthesis rate, leading to drop in total sugar level [30]. The biochemical findings highlight the fluctuation in values of significant biochemical parameters such as total proteins, sugars, and phenols, superoxide dismutase, and hydrogen peroxide during the *A. candida* infection in *B. juncea* [45].

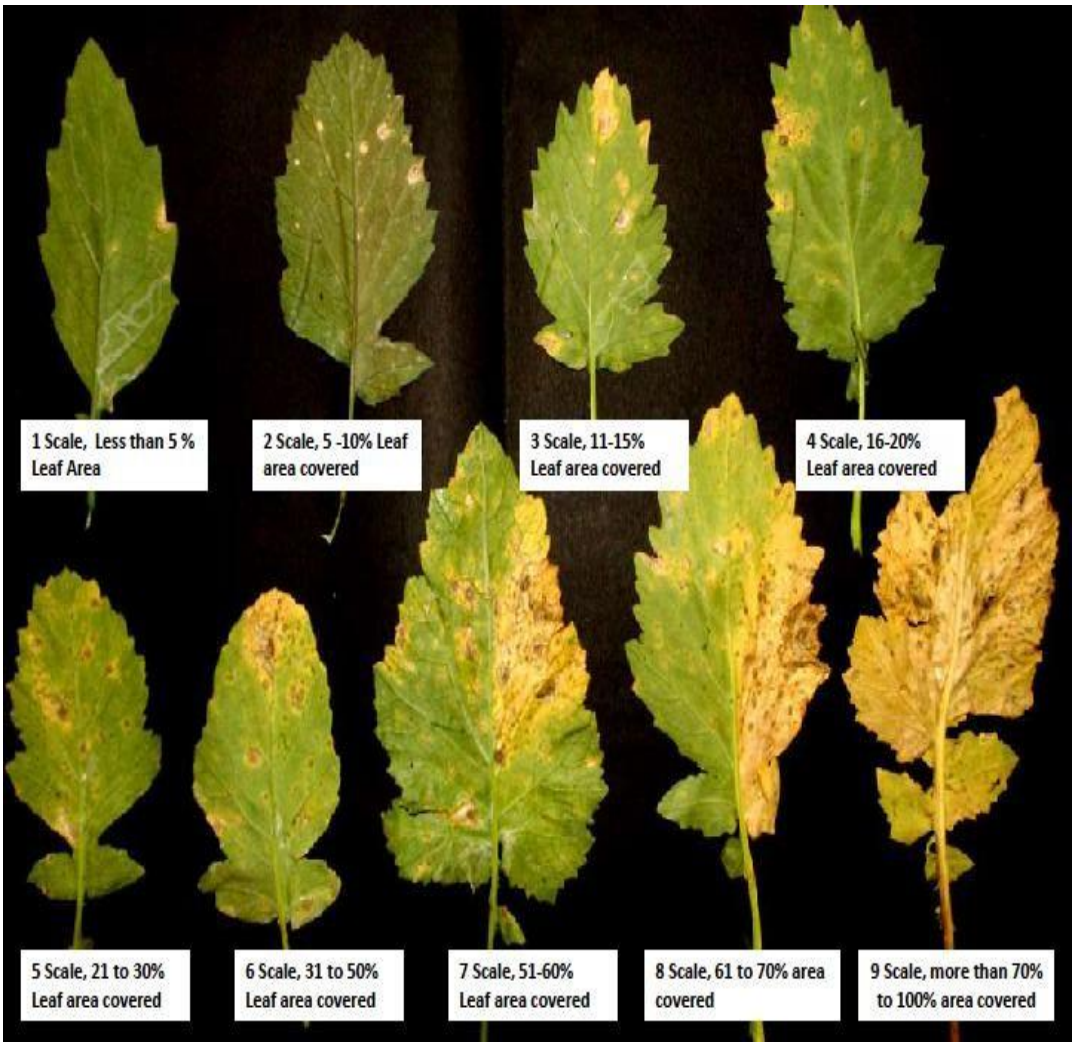

Fig. 3. Categorizations of reactions of brassica genotypes against alternaria blight (*Alternaria brassicicola*)

Table 5. Biochemical responses of *Brassica* genotypes in healthy and diseased leaves

S. No.	Genotypes	MDA (mM/g)		Chlorophyll (mg/g)		Amino acid (ug/mg)		Protein (%)		Reducing sugar (ug/mg)		Non-reducing sugar (ug/mg)		Total sugar (ug/mg)		Phenol (ug/mg)		Proline (Umol/g)	
		Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
1	RB-50	3.4	4.2	1.1	0.75	11.3	12.5	21.36	12.36	12.54	14.6	27.11	13.5	39.65	28.1	2.14	1.75	1.2	5.4
2	Pusa Bold	3.6	5.3	1.2	0.95	11.6	12.6	23.23	15.32	14.65	13.25	24.24	17.4	38.89	30.65	2.19	1.79	1.3	5.9
3	Varuna	3.8	5.7	1.2	0.65	11.8	13.5	23.52	15.23	16.32	14.26	23.33	19.39	39.65	33.65	2.25	1.65	2.5	5.4
4	Rohini	4.6	5.6	1.3	0.63	12.6	13.9	22.25	14.36	14.56	14.96	24	19.36	38.56	34.32	2.24	1.76	2.5	6.2
5	Kranti	4.5	6.3	1.2	0.85	13.1	15.3	26.35	16.65	15.58	17.65	25.98	16.6	41.56	34.25	2.29	1.72	3.2	6.3
6	RH-725	4.8	6.5	1.2	1.25	12.6	14.6	24.65	14.52	13.68	16.42	28.67	13.47	42.35	29.89	2.36	1.63	1.2	5.3
7	Maya	3.6	4.8	1.3	1.15	13.5	16.3	25.32	15.36	14.87	15.23	26.78	17.55	41.65	32.78	2.27	2.22	1.5	5.9
8	Vardan	3.4	4.2	1.4	1.12	13.4	14.2	25.36	17.23	16.48	18.39	27.98	21.17	44.46	39.56	2.64	2.28	2.4	6.1
9	Vasundhara	3.5	5.6	1.1	1.14	12.6	12.6	21.56	15.65	17.87	19.34	25.49	18.22	43.36	37.56	2.31	1.85	3.5	6.2
10	SwarnJyoti	4.5	6.6	1.2	1.16	12.8	13.5	24.24	13.87	16.12	11.58	28.35	25.07	44.47	36.65	2.54	1.87	3.4	5.8
11	Pusa Jagannath	4.9	6.4	1.3	1.46	13.6	14.6	28.32	13.68	12.98	11.35	32.67	25.1	45.65	36.45	2.13	1.64	3.5	5.7
12	Pusa JaiKisan	4.6	5.5	1.4	1.25	12.8	15.5	25.56	17.25	13.87	14.35	25.69	21.43	39.56	35.78	2.15	1.94	3.8	6.7
13	Albeli	3.7	5.3	1.4	1.32	13.8	15.9	24.63	16.56	16.56	15.98	23.72	21.5	40.28	37.48	2.65	1.96	3.4	5.4
14	Sej-2	3.1	5.1	1.2	1.36	13.7	12.5	26.78	15.26	17.47	15.53	23.07	25.34	40.54	40.87	2.45	2.03	2.6	6.1
15	Shraddha	3.6	4.2	1.4	1.34	12.6	13.6	27.25	17.36	18.62	17.69	28.27	23.89	46.89	41.58	2.02	2.31	3.5	6.8
16	DMH 1	4.2	5.3	1.3	1.31	11.4	12.4	24.23	14.14	19.36	18.36	28.33	11.87	47.69	30.23	2.78	2.1	2	5.2
17	L-4	4.1	4.9	1.5	1.26	11.6	13.8	25.65	14.36	20.65	19.35	27.7	12.79	48.35	32.14	2.65	2.13	3.5	5.4
18	JMWR-908-1	3.9	5.6	1.2	1.24	11.5	14.5	26.35	15.25	22.85	15.36	24.69	21.29	47.54	36.65	2.68	2.36	2.4	5.6
19	RGN-73	4	5.9	1.2	1.42	12.8	13.6	28.64	13.35	23.65	14.87	24.99	20.55	48.64	35.42	2.34	2.16	3.2	6.1
20	NRC-HB-101	3.5	4.3	1.6	1.23	12.9	15.4	24.23	16.35	23.47	15.36	16.11	21.05	39.58	36.41	2.64	2.63	2.8	5.2
21	NRC-HB-506	3.6	5.6	1.7	1.15	12.8	15.9	28.24	14.45	20.14	17.68	21.44	10.97	41.58	28.65	2.58	1.79	3.7	6.4
22	RVM-3	3.7	6.5	1.6	1.41	13.5	13.7	24.69	16.56	21.16	14.69	26.8	14.96	47.96	29.65	2.14	1.86	2.6	5.3
23	RH-749	3.6	6.4	1.4	1.21	13.8	16.5	25.31	14.32	23.25	19.32	24.89	10.16	48.14	29.48	2.63	1.76	3.2	6.8
24	NRC DR-2	3.9	6.2	1.2	1.31	11.3	15.8	23.35	15.63	26.36	12.15	14.51	20.44	40.87	32.59	2.42	1.64	2.5	5.3
25	DRMR IJ-31	3.8	4.2	1.1	1.42	12.6	14.8	28.53	17.75	25.54	14.56	14.82	19.65	40.36	34.21	2.12	1.82	3.5	6.4
26	China	4.8	5.8	1.1	1.24	13.4	12.6	24.13	13.35	22.63	13.41	18	20.06	40.63	33.47	2.45	1.56	2.8	5.4
27	GSL-1	4.5	6.8	1.3	1.26	11.3	14.4	22.24	14.21	20.15	12.17	27.42	26.11	47.57	38.28	2.17	1.54	3.7	6.4
28	GSC-7	3.6	8.5	1.1	1.35	13.4	16.9	22.36	14.23	24.64	13.65	21.9	25.89	46.54	39.54	2.58	1.63	2.7	6.5
29	PC-5	3.5	7.4	1.6	1.34	12.8	13.3	24.89	17.65	22.36	17.95	24.27	16.3	46.63	34.25	2.65	2.1	3.5	5.2
30	PC-6	4.2	7.6	1.3	1.31	12.7	15.8	24.56	14.98	20.45	18.56	18.78	17.31	39.23	35.87	2.34	2.45	2.1	6.3
31	RP-9	4.3	7.2	1.5	1.25	12.3	14.7	21.21	17.21	22.35	12.22	18.19	29.37	40.54	41.59	2.56	2.32	3.2	5.4
32	Kiran	4.1	4.2	1.2	1.24	13.6	12.9	28.87	14.23	26.98	15.24	19.41	27.11	46.39	42.35	2.54	2.62	2.4	6.2
33	JTC-1	4.6	4.6	1.4	1.32	12.5	13.8	21.23	13.35	24.65	14.68	17.71	28.61	42.36	43.29	2.78	2.46	3.6	5.7
34	JM-1	3.9	6.4	1.2	1.34	11.4	16.8	27.25	16.32	13.34	19.68	27.31	19.54	40.65	39.22	2.89	1.75	2.7	6.3
35	JM-2	3.1	6.1	1.1	1.36	12.5	13.7	26.32	15.65	14.25	20.23	28.43	9.98	42.68	30.21	2.87	2.31	3.7	6.2
36	JM-3	3.5	5.6	1.6	1.43	12.6	16.2	25.23	14.32	15.26	11.12	31.09	23.44	46.35	34.56	2.67	2.1	2.4	5.8
37	RVM-1	3.6	5.4	1.2	1.45	11.4	14.3	27.65	15.45	18.35	13.35	26.52	25.01	44.87	38.36	2.64	2.14	3.5	5.7
38	RVM-2	3.8	7.3	1.6	1.25	12.7	14.7	23.45	16.78	14.35	14.56	34.3	17.98	48.65	32.54	2.24	2.14	2.1	6.7
39	PM-25	4.8	5.2	1.1	0.57	12.8	15.6	22.36	13.23	13.65	12.56	25.86	16.76	39.51	29.32	2.13	1.65	2.8	6.8
40	PM-26	3.3	5.8	1.2	0.75	13.6	12.5	23.23	13.25	13.25	12.69	26.98	17.67	40.23	30.36	2.15	1.79	2.8	5.3
41	PM-27	4.4	5.4	1.2	0.87	12.4	13.9	21.65	14.32	14.29	14.15	28.03	16.97	42.32	31.12	2.14	1.69	2.5	6.5
42	PM-28	4.6	8.3	1.3	0.97	12.6	16.8	25.67	15.25	14.95	16.16	26.07	20.37	41.02	36.53	2.65	2.23	3.6	5.1
43	PM-30	4.3	6.2	1.3	0.95	12.8	15.6	24.96	14.25	15.56	15.63	29.67	19.82	45.23	35.45	2.14	2.25	3.4	6.4
44	Pusa Vijay	4.6	6.1	1.2	0.65	11.6	13.8	21.35	12.23	16.35	14.25	24.97	22.23	41.32	36.48	2.32	2.24	3.1	5.3
45	JMM-927	4.6	5.2	1.3	0.75	11.5	15.9	26.64	13.65	14.68	15.65	26.71	13.91	41.39	29.56	2.56	2.14	2.1	6.5

S. No.	Genotypes	MDA (mM/g)		Chlorophyll (mg/g)		Amino acid (ug/mg)		Protein (%)		Reducing sugar (ug/mg)		Non-reducing sugar (ug/mg)		Total sugar (ug/mg)		Phenol (ug/mg)		Proline (Umol/g)	
		Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
46	JMM-991	3.6	5.7	1.4	1.12	11.9	12.5	27.14	14.63	16.24	18.65	19.21	10.97	35.45	29.62	2.31	2.16	2.6	5.1
47	RMM-10-01-01	4.7	6.3	1.5	1.13	12.9	13.6	22.35	15.98	14.32	16.32	27.03	13.92	41.35	30.24	2.14	1.69	3.5	6.8
48	RMM-12-01-18	3.7	6.8	1.6	1.15	13.6	14.8	21.98	16.24	22.36	15.21	19.77	15.26	42.13	30.47	2.78	1.67	2.4	5.7
49	RMM-12-03-18	3.5	7.1	1.3	1.16	13.7	15.6	27.51	15.89	23.14	17.69	23.11	17.78	46.25	35.47	2.45	2.27	2.6	6.4
50	WRR-5	3.2	7.8	1.2	1.25	12.7	16.4	26.54	16.35	22.65	18.58	22.6	20.11	45.25	38.69	2.36	2.68	2.4	5.2
51	WRR-6	3.1	4.8	1.6	0.98	12.4	15.2	22.34	14.65	14.65	19.65	28.56	19.93	43.21	39.58	2.14	1.68	3.4	6.4
52	WRR-7	4.8	6.7	1.5	0.87	13.5	14.5	28.22	15.25	15.23	14.65	28.02	20.5	43.25	35.15	2.21	1.57	3.8	5.8
53	WRR-8	3.5	6.5	1.6	0.94	11.6	12.6	21.35	14.35	12.33	19.65	28.99	12.49	41.32	32.14	2.65	2.12	2.7	6.3
54	WRR-9	3.2	5.2	1.2	0.67	11.9	13.7	25.21	15.65	17.98	15.26	20.58	21.21	38.56	36.47	2.96	2.15	2.6	6.7
55	WRR-10	3.1	4.6	1.5	1.13	12.3	13.8	24.32	14.23	17.89	17.32	24.32	18.93	42.21	36.25	2.19	2.64	2.5	5.6
56	WRR-11	4.1	8.6	1.4	1.15	13.5	14.5	28.54	17.69	18.45	21.21	21	14.11	39.45	35.32	2.58	2.13	3.1	6.9
57	WRR-12	4.2	4.6.	1.4	1.14	11.4	12.9	21.53	14.56	15.65	20.35	23.1	15.79	38.75	36.14	2.1	2.78	3	5.2
58	WRR-13	4.9	5.7	1.6	1.45	12.6	13.8	22.25	16.78	14.25	20.69	24.07	18.96	38.32	39.65	2.13	2.45	3.2	6.3
59	WRR-14	3.4	6.8	1.5	1.23	11.3	14.6	26.23	14.98	16.35	16.68	29.3	23.88	45.65	40.56	2.15	2.15	3.6	6.4
60	WRR-15	3.6	6.7	1.4	1.23	11.8	15.5	21.36	12.63	17.25	22.45	29.31	12	46.56	34.45	2.16	2.65	2	6.8
61	WRR-16	3.2	5.6	1.6	1.45	12.5	12.6	24.25	13.56	26.35	17.89	20.97	19.68	47.32	37.57	2.89	1.65	2.4	5.8
62	WRR-17	4.1	5.9	1.2	1.23	13.4	15.8	21.24	14.26	26.54	15.98	22.11	20.5	48.65	36.48	2.47	1.98	3.5	5.9
63	WRR-18	3.5	5.7	1.3	1.35	13.6	16.8	21.98	16.24	25.96	12.63	16.49	19.52	42.45	32.15	2.46	1.89	3.2	6.7
64	WRR-19	4.3	5.4	1.6	1.36	12.5	15.5	22.65	17.65	25.63	16.47	15.58	20.11	41.21	36.58	2.49	1.78	3.6.	6.4
65	WRR-20	4.5	4.5	1.6	1.25	13.4	16.6	28.56	14.96	13.21	15.56	27.05	22.9	40.26	38.46	2.13	2.25	2.5	5.4
66	WRR-21	3.5	4.9	1.5	1.36	12.8	14.6	22.14	13.44	14.21	21.36	26.02	18.29	40.23	39.65	2.17	2.13	2.8	5.2
67	WRR-22	3.9	6.9	1.4	1.42	11.8	15.3	23.54	16.54	16.35	18.65	26.61	15.6	42.96	34.25	2.13	2.15	3.9	6.2
68	WRR-25	4	7.8	1.6	1.32	11.2	12.9	26.45	14.25	12.63	15.24	34.35	24.32	46.98	39.56	2.36	2.14	2.9	5.1
69	WRR-26	3.6	7.6	1.2	1.31	13.7	13.4	26.32	13.21	12.85	14.24	35.11	27.41	47.96	41.65	2.47	2.31	3.5	6.2
70	WRR-27	3.4	5.8	1.3	1.25	12.3	13.5	22.69	13.98	17.32	16.56	29.26	23.02	46.58	39.58	2.15	2.41	2.7	5.1
71	WRR-28	3.8	7.6	1.3	1.45	13.9	16.8	27.52	13.78	18.89	18.78	21.34	20.69	40.23	39.47	2.63	2.14	3.8	6.5
72	WRR-29	4.1	7.4	1.2	0.98	12.6	14.5	26.32	13.85	26.21	17.89	14.04	18.65	40.25	36.54	2.56	2.36	2.1	6.4
73	WRR-30	4.6	6.2	1.6	0.97	13.5	15.8	24.87	13.21	16.45	15.45	31.2	16.79	47.65	32.24	2.32	2.25	2.2	5.8
74	WRR-31	4.2	4.6	1.4	0.95	13.6	13.7	25.52	14.23	16.25	17.65	26.1	18.49	42.35	36.14	2.38	2.31	3.2	6.4
75	WRR-32	4.1	4.9	1.4	1.35	11.5	13.9	26.35	15.65	14.32	16.45	28.04	19.02	42.36	35.47	2.25	2.12	2.1	5.2

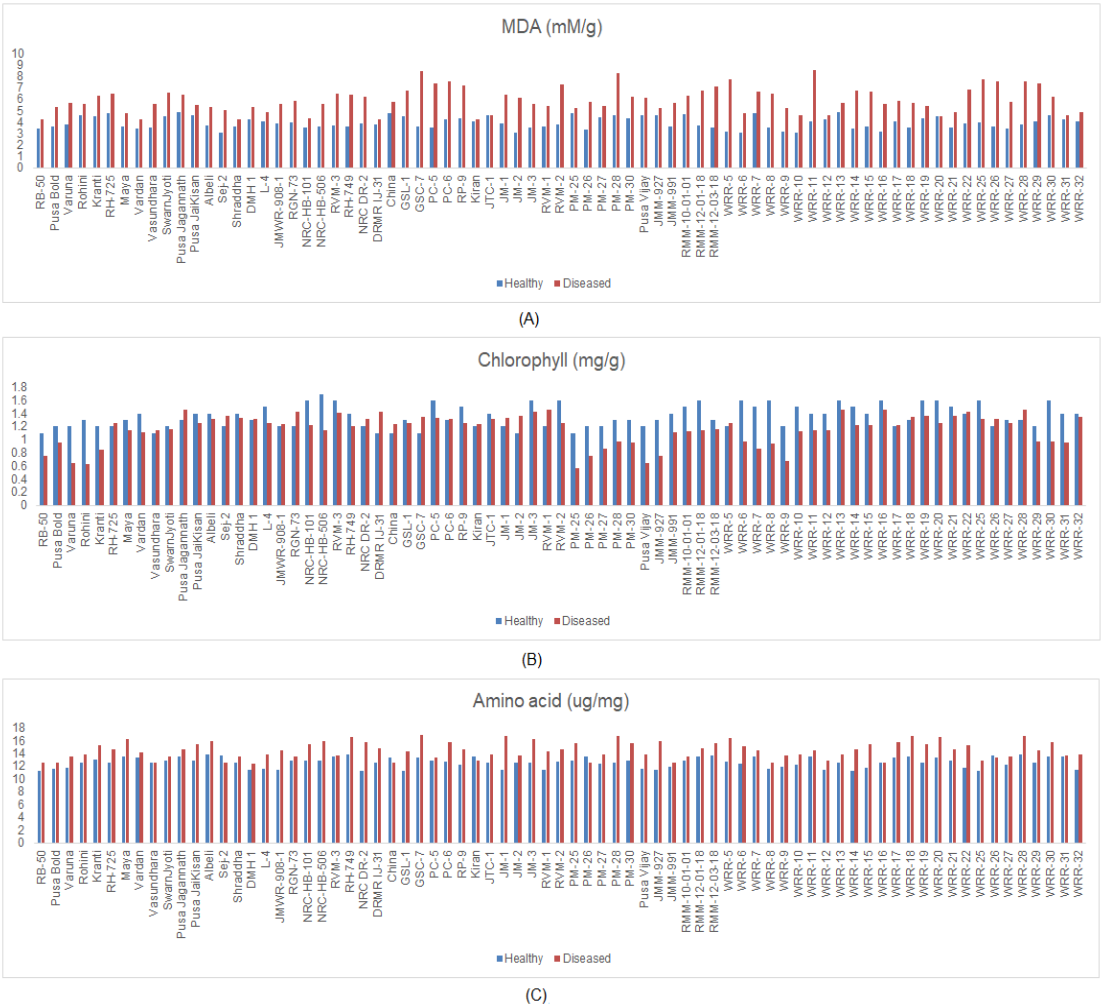

Fig. 4. MDA (A), chlorophyll (B), and amino acid (C) responses of *Brassica* genotypes under healthy and diseased leaves

The total phenol content of leaves reduced as the severity of the white rust increased, although this was not the case during the first infection. Instead, it declined over time by 0.6% as the disease severity increased by 1%. Similarly total phenol, the amount of o-dihydroxyl phenol was higher in the leaves of *Brassica juncea* resistant genotypes compared to susceptible ones, and it was similarly higher at an advanced stage of crop growth. The amount of phenol in the leaves was reduced by the disease's severity. The regression analysis revealed that the 0.61 percent fall in o-dihydroxyl phenol content in leaves was brought on by a 1% rise in white rust severity. The resistant genotype's greater levels of total phenol and o-dihydroxyl phenol content may encourage wax deposition on the surface, acting as a barrier to the pathogen's penetration. White rust resistant genotypes have higher amounts of total phenol and o-dihydroxyl phenols than susceptible genotypes and less of these compounds in infected leaves. There are greater total phenol component concentrations in resistant cultivars. In comparison to the susceptible genotypes, the total soluble sugar and reducing sugar levels were higher in the genotypes resistant to white rust. The total soluble sugar and reducing sugar content of leaves fell by 0.47 percent and 0.50 percent, respectively, with each 1% rise in the severity of fungal infection. However, the initial severity level of the disease (4-6%) had no effect on these contents. The sugar contents decreased as levels of *Albugo candida* infection rose in genotypes of *Brassica alba* that were resistant to white rust and had higher levels of total soluble and reducing sugar than susceptible Varuna (*Brassica juncea*). As plants grew older, decreasing sugar and total soluble sugar levels fell in rapeseed-mustard genotypes.

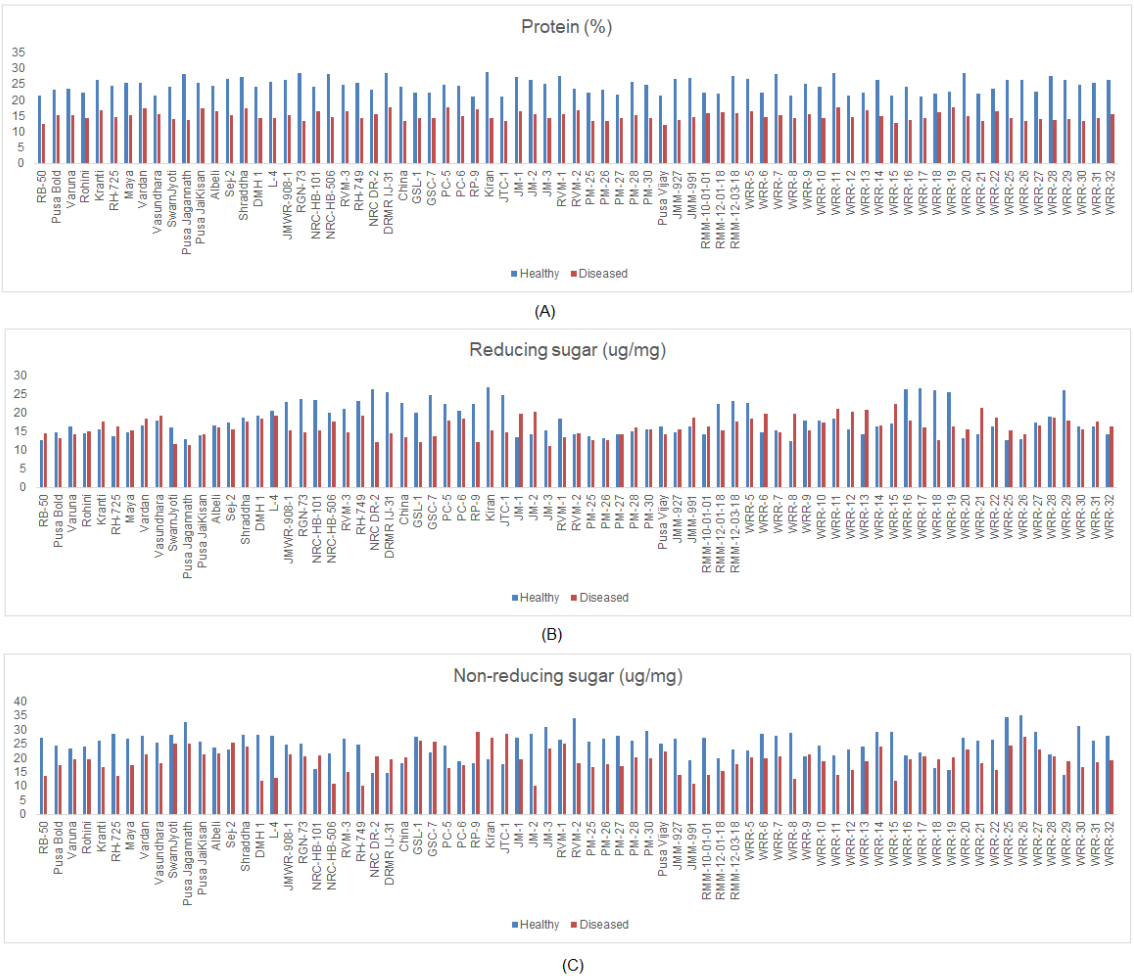

Fig. 5. Protein (A), Reducing sugar (B) and Non-reducing sugar (C) responses of *Brassica* genotypes under healthy and diseased leaves

Phenolic compounds and associated oxidative enzymes are regarded to be the utmost imperative biochemical parameters for disease resistance [26]. Phenolics acts as potent antioxidants and ROS scavengers in plant tissues under biotic stress and an enhanced level has also been exhibited in stressed plants as a resistance machinery against biotic stress. In host-parasite interaction, the concentrations/activities of these osmolytes/ enzymes and their reaction products are recognized to have direct association with the mechanism of host resistance [30].

The accumulation of total phenols, total sugar content in resistant was higher than susceptible genotypes (Fig. 6). The result was clearly demonstrated their agreement with general trend of increasing resistance in host tissues in which resistance of host tissues are increased with accumulation of total phenols and sugars content [46]. The increased level of reducing and non-reducing sugars contents linked with higher resistance observed in this investigation and the decreased value after infection [26] . The co-existence of phenols and free-sugars resulted in glycosylation of phenolics by free sugars to form phenolic glycosides, leads to more proficient expression of resistance due to more solubility in cell sap [30].

3.3 Heatmap Clustering to show level of Expression of Biochemical Parameters and Correlation Analysis among 75 Mustard Genotypes

The heat map represents the level of expression of different biochemical parameters. The 75 mustard genotypes can be largely grouped into three major clusters based on their similarity

taking into consideration all nine biochemical parameters including MDA, chlorophyll, amino acid, protein, reducing and non-reducing sugar, total sugar, phenol and proline contents (Fig. 7). Cluster I consist 19 mustard genotypes including Kiran, JTC-1, RP-9, GSC-7, RMM-12-01, China, WRR-18, NRC-DR-2, WRR-29, DRMR-IJ-31, WRR-19, NRC-HB-101, WRR-17, WRR-16, WRR-5, RMM-12-03, PC-5, RGN-73 and JMWR-908-1. Cluster II, the largest cluster comprised with four subclusters. Cluster IIA contained 10 mustard genotypes including WRR-26, WRR-25, GSL-1, RVM-1, WRR-27, WRR-14, Shraddha, Pusa Jagannath, JM-3 and Swarn Jyoti. Whilst cluster IIB confined with 7 mustard genotypes including WRR-30, RVM-2, WRR-15, RH-749, RVM-3, L-4 and DMH-1.Whereas cluster IIC had 13 mustard genotypes *viz*., WRR-8, JM-2, RB-50, RMM-10-01, JMM-927, RH-725, PM-27, PM-26, PM-25, Rohini, Varuna, Pusa Bold and Pusa Vijay. Cluster IID contained 23 mustard genotypes including WRR-11, WRR-28, WRR-9, PC-6, JM-1, WRR-6, Vardan, WRR-20, PM-28, Albeli, Pusa Jai Kisan, WRR-32, WRR-7, PM-30, WRR-31, WRR-10, Vasundhara, Maya, WRR-22, Kranti, WRR-21, WRR-13, WRR-12 and Sej-2. Cluster IV, the smallest cluster has only 2 mustard genotypes *i.e*., JMM-991 and NRC-HB-505 which were significantly different from all other mustard genotypes. In the experiment of Meena *et al*. [16], the 26 genotypes of Indian mustard were broadly classified into four major clusters based on their likeness considering biochemical parameters to confirm the induced tolerance to Alternaria blight. Cluster I consisted 16 genotypes, cluster II comprises three genotypes, cluster III consisted 6 genotypes while cluster 4 contained only single genotype which was considerably dissimilar from all others.

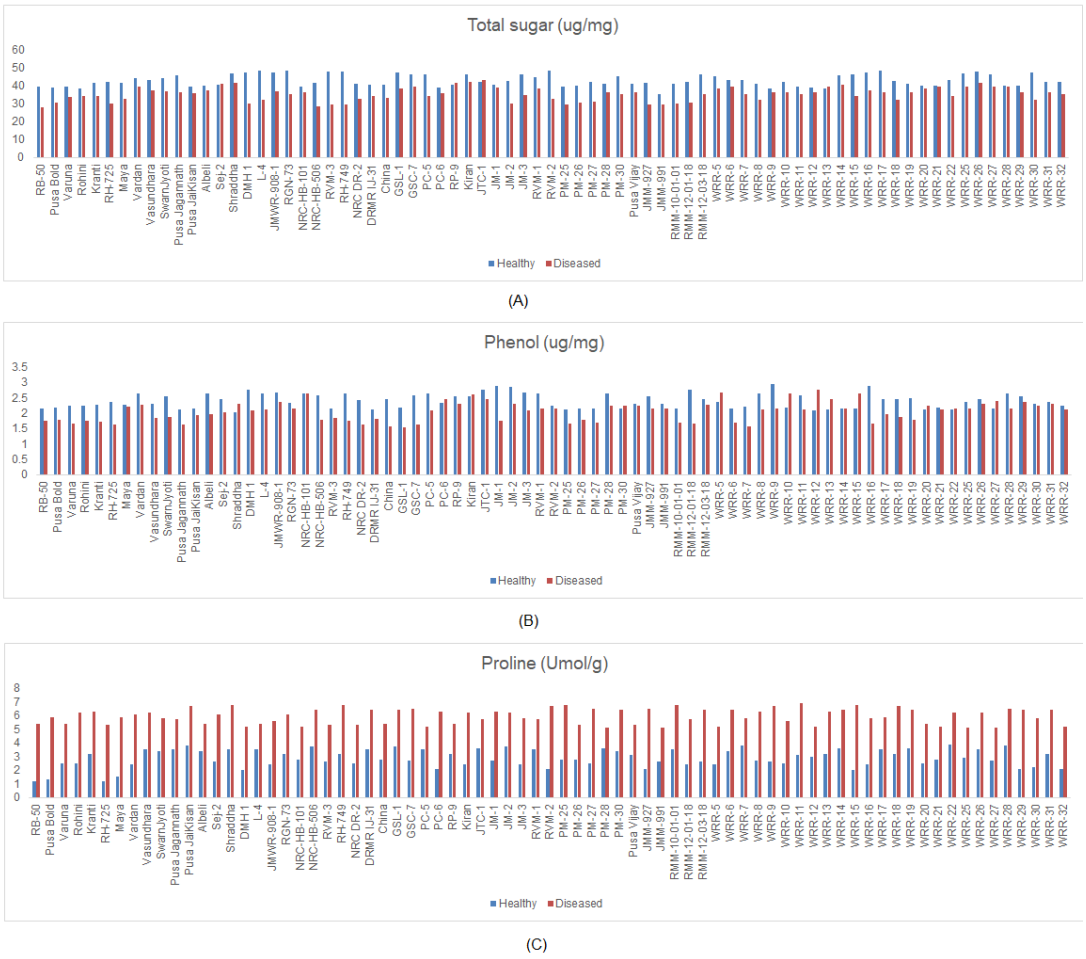

Fig. 6. Total sugar (A), phenol (B) and proline (C) responses of *Brassica* genotypes under healthy and diseased leaves

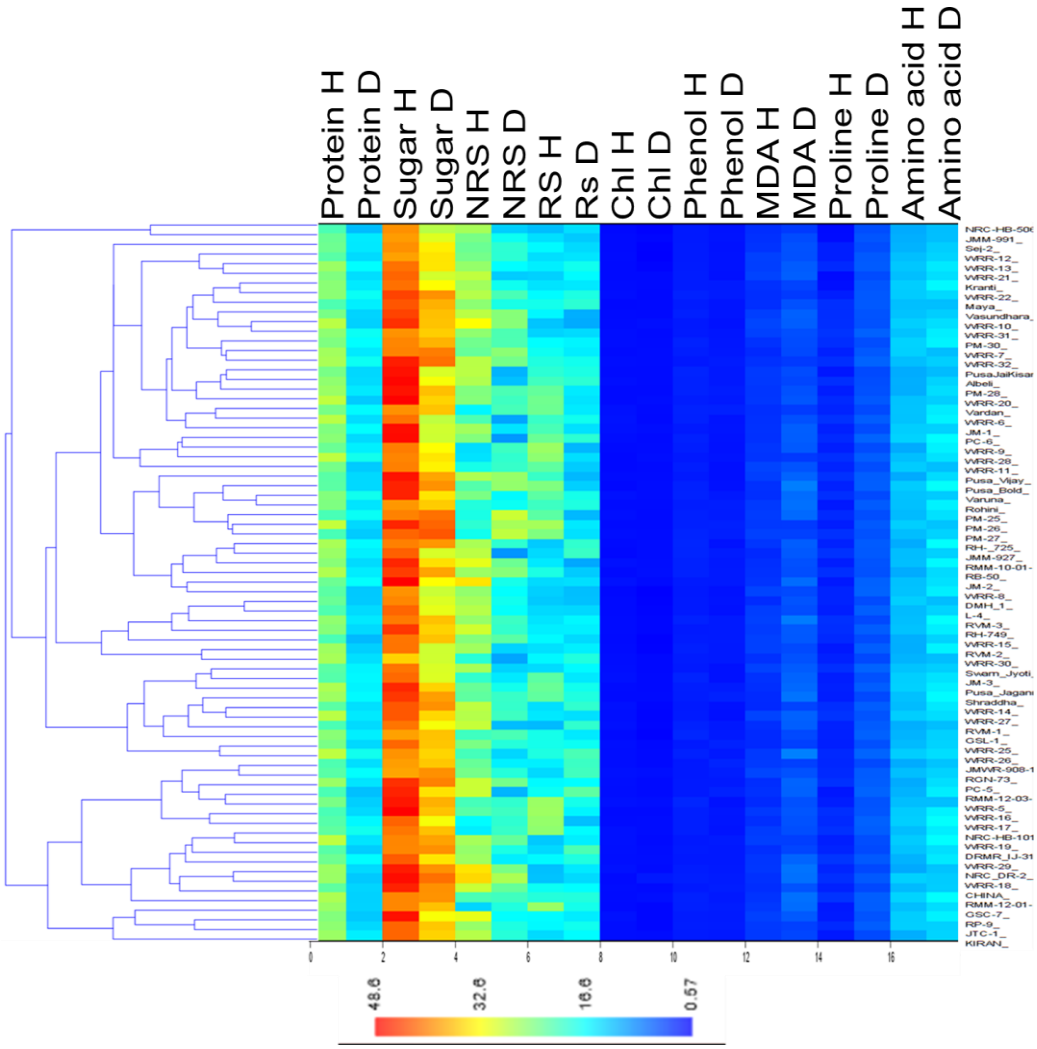

Fig. 7. Dendrogram and Heat Map of 75 mustard genotypes for biochemical responses

4. CONCLUSION

In the quest for developing resistance against fungal infections in Brassicaceae cultivars, the role of genetic management has proven pivotal in shaping the dynamics of host-pathogen interactions. Beyond the realm of host genetics, the study emphasizes the significant impact of biochemical constituents on the severity of diseases. Regardless of their tolerance status, all biochemical constituents were found to influence the plant's response to fungal pathogens.

The disease indexing revealed specific mustard genotypes that exhibited exceptional resistance against key fungal pathogens. Mustard genotypes L-4, GSC-7, and PC-6 were identified as immune against Alternaria brassicae, while Maya, L-4, China, GSL-1, GSC-7, PC-5, PC-6, and RP-9 demonstrated immunity against *Albugo candida*. Additionally, L-4 and PC-5 were classified as immune against Erysiphe cruciferarum. These immune genotypes, characterized through disease indexing, showcased their remarkable ability to resist fungal infections.

The in-depth study of biochemical constituents provided insights into the mechanisms underlying the resistance of these genotypes. The immune genotypes exhibited lower susceptibility to pathogens by accumulating higher levels of osmolytes in diseased conditions. This accumulation of osmolytes in response to fungal infections highlighted the tolerance capacity of these genotypes.

The identified immune genotypes, *namely* L-4, GSC-7, PC-6, Maya, China, GSL-1, PC-5, and RP-9, hold immense potential for further

exploration in Phyto pathological studies and fungal resistance breeding. The broader implications of these findings extend to the development of mustard varieties with enhanced resistance, contributing to sustainable and resilient agricultural practices in the face of fungal challenges.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERRENCES

1. Naseem M, Kushwaha C, Juneja S Anand S. Influences of growth stages on peroxidase and phenol production in Indian mustard exposed to *Alternaria brassicicola* infections. The Pharma Innovation Journal 2023; 12(3): 2305-2308.
2. Shrivastava A, Tripathi MK, Solanki RS, Tiwari S, Tripathi N, Singh J, Yadav R. Genetic correlation and path coefficient analysis of yield attributing parameters in Indian mustard. Curr. J. Appl. Sci. Technol. 2023a;42(7):42-58.
DOI: 10.9734/CJAST/2023/v42i74079
3. Tarar, M. Biochemical, Biotechnical significance of mustard and its role in agricultural based industries. Sch Bull. 2021; 7(7):185-189.
4. Shyam C, Tripathi M K, Tiwari S, Ahuja A, Tripathi N, Gupta N. *In vitro* regeneration from callus and cell suspension cultures in Indian mustard [*Brassica juncea* (Linn.) Czern & Coss] International Journal of Agricultural Technology. 2021a;17(3):1095-1112.
5. Rajpoot NS, Tripathi MK, Tiwari S, Tomar RS, Kandalkar VS. Characterization of Indian mustard germplasm on the basis of morphological traits and SSR markers. Curr J Appl Sci Technol. 2020;39:300-311.
6. Shyam C, Tripathi MK, Tiwari S, Tripathi N. Genetic components, and diversity analysis in Indian mustard [*Brassica juncea* (Linn.) Czern & Coss] based on different morpho-physiological traits. Current Journal of Applied Science and Technology. 2021b;40 (20):34-57. DOI: 10.9734/CJAST/2021/v40i2031462
7. Shrivastava A, Tripathi MK, Singh P, Tiwari S, Tripathi N, Tiwari PN, Parihar P, Singh J, Chauhan S. Disease indexing of Indian mustard genotypes against *Alternaria* blight disease. The Pharma Innovation Journal; 2023b;12(4): 08-13.
8. Shyam C, Tripathi MK, Tripathi N, Tiwari S, Sikarwar R S. Genetic variations in fatty acids and oil compositions among 188 Indian mustard *Brassica juncea* (Linn.) Czern & Coss genotypes. Curr J Appl Sci Technol. 2022a;40(46):9-28
DOI: 10.9734/CJAST/2021/v40i4631629
9. Shyam C, Tripathi MK., Tripathi N, Tiwari S, Sikarwar RS. Analysis of genetic differences in fatty acids and oil contents among *Brassica juncea* (Linn.) Czern & Coss genotypes. In book: Research Developments in Science and Technology. 2022b;1:127-149.
DOI:10.9734/bpi/rdst/v1/6010F
10. Shyam C, Tripathi MK, Tiwari S, Ahuja A, Tripathi N, Gupta N. Plant regeneration in Indian mustard [*Brassica juncea* (Linn.) Czern & Coss]: Experimental investigation. In book: Current Topics in Agricultural Sciences. 2021c;3:120-135.
DOI: 10.9734/bpi/ctas/v3/2118C
11. Shyam C, Tripathi MK, Tiwari S, Tripathi N and Sikarwar RS. Morpho-physiological variations and genetic components analysis in *Brassica juncea* (Linn.) Czern & Coss. In book: Research Developments in Science and Technology. 2022d;1:98-126,
DOI: 10.9734/bpi/rdst/v1/6009F
12. Verma K, Tripathi MK, Tiwari S, Tripathi N. Analysis of genetic diversity among *Brassica juncea* genotypes using morpho-physiological and SSR markers. Int J Curr Microbiol App Sci. 2021a;10(01):1108-1117.
13. Rajpoot NS, Tripathi MK, Tiwari S, Tomar RS, Tripathi N, Sikarwar RS, Tomar SS. Morphological and molecular characterization of Indian mustard germplasm lines. Research Developments in Science and Technology. 2022;4:151-165
Available:https://doi.org/10.9734/bpi/rdst/v4/2307B
14. Shyam C, Tripathi MK, Tiwari S, Tripathi N, Ahuja A. Molecular characterization and identification of *Brassica* genotype(s) for low and high erucic acid content using SSR markers. Global J Biosci Biotechnol. 2020;9(2):56-66.
15. Shyam C, Tripathi MK, Tripathi N, Tiwari S, Sikarwar RS. Identification of low and high erucic acid containing genotype(s) in Indian mustard employing molecular markers. In book: Recent Progress in Plant and Soil Research, 2022c; 5: 18-36.

DOI: 10.9734/bpi/rppsr/v5/15384D
16. Meena PD, Sujith Kumar MS, Meena HS, Jambhulkar S, Gohartaj, Pathak D, Srivastava S, Gupta R, Singh D, Gurung B, Rai PK. Confirmation of induced tolerance to Alternaria blight disease in Indian mustard (*Brassica juncea* L.) Appl. Biochem. Biotechnol. 2020;192(3):965-978. DOI: 10.1007/s12010-020-03362-2
17. Shyam C, Tripathi MK. Biochemical studies in Indian mustard [*Brassica juncea* (Linn) Czern & Coss] for fatty acid profiling. Int. J. Chem. Stud. 2019;7(4):338-343.
18. Santos FJ, Galceran MT. The application of gas chromatography to environmental analysis. TrAC Trends in Analytical Chemistry. 2002;21(9-10):672-685.
19. Baghel R, Sharma AK, Tiwari S, Tripathi MK, Tripathi N. Genetic diversity analysis of Indian mustard (*Brassica spp*.) germplasm lines using SSR molecular markers. Int J Curr Microbiol App Sci. 2020;9(12):137-143.
20. Tripathi MK, Tomar SS, Tiwari VK, Awasthi D, Gupta JC. Heterosis in Indian mustard [*Brassica juncea* (L) Czern and Coss]. Prog. Res. 2015;10 (Special-VI):3376-3379.
21. Barfa D, Tripathi MK, Kandalkar VS, Gupta JC, Kumar G. Heterosis and combining ability analysis for seed yield in Indian mustard [*Brassica Juncea* (L) Czern & Coss]. Eco. Env. & Cons. 2017;23(February Suppl.) :75-83.
22. Shyam C, Tripathi MK, Tiwari S, Tripathi N, Solanki RS, Sapre S, Ahuja A, Tiwari S. *In vitro* production of somaclones with decreased erucic acid content in Indian mustard [*Brassica juncea* (Linn.) Czern & Coss. Plants. 2021c;10:1297. Available:https://doi.org/10.3390/plants1007 1297
23. Shrivastava A, Tripathi MK, Tiwari S, Tripathi N, Tiwari PN, Bimal SS, Rajpoot P, Chauhan S. Evaluation of genetic diversity in Indian mustard (*Brassica juncea* var. rugosa) employing SSR molecular markers. Plant Cell Biotechnology and Molecular Biology. 2023d;24(3-4):10-21.
24. Dange SRS, Patel RL, Patel SI, Patel KK. Effect of planting time on the appearance and severity of white rust and powdery mildew diseases of mustard. Indian J. Agric. Res. 2003;37(2):154-156.
25. Meena PD, Awasthi R P, Chattopadhyay C, Kolte SJ, Kumar A. Alternaria blight: A chronic disease in rapeseed-mustard. Journal of Oilseed Brassica. 2010; 1: 1–11.
26. Mishra KK, Kolte SJ, Nashaat NI, & Awasthi RP. Pathological and biochemical changes in *Brassica juncea* (mustard) infected with *Albugo candida* (white rust). Plant Pathology. 2009;58(1):80-86.
27. Shrivastava A, Tripathi MK, Tiwari S, Singh P, Tripathi N, Tiwari P N, Parihar P, Singh J, Chauhan S. Screening of Indian mustard genotypes against white rust disease based on disease indexing. Biological Forum – An International Journal. 2023c;15(4):268-272.
28. Shrivastava A, Tripathi M K, Tiwari S, Tripathi N, Tiwari P N, Singh P, Parihar P, Yadav R, Chauhan S, Singh J. Selection of powdery mildew resistant *Brassica* genotypes based on disease indexing and microsatellite markers. Current Journal of Applied Science and Technology. 2023;42 (16):54-66.
29. Nirwan S, Sharma AK, Tripathi RM, Pati AM, Shrivastava N. Resistance strategies for defense against *Albugo candida* causing white rust disease. Microbiological Research. 2023;127317.
30. Mallick SA, Kumari P, Gupta M, Gupta S. Effect of Alternaria blight infection on biochemical parameters, quantity and quality of oil of mustard genotypes. Indian Journal of Plant Physiology. 2015;20:310-316.
31. Naservafaei S, Sohrabi Y, Moradi P, Mac Sweeney E, Mastinu A. Biological response of *Lallemantia iberica* to brassinolide treatment under different watering conditions. Plants. 2021;10(3):496.
32. Arnon DI. Copper enzymes in isolated chloroplasts, polyphenoxidase in *Beta vulgaris*. Plant Physiol. 1949;24:1-15.
33. Moore S, Stein WH. Photometric ninhydrin method for use in the chromatography of amino acids. Journal of Biological Chemistry. 1948;176: 367-388.
34. Lowry OH, Rosenberg NJ, Farr A L, Randall RJ. Protein measurement with the Folin–Phenol reagent. Journal of Biological Chemistry. 1951;193: 265–275.
35. DuBois M, Gilles KA, Hamilton JK, Rebers PT, Smith F. Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 1956; 28(3):350-356.
36. Miller LG. Use of dinitrosalicylic acid

reagent for the determination of reducing sugars. Analytical Chemistry. 1959;31:426–428.
37. Bates IS, Waldren RA, Teare ID. Rapid determination of free proline for water-stress studies. Plant and Soil. 1973;39:205-207.
38. Swain T, Hillis WE. The phenolic constituents of Prunus domestica. I.—The quantitative analysis of phenolic constituents. Journal of the Science of Food and Agriculture. 1959;10(1):63-68.
39. Petrie GA. Diseases of *Brassica species* in Saskatchewan, 1970-72.Staghead and aster yellows. Canadian Pl. Dis. Surv. 1973;53: 19-25.
40. Ghosh S, Mazumder M, Mondal B, Mukherjee A, De A, Bose R., ... Basu D. Morphological and SSR marker-based genetic diversity analysis of Indian mustard (*Brassica juncea* L.) differing in *Alternaria brassicicola* tolerance. Euphytica. 2019;215, 1-19.
41. Nanjundan J, Manjunatha C, Radhamani J, Thakur A K, Yadav R, Kumar A, ... Singh D. Identification of new source of resistance to powdery mildew of Indian mustard and studying its inheritance. The Plant Pathology Journal. 2020;36(2):111.
42. Nirwan S, Chatterjee A, Tewari AK, Agnihotri A, Sharma P, Abdin MZ, Shrivastava N. Pathotyping of white rust pathogen *Albugo candida* on Brassicaceae hosts in India. Indian Phytopathology. 2022;*75*(2): 383-393.
43. Kong W, Liu F, Zhang C, Zhang J, Feng H. Non-destructive determination of Malondialdehyde (MDA) distribution in oilseed rape leaves by laboratory scale NIR hyperspectral imaging. Sci Rep. 2016;6:1-8.
44. Rani A, Devi P, Jha UC, Sharma KD, Siddique KH, Nayyar H. Developing climate-resilient chickpea involving physiological and molecular approaches with a focus on temperature and drought stresses. Fron Plant Sci. 2020;10:1759.
45. Chatterjee A, Nirwan S, Mohapatra S, Sharma P, Agnihotri A, Shrivastava N. Biochemical aspects of pathogenic variability in white rust infected Indian mustard. Mycologia. 2022;114(4):757-768.
46. Kumar R, Thakral NK, Gupta SK, Kaushik CD. Alterations in sugars and phenols in *Brassica juncea* during interaction with white rust (*Albugo candida*). Cruciferae-Newsletter. 2002;24 :91-92.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/114560