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RESEARCH ARTICLE

Post-infectional biochemical changes in maize leaves affected by banded leaf and sheath blight disease

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ABSTRACT: Total phenol content, peroxidase and polyphenol oxidase contents were estimated in maize germplasm affected by banded leaf and sheath blight disease. In comparison to healthy plants, the increase in total phenol content in diseased plants was maximum in extra early germplasm (362.3%) followed by inbreds (353.06%). Contrarily, peroxidase level increase was maximum in early maturity germplasm (87.5%) followed by inbred (86.0%). The relative increase in polyphenol oxidase was maximum in early maize germplasm (70.8%) followed by inbreds (63.7%). These are secretions which require trigger by means of pathogen attack, elicitor response and abiotic stresses. It appears that higher phenol, peroxidase and polyphenol oxidase activities in maize germplasm plays a vital role in inducing resistance against banded leaf and sheath blight (BLSB) disease in maize.

Key words: BLSB, polyphenol oxidases, *Rhizoctonia solani,* total phenol

The banded leaf and sheath blight (BLSB) disease on maize caused by *Rhizoctonia solani.* f. sp. *sasakii* is one of the important diseases of maize. It has rapidly gained economic importance in several parts of the world (Kumar and Singh, 2004). In India, the disease has been reported from states of Himachal Pradesh, Uttar Pradesh, Haryana, Punjab, Madhya Pradesh, Rajasthan, West Bengal, Meghalaya, Assam and Orissa (Rani *et al.,* 2013). It has a potential to inflict economic losses up to 100 per cent (Sharma *et al.,* 2002). This disease is more prevalent in humid weather with temperature around 28°C (Singh and Shahi, 2012). Its soil borne nature and scarcity of resistant germplasm make its management a challenge for pathologists across the globe.

Like many plant species, maize employs a diverse array of defenses that minimizes losses during pathogen attack. Besides pre-existing physical and chemical barriers, a variety of defense mechanisms are activated upon pathogen attack (Huang *et al*., 2008). Biochemical changes in many plant-pathogen interactions are accompanied by the rapid increase in phenolic compounds and related enzymes, often termed the hypersensitive response (Mondal *et al*., 2012). It is revealed from certain studies on biochemical changes during pathogenesis that certain defense biomolecules such as phenols, sugars as well as enzymes like peroxidase, polyphenols are formed to increase in levels so as to after resistance against the pathogen (Jiang *et al., 2*009). Such changes can be attributed to a variety of mechanisms of defense as exhibited by the host during pathogenesis (Jayaraj *et al.,* 2010). Different types of chemical changes in infected host tissues have been reported in many host-pathogen systems but there is no report seems to be available for maize. However, changes in these biochemical parameters in the germplasm lines of maize and their associated BLSB resistance are unknown. Thus, the objective of the present work was to study some biochemical parameters in maize leaves infected by the *Rhizoctonia solani.* f. sp. *sasakii*.

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MATERIALS AND METHODS

Seeds of the various germplasm groups *viz*. early maturity, extra early maturity, late maturity, medium maturity, QPM (quality protein maize), inbreds, hybrids and sweet corn were sown in 4 m rows, maintaining row to row and plant to plant distance at 60 and 20 cm. respectively. Two plot of each variety was inoculated by inserting 4 barley grain with fungal growth and sclerotia bodies of *R. solani* between the rind and leaf sheath of three leaves of each of the plant, based on the method suggested by Ahuja and Payak (1978). Un-inoculated plot of each variety served as check against each treatment. After 15 days of inoculation, the samples were taken from diseased and healthy plants of all trials to estimate the activities of Peroxidase, Polyphenol oxidase and the contents of total phenols. The infected as well as healthy leaf samples were collected and placed in a thermocol box kept moist and brought to the laboratory for biochemical analysis.

Extracts were prepared by weighing 200 mg of the sample, homogenized in 10.0 ml of ice-cold phosphate buffer (0.1M, pH =6.5) in pre-chilled mortar-pestle. The homogenate was centrifuged at 2°C at 10,000 rpm for 15 minutes in a refrigerated centrifuge. The clear supernatant obtained was collected and separated into two 5 ml portions. One 5 ml portion was kept on ice under refrigerated conditions and used for estimation of the activities of peroxidase and polyphenol oxidase. The other 5 ml portion was kept at room temperature and used for estimating the contents of total phenols.

Estimation of total phenol content

Total phenol content was estimated by the procedure given by Malick and Singh (1980). An aliquot of 2.0 ml was taken from the other 5 ml portion of the extract maintained at room temperature. 2.0 ml of ethanol was mixed to it and the tubes kept in a boiling water bath until all liquid got evaporated. The residue thus obtained was dissolved in 5 ml distilled water. Assay was done by taking 1.0 ml of this aqueous extract, 2.5 ml of distilled water, 0.5 ml of Folin- Ciocalteau reagent (1:1 diluted) and 1 ml of 35 per cent Na_2CO_3 were mixed in test tube. The tubes were properly shaken and allowed to stand at room temperature for one hour along with the suitable blank. The intensity of blue colour was read at 650 hm in a spectrophotometer. A standard curve was prepared using Catechol as the standard 100mg/ml) to know the content in unknown samples.

Estimation of peroxidase activity

Peroxidase activity was estimated by the protocol given by Manoranjankar and Mishra (1976). Here again, the first 5 ml portion of the crude extract preparation kept under 0^0-4^0 C was used. 3.0 ml of the assay mixture for Peroxidase activity estimation comprised of 2.3 ml of 0.1M Phosphate buffer (pH 6.5), 0.5 ml of Guaicol substrate, 0.1 ml of the enzyme extract and finally 0.1 ml of H_2O_2 (5%) to start reaction. The assay components were quickly mixed and transferred to spectrophotometer cuvette for recording changes in absorbance at 15-second intervals for a maximum time of 3 minutes. Each observation was recorded for the Peroxidase activity against a substrate blank. Enzyme activity was calculated based on change in absorbance per minute per milliliter of the enzyme in reaction mixture. As the substrate got transformed into product, a colorless to dark brown oxidation product was formed by three minutes time.

Estimation of poly phenol oxidase activity

Estimation of poly phenol oxidase activity was done as per earlier protocol (Manoranjankar and Mishra, 1976). The first 5 ml portion of the crude extract preparation was used. The assay mixture for PPO activity comprised of 1.5 ml of phosphate buffer (0.1M, pH=6.5), 1.0 ml of catechol (50 M) as substrate followed by 0.5 ml of the undiluted crude enzyme extract added in a clean, dry test tube. The assay mixture was incubated for 5 min at 25°C. Thereafter, the reaction was stopped by adding 0.5 ml of 5% H_2SO_4 (v/v) solution. The yellow colour of product formed was read spectrophotometrically at a wavelength of 410 nm on a spectrophotometer against a substrate blank. One unit of enzyme activity was calculated as the change in absorbance per min per milliliter of the crude extract.

RESULTS AND DISCUSSION

Total phenol content

It is evidence from the data that there is more than 100% increase in total phenol content in diseased plants in all the germplasm of maize (Table 1). Maximum percent increase in total phenol content was observed in extra early with 382.35% followed by Inbreds where, 353.06% increase was recorded. Minimum present increase was observed in local susceptible check Surya with 102.5%. This increase shows elicitation of biochemical defense by host tissue. Synthesis of phenolics in plants is altered due to wounding and infection by pathogens (Dubeler *et al.,* 1997). Conversion of phenols to non-toxic compounds is widespread among microorganisms. Pathogens achieve this by producing oxidative enzymes. The presence of a high concentration of phenolic compounds is considered to be one of the major factors for an incompatible host pathogen interaction. After infection by a pathogen, plant cells synthesize phenol oxidising enzymes that oxidise phenols into toxic quinines, which play a crucial role in disease resistance (Jiang *et al., 2*009; Ashry and Mohamed, 2011). Siqueira *et al.* (1991) discussed the role of phenolic compounds in host-pathogen interaction, particularly in disease resistance. The results suggested that the accumulation of total phenol was higher in infected genotypes as compared to healthy ones.

Peroxidases activity

This oxido-reductase has been recognized as an antioxidant enzyme and hence, its activity has been found to increase, thereby exhibiting high level of resistance shown by the host. This result on POX activities is presented in table 1 that shows an increasing trend in the diseased material. Maximum percentage increase *i.e.* 87.5% was found in early maturity group of entries followed by Inbreds. QPM has also shown good amount of peroxidase. Lowest peroxidase *i.e.* 39.13% was obtained in local susceptible check Surya. Increase in Peroxidase activity is also reported when host is challenged by pathogen. This increase also shows defense response of host. This enzyme is termed as defense enzyme and increase in quantity also exhibits the level of resistance in host. Increase in Peroxidase activity has been reported when host tissues are challenged by pathogen (Li *et al*., 2009). This increase is an outcome of host creating an antioxidation state defense response (Deborah *et al*., 2001). These studies indicate that Inbreds are genetically strong with dominant resistant genes responsible for defense enzymes secretions. These inbreds are potential donors of such genes and these can be used for further breeding programme in resistance breeding.

Poly phenol oxidases activity

The maximum percentage increase in poly phenol oxidases activity was observed in early maturity group of entries with. 70.80% followed by Inbreds (Table 1). Again the trend shows that late maturity group shows least poly phenol oxidases activity which indicates more prone to diseases and less biochemical defense. Even the local susceptible check has more amount of poly phenol oxidases activity as compared to hybrids. The inbred germplasm are again found worthy as these can

Table 1. Variation in total phenol, peroxidases and poly phenol oxidases (mg 100 mg^{-1} fresh wt. leaf) in different groups under artificially inoculated (diseased) and uninoculated (healthy) conditions

Group/variety	Total phenol content*			Peroxidases (PO)*			Poly phenol oxidases (PPO)*		
	Healthy	Diseased	% variation	Healthy	Diseased	% variation	Healthy	Diseased	% variation
Surya	40.0	81.0	**41(102.5)**	23.0	32.0	**9(39.13)**	0.81	1.06	0.25(30.86)
Late maturity	43.0	116	73(169.76)	20.0	31.0	11(55.00)	0.56	0.67	**0.11(19.64)**
QPM	46.0	147	101(219.56)	18.0	30.0	12(66.66)	0.63	0.86	0.23(36.50)
Hybrid	45.0	141	96(213.33)	22.0	33.0	11(50.0)	1.01	1.32	0.31(30.69)
Inbred	49.0	222	173(353.06)	15.0	28.0	13(86.60)	1.13	1.85	0.72(63.71)
Extra early	51.0	246	**195(382.35)**	25.0	35.0	10(40.0)	1.37	2.34	**0.99(70.80)**
Early maturity	41.0	106	65(158.53)	16.0	30.0	**14(87.5)**	0.90	1.24	0.34(37.77)
Medium	47.0	191	144(306)	17.0	28.0	11(67.70)	0.70	0.91	0.21(30.00)
		CD at 5%			CD at 5%			CD at 5%	
P (Healthy)		6.56			1.24			0.05	
T (Diseased)		3.28			0.62			0.02	
P xT (Healthy x Diseased)		9.28			1.76			0.07	
C.V.%		5.54			4.22			4.32	

*Mean of three replications.

be used for breeding for resistance for developing good and potential hybrids of maize. However, there is less percent increase as compared to total phenols, but this increase recorded is also commendable. Several reports of this kind of studies are available, Deborah *et al.* 2001 concluded on the basis of increase in peroxidase, polyphenol oxidase, phenolics and lignin's were increased significantly in response to inoculation with non pathogen compared to inoculation with pathogen. The oxidative enzymes such as polyphenol oxidase and peroxidase are known to be associated with the browning of host tissues (Khan *et al.,* 2001). They are also capable of oxidizing phenolics and related compounds, thus increasing their toxicity. This enzyme plays active role in inhibiting mycelial elongation, penetration, colonization and in spore producing fungi, they may inhibit spore germination too (Usenik *et al.,* 2004). The alteration in the oxidative enzyme level in maize infected with *R. solani* may be due to injury of the host tissues by fungal hyphae leading to the oxidation of phenolic compounds, however, the amount of oxidation products is insufficient to stop the invasion of mycelium in the leaf tissues (Liu *et al.,* 2012).

In conclusion biochemical defense is a powerful mechanism in plants which helps in resistance against disease. Accumulation of these substances provides workable protection against diseases. The concentration of total phenols, poly phenol oxidases and peroxidases varies in healthy and diseased plant as well as in different genotypes. This work it clear that biochemical defense is triggered in response to pathogen attack however, they were unable to totally inhibit the pathogen because of more virulence in pathogen. Further work needs to be done with resistant and susceptible maize varieties to establish the role of various biochemical constituents in disease resistance.

REFERENCES

Ahuaja, S.C. and Payak, M.M. (1978). A field inoculation technique for evaluating maize germplasm to banded leaf and sheath blight. *Indian Phytopath.* **31:** 517-552.

Ashry, N.A. and Mohamed, H.I. (2011). Impact of secondary metabolites and related enzymes in flax resistance and or susceptibility to powdery mildew. *World J. Agri. Sci.* **7**: 78-85.

Deborah, S. D. Palaniswami, A., Vidhyasekaran, P. and Velazhahan, R. (2001). Time-course study of the induction of defense enzymes, phenolics and lignin in rice in response to infection by pathogen and non-pathogen. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz.* **108:** 204-216.

Dubeler, A., Voltmer, G., Gora, V., Lunderstadt, J. and Zeeck, A. (1997). Phenols from *Fagus sylvatica* and their role in defense against Cryptococcus fagisuga. *Phytochem.* 45: 51-57.

Huang S.W., Wang, L., Wang, Q.Y., Tang, S. Q., Chen, H.Z., Zhu, DF. (2008). Rice sheath blight pathogen impacts the activities of disease resistance-related enzymes in leaves of different rice varieties. *Chinese J. Rice Sci.* **22:** 219-222.

Jayaraj, J., Ranganathan B., Ramalingam R., Muthukrishnan, S. Rethinasamy V. (2010). Oxalic acid-induced resistance to *Rhizoctonia solani* in rice is associated with induction of phenolics, peroxidase and pathogenesis-related proteins. *J. Plant Interactions* **5**: 147-157.

Jiang, Y.F., Cheng, W.D., Qin, L.Q., Tan, X.J., Zhou, J.G., Xie, H.X., Chen, L. (2009). Physiological mechanism of exogenous salicylic acid induced resistance to sheath blight of maize (*Zea mays L.). Guangxi Agri. Sci.* **40:** 242-245.

Khan, A.J., Deadman, M.L., Srikandakumar, A., Maqbali, Y.M. Al, Rizvi S.G. and Sabahi, J. (2001). Biochemical Changes in Sorghum Leaves Infected with Leaf Spot Pathogen, *Drechslera sorghicola. Plant Pathol. J.* **17**: 342-346

Kumar, R. and Singh, I. S. (2004). Genetic control of banded leaf and sheath blight (*Rhizoctonia solani* f. sp. *sasakii*) in maize (*Zea mays L.*). *Cereal Res. Commun.* **32**: 309-316.

Li, L., Yi, M.Y., Ming, Z.Z., Fei, L.P., Tang, P.G., Jun, Z.M. (2009). Functional effects of different defense enzymes on banded leaf and sheath blight of maize. *J. Maize Sci.* **17**: 99-102 and 106.

Liu, Y., Wang, R., Hua, L,M., Xie, Z.J. (2012). Effects of N applied rate on leaf senescence and protective enzyme system at later growth stage of maize. *J. Maize Sci.* **20:**124-127.

Malick, C.P. and Singh, M.B. (1980). *Plant Enzymology and Histoenzymology*. Kalyani Publication, New Delhi, 286.

Manoranjankar, M and Mishra, D. (1976). Catalase Peroxidase and PPO activities during leaf senescence. *Plant Physiol.* **57**: 315-319.

Mondal, A., Dutta, S., Nandi, S., Das, S. and Chaudhuri, S. (2012). Changes in defence-related enzymes in rice responding to challenges by *Rhizoctonia solani. Arch. Phytopath. Plant Protec.* **45:**1840-1851.

Rani, V.D., Reddy, P.N., Devi, G.U. (2013). Banded leaf and sheath blight of maize incited by *Rhizoctonia solani* f.sp *sasakii* and its management- A review. *Int. J. Appl. Biol. Pharma Tech*. **4:** 52-6

Sharma, R. R., Gour, H. N., Bora, K. K. and Gaur, R. B. (2002). Lipid peroxidation and defense related enzymes in maize infected by *Rhizoctonia solani*. *Indian Phytopath.* **55:** 486-487.

Singh, A. and Shahi, J.P. (2012). Banded leaf and sheath blight: an emerging disease of maize. *Maydica* **57**: 215-219.

Siqueira, J. O., Nair, M. G., Hammerschmidt, R. and Safir, G. R. (1991). Significance of phenolic compounds in plant soil microbial system. *Rev. Plant Sci.* 10:63-131.

Usenik V, Mikulic-Petkovsek M, Solar, A., Stampar, F. (2004) Flavonols of leaves in relation to apple scab resistance. *Z. Pflanzenkr. Pflanzenschutz* 111: 137- 144.

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