P: ISSN NO.: 2394-0344 RNI No.UPBIL/2016/67980 VOL-4* ISSUE-1* (Part-2) April- 2019
E: ISSN NO.: 2455-0817 *Remarking An Analisation*

Brown Rust (*Puccinia recondita Rob. Ex. Desm. F. Sp. Tritici*) Development in Relation to Nitrogen Fertilization as Determined through Effect on Phenolics and Peroxidase Metabolism in Wheat Leaves

Archana Singh
Associate Professor,
Deptt. of Botany,
M.S.J. Govt. Post Graduate College,
Bharatpur, Rajasthan, India

Abstract

Appreciable interactions in total phenolics were observed in case of third leaf as compared to first and flag under various fertilization regimes in both susceptible (cv. Lal Bahadur) and the resistant cultivar (Raj 3765) at 72h, 96h and 120h of progressive brown rust infection. In the first leaf showed leaf shower lesser alteration at 72h and 96h stages but phenolics showed increasing trend towards 120h stage. The resistant cultivar reflect reflected higher levels of phenolics in flag leaf and specially to higher fertilization (N90) levels in general at all the three determined stages of disease dynamics.

Various N- fertilization regimes were found to affect leaf peroxidase activity differently in first, third and flag leaf in both susceptible and resistant cultivars. Higher profile of peroxidase activity was noticed in third leaf and flag leaf under No fertilization application condition irrespective of the nature if interacting cultivar at different stages of rust development. The resistant (cv. Raj 3765) however showed higher peroxidase activity profile in flag leaf as compared to the suspectible cv. Lal Bahadur.

Keywords: Brown *Rust,* Nitrogen- Fertilization*,* Phenolics *And* Peroxidase Activity.

Introduction

Brown Rust (Puccinia recondita Rob. Ex. Desm. F. Sp. Tritici) is one of the most wide spread disease of wheat (Triticum aestivum) in the world (Statter, 1988). During infection the resistant host plant defends itself against potential pathogen by means of a number of physical and chemical factors which may be already present in the host or may be produced in response to infection (Rana et al 2005). Phenols have been implicated as active resistant factors in defense mechanism of many host-parasite interactions and are present in higher amounts in resistant plants (Motta et al 2005). The phenolics substances may be responsible for the delay and decline in number of spore germination, appresoria formation, penetration and colonization at the onset of infection (Raman 2007). Increase in total peroxidase activity are often found during infestation of higher plants by pathogens with the greatest increase associated with a host responsible classified as resistance. Increased activity of peroxidase (pox) has been reported in plants treated with biotic and abiotic inducers of resistance (Huang and Backhouse 2005, Raghvendra et al 2007). The application of nitrogen fertilizer is well known to increase gain yield, if somehow or the other the rust disease may also interplay this remains the nature of the present studies.

Nawar and Kutti (2003) stated that there are positive relationships between peroxidase and resistance development in plants Caruso et al (2001) experimentally supported the idea that peroxidases play a defense role against invading pathogen.

Aim of the Study

How the release or accumulation of specific biochemical products may directly be taken as the earlier characteristics of disease development and progression in relation to their resistance or susceptibility. The study was carried out in contact of different nitrogen fertilization on first, third and flag leaf due to the peculiar physiology of leaves.

Material and Methods

1. Raising of crops: The crop was raised in earthen pots (height 30 cm, diameter 20 cm) filled with sterile coarse sand (pH 8.3) in polyethylene bags. Two wheat variety viz. Lal Bahadur (susceptible) and Raj 3765 (resistant) were taken for conducting studies during the usual winter season (December- March).
2. N- fertilization: The pots were supplemented by different doses of Urea (with 46.5%N) so as to give 0 (no urea), 30 and 90 kg of nitrogen per hectare of field area calculated on the basis of the weight of the sand at the sowing time.
3. Rust Inoculation: The first, third and later the flag leaves were inoculated artificially by rubbing uredospore powder of the brown rust on the lower surface of the leaves. The rust inoculums was the mixture of races 12-2, 77-2, 77-5 and 104-2 are was procured for Regional Research Station of the Directorate of Wheat Research, Flowerdale, Shimla (India). During inoculation the plants were covered with polyethylene bags (40x 80cm) to ensure enough humid environment.
4. Sampling upon rust inoculation the polyethylene bags were removed after 24h and the leaf samples were collected 0 (just before inoculation), 72, 96 and 120h after attempted inoculation.
5. Biochemical estimation: The biochemical estimation of total phenolics was done by the method of Swain and Hillis (1959). For Peroxidase assay the modified method of Shanon et al (1968) was employed. For assaying specific activity of peroxidase 1.0g of leaf was homogenized in 10ml of 0.05 M Tris-HCL buffer (pH 7.6) in a chilled pestle and mortar by using acid washed sand as an abrasive. The homogenate was centrifuge at 20,000x g for 20 min at 4°C and the supernatant were used for enzyme assay and protein content estimation. O.D. 0.01+1Unit. One unit of enzyme activity was taken as an O.D. equivalent to 0.01/min and for specific activity units it was expressed as units of activity/mg of protein in fresh tissue.

Result and Discussion

A zigzag pattern of peroxidase activity was observed ranging between (1-3 units) in first leaf in both susceptible and resistant cultivars (fig.1). While coming to third leaf, the susceptible cultivar (Lal Bahadur) reflected much higher levels of peroxidase activity at different stages of determination following rust inoculation under zero nitrogen application treatments. More or less a similar pattern was noticed in case of the resistant cultivar (Raj 3765) too.

Metabolically active flag leaf picture was also activated was also activated a big higher levels of peroxidase activity under zero nitrogen application treatment in both the cultivar at different stages of determination, but this time the overall enzyme activity profile was found doubled in the resistant cultivar under all nitrogen fertilization doses.

As regard to phenolics contents, the first, third and flag leaf of the susceptible variety (Lal Bahadur) showed comparative higher levels of phenolics at the nil nitrogen application as compared to higher nitrogen doses. In contrast to the resistant variety Raj 3765 (except more or less in third leaf) had more of phenolics under higher nitrogen application doses featuring a sort of resistance characteristics.

Comparatively lower level of phenolics seems to favour rust infection in the susceptible variety under nitrogen treatment. As the resistance to pathogens is very often ascribed to higher concentrations of fungi toxic phenolic substances and their oxidation products (Farakas and Kiraly 1962). The resistant variety Raj 3765 appeared to impart resistance through higher level of phenolics under higher nitrogen fertilization. The distinction of their two wheat cultivars in relation to nitrogen fertilization under impact of progressive leaf rust infection appeared that in susceptible variety, application of nitrogen resulted in produced phenolic contents which considered as toxic metabolites to the invading pathogens.

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 response to pathogen, particularly to fungi (Almagro et al, 2009).

The flag leaf analysis showed that the resistant cultivar (Raj 3765) had higher phenolic level. The rust infection progressed beyond 4^{th} day and that too high under N90 treatment illustrating increased level under disease impact.

All the observations were taken from three replications and data was subjected to one way analysis of variance (ANOVA) was used to show significance of difference with respect to control. In all experiments of value was found to be lower than 0.05 which indicate that differences were statistically significance.

Related studies on increased disease severity with increasing nitrogen levels have also been reported by Ojha and Mehta (1970), Damen et al (1989) and Johnston (1992) etc. Higher phenolic contents have been told as the basic characteristics of resistance in various plants-pathogen interactions.

The enhanced activity of peroxidase in infected seeds might result in augmented role of oxidation of phenolic substances. This favours the formation of toxic quinines. These substances participate in the defense reaction of the host. An overall higher peroxidase activity profile during course of initial rust progression (in both susceptible and resistant interactions) might be ascribed to increase de novo synthesis of proteins, as a result of metabolic interactions in the infected tissues. It is due to the result of increased amount of protein, nitrogen and amino acids.

Change 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 seedling might be that peroxidases participate in defensive lignifications and synthesis of phenolic compounds. As regard to specific nature of progressive rust infection in susceptible and resistant interaction, the third and flag leaf analysis revealed some interesting observations. The quantum dynamics of enzyme activity was observed in resistant – interaction, presenting a passive dynamics. Considering higher levels of peroxidase and phenolics at N0 level in the susceptible interaction may be indexed in first and third leaves as resulted in reduction of phenol synthesis. However, in our studies the most favourable point is to determine the genetic set up of the wheat genotypes. Resistant interaction showed increased level of phenolics and peroxidase at different levels of nitrogen fertilization. Such an excitement of this paired analysis might be assigned to the inbuilt resistant in cultivar Raj 3765. Peroxidase activity enhanced due to the oxidation of phenolics. Which has been resulting in production of toxic quinines. It has been assigned a characteristic of resistance in host-parasite interaction (Friend 1980, Farakas and Kiraly 1962, Kosuge 1969 and Frick 1976). Under rust interaction in Wheat, increased peroxidase activities have been reported in accordance with disease progression index (Singh 2007).

Changes in the activity of peroxidase play a role in the regulation of metabolic pathways in diseased and injured tissues. The role of peroxidase enzyme shows the defensive lignifications and synthesis of the phenols. Sudhagar et al (2000) also supported this view. The increased activity of peroxidase in maize infected with Helminthosporium maydis and H. turcicum was also observed by Sukhwal and Purohit (2003).

Increased nitrogen application seemed to enhance rust susceptibility in generation irrespective of the genetic resistance of cultivars. However, the resistant cultivar was found to have an overall higher phenolics-peroxidase activity particularly at metabolically active flag leaf. Thus the flag leaf tissue might be spotted as evidence to determine plant resistance.

Biochemical examination by Singh (2007) showed higher peroxidase activity in the infected seedlings of pearlmillet by Curvularia penneseti 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 of maturing level.

Conclusion

The increase in phenolics and specific activity of peroxidase enzyme in relation to disease incidence shows that these metabolic changes plays at important roles in defense mechanism.

References

Almagro, L; Ros, G; Belchi-Navarro, S; Bru, R; Barcelo, AR and Pedren, M.A. 2009, Class III Peroxidases in plant defense reactions, J. Experi. Bot, 60: 377-390.

Caruso, C; Chilosi, G; Leonard, L; Bertin, L; Magro, P; Buonocore, V and Caporale, C, 2001. A basic Peroxidase from Wheat Kernel with antifungal activity, Phytochemistry. 58: 743-750.

Farkas, G.L and Kiraly, Z. 1962. Role of Phenolic compounds in the Physiology of Plant diseases anddisease resistance. Phytology, z, 44: 105-150.

Friend, J. 1980.Role of Phenolic compounds in desease resistance of plants to fungal pathogen. In: Recent Advance in Biology of Microorganism. Ed. Bilgrami and Vyas. Vol., PP 353-363.

Huang, L.D and Backhouse, D, 2005, Induction of defense responses in roots and mesocotyles of orghum seedlings by inoculation with Fusarium thapsinum and F. proliferation, J. Phytopathol, 153: 522-529.

Johnston, H.W. 1992. Role of crop sequence in suppressing fungal pathogens and the yield response of winter Wheat to nitrogen. In Proceedings of the 39th Annual meeting of the Canadian Pest Management Society, Brandon, Manitoba, Canada. (Edited by Holm, F.A.) Agassiz, Canada; Agassiz Research Station, Agriculture Canada 33-37.

Joshi, LH; Gupta, PP; Gupta, V and Kumar, S. 2004 Biochemical factors in cluster bean that impart Alternaria blight resistance, J. Mycol. Pl. Patrol. 34(2): 581-583.

Motta L.B; Kraus J.E; Salatino A and Salantino MLF, 2005, Distribution of metabolites in galled and nongalled foliar tissues of Tibouchina pulchra Biochem, Systematics and Ecol. 33 (10) 971-981.

Nawar, HF and Kuti, JD (2003), Wyerone acid Phytrolexin synthesis and Peroxidase activity asa markers for resistance of broad beans to chocolate spot disease, J.phytopathol. 151; 564-570.

Raghvendra,V.B, Lokesh,S; Govindappa, M and Vasanth, KT, 2007. Dravya: As an organic agent for the management of seed burne fungi of sorghum and its role in the induction of defense enzymes, Pest, Biochem, Physiol. 89: 190-197.

Raman A, 2007, Insect induced plant galls of India: unresolved questions, Curr, Sci, 92 (6) 748-757.

Rana A, Chauhan S and Chauhan SVS, 2005, Ultrastructutal and Biochemical Changes in Alstonia scholaris L. Leaf galls induced by Pauropsylla tuberculata Crawl in: Kumar S Ed Plant Science Research in India: Challenges and Prospects Botanical Survey of India, Dehradun 139-150.

Singh, A. 2007. Biochemical, histochemical and Isoenzyme Peroxidase studies on Pearl millet seedlings infected by Curvularia penneseti. J. Phytol, Res 20(1): 47-54.

Shanon, L.M; Key, E; Law, J.Y.1968. Peroxidase Isoenzymes from HorseRadish roots: isolation and physical properties. J. Biol. Chem. 241: 2166-2172.

Sudhagar, R; Sassikumar, D and Muralidharan, V.2000. Biochemical changes in Groundnut Genotypes Consequent to infection with the rust Pathogen puccinia arachidis. Tropl. Agric. Res, 12: 199-204.

Sukhwal, R and Purohit, SD. 2003. Accumulation of Phenolics and Changes in activity of oxidative enzymes in Maize infected with Helminthosporium species.J. rhycol.Pl.Pathol. 33. (2): 236-239.

Swain, T; Hillis, W.E.1959.The Phenolic constituent of Prunus domestica L: The Quantative Analysis of Phenolic Constituents. J. Sci. Food. Agr. 10: 63-68.

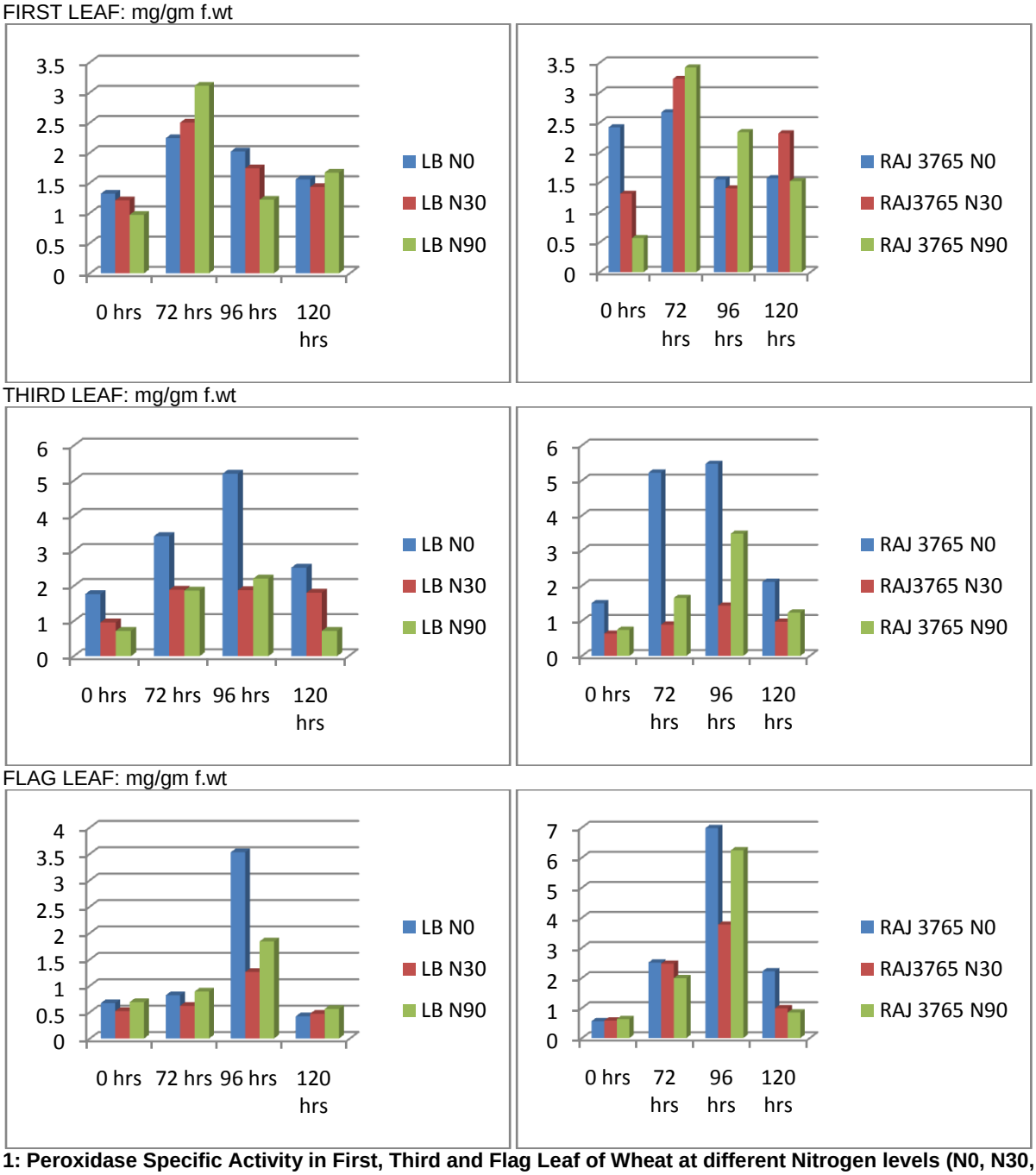

Fig 1: Peroxidase Specific Activity in First, Third and Flag Leaf of Wheat at different Nitrogen levels (N0, N30, N90). The variety of treatment LB= Lal Bahadur, The Susceptible Variety, Raj 3765= The Resistant Variety. FIRST LEAF: mg/gm f.wt

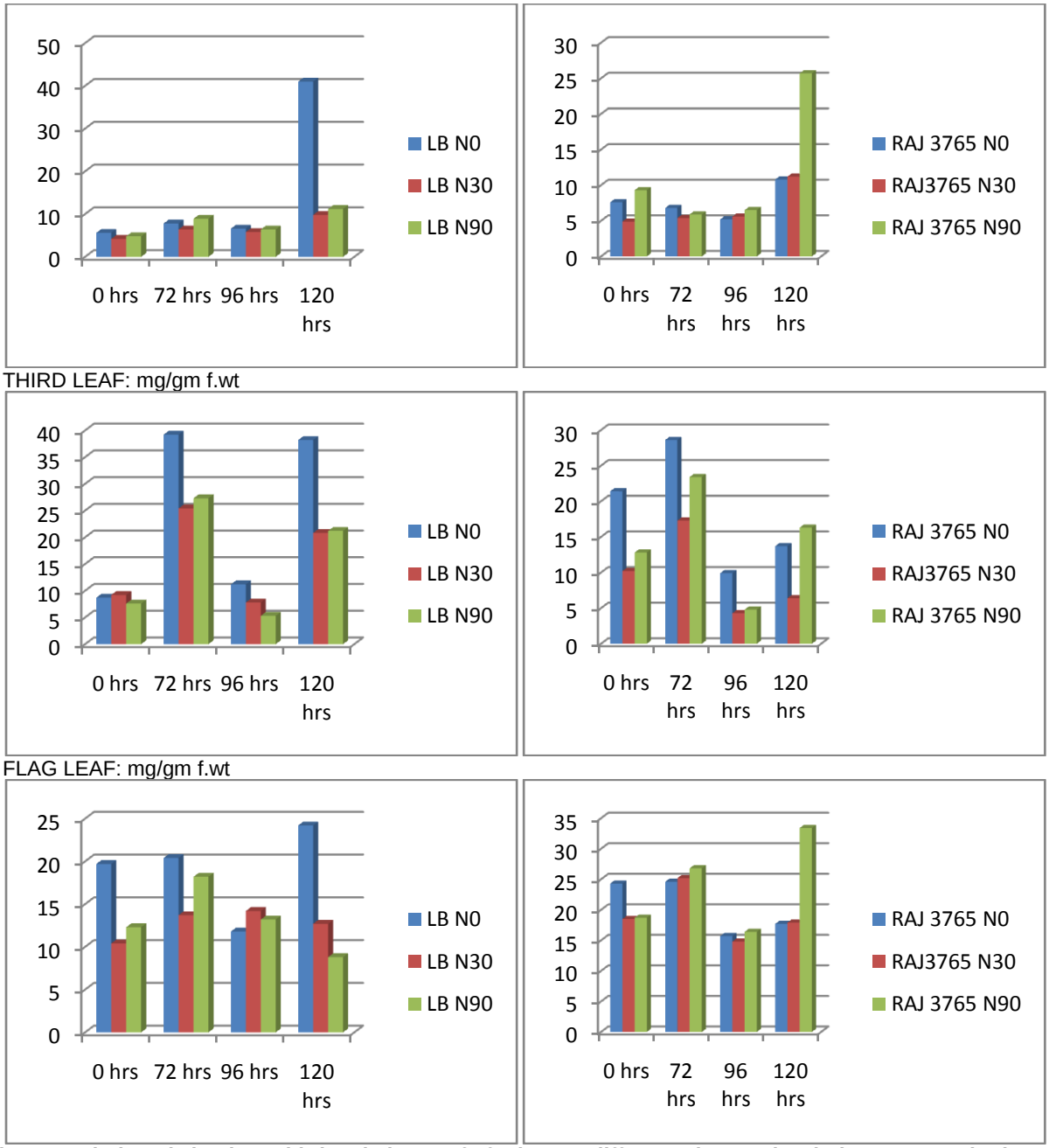

Fig 2: Total Phenols in First, Third and Flag Leaf of Wheat at different Nitrogen levels (N0, N30, N90). The variety of treatment LB= Lal Bahadur, The Susceptible variety, RAJ 3765= The Resistant Variety