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ALTERED SPECIFIC ACTIVITES OF PEROXIDASE AND IAA OXIDASE DUE TO INFECTION OF *DRECHSLERA GRAMINEA* IN BARLEY (*HORDEUM VULGARE* L.)

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ABSTRACT

This study was planned to determine the alterations in specific activities of two oxidative enzymes Peroxidase (E.C.1.11.1.7) and IAA Oxidase (E.C.1.13.16) in barley due to *Drechslera graminea*, causal agent of Leaf Stripe disease with the objective to investigate the role of these oxidative enzymes in host pathogen interactions. Healthy, naturally infected and artificially inoculated seeds and their seedlings at different time intervals after sowing of barley were used. Estimations of enzyme activities were done by using standard protocols. In seeds, specific activities of Peroxidase and IAA oxidase increased as the severity of infection increased thus it was highest in heavily infected among categories of naturally infected seeds. In all categories of seedlings healthy, naturally infected and artificially inoculated, peroxidase activity increased throughout from 10 to 30 days. Specific activity of IAA oxidase decreased initially upto 20 days and then increased in healthy (control) and artificially inoculated seedlings whereas in all categories of naturally infected seedlings, IAA oxidase specific activity increased throughout from 10th to 30th day of sowing.

INTRODUCTION

Barley has become the integral part of the diet of large chunk of human beings on almost whole of the globe. The family Gramineae of which barley (*Hordeum vulgare* L.) is an important member largely encompasses the group of cereals which have been serving the major source for diet of human beings and feed for animals. In India average yield of crop is lower than several other countries due to poor crop management and the ravages caused by destructive pests and diseases. In India it is reported to be attacked by a number of pathogenic fungi. *Drechslera graminea* (Rabenh. ex. Schlecht) Shoemaker (sexual *Pyrenophora graminea*) is the causal agent of barley stripe. Among the fungal diseases, stripe disease is a major disease in our country causing losses as high as 70 to 72 per cent under epiphytotic conditions (Pant and Bisht, 1983). In India, the stripe disease has been reported as early as in 1918 first by Butler and later by Shaw (1921) and Mitra (1931). Analysis of enzymes is an essential feature of the biochemical organization of living things. The existence of multiple forms of peroxidase in plants has been known for a number of years, but the relationship of individual isozymes to specific biological functions is not clear. Increases in total peroxidase activity are often found during infection of higher plants by pathogens[1] with the greatest increases associated with a host response classified as resistance. Increased activity of peroxidase (POX) has been reported in plants treated with various biotic and abiotic inducers of resistance (Huang and Backhouse, 2005; Raghvendra *et al.*, 2007). In the present study, biochemical changes were estimated on the basis of enzyme activities of peroxidase (POX) and IAA oxidase (IAAO). Other investigators such as Nawar and Kuti (2003) stated that there are positive relationships between peroxidase (enzymes and isozymes) and resistance development in plants. Furthermore, Caruso *et al.* (2001) experimentally supported the idea that peoxidases play a defence role against invading pathogens. IAA oxidase activity is related to IAA metabolism, which is directly concerned with the expression of resistance by host cells. The naturally occurring auxin is indole acetic acid (IAA). Pathogenic diseases directly alter auxin levels then need for IAA oxidase to maintain the auxin concentration become necessary. Pathogens, not only increases levels of IAA in their hosts, but produces IAA. In some diseases, however, increased levels of IAA are totally or partly due to the decreased degradation of IAA through the inhibition of IAA oxidase.

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MATERIALS AND METHODS

Material

Seeds of healthy, naturally infected (three categories weakly, moderately and heavily) and artificially inoculated with *Drechslera graminea* of sample acc. No. Br32 and their seedlings at 10th day, 20th day and 30th day from sowing, were taken for conducting studies. Artificial inoculation of *Drechslera graminea* was made in healthy seeds of acc. No. Br32.

Raising of crop: The crop was raised in earthen pots (height 30 cm, diameter 20 cm) filled with sterile coarse sand (pH 8.3). Seedlings were harvested at different time intervals for conducting the studies.

Peroxidase

Preparation of crude enzyme extract

1 g of tissue (seeds or seedlings) was washed with distilled water, blotted to surface dry and homogenized in 10 ml of 0.05 M Tris buffer (PH 7.6) in chilled pestle mortar using acid washed sand as an abrasive. The homogenate was filtered through 4 layers of muslin cloth and filtrate centrifuged at 12,000 rpm for 20 minutes at 4˚ C in refrigerated centrifuge and the supernatant so obtained was then used for enzyme assay.

Estimation of Peroxidase specific activity

Peroxidase activity was assayed by the modified method of Shanon *et a*l. (1968). The reaction mixture contained 0.2 ml of 0.2 % O-dianisidine in methanol, 0.1 ml of 0.2 % H_2O_2, 3.6 ml of 0.05 M phosphate buffer (PH 6.5) and 0.1 ml of properly diluted enzyme extract. The enzymatic reaction was initiated by addition of H_2O_2 and the change in absorbance was followed upto 2 min at 430 nm in a spectrophotometer Systronic 106. The absorbance change indicates the level of peroxidase activity and this data is compared among samples. Repeated peroxidase activity assay necessary to complete all samples are required.

Sample data is then statistically analyzed. The enzyme peroxidase activity was expressed on a plant protein basis. Units of peroxidase are divided by the soluble protein in the sample (in micrograms) to give the specific activity. Peroxidase specific activity was expressed as change in Δ OD min^{-1} mg^{-1} protein.

IAA oxidase

Preparation of Crude enzyme extract

500 mg tissue (seeds or seedlings) was washed with distilled water, blotted to surface dry and homogenized in 10 ml phosphate buffer (PH 6.4) in a chilled pestle mortar. The homogenate was filtered through 4 layers of muslin cloth and filtrate was centrifuged at 12,000 rpm for 20 minutes at 4 ˚ C. The supernatant so obtained was then used for enzyme assay.

Estimation of IAA oxidase specific activity

IAA oxidase specific activity was estimated according to the method of Sequeria and Minco (1966). The activity of IAA oxidase was determined by the rate of disappearance of IAA. The reaction mixture contained 0.25 ml of 1mM DCP (Dichlorophenol), 0.75ml of a mixture of 1 mM IAA and 0.5 mM $MnCl_2.H_2O$, 3.25 ml of 0.02 M Phosphate buffer (PH 5.5) and 1 ml of enzyme extract. The mixture was shaken and kept 1 hour for incubation at 32˚ C. After the incubation period was over, 5 ml of assay mixture was pipetted out at different time intervals such as 0, 30 and 60 min and 1 ml of Salper's reagent was added. The pink colour developed and optical density of each reaction mixture was then recorded at 530 nm in Systronic 106 spectrophotometer. The control treatment was carried out in an identical manner except that the enzyme was added after the addition of Salper's reagent. The unit of IAA oxidase enzyme was defined as µg of IAA destroyed per ml of enzyme in min. Specific activity of IAA oxidase enzyme is expressed as change in Δ OD min^{-1} mg^{-1} protein.

Statistical Analysis: All experiments were performed in 3 different sets with each set in triplicates. The data are expressed as mean, ± SEM (standard error of the mean).

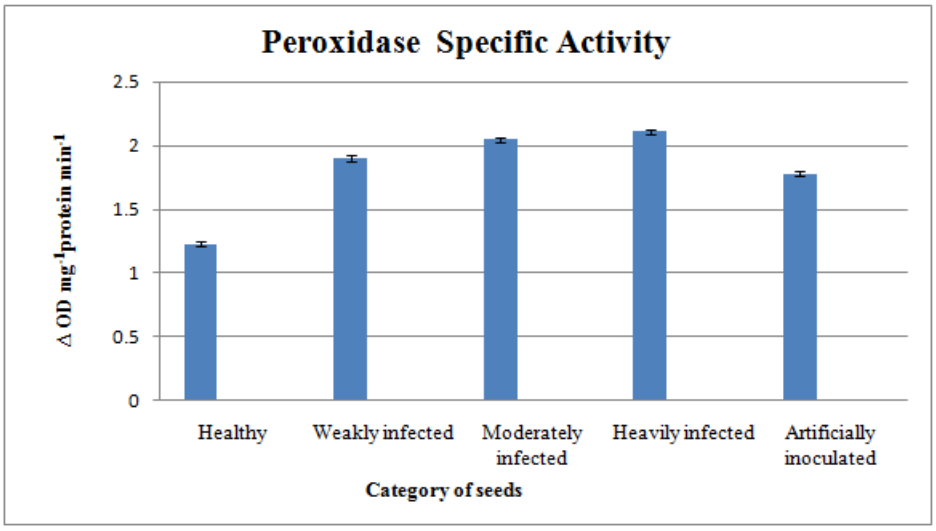

Figure 1. Specific activity of Peroxidase in seeds of healthy (control), naturally infected (weakly, moderately and heavily) and artificially inoculated of sample acc. No. Br32

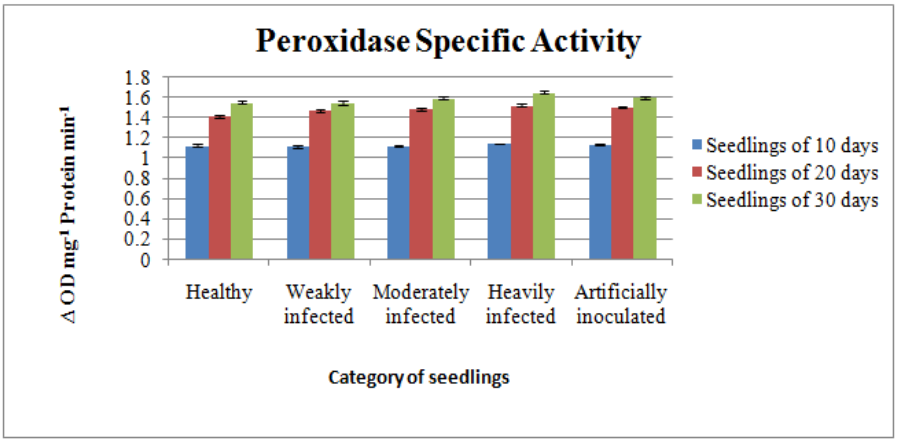

Figure 2. Specific activity of Peroxidase in seedlings of healthy (control), naturally infected (weakly, moderately and heavily) and artificially inoculated on 10th, 20th and 30th day after sowing (sample acc. No. Br32)

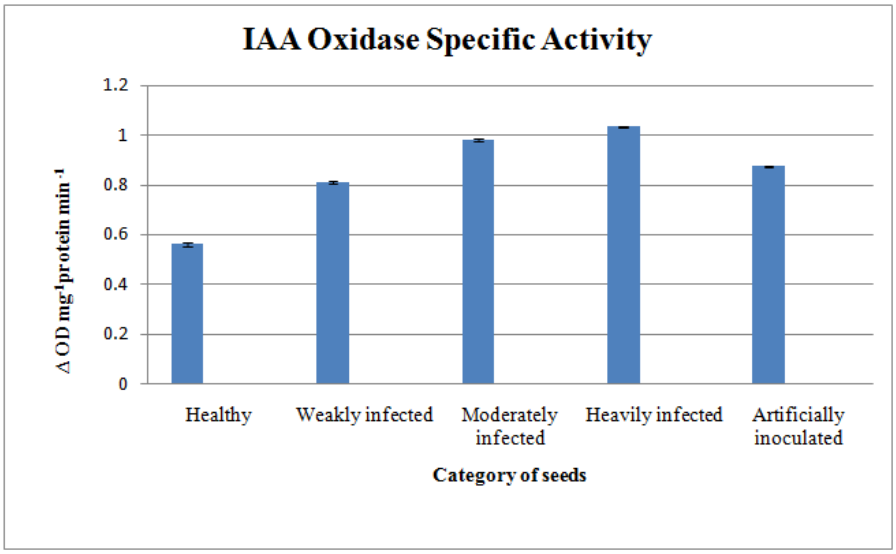

Figure 3. Specific activity of IAA Oxidase in seeds of healthy (control), naturally infected (weakly, moderately and heavily) and artificially inoculated of sample acc. No. Br32

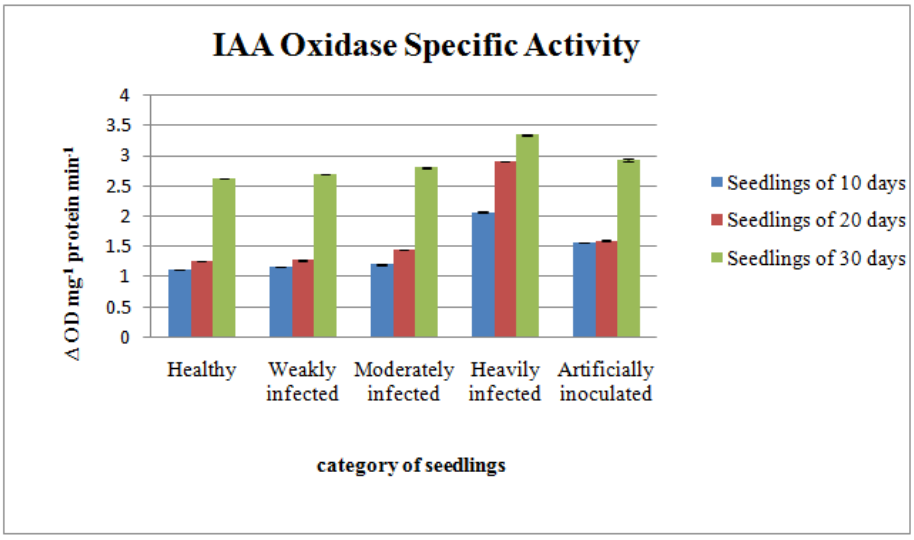

Figure 4. Specific activity of IAA Oxidase in seedlings of healthy (control), naturally infected (weakly, moderately and heavily) and artificially inoculated on 10th, 20th and 30th day of sowing (sample acc. No. Br32)

Statistical analysis of data was done by using Graph pad prism 5 statistical software in a completely randomized design. All data obtained by subjected to one way analysis of variance (ANOVA). Values of ρ which were ≤0.05 were considered as significant. Graphs were drawn by using Microsoft Excel software.

RESULTS AND DISCUSSION

Peroxidase (E.C. 1.11.1.7)

Seeds

Result of our present investigation revealed that peroxidase specific activity increased as the severity of infection increased thus it was highest in heavily infected among categories of naturally infected seeds. Healthy (control) seeds showed lowest activity of peroxidase while in artificially inoculated seeds, it was in between of healthy and weakly infected seeds.

Seedlings

In all categories of seedlings healthy, naturally infected (weakly, moderately and heavily) and artificially inoculated, peroxidase activity increased throughout from 10 to 30 days of sowing. Infected seedlings showed higher activity of peroxidase than healthy seedlings. It was maximum in heavily infected seedlings at 30th day of sowing. All experiments were performed in triplicates. One way analysis of variance (ANOVA) was used to show significance of difference with respect to control. In all experiments ρ value was found to be lower than 0.05 which indicate that differences were statistically significant.

Peroxidase play a central role in the biosynthesis of plant cell wall components, including lignin, suberin, and cross linked extensions that are linked with plant defense responses to pathogen, particularly to fungi (Almagro *et al.*, 2009). This enzyme has ubiquitous distribution in plants and therefore been studied extensively in many plant-pathogen interactions.
The enhanced activity of peroxidase in infected seeds might result in augmented role of oxidation of phenolic substances. This favors the formation of toxic quinones. These substances participate in the defence reaction of the host. Friend (1980) and Saini (1988) also supported the view that during infection oxidative enzymes like peroxidase increased for oxidation of phenols to toxic quinones, which play an important role in defence of the host to fungal infection. Joshi *et al*. (2004) also reported that activity of peroxidase increased with the increase in disease intensity in infected cluster bean to *Alternaria cucumerina* var. *cyamopsidis*. This indicated that this enzyme play an important role in defence mechanism against *Alternaria* blight in cluster bean and contributes toward imparting resistance.

Changes in the activity of peroxidase play a role in the regulation of metabolic pathways in diseased and injured tissues. Reason of increased activity of peroxidase in infected seedlings might be that peroxidases participate in defensive lignification and synthesis of phenolic compounds, which are effective against pathogens. Sudhagar *et al*. (2000) reported the changes in the activities of the defense enzymes viz., phenyl alanine ammonia lyase and peroxidases at 80th and 90th day in resistant and susceptible genotypes of groundnut consequent to infection with the rust pathogen, *Puccinia arachidis* Speg. The level of peroxidase activity increased and prolonged in both 80 and 90 days after sowing in susceptible genotypes while in resistant genotypes the levels were high initially and declined at 90 days after sowing. The prolonged increase of peroxidase in susceptible genotypes may be due to increase of reactive oxygen species such as super oxides radical (0_2^-) hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) which are maintained at lowest level in plant system is instigated by pathogenic attack and accumulates to toxic concentrations and corroborated with the severity of infection (Bolwell and Wojtaszek, 1997). Similar observations were also made by Shimoni *et al.* (1991) and Velazhahan and Vidyasekaran (1994). Biochemical examination by Singh (2007) showed higher peroxidase activity in the infected seedlings of pearlmillet by *Curvularia penneseti* as compared to healthy. Reason might be that peroxidase showed the multifacial involvement in infection process ranging from secondary phenol metabolism to lignin biosynthesis. Such phenomenon was recognized as the primary reflection of brown spot disease establishment in young seedlings and later on at maturity level.

IAA Oxidase (E.C. 1.13.16)

Seeds

Result of present investigation revealed that IAA oxidase specific activity showed same response as peroxidase specific activity to the severity of infection. It increased as the severity of infection increased thus it was highest in heavily infected among categories of naturally infected seeds. In artificially inoculated seeds, IAA oxidase specific activity was in the middle of weakly infected and moderately infected seeds. It was lowest in healthy (control) seeds.

Seedlings

IAA oxidase specific activity increased throughout from 10th to 30th day of sowing in healthy (control), all categories of naturally infected as well as in artificially inoculated seedlings. Heavily infected seedlings showed highest specific activity of IAA oxidase. All experiments were performed in triplicates. One way analysis of variance (ANOVA) was used to show significance of difference with respect to control. In all experiments ρ value was found to be lower than 0.05 which indicate that differences were statistically significant. IAA oxidase activity is related to IAA metabolism, which is directly concerned with the expression of resistance by host cells. Enhanced specific activity of IAA oxidase in infected seeds might be due to some diseased tissue related to breakdown processes of IAA contents. Similar report has been presented by Singh in 2007. The drastic increase in IAA oxidase activity initially, followed by decrease and again increase showed the differential changes during progressive infection of *Curvularia penneseti* in pearl millet, which may play an important role in disease establishment of infection.
Reason for enhanced activity of IAA oxidase in infected seedlings might be that IAA or auxin contents increases at the infection sites. Hyperauxiny is not conducive to the growth of pathogen and hence the pathogen produces auxin degrading enzyme for its survival. Indole acetic acid oxidase (IAA

oxidase) is one of the auxin degrading enzymes. Since auxin content decreases in the infected host tissue due to the action of IAA oxidase. Singh (1998) observed that IAA oxidase activity decreases at the later stage of fungal infection. Decrease in fungal infection may be due to the presence of an inhibitor produced by the fungus or may be due to some parasitic induction. Sukhwal and Purohit (2003) reported increased activity of IAA oxidase in three varieties Ganga-5, VL-42 and Malan of maize infected with Helminthosporium leaf blight as compared to healthy leaves. Increased activity of IAA oxidase in all three varieties of maize infected with *Helminthosporium maydis* and *H. turcicum* may be related to increased peroxidase activity. The view that peroxidase is responsible for some IAA oxidase activity and IAA oxidase activity was related to oxidase function of peroxidase, has been supported by many researchers (Srivastava and Van Huystee, 1973; Shinshin and Noguchi, 1975).

Singh *et al*. (2011) reported that IAA oxidase activity was maximum in diseased inflorescence compared to healthy inflorescence, healthy leaves and diseased leaves of Brassica. Higher IAA oxidase was found to be in infected leaves compared to healthy leaves. Reason of increased activity of IAA oxidase might be that during late phase of systematic infection auxin content decreases and records well below that the normal tissue level. This may be attributed to reduced concentration and conversion of auxin precursors like tryptophan or increased synthesis of IAA oxidizing enzymes. Increased level of suggested that the enzymes oxidized IAA rapidly causing reduction in auxin. Brindha *et al*. (2012) investigated the Indole acetic acid oxidase IAAO activity from *Alternaria cepulae* infected leaves of onion. From the experiments conducted, IAAO activity is maximum after the severity of the leaf blight disease. The fact that micro organism produce IAA oxidase in infected plants by parasites is to influence the auxin concentration. IAAO activity was started only after the 20th day after inoculation. When the hyperauxiny is developed in the infection site, the need for IAA oxidase to maintain the auxin concentration become necessary. Before the blight infection IAA was not present at the infection site, but when the disease become severe the translocation of IAA which stimulates the parasite to escape from the situation of hyperauxiny, hence the production of IAAO from 16th day onwards is to degrade IAA concentration in the blight area.

Conclusion

Specific activities of Peroxidase and IAA oxidase enzymes increased in diseased tissues as the disease response because these enzymes play important roles in defence mechanism.

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