© 2023, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences
Volume 14; Issue 02 (Mar-Apr 2023); pp 397–401

Open Access

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Full Length Research Article

Evaluation of Impact of Infection with *Pseudomonas syringae* pv. *syringae* on Biochemical Constituents and Enzymatic Activity in Pearl Millet [*Pennisetum glaucum* (L.) R. Br.] Seeds

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Abstract

It is crucial to provide access to affordable indigenous food sources as alternative to traditional food in order to combat under nutrition and improve food security. Pearl millet crop suffers from many serious diseases of which bacterial brown spot disease caused by *Pseudomonas syringae* pv. *syringae* (*Pss*) is an important disease in pearl millet crop. The current study, the biochemical as well as physiological alterations brought about by naturally occurring *Pss* infection in seed samples of pearl millet collected through various areas of Marwar region in Rajasthan were evaluated. Diseased seed samples had considerably higher protein value compared with healthy check samples but lower values of moisture, crude fiber, crude fat, total carbohydrate and total soluble sugar content. The seed samples infected with *Pss* showed a significantly rise in total phenols and in defense enzymes viz catalase, polyphenoloxidase and peroxidase. The findings show that *Pss*-host interaction disrupts host metabolism and several protective enzymes as well as secondary metabolites produce symptoms on pearl millet seeds. The results show the change in biochemical constitutes of seeds due to *Pss* infection in pearl millet.

***Key words*:** Food security, *Pseudomonas syringae* pv. *syringae* (*Pss*), Pearl millet, Marwar region, Defense enzymes

Asia and Africa continents are where pearl millet is most commonly farmed. It is one of the ancient edible crops in the world and is native to the Sahalian region of African continent [1]. Rajasthan, Maharashtra, Gujarat, Haryana and Uttar Pradesh are major producing states in India [2]. In Rajasthan, Barmer district is a largest sowing area but Alwar district is major producing area of pearl millet. Depending on the location and people's dietary preferences, it is consumed in numerous cuisine forms in India. In the recent years, interest of people are being increased for using pearl millet due to absences of gluten [3] presence of minerals (Ca, Fe, Zn, Mg), vitamins C & B-complex and low glycemic index [4] useful phenolic acid (trans-cinnamic, gentisic, caffeic, p-coumaric) [5] and high level of fiber [6]. The seeds are remarkable source of natural antioxidants for preventing contagious as well as non-contagious diseases and supporting good health due to the abundance of bioactive chemicals and phenols [7-9].

There are many pathogens that affects pearl millet but *Pseudomonas syringae* pv. *syringae* (*Pss*) causing bacterial brown spot or brown spot disease is one of the most significant one. The oozing, yellow to brown rotting on margins of leave and necrotic symptoms on the plant are the major symptoms which lead to a significant yield loss [10-11]. Changes in the host plant's metabolism are common occurrence following pathogen invasion and these changes are linked to certain important biochemical abnormalities in tissues of host. Additionally, plants produce enzyme like pathogen related (PR) protein and derivatives of phenolic substances in an effort to defend their cells against the pathogens by initiating a series of defense response mechanism [12]. It is clear from the bio-physiological changes in the host that the changed host metabolism caused by pathogenic infection can be detected including defense-enhancing enzyme production viz. catalase, peroxidase, polyphenol oxidase and other secondary metabolites [13-14].

It becomes necessary to quantify the ash, moisture, fiber, fat, protein, carbohydrate and enzyme levels in order to have proper incite of the interaction between host and the pathogen. Therefore, an effort has been undertaken to research the modifications in biochemical constituents of healthy and diseased pearl millet seeds having *Pss* infection.

Received: 27 Dec 2022; Revised accepted: 18 Feb 2023; Published online: 16 Mar 2023

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Citation: Khorwal S, Agrawal, Sharma S, Agrawal M. 2023. Evaluation of impact of infection with *Pseudomonas syringae* pv. *syringae* on biochemical constituents and enzymatic activity in pearl millet [*Pennisetum glaucum* (L.) R. Br.] seeds. *Res. Jr. Agril Sci*. 14(2): 397-401.

MATERIALS AND METHODS

Isolation and categorization

Seed samples of pearl millet collected from Jodhpur, Barmer, Jaisalmer, Jalore, Nagaur and Pali districts of Rajasthan, India were categorized into healthy (accession numbers PM-13, PM-44 and PM-92) and infected (accession numbers PM-17, PM-32 and PM-67) on the basis of exomorphic symptoms such as shriveling, rotting and browning on seed coat on investigation of dry seeds. After being sterilized with solution of 2% of sodium hypo chlorite, the samples were placed directly on nutrient agar (NA) medium and King's B agar medium, kept in an incubator at 28±2 °C for 2 days. Group of bacterial cells exhibiting *Pss*-specific characteristics were purified on NA by using the technique of re-streaking. The pure colonies were subjected to biochemical assays and confirmed by host-pathogen test on pearl millet and other plants.

Biochemical changes

Three seed samples each of healthy (check) and diseased pearl millet was used for estimation of biochemical changes.

The standard methods were used [15-18] for estimation of the content. 10 g powder of seeds of pearl millet was taken and dried in hot air oven at 110 °C. Crude fat estimation action was done in moisture free 2 g powder of seeds by Soxhlet apparatus through solvent extraction method. Crude fiber was estimated by using 2 g moisture and fat free powder of seeds using alkali wash method. Kjeldahl method was used for estimation of nitrogen content present in dry seed powder.

Ash was estimated by dry ashing method using muffle furnane. The total content of carbohydrates was calculated by difference = 100 – (ash% + crude fat% + moisture% + total protein% + crude fiber%) [2] [16] [19].

Total phenol content estimation by Folin's Ciocalteau phenol reagent method was done. The procedure based on the production of a blue color as a result of the interaction between phenol and oxidizing agent phosphomolybdate. In a spectrophotometer, the reaction's absorbance of blue color at 670 nm was measured. Similarly, a blank was prepared by using of distilled water. A standard graph and solution of standard phenol (50µg/ml) were used to calculate the quantity of total phenol content [16] [20-21].

Enzymatic activity

Each sample of 300 mg of incubated seeds using standard blotter method were milled in 3 ml of 0.1 M sodium phosphate buffer at P^H 6.5. The supernatant from the centrifugation of homogenate for 15 min at 10000 rpm for 4 °C was utilized for an enzyme assay. The solution of 0.2 ml hydrogen peroxide (0.2M) was added into cuvette, measurement of the sample taken at 470 nm in spectrophotometer. The initial absorbance A_0 was measured and then again at intervals of 30 sec. to record activity of peroxidase [22-23].

Similarly, the change caused by the oxidized catechol was recorded at 420 nm every 30 sec. for five minutes to estimate polyphenol oxidase activity [22] [24-25].

A method described by [26] was used to assess the enzyme catalase. The solution of 0.1 M of 2.7 ml phosphate buffer at pH 6.5 and stored at 4 °C. The enzyme was measured by using of spectrophotometer at 250 nm for 2 min on every 15 second interval.

RESULTS AND DISSCUSION

The samples taken from diverse fields of the Marwar region Rajasthan showed that the pearl millet seeds had varying degrees of infection and showed substantial alteration in their biochemical constituents. In the current investigation, the three selected infected seed samples had lower moisture contents (4.90) than the healthy (check) samples (6.41) (Table 1). The three pearl millet seed samples that were affected with the disease had a greater protein content in comparison to check healthy samples (Table 1).

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

			Biochemical constituents							
S. No.	Sample accession No.	Replicates of samples	Moisture (%)	Crude fiber (%)	Crude fat (%)	Crude protein (%)	Ash (%)	Total carbohydrate (%)	Total soluble sugar (%)	Phenol (%)
					Check samples					
(i)	PM-13	R_1	6.54	2.97	1.73	7.71	1.08	61.42	48.25	1.86
		R_2	6.29	2.66	2.19	7.54	1.21	62.23	49.49	2.71
		R_3	5.83	3.22	1.48	7.87	1.34	63.75	51.57	2.68
(ii)	PM-44	R_1	4.69	3.96	2.29	6.31	1.17	59.32	43.26	1.82
		R_2	4.91	4.17	2.47	6.12	1.34	57.21	45.97	1.64
		R_3	5.11	3.28	1.63	5.94	0.91	58.93	45.89	1.95
(iii)	PM-92	R_1	7.75	4.57	2.70	6.67	1.91	67.12	53.41	1.79
		R_2	8.49	4.92	1.95	6.54	2.13	65.44	52.37	2.14
		R_3	8.12	5.13	2.61	7.29	1.61	65.31	54.19	1.63
	Mean		6.41	3.88	2.12	6.89	1.41	62.30	49.38	2.02
	SD		1.428	0.889	0.442	0.726	0.399	3.376	3.802	0.410
	SEM		0.476	0.296	0.147	0.242	0.133	1.125	1.267	0.137
					Infected seed samples					
(i)	PM-17	R_1	4.41	1.62	1.15	10.25	1.72	47.11	38.83	2.92
		R_2	4.74	1.85	1.21	9.87	2.29	49.93	37.95	3.52
		R_3	5.06	2.32	1.49	10.32	1.93	48.84	38.59	3.24
(ii)	PM-32	R_1	6.29	1.96	1.29	6.91	1.19	52.62	40.86	2.42
		R_2	5.31	2.32	0.84	7.25	1.66	53.41	42.11	1.91
		R_3	5.64	1.83	0.97	7.47	1.49	52.96	41.34	2.72
(iii)	PM-67	R_1	4.25	3.17	1.50	8.67	2.31	46.12	34.71	2.79
		R_2	3.89	2.64	1.76	8.32	1.27	44.33	35.39	3.34
		R_3	4.51	2.79	2.03	9.71	2.36	44.41	34.85	2.83

Mean	4.90	2.28	1.36	8.75	1.80	48.86	38.29	2.85
SD	0.753	0.514	0.377	1.340	0.448	3.600	2.830	0.492
SEM	0.251	0.171	0.126	0.447	0.149	1.200	0.943	0.164
Difference (2-1)	-1.51	-1.60	-0.76	1.86	0.39	-13.44	-11.09	0.83
SEd	0.538	0.342	0.194	0.508	0.200	1.645	1.580	0.213
t- Value	2.815	4.669	3.905	-3.671	-1.955	8.172	7.017	-3.888
DF	16	16	16	16	16	16	16	16
P	0.012	0.000	0.001	0.002	0.048	0.000	0.000	0.001

SD- Standard deviation; **SEM-** Standard error of mean; **SEd-** Standard error of difference; **DF-** degree of freedom

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

S. No.	Sample accession No.	Replication of samples	Enzymatic activity: Peroxidase	Polyphenoloxidase	Catalase
		Check (Healthy seed samples)			
1	PM-13	R_1	0.776	0.043	0.328
		R_2	0.947	0.021	0.295
		R_3	0.832	0.034	0.341
2	PM-44	R_1	0.610	0.031	0.457
		R_2	0.665	0.059	0.463
		R_3	0.583	0.026	0.449
3	PM-92	R_1	1.198	0.033	0.211
		R_2	1.251	0.051	0.194
		R_3	1.173	0.064	0.236
	Mean		0.893	0.040	0.330
	SD		0.262	0.015	0.107
	SEM		0.087	0.005	0.036
		Sample naturally infected			
1	PM-17	R_1	1.315	0.469	1.248
		R_2	1.617	0.527	1.574
		R_3	1.421	0.374	1.423
2	PM-32	R_1	2.983	0.722	1.057
		R_2	2.861	0.569	1.113
		R_3	3.103	0.647	1.147
3	PM-67	R_1	2.121	0.313	2.031
		R_2	1.703	0.426	1.989
		R_3	1.896	0.388	1.763
	Mean		2.113	0.493	1.483
	SD		0.696	0.135	0.376
	SEM		0.232	0.045	0.125
	Difference (2-1)		1.221	0.453	1.152
	SED		0.248	0.045	0.130
	t- Value		-4.925	-9.998	-8.838
	Df		16	16	16
	P		0.000	0.000	0.000

SD- Standard deviation; **SEM**- Standard error of mean; **SEd**- Standard error of difference; **DF**- degree of freedom

The crude fat content of seed samples analyzed has decreased. This decrease may be due to the lipid molecule being break down by lypolytic activity. Similar research has been published on Pss infected sunflower plant [27] and in barley [28]. In the current investigation, the infected seed samples had lower crude fiber contents than the healthy (check) samples (Table 1). Similar kind of results observed in various microbe infected pearl millet [29] and in Ajwain seeds compromised with pathogenic attack [30]. The lower value of crude fiber may be due to decomposition of cellulose during cellulolytic activity [31]. The infected seed samples had lower in total carbohydrate and total soluble sugar contents than the healthy (check) samples (Table 1). The ability of the pathogen to regulate itself own unique method to consume host carbohydrates consequently, the ratio of sucrose to hexose may gradually decline [32]. Similar carbohydrate reduction outcomes were observed in peanut [33] and cowpea [34]. The decline in total soluble sugar in *S. orientale* L. have been also reported [35].

The infected seed samples had increase in total phenols than the healthy (check) samples (Table 1). In earlier investigation, a protein that help in defense mechanism also known as pathogen related (PR) protein synthesized the host by pearl millet **[**36]. PR proteins commonly known as first line defense immune response and increases in production during biotic stress condition [12]. It was also observed in bacterial infected *Capsicum annuum* [37]. Phenolics are naturally existing plant molecules that are widely known for their antibacterial activities and ubiquitination [38]. Increase in phenolic substances was found to be significantly greater in infected pearl millet seed samples than in check samples (Table 1). Numerous studied have examined the rise in phenolic components in crop caused by microbes' infection. Phenolic compounds secreted against *Pss* and pathovars of *Pseudomonas syringae* in infected plants such as in sweet cherry [39] and in *Arabidopsis thaliana* [40] have been reported.

An efficient plant defense system against pathogen attack is demonstrated in the current investigation by study on enzymatic activity. The infected seed samples of seeds showed noticeably higher catalase (1.483), peroxidases (2.113) and polyphenyl oxidases (0.493) as compared to healthy check samples (0.330), (0.893) and (0.040) respectively (Table 2). Catalase (CAT), polyphenol oxidase (PPO), peroxidase (POD), and chitinase are most significant defense enzymes found to defend plants from biotic stress [41]. Stressed plants go through a number of inherent equilibrium for initial stress step sensing, including signal cascade, expression of genes and metabolic alterations of defense chemicals to avoid unfavorable situations [42]. Additionally, increased phenylalanine lyase (PAL) and superoxide dismutase (SOD) activity in infected cells as well as the accumulation of phenolic contents in highly diseased tissue [43]. Similar findings have been also found in tobacco [44], soyabean [25] and rice [45] due to the involvement of oxidative enzymes in the defense system that caused these plants to react against pathogenic infection. The high CAT, PPO and POD activities can be attributed to their probable function in the first line of defense, which alters secondary metabolism and eventually lead in order to resist the pathogens [46]. These enzymes have been found located in the seed coat, endosperm and cotyledon of seeds [47-49].

CONCLUSION

The pathogen's capacity to successfully enter host cells to occupy the space of apoplastic region as well as cytosol is used evaluated the effects of infection on the host crop and emergence a chain reaction of bio-physiological changes in the crop's overall system that also activates its defenses. Each form of host-pathogen interaction type affects the start and appearance of every defensive molecule in response to each pathogen invasion. These protective and defensive biochemical investigations together with research on other biochemical constituents (crude fiber, fat, carbohydrate, total soluble sugars) can be used as indicator to track infections. The present research work has shown that the chemicals that actively participate in the defensive mechanism of pearl millet seeds against *Pss* are total protein, phenolic compounds, PPO, POD and CAT. Therefore, it can be concluded that when *Pss* comes into contact with seeds of pearl millet, there is correlation between variance in these biochemical constituents, enzymes and the development of disease.

Acknowledgment

Head, Department of Botany, University of Rajasthan, Jaipur and UGC-NFSC fellowship for the financial support.

LITERATURE CITED

1. Oumar I, Mariac C, Pham Jl, Vigouroux Y. 2008. Phylogeny and origin of pearl millet [*Pennisetum glaucum* (L.) R. Br.] as revealed by microsatellite loci. *Theor. Appl. Genetics* 117: 489-497.
2. Verma JP, Kewat RN, Singh P, Singh RP, Kumar A, Mishra KK. 2018. Biochemical composition of pearl millet germplasm collected from Eastern UP. *Jr. Pharmacogn. Phytochem.* 7(2): 1888-1889.
3. Bhardwaj R, Kaur M, Goyal M, Sohu RS, Satyvathi CT. 2014**.** Pearl millet for health and nutritional security. *Electron. Jr. Plant Breed.* 5(3): 573-576.
4. Nambiar VS, Dhaduk JJ, Sareen N, Sahu T, Desai R. 2011. Potential functional implication of pearl millet (*Pennisetum glaucum*) in health and diseases. *Jr. Appl. Pharm. Sci.* 1(10): 62-67
5. Slama A, Cherif A, Sakouhi F, Boukhchina S, Radhouane L. 2019. Fatty acids, phytochemical composition and antioxidant potential of pearl millet oil. *J. Consum. Prot. Food Saf.* 1-7.
6. Rustagi S, Khan S, Jain T. 2022. Comparative study of nutritional and functional characteristics of pearl millet, buckwheat, amaranth and unripe banana flours for gluten-free bakery products. *Food Res.* 6(3): 411-419.
7. Salar RK, Purewal, SS, 2017. Phenolic content, antioxidant potential and DNA damage protection of pearl millet (*Pennisetum glaucum*) cultivar of North Indian region. *Food Meas.* 11: 126-133.
8. Ibidapo O, Henshaw F, Shittu T, Afolabi W. 2019. Bioactive components of malted millet (*Pennisetum glaucum*), soy residue "okara" and wheat flour and their antioxidant properties. *Int. Jr. Food Prop.* 22(1): 1886-1898.
9. Mawouma S, Condurache NN, Turaturica M, Constantin OE, Croitora C, Rapeanu G. 2022. Chemical composition and antioxidant profile of sorghum [*Sorghum bicolor* (L.) Moench] and pearl millet [*Pennisetum glaucum* (L.) R. Br.] cultivated in the far-North region of Cameroon. *Food* 11: 1-13.
10. Frederickson DE, Monyo ES, King SB, Odvody GN, Claflin LE. 1999. Presumptive identification of *Pseudomonas syringae*, the cause of foliar leaf spots and streaks on pearl millet in Zimbabwe. *Jr. Phytopathology* 147: 701-706.
11. Singh R, Tomar A, Chandel SS, Dhwaj M, Chauhan S. 2021. Pearl millet (Bajara): common diseases. Diseases of Nationally Important Field Crops, Today, Tomorrow's Printers and Publishers, New Delhi, India. pp 189-200.
12. Gupta G, Paul S, Singh S, Pietramellara G, Pathan SI, Danish S, Jabborova D, Datta R, Jha PN. 2022. Exploring functional diversity and community structure of diazotrophic endophytic bacteria associated with *Pennisetum glaucum* growing under field in semi-arid region. *Land.* 11(991): 3-24.
13. Kumar A, Mali PC, Manga VK. 2010. Changes of some phenolic compounds and enzyme activities of infected pearl millet cause by *Sclerospora graminicola*. *Int. Jr. Plant Physiol. Biochem.* 2(1): 6-10.
14. Mormouzi I, Ali K, Harhar H, Gharby S, Sayah K, Madani NE, Cherrah Y, Faouzi MEA. 2018. Funtional composition, antibacterial and antioxidative properties of oil and phenolic from Morroccan *Pennnisetum glaucum* seeds. *Jr. Saudi Soc. Agric. Sci.* 17: 229-234.
15. AOAC. 1995. Official methods of analysis. (Ed. Hornitz, W.), Association of official analytical chemists. Washington DC. pp 1899.
16. Raghuramulu N, Madhavan NK, Kalyansundaram S. 2003. A manual of laboratory techniques 2nd Edition. NIN Press, National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India. pp 424.
17. Govindraj M, Selvi B, Rajarathinam S, Sumathi P. 2011. Genetic variability and heritability of grain yield component and grain mineral concentration in India's pearl millet (*Pennisetum glaucum* (L.) R. Br.) accessions. *African. Jr. Food Agric. Nutr. Dev.* 11(3): 4758-4771.
18. Chhabra N, Kaur A. 2017. Studied on physical and engineering characterstics of maize, pearl millet and soybean. *J. Pharmacogn. Phytochem.* 6(6): 1-5.

19. Yamme FW, Wills AJ. 1954. The estimation of carbohydrate in plant extract by anthron. *Jr. Biochem.* 5(7): 505-514.
20. Sadasivam S, Manickam A. 1992. Biochemical methods for agricultural sciences. Wiley Eastern Limited. Tamil Nadu Agricultural University, Coimbatore, India. pp 246.
21. Berwal MK, Chung LK, Goyal P, Kumar R. 2016. Variability in total phenol content of pearl millet genotype: inbreeds and designated B-lines. *Jr. Agric. Ecol.* 1: 41-49.
22. Mahatma MK, Bhatnagar R, Rawal P. 2008. Changes in enzymes and proline levels in leaves of downy mildew resistant and susceptible pearl millet genotypes. *Jr. Mycol. Plant Pathol.* 38(2): 277-281.
23. Manjula S, Murali M, Thrivini MC, Amruthesh KN. 2014. Enhanced defence enzyme activity by seed treatment with partially purified ns-LTPs in Pearl millet against downy mildew disease. *Jr. Chem. Biol. Res.* 31(2): 929-941.
24. Arun K, Mali PC, Manga VK. 2010. Changes of some phenolic compounds and enzymes activities on infected pearl millet caused by *Sclerospora graminicola*. *Int. Jr. Plant Physiol. Biochem.* 2(1): 6-10.
25. Yuldashov UX, Matniyazova HX, Azimov AA, Sherimbetov AG, Khamdullaev SHA, Rasulova OO, Shavkiev JSH. 2021. Dependence of peroxidase (PO) and polyphenol oxidase (PPO) enzyme activity on plant productivity under the influence of phytopathogen Micromycetes in soyabean plant [*G. max* (L.) Merr.]. *Plant cell biotechnol. Mol. Biol.* 22(33/34): 293-303.
26. Mahadevan A, Sridhar R. 1986. Methods in Physiological Plant Pathology. Sivakami Publication, Madras, India. pp 183.
27. 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. IARI, New Delhi. pp 1131-1132.
28. Sharma S, Agrawal K, Khorwal S, Agrawal M. 2023. Assessment of effect of *Pseudomonas syringae* pv. *syringae* infection on biochemical constituents and enzymatic activity of barley (*Hordeum vulgare* L.) seeds. *Res. Jr. Agril. Sci.* 14(1): 58-63.
29. Ojokoh AO, Fayemi OE, Ocloo FCK, Nwokolo FI. 2015. Effect of fermentation on proximate composition physiochemical and microbial characteristics of pearl millet [*Pennisetum glaucum* (L.) R. Br.] and Acha [*Digitaria exilis* (Kippist) Stapf] flour blends. *J. Agric. Biotech. Sustain. Dev.* 7(1): 1-8.
30. Agrawal M, Saxena S, Nagar P, Agrawal K. 2020. Impact on the seed quality of Ajwain [*Trachyspermum amii* (L.) Sprague] stored under different storage condition. *Indian Jr. Agric. Res.* 1: 1-7.
31. Villares A, Moureau C, Bennati-Granier C, Garajova S, Foucat L, Falourd X, Saake, B, Berrin J, Cathala B. 2017. Lytic polysaccharide monooxygenase disrupt the cellulosic fibers structure. *Sci. Reporter* 7: 40262.
32. Herbers K, Takahata Y, Melzer M, Mock HP, Hajirezaei M, Sonnewald U. 2000. Regulation of carbohydrate partition during the interaction of potato virus Y with tobacco. *Mol. Plant Pathol.* 1(1): 51-59.
33. Nakrani BR, Patel DB. 2018. Effect of different storage period of groundnut seeds of different varieties for biochemical changes due to *Aspergillus flavus*. *J. Pharmacogn. Phytochem.* 7(4): 2572-2575.
34. Amadioha AC, Nwazuo ED. 2017. Biochemical composition of seed and husk of cowpea [*Vigna unguiculata* (L.) Walp] infected by *Colletotrichum destructivum* O'Gara in storage. *Annu. Res. and Rev. Biol*. 31(1): 1-7.
35. Lubaina AS, Murugan K. 2013. Ultrastructural changes and oxidative stress markers in wild and cultivar *Sesamum orientale* L. following *Alternaria sesame* (Kawamura) Mohanty and Behera. Inoculation. *Indian Jr. Exp. Biol.* 51: 670-680.
36. Shailasree S, Ramachandra KK, Shetty SH. 2007. ß- amino butyric acid induced resistance in pearl millet to downy mildew is associated with accumulation of defence related protein. *Australasian Plant Pathol.* 36: 204-211.
37. Ramzan M, Sana S, Javaid N, Shah AA, Ejaz S, Malik WN, Fahad S. 2021. Mitigation of bacterial spot disease induced biotic stress in *Capsicum annuum* L. cultivar via antioxidant enzyme and isoforms. *Sci. Rep.* 11(2): 9445.
38. Lattanzio V, Lattanzio VM, Cardinali A. 2006. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. *Phytochem: Adv. Res.* 661(2): 23-67.
39. Mo YY, Geibel M, Bonsall RF, Gross DC. 1995. Ananlysis of Sweet Cherry (*Prunus avium* L.) leaves for plant signal that activate the syrB gene required for synthesis of phytotoxin, syringomycin by *Pseudomonas syringae* pv. *syringae*. *Plant Physiol.* 107: 603-612.
40. Soylu S. 2006. Accumulation of cell wall bounded phenolic compound and phytoalexin in *Arabidopsis thaliana* leaves following inoculation with pathovars of *Pseudomonas syringae*. *Plant Sci.* 170: 942-952.
41. Mondal A, Dutta S, Nandi S, Das S, Chaudhuri S. 2012. Changes in defense-related enzymes in rice responding to challenges by *Rhizocotinia solani*. *Arch. Phytopathol. Pflanzenschutz.* 45(15): 1840-1850.
42. Singh DP, Singh V, Gupta VS, Shukla R, Sharma BK, Patel JS. 2020. Microbial inoculation in rice regulates anti-oxidative reactions and defense related genes to mitigate drought stress. *Sci. Rep.* 10: 1-17.
43. Thripathi R, Vishunavat K, Tewari R, Kumar S, Tantiana M, Corato UD, Keswani C. 2022. Defense inducer mediated mitigation of bacterial canker in tomato through alternation in oxidative stress marker. *Microorganisms* 10: 1-19.
44. Polidoros AN, Mylona PV, Scandalios JG. 2001. Transgenic tobacco plants expressing the maize *Cat2* gene have altered catalase levels that affect plant-pathogen interactions and resistance to oxidative stress. *Transgenic Res.* 10: 555-569.
45. Elsharkawy MM, Sakran RM, Ahmad AA, Behiry SI, Abdelkhalek A, Hassan MM, Khedr AA. 2022. Induced systemic resistance against sheath blight in rice by different *Pseudomonas* isolates. *Life* 12(349): 1-13.
46. Biswas K, Adhikari S, Tarafdar A, Kumar R, Saha S, Ghosh P. 2020. Reactive oxygen species and antioxidant defense system in plant: role and cross talk under biotic stress. Sustainable Agriculture in Era of Climate Change. Springer Cham, Springer Nature Switzerland. pp 265-292.
47. Olcer H, Mecit B. 2012. Polyphenol oxidase activities in embryo and endosperm of wheat seeds during germination under boron stress. *Fresenuis Environ. Bulletin* 21(10): 2936-2941.
48. Mendoza CC, Hernandez-Figueroa KI, Sanchez E. 2018. Antioxidant capacity and phytonutrient content in seed coat and cotyledon of common beans (*Phaseolus vulgaris* L.) from various regions in Mexico. *Antioxidant* 8(5): 1-19.
49. Shi Z, Li Z, Yang X, He Y, Wang X. 2022. Production of endo-ß-mannose, superoxide, radicals, hydrogen peroxide and seed germination in the micropylar endosperm of pepper seed during germination is determined by endosperm itself. *Sci. Hortic.* 294: 1-8.