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Assessment of Effect of *Pseudomonas syringae* pv. *syringae* Infection on Biochemical Constituents and Enzymatic Activity of Barley (*Hordeum vulgare* L.) Seeds

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Abstract

Basal kernel blight disease of barley due to *Pseudomonas syringae* pv. *syringae* (Pss) is known for remarkable crop losses worldwide. Current investigation was carried out for assessment of physiological and biochemical changes induced due to natural infection of Pss in barley seed samples. It was observed that moisture, fibre, ash and total carbohydrates were variable with significant values in all infected samples. Total phenols increased significantly in all Pss infected seed samples. Notably protein content was increased significantly in the infected samples, whereas crude fat, crude fibre and total carbohydrates decreased significantly. However, the enzymes such as polyphenoloxidase (PPO), catalase (CAT) and peroxidase (POD) were remarkably higher in the infected samples. The results suggest that interaction of Pss with host metabolism interferes with biochemical pathways and production of various defensive enzymes and other products in process to bring disease symptoms on the barley. It is concluded that this study will serve as a pool of information to understand the response of barley to Pss infection on biochemical basis.

***Key words*:** Antioxidative enzymes, Barley, Biochemical changes, *Pseudomonas syringae* pv. s*yringae*

Barley is known as a member of the oldest crops and most consumed globally. Barley is mainly used in production of malt and as an animal feed. However, in the recent era people's interest is being increased for using barley as an important component of healthy foods because of the presence of bio-actives reported in it such as tocols and β-glucans [1] and phenolics [2]. In the present decades, barley is utilized for production of well-designed foods [3]. The plenty of phenols present has made barley an exceptional dietary source of natural anti-oxidants for prevention of many diseases and promoting good health [4].

Barley is also hosted by many pathogens; one of the important bacterial pathogen is *Pseudomonas syringae* pv. *syringae* (Pss) which causes kernel blight with typical necrotic symptoms on the plant [5-6]. Barley is an important common rabi cereal of India, particularly grown in Bihar, Haryana, Himachal Pradesh, Jammu and Kashmir, Madhya Pradesh, Punjab, Rajasthan and Uttar Pradesh states. These are reported to face a lot of field loss due to this disease [7]. As a normal phenomenon after pathogen invasion, changes in metabolism of host plant occurs which are associated with some significant biochemical alterations in the host tissues. It is also noted that plants try to protect their cells against pathogen by launching a cascade of defence response pathways by producing enzymes chemicals like pathogenesis related (PR) proteins and sugars [8]. It is evident that the altered host-metabolism due to pathogenic infection is identified by the physio-chemical alterations in the magnitude and actions of defensive enzymes viz. Peroxidase (POD), polyphenol oxidase (PPO) and enormous oxidative enzymes [9-10].

For an appropriate understanding of the host-pathogen interactions it becomes mandatory to estimate quantitatively the proteins, carbohydrates, enzymes, etc., so as to make significant conclusions on it. Hence, an attempt has been made to study the changes in the biochemical profile of healthy and diseased barley seeds after infection of Pss.

MATERIALS AND METHODS

Isolation and categorization

Farm seed samples of barley were grouped as healthy (accession numbers Hv-17-09, Hv-17-35, Hv-17-48, Hv-17-83)

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and diseased (accession numbers Hv-17-21, Hv-17-53, Hv-17-71, Hv-17-97) on the basis of blemishing and presence of wrinkles on seed pericarp in dry seed examination. Both the categories of samples were sterilized with 2% sodium hypochloride (NaOCl), transferred directly on nutrient agar (NA) medium and placed in incubator for the next 2-3 days at 25±2 °C. Clusters of bacteria with distinctive features of Pss were refined on NA by the method of serial dilution and streaking. Thus, obtained pure colonies were exposed to various biochemical tests and confirmed by pathogenicity tests on barley and other host plants.

Biochemical changes

For estimation of biochemical changes following procedures were followed using four barley seed samples each of healthy (control) and infected samples:

Moisture

For moisture estimation 10 g of the ground barley seeds from each sample were taken and moisture was estimated by oven drying method [11-12]. The moisture content in percentage was calculated using following formula:

$$\text{Moisture (\%)} = \frac{\text{Initial weight – final weight (after drying)}}{\text{Weight of the sample}} \times 100$$

Crude fat

The crude fat estimation of each sample was completed as crude ether extract of the dry material (2 g) in a Soxhlet continuous extraction apparatus by solvent extraction method [11-12]. The crude fat content (%) was calculated as:

$$\text{Crude fat (\%)} = \frac{\text{Weight of ether extract}}{\text{Weight of the sample}} \times 100$$

Crude fibre

For crude fibre estimation, 2 g material from moisture and fat free sample was taken and 200 ml of acid was added to it. Further, it was first boiled with acid and then with alkali for 30 min every time. After cooling it was filtered to a pre-weighed ash dish (W1) and dried in oven at 100 °C for 2 h, cooled down and weighed (W2). Then the sample was burned at ignition point for 30 min at 600 °C, cooled and reweighed (W3) [11-12].

The percentage of crude fibre in diseased and healthy (control) seed sample was calculated as:

$$\text{Crude fibre (\%)} = \frac{(W_2 - W_1) - (W_3 - W_1)}{Weight\ of\ the\ Sample} \times 100$$

Crude protein

Quantitative estimation of crude protein in each sample was estimated by Folin-lowry method [13]. The absorbance of the complex molecule formed in this reaction was measured spectrophotometrically at 660 nm [14].

Ash

Per cent ash content was estimated by using methods given in literature [11-12]. 5 g of the grounded seed sample was taken into a pre-weighed crucible (W1) and burnt entirely by heating at a low flame and then left for ignition in a muffle furnace at 600 °C for about 3-5 h. This crucible was cooled down in a desiccator and weighed (W2). The ash content was calculated by using following formula:

$$\text{Ash content (\%)} = \frac{(W_2 - W_1)}{Weight\ of\ the\ Sample} \times 100$$

Total carbohydrates

The total carbohydrate content for each sample was calculated by difference using following formula [12]:

Total carbohydrate (%) = 100 - (moisture% + crude fat% + crude fibre% + total proteins% + ash%)

Total phenols

For estimation of total phenol content, *Folins ciocalteau* phenol reagent method was used and it was measured quantitatively at 650 nm in spectrophotometer. The amount of phenol was calculated by plotting absorbance of sample on standard graph prepared by using standard phenol solution (50 µg/ml) [15].

Enzymatic activity

Peroxidase (POD)

From naturally infected and healthy (control) seeds of barley samples (incubated for 7 days using moistened blotter method), 300 mg was taken and ground in 3 ml solution of 0.1M of phosphate buffer (pH 6.5). The supernatant was taken after centrifugation at 10000 rpm for about 15 min maintained at 4 °C and enzyme assay was performed in it and the OD was taken at 430 nm [16-17].

Polyphenol oxidase (PPO)

Some seeds of barley were incubated for 7 days using moistened blotter method and 3 ml (0.1 M) phosphate buffer pH (6.0) was used to crush it. Centrifugation was applied to the homogenate same as for POD. Enzyme assay was done in the supernatant and OD was taken at 495 nm [16-17].

Catalase (CAT)

Enzyme catalase was estimated by the procedure mentioned by Mahadevan and Sridhar [18]. The reaction mixture containing 2.7 ml (0.1 M) phosphate buffer (pH 6.5) stored at 4 °C was used. The enzyme was measured by spectrophotometer at 230 nm at 15 s interval for 2 min. Statistical analysis: Statistical analysis was performed for each factor and exposed to independent sample t-test. The mean values were matched for importance.

RESULTS AND DISCUSSION

All the barley seed samples displayed variability in the extent of infection with significant changes in biochemical constituents in the samples collected from fields. Different percentage of infection observed for barley seed samples. Ac. nos. Hv-17-21, Hv-17-53, Hv-17-71, Hv-17-97 was 53.50%, 49.50%, 66.75%, and 61.25% respectively. A total of 19 isolates were identified as *Pseudomonas syringae* strain in primary screening on agar medium and 11 isolates were confirmed as Pss based on biochemical tests and host sensitivity confirmation by pathogenicity reactions on barley and other host plants.

In three seed samples Hv-17-21, Hv-17-71 and Hv-17-97 moisture content was lower in comparison to the healthy samples tested whereas in Hv-17-53 the moisture content was increased (Table 1). It was found in the present study that infected seeds were shrivelled and less viable. The decrease in the fat content in seed samples of barley studied due to *P. syrinage* pv. *syringae* (Table 1), may be induced due to lipolytic activity of the pathogen. Similar investigations have been reported by various researchers in plants infected with *P. syringae* pv. *syringae* in sunflower [19] and *R. solanacearum*in cluster bean [20]. It is also supported by the fact that during invasion of bacterial and other microbial pathogens plants start a defence signaling cascade which includes fatty acid derivatives to produce protective biopolymers such as proteins and fats [21-22].

In the present findings, total proteins have increased in three diseased seed samples as compared to healthy (Table 1) with the fact that is stated earlier in individual studies on

different plants which produce PR proteins to overcome the bacterial load. To escape infection, plant cells exploit mechanisms in which they alter CWI (cell wall integrity) and on the other hand pathogens produce effector proteins which eventually increases total protein content of the cell [23]. The crude fibre content of all the seed samples infected with Pss was significantly low in comparison to the control samples (Table 1). Ash content was found higher in the infected samples of barley as compared to healthy samples in three seed samples (Table 1). It is an important fact that the degradation of host cell wall during plant–pathogen interactions have a major role for establishment of virulence by some bacteria and fungi [24]. Additionally, glucanases and lytic polysaccharide monooxygenases are known to digest some amount of the crystalline cellulose [25]. This fact supports the results obtained for decreased crude fiber in the present study. Similar pattern of results was also observed by Lahouar *et al.* [26] in barley seeds compromised with biotic stress. The results obtained for ash and crude fibre are in accordance with the fact that cellulolytic activities and toxic effects induced by pathogens as well as aging of plant tissues due to biochemical injury has been identified as a reason to cause a proportional change in ash content of various plants [27]. This might have occurred due to cellulolytic activity of cellulases and hemicellulases which generally happens after fungal and bacterial infections [28].

In the present study, the carbohydrate content was variably lower in three infected seed samples of barley (Table 1). The results of the study are explained by the fact that some pathogens enhance sugar metabolism that lead to a significant adjustment in the synthesis of carbohydrates in the cells diseased with pathogen. This may also contribute to the declined in cytoplasmic carbohydrates [29-30]. It is also noticed that every pathogen has differential regulation and its own specialized mechanism to take over host carbohydrates. It is also reported earlier that pathogen infection increases carbohydrate assimilation and their conversion in amino acids which are used for protein synthesis [31]. It is noticed in the present study also that carbohydrate content is reduced and protein is increased substantially.

Phenolics are naturally occurring compounds of plants; well characterized for their antimicrobial properties and famous for their role as precursors to structural macromolecules such as lignin [32]. Accumulation of the phenolic compounds in the infected seed samples of barley was found significantly higher in comparison to healthy control (Table 1). In many of the plants increase in phenolic compounds due to pathogen infection has been studied; as in tomato seeds infected with blight [33]. Researchers suggested that these have a significant role as signaling molecules which activate genes of defence mechanism in plants [34-35].

Means of replicates of moisture, crude fat, crude fibre, crude protein and ash are presented in (Fig 1). Similarly, means of replicates of total carbohydrate and phenol are presented in (Fig 2-3), respectively.

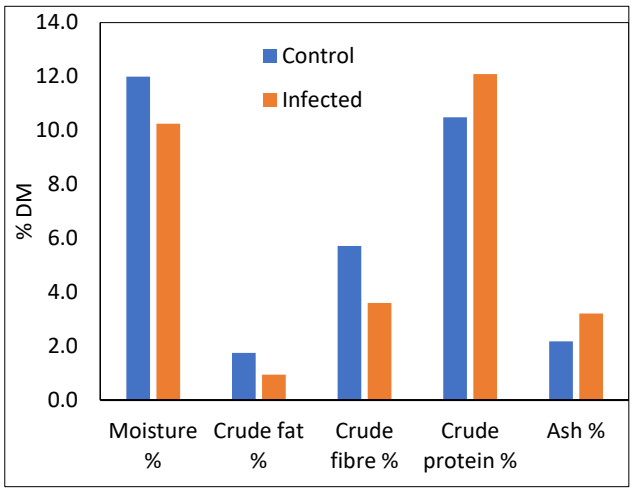

Fig 1 Changes in biochemical constituents of barley seeds naturally infected with Pss

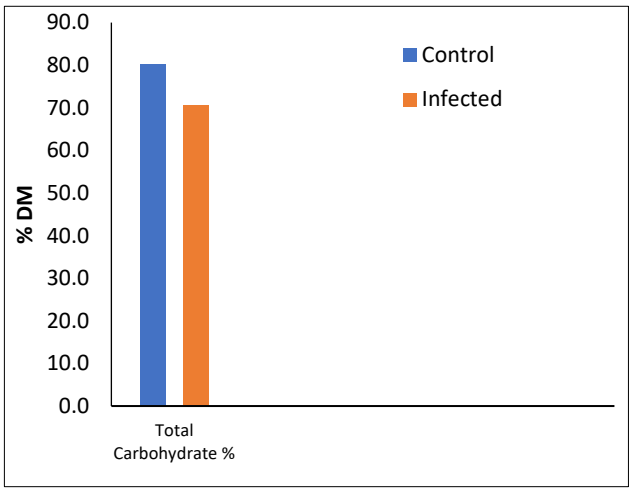

Fig 2 Changes in total carbohydrate content of barley seeds naturally infected with Pss

Table 1 Changes in biochemical constituents of barley seeds due to *Pseudomonas syringae* pv. *syringae* infection

Test samples	Replicates of test samples	Moisture %	Crude fat %	Crude fibre %	Crude protein %	Ash %	Total Carbohydrate %	Phenol %
Check (Healthy seed samples)								
Ac. No. Hv-17-21	R1	10.28	2.72	5.82	11.86	2.46	78.82	2.11
	R2	11.4	1.84	5.86	10.52	2.62	81.46	2.02
	R3	10.56	2.04	5.35	12.21	2.51	79.39	2.64
Ac. No. Hv-17-53	R1	11.68	1.93	5.4	9.67	1.96	77.38	1.86
	R2	10.39	1.65	5.3	10.84	2.06	78.36	1.44
	R3	11.54	1.21	5.2	10.57	2.38	79.61	1.67
Ac. No. Hv-17-71	R1	11.47	2.05	6.05	8.12	2.17	79.37	1.27
	R2	12.86	1.89	5.94	9.84	2.11	80.16	1.93
	R3	12.05	1.97	5.98	9.28	2.13	81.27	2.41
Ac. No. Hv-17-97	R1	14.11	1.35	5.95	10.99	1.99	82.37	1.94
	R2	13.85	1.18	5.87	11.53	1.91	80.83	2.68
	R3	13.69	1.21	5.92	10.48	1.83	81.68	2.38
Mean	-	11.990	1.753	5.720	10.492	2.177	80.058	2.029
SD	-	1.350	0.457	0.309	1.147	0.256	1.499	0.444
SEM		0.389	0.132	0.089	0.331	0.073	0.432	0.128

		Infected seed samples						
Ac. No. Hv-17-09	R1	9.04	1.37	3.93	13.26	2.68	67.31	4.02
	R2	8.52	0.93	3.98	12.68	2.73	70.25	3.96
	R3	8.91	1.02	3.62	12.92	2.82	68.11	4.11
Ac. No. Hv-17-35	R1	13.47	0.99	3.75	13.78	2.98	85.89	4.07
	R2	14.48	0.83	3.65	14.39	2.59	85.71	3.28
	R3	14.45	1.06	3.71	12.93	2.75	84.63	3.19
Ac. No. Hv-17-48	R1	9.38	1.01	3.85	7.92	3.75	65.88	3.17
	R2	8.49	0.92	3.11	8.98	3.76	61.69	3.46
	R3	7.11	1.12	3.19	8.79	3.89	62.43	3.61
Ac. No. Hv-17-83	R1	9.85	0.72	3.51	13.57	3.98	65.55	3.95
	R2	9.29	0.55	3.45	13.83	2.82	64.85	4.01
	R3	10.04	0.75	3.53	12.04	3.77	65.01	4.69
Mean		10.252	0.939	3.606	12.090	3.210	70.609	3.793
SD		2.467	0.212	0.269	2.224	0.558	9.216	0.456
SEM		0.712	0.061	0.077	0.642	0.161	2.660	0.131
Difference (2-1)		1.737	0.814	2.113	-1.598	-1.032	9.449	-1.764
SED		0.812	0.145	0.118	0.722	0.177	2.695	0.183
t Value		2.140	5.594	17.839	-2.212	-5.823	3.506	-9.595
Df		22	22	22	22	22	22	22
P		0.044**	0.000*	0.000*	0.038**	0.000*	0.002**	0.000*

SD – Standard deviation; SEM – Standard error of Mean; SED – Standard error of Difference;
*Significant at 1% (P ≤ 0.01); **Significant at 5% (P ≤ 0.05)

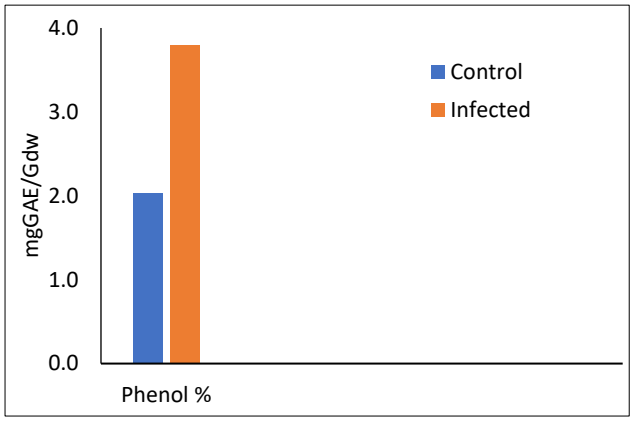

Fig 3 Changes in phenol content of barley seeds naturally infected with Pss

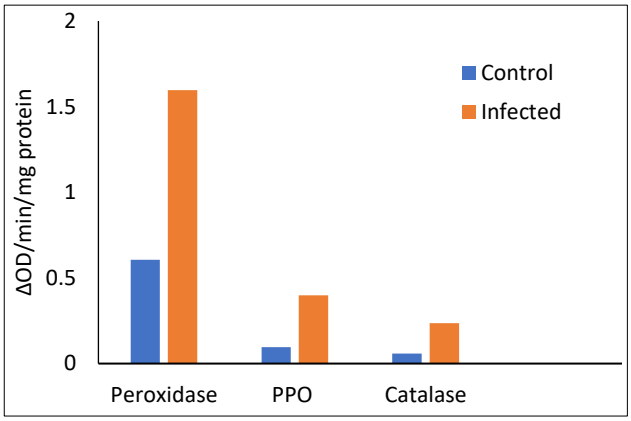

Fig 4 Changes in enzymatic activity of barley seeds naturally infected with Pss

Defence-related enzymes are part of effective protective system of plants against pathogen attack. The most important defensive molecules determined to protect plants against biotic stress are catalase (CAT), peroxidase (POD) and polyphenol oxidase (PPO) [36]. POD and PPO are the key antioxidant enzymes which play an important role in preventing membrane lipid peroxidation as well as oxidative stress of cells when plant encounters a deadly pathogen [37]. In the present study, the infected seed samples displayed considerably greater peroxidases, polyphenyloxidases and catalases (Table 2) in all of the infected samples explored. For coffee also, a rise in activities of POX, PAL and SOD was reported along with accretion of phenolics in the infected cells [38]. Many other similar studies have been reported on banana, rice, and grape for response of these plants against pathogens in relation to interference of oxidative enzymes in defence mechanism provoked [39-41].

The increased PPO, POD and CAT activities are explained by the fact that these have potential role in first line of defense of plant which leads to modifications in secondary metabolism ultimately resulting in eradication of the pathogen [42]. The present findings are in accordance with the fact that Polyphenol oxidases, peroxidases, and catalases are known to have shared properties related to seed defence. These enzymes are heat-stable and present in the pericarp of seeds [43-44].

Mean of replicates of peroxidase, PPO and catalase activity of barley seeds infected with Pss is presented in (Fig 4).

CONCLUSION

The consequences of a pathogen infection in a plant are assessed by its ability to effectively invade the host cells, occupy the apoplastic space and start a cascade of biochemical and physiological changes in the entire system of plant which evokes plant's defence system also. The initiation and the manifestation of all defence molecules in response to any pathogen invasion depend on every plant pathogen interaction type. Biochemical studies on these defensive and anti-oxidative enzymes as well as other biochemical components can also be applied as markers to trace early infection. Our findings have indicated the fact that the phenolic compounds, protein, POD, CAT, and PPO are the molecules that play a dynamic role in barley seed's defence mechanism to control *P. syringae* pv. *syrinage*. Thus, it can be concluded that there is a correlation between changes of these biochemicals and disease development in barley seeds when they are encountered by Pss. It is expected that this finding will help in better understanding of nature and mechanisms of pathogenic effects of Pss on seed quality of barley and also in selection of resistant varieties.

Table 2 Changes in enzymatic activity in seeds of barley naturally infected with *Pseudomonas syringae* pv. *syringae*

Test samples	Replicates of test samples	Peroxidase	PPO	Catalase
		Check (Healthy seed samples)		
Ac. No. Hv-17-09	R1	0.729	0.027	0.246
	R2	1.018	0.015	0.102
	R3	0.537	0.036	0.075
Ac. No. Hv-17-35	R1	0.58	0.09	0.01
	R2	0.62	0.10	0.03
	R3	0.69	0.08	0.02
Ac. No. Hv-17-48	R1	0.56	0.18	0.05
	R2	0.52	0.19	0.03
	R3	0.59	0.15	0.04
Ac. No. Hv-17-83	R1	0.51	0.07	0.033
	R2	0.47	0.16	0.029
	R3	0.45	0.06	0.028
Mean	-	0.606	0.096	0.057
SD	-	0.153	0.060	0.064
SEM		0.044	0.017	0.018
		Infected seed samples		
Ac. No. Hv-17-21	R1	1.712	0.071	0.593
	R2	1.964	0.083	0.294
	R3	1.231	0.086	0.357
Ac. No. Hv-17-53	R1	1.86	0.56	0.17
	R2	1.59	0.62	0.11
	R3	1.68	0.66	0.13
Ac. No. Hv-17-71	R1	1.94	0.44	0.17
	R2	1.99	0.52	0.27
	R3	1.72	0.58	0.19
Ac. No. Hv-17-97	R1	0.93	0.38	0.18
	R2	1.21	0.48	0.17
	R3	1.33	0.31	0.19
Mean		1.596	0.399	0.235
SD		0.345	0.215	0.132
SEM		0.099	0.062	0.038
Difference (2-1)		-0.990	-0.302	-0.177
SED		0.109	0.064	0.042
t Value		-9.078	-4.679	-4.167
Df		22	22	22
P		0.000*	0.000*	0.000*

SD – Standard deviation; SEM – Standard error of Mean; SED – Standard error of Difference; *Significant at 1% (P ≤ 0.01)

LITERATURE CITED

1. Ghafoor K. 2015. Optimized extraction of phenolic compounds from barley (*Hordeum vulgare* L.) seed and their radical scavenging properties. *Jr. Food Proc. and Pres.* 39(6): 793-799.
2. Liu Q, Yao H. 2007. Antioxidant activities of barley seeds extracts, *Food Chemistry* 102(3): 732-737.
3. Holtekjolen AK, Baevere AB, Rodbotten M, Berg H, Knutsen SH. 2008. Antioxidant properties and sensory profiles of breads containing barley flour. *Food Chemistry* 110(2): 414-421.
4. Madhujith T, Shahidi F. 2009. Antioxidant potential of barley as affected by alkaline hydrolysis and release of insoluble-bound phenolics. *Food Chemistry* 117(4): 615-620.
5. Gangwar OP, Bhardwaj SC, Singh GP, Prasad P, Kumar S. 2018. Barley disease and their management: An Indian perspective. *Wheat and Barley Research* 10(3): 138-50.
6. Yoshioka R, Uematsu H, Takikawa Y, Hiroshi K, Yasuhiro I. 2020. PCR detection of *Pseudomonas syringae* pv. *syringae*, the causal agent of bacterial black node in barley and wheat, using newly designed primer sets. *Jr. Gen. Plant Pathology* 86: 387-392.
7. Verma RPS, Kumar V, Sarkar B, Kharub AS, Kumar D, Selvakumar R, Malik R, Sharma I. 2012. Barley cultivars released in India: Names, parentages, origins and adaptations. *Res. Bulletin No. 29, DWR, Karnal, India*. pp 26.
8. Staskawicz BJ, Ausubel FM, Baker BJ, Ellis JG, Jones JD. 1995. Molecular genetics of plant disease resistance. *Science* 268(5211): 661-667.
9. Percival GC. 2001. Induction of systemic acquired disease resistance in plants: Potential implications for disease management in urban forestry. *Jr. of Arboriculture* 27(4): 181-192.
10. Song F, Goodman RM. 2001. Molecular biology of disease resistance in rice. *Physiological and Molecular Plant Pathology* 59: 1-11.
11. AOAC. 1995. *Official Methods of Analysis*. (Eds.) Hornitz W. Association of official analytical chemists. Washington DC, 1899.
12. Raghuramulu N, Madhavan NK, Kalyansundaram S. 2003. *A Manual of Laboratory Techniques*. 2nd Edition. NIN Press, National Ins. of Nutrition, Indian Council of Medical Research, Hyderabad, India. pp 424.

13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin-Phenol reagents. *Jr. Biol. Chemistry* 193: 265-275.
14. Cutler P. 2004. Protein purification protocols. 2nd Edition. Humana Press Inc. 999. Riverview Drive, Suite 208 Totowa, New Jersey 07512: 484.
15. Sawhney SK, Singh R. 2000. *Introductory Practical Biochemistry*. Narosa Publishing House, New Delhi. pp 452.
16. Malik CP, Singh MB. 1980. *Plant Enzymology and Histo-enzymology: A Text Manual*. Kalyani Publishers, New Delhi (1980).
17. Mahatma MK, Bhatnagar RP, Rawal P. 2008. Changes in enzymes and proline levels in leaves of downy mildew resistant and susceptible pearl millet genotypes. *Jr. Mycol. Plant Pathology* 38(2): 277-281.
18. Mahadevan A, Sridhar R. 1986. *Methods in Physiological Plant Pathology*. 3rd Edition. Sivakami Publication, Madras. India. pp 183.
19. Godika S, Agarwal K, Singh T. 2000. Histopathological and biochemical changes in seeds of sunflower infected with *Pseudomonas syringae*. *In*: Proceeding of *Indian Phytopathol.*, International conference. Integrated plant Disease Management of Sustainable Agriculture. Vol. II. New Delhi: IARI; 2000 1131-32.
20. Jain R, Agrawal K. 2011. Incidence and seeds transmission of *Xanthomonas axonopodis pv. cymopsidids* in cluster bean. *Jr. of Agri. Tech.* 7(1): 197-205.
21. Pinot F, Beisson F. 2011. Cytochrome P450 metabolizing fatty acids in plants: characterization and physiological roles. *The FEBS Journal* 278: 195-205.
22. Singh A, Sarma BK, Upadhyay RS, Singh HB. 2013. Compatible rhizospheres microbes mediated alleviation of biotic stress in chickpea through enhanced antioxidant and phenylpropanoid activities. *Microbiol. Research* 168: 33-40.
23. Bellincampi D, Cervone F, Lionetti V. 2014. Plant cell wall dynamics and wall-related susceptibility in plant–pathogen interactions. *Front. Plant Sci.* 5: 228.
24. Lorrai R, Ferrari S. 2021. Host cell wall damage during pathogen infection: Mechanisms of perception and role in plant-pathogen interactions. *Plants* 10(2): 399.
25. Villares A, Moreau C, Bennati-Granier C, Garajova S, Foucat L, Falourd X, Saake B, Berrin J, Cathala B. 2017. Lytic polysaccharide monooxygenases disrupt the cellulose fibers structure. *Sci. Reporter* 7: 40262.
26. Lahouar L, Amira EA, Ghrairi F, Chahdoura H, Salem BH, Felah ME, Achour L. 2014. Phytochemical content and antioxidant properties of diverse varieties of whole barley (*Hordeum vulgare* L.) grown in Tunisia. *Food Chemistry* 145: 578-583.
27. Astegar ZR, Sedghi M, Khomari S. 2011. Effects of accelerated aging on soybean seed germination indexes at laboratory conditions. *Not. Sci. Biol.* 3: 126-129.
28. Kaur B, Purkayastha S. Dilbaghi N, Chaudhury A. 2005. Characterization of *Xanthomonas axonopodis* pv. *cyamopsidis*, the bacterial blight pathogen of cluster bean using PCR-based molecular markers. *Journal of Phytopathology* 153(7/8): 470-479.
29. Morkunas I, Stobiecki M, Marczak Ł, Stachowiak J, Narożna D, Remlein-Starosta D. 2010. Changes in carbohydrate and isoflavonoid metabolism in yellow lupine in response to infection by *Fusarium oxysporum* during the stages of seed germination and early seedling growth. *Physiol. Mol. Pl. Pathology* 75(1/2): 46-55.
30. Kawakami A, Yoshida M. 2012. Graminan breakdown by fructan exohydrolase induced in winter wheat inoculated with snow mold. *Plant Physiology* 169(3): 294-302.
31. Sinha A, Srivastava M. 2010. Biochemical changes in mungbean plants infected by *Mungbean yellow mosaic virus*. *Int. Jr. Virology*, 6(3): 150-157.
32. Hung PV. 2014. Phenolic compounds of cereals and their antioxidant capacity. *Crit. Rev. in Food Sci. and Nutri.* 56(1): 25-35.
33. Anupama N, Murali M, Sudisha J, Amruthesh KN. 2014. Crude oligosaccharides from *Alternaria solani* with *Bacillus subtilis* enhance defense activity and induce resistance against early blight disease of tomato. *Asian Jr. Sci. and Tech.* 5(7): 412-416.
34. Jayaraj J, Bhuvaneswari R, Rabindran R, Muthukrishnan S, Velazhahan R. 2010. Oxalic acid-induced resistance to *Rhizoctonia solani* in rice is associated with induction of phenolics, peroxidase and pathogenesis-related proteins. *Jr. Plant Interact.* 5(2): 147-157.
35. Singh HP, Kaur S, Batish DR, Kohli RK. 2014. Ferulic acid impairs rhizogenesis and root growth, and alters associated biochemical changes in mungbean (*Vigna radiata*) hypocotyls. *Jr. Plant Interact.* 9: 267-274.
36. Han YQ, Wen JH, Peng ZP, Zhang DY, Hou ML. 2018. Effects of silicon amendment on the occurrence of rice insect pests and diseases in a field test. *Journal of Integrative Agriculture* 17(10): 2172-2181.
37. Foyer C, Noctor G. 2003. Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiol. Plant.* 119(3): 355-364.
38. Possa KF, Silva JAG, Resende MLV, Tenente R, Pinheiro C, Chaves I, Planchon S, Monteiro A CA, Renaut J, Carvalho MAF, Ricardo CP, Guerra-Guimarães L. 2020. Primary metabolism is distinctly modulated by plant resistance inducers in *Coffea arabica* leaves infected by *Hemileia vastatrix*. *Front. Pl. Sci.* 11: 309.
39. Sun D, Lu X, Hu Y, Li W, Hong K, Mo Y, Cahill DM, Xie J. 2013. Methyl jasmonate induced defense responses increase resistance to *Fusarium oxysporum* f. sp. *cubense* race 4 in banana. *Sci. Hortic.* 164: 484-491.
40. Qin X, Xiao H, Xue C, Yu Z, Yang R, Cai Z, Si L. 2015. Biocontrol of gray mold in grapes with the yeast *Hanseniaspora uvarum* alone and in combination with salicylic acid or sodium bicarbonate. *Postharvest Biol. Technology* 100: 160-167.
41. Han Y, Li P, Gong S, Lang Y, Wen L, Hou M. 2016. Defense responses in rice induced by silicon amendment against infestation by the leaf folder *Cnaphalocrocis medinalis*. *PLoS One* 11: e0153918.
42. Guerra-Guimarães L, Tenente R, Pinheiro C, Chaves I, Silva MC, Cardoso FMH, Planchon S, Barros DR, Renaut J, Ricardo CP. 2015. Proteomic analysis of apoplastic fluid of *Coffea arabica* leaves highlights novel biomarkers for resistance against *Hemileia vastatrix*. *Front. Plant Science* 6: 478.
43. Almagro L, Gomez RLV, Belchi-Navarro S, Bru R, Ros BA, Pedreno MA. 2009. Class III peroxidases in plant defence reactions. *Jr. Exp. Botany* 60: 377-90.
44. Jerkovic A, Kriegel AM, Bradner JR, Atwell BJ, Roberts TH, Willows RD. 2010. Strategic distribution of protective proteins within bran layers of wheat protects the nutrient-rich endosperm. *Plant Physiology* 152(3): 1459-1470.