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ALTERED ACTIVITY PROFILE OF IAA OXIDASE IN FIRST, THIRD AND FLAG LEAF OF WHEAT DURING INTERACTION WITH *PUCCINIA RECONDITA* AT DIFFERENT NITROGEN LEVELS

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IAA oxidase activity was conducted on susceptible Lal Bahadur and Resistant Raj 3765 wheat cultivars at first third and flag leaf stages. Inoculated with *Puccinia recondita* at different nitrogen levels i.e. N_0, N_{30}, N_{90}. The first leaf of susceptible variety showed a drastic decrease in the IAO activity at 72h at (N0, N30 and N90) nitrogen levels, whereas the third and Flag leaf at lower nitrogen levels (N0+N30) showed reverse trend. The resistant interaction reflected significant increase in IAO at 72 irrespective of the nitrogen levels. The differential changes observed during progressive rust infection illuminate the issue of host-pathogen correlation with IAO activity during disease causation and establishment.

Keywords IAA Oxidase Activity; Nitrogen levels; *Puccinia recondita*; Wheat.

Introduction

The physical characteristics are mechanical barriers, which prevent the entry and spread of pathogen. The chemical factors, which are toxic to the pathogen, inhibit its growth and activity in the host. Similarly, the pathogen may produce certain compounds against the host. The present study reports the changes in the activity of the IAA oxidase of the susceptible Lal Bahadur and Resistant Raj. 3765 wheat leaves infected with brown rust pathogen, *Puccinia recondita* F. Sp. tritici Rob. ex Desm. The pathogen race was mixture of 12-2, 77-2, 104-2 procured from Directorate of wheat Research, Regional station Flowerdale, Shimla (India).

In the present studies, the total nitrogen concentration was given initially in one dose in plastic pots at the time of sowing (not in the split dose as in the case of field). The studies were conducted at early stages of plant growth (i.e. first and third leaf) and Later stage of plant growth (i.e. flag leaf). First, third and flag leaf and critical stages during the growth of the wheat plant, first leaf arises during the germination of seed and third leaf emergence after about 15 days prior to the tillering of the plant whereas the flag leaf arises in mature plant and contributes about 25% in yield production. The study was performed in susceptible as well as resistant cultivars at all stages to see if any correlation exists between levels of nitrogen doses, stages of the plant and resistance toward the test pathogen.

Materials and Methods

Wheat seeds of resistant and susceptible cvs were sown in polyethylene bags containing mixture of sand and different concentrations of Urea with 46.5% of nitrogen, the pH of sand was 8.3, The concentration of nitrogen (Urea) was 0 Kg/hectare, 30 kg/hectare and 90 Kg/hectare.

First, third and flag leaves of wheat plants previously sprayed with water, were inoculated with urediospores mass powder, containing spore mass 10 mg/ 100 leaves approximately. The inoculation of the leaves was carried out in the evening hours (4-6 p.m.) and the plastic pots carrying plants were subsequently watered and covered with wet polyethylene bags for maintenance of sufficient humid environment (100% R.H. overnight) favourable for rust infection. After 24h, the bags were removed, for sampling, the inoculated wheat leaves were harvested and collected at various time intervals i.e. oh, 72h, 96h and 120h, After inoculations, the fresh leaves were collected prior to inoculation served as 0h sampling i.e. control. The samples collected were subjected to analysis of IAA oxidase specific activity, assayed spectrophotometrically. Some seedlings were left after the inoculation and transferred to green house upto 10-12 days to confirm the infection by formation of Urediospores of brown rust.

Results and Discussion

Physical changes during disease development Following the rust infection wetted water streak appeared along the veins on the leaf upto 24h after inoculation and through successive stages. There was an appearance of more clearer fleaks towards 72h after inoculation and ultimately two weeks of infection, different interaction response was noticed in nitrogen levels (N_0, N_{30} and N_{90}) (Table 1).

IAO Activity during disease development (Fig. 1) Leaf samples were collected as described under methods and IAO activity were determined and for the sake of convenience the results are given for each leaf stage separately.

First Leaf - The susceptible var. showed at all the three-nitrogen fertilizer level, a drastic decrease in the IAO activity at 72h, Later on, the activity level was found more or less constant throughout the period of determination. The resistant var. showed more or less similar trend in IAO activity with more variations.

Third Leaf-At the third leaf stage the susceptible var. at lower nitrogen levels (N_0 + N_{30}) showed initial sharp increase in IAO activity followed by drastic decrease at 96h. Again it tended to show minor recovery. But at the N_{90} level the IAA oxidase activity gradually decrease upto 96h followed by recovery at 120h. The resistant interaction reflected significant increase in IAA oxidase activity at 72h irrespective of the nitrogen levels. Afterward it declined upto 96h at N_0, N_{90} levels and upto 120 h at N_{30} level. However in the former (at N_0, N_{90}) it recovered at 120 h.

Flag Leaf- During the flag leaf brown rust interaction, the susceptible var. showed increase of IAO activity during the initial period of 72h. The activity levels declined at 96h except the N_0 level where continued increase was recorded up to 96h and a sharp decline at 120h. However, in N_{30} and N_{90} levels the IAA oxidase activity become more or less stable at 120h.

The resistant var. also showed an increase in the enzyme activity at the initial stage of determination (i.e. 72h) Later on a continuous steep decrease was found at the high nitrogen level as compared to low nitrogen level. At the final stage of determination (i.e. 120h) the N_0 level reflected much higher activity profile as compared to the other two-nitrogen fertilizer levels.

The brown rust infection seemed to progress through different stages of infection including germination, appresorium formation, penetration of the host, haustorial development in the host cell and formation of secondary hyphae etc. in succession. The appearance of different interaction symptoms were noted after two week, indicated that the morphogenesis of the invading pathogen during the infection process may be specifically inhibited according to the particular incompatible genotype (resistant) inoculated in the host-pathogen interaction.[2,3] The residual leaf mass was found to decrease as the infection progressed in all the interactions upto 48h but this trend was reversed in resistant interaction (Race-63) at 72h and remained almost constant in the remaining two interactions.[3] In the present study, disease progression index showed relatively much increase in Lal Bahadur and Raj 3765 at N_{90} level was followed by N_0 and N_{30}. The former var. showed formation of pustules in the leaves after two weeks but later not showed the pustule formation after two weeks (Table 1) .

IAA metabolism is directly concerned with the expression of resistant by host cells. The high rates of decarboxylation of exogenous IAA may be only fortuitous expression of other metabolic activities that are actually concerned directly with resistance but that can govern simultaneously metabolism of exogenous compounds unrelated to resistance or susceptibility. Although emphasis has been placed on IAA in cereal rust disease[4]. It has been assumed that IAA oxidase controls IAA concentrations. The increase in IAA is associated with growth disturbances and reduction in IAA oxidase activity in homogenates of infected tissues.

In the first leaf, a drastic fall in IAA oxidase activity at 72h was characteristic of the progressive rust infection where the IAA contents may be in very high concentration which may continue to occupy the prime position even after infection of the rust pathogen resulted into decrease in IAA oxidase specific activity. Although this study does not permit any definite conclusion regarding the specificity of the susceptible and resistant varieties. However, it was evident in susceptible, particularly in resistant var. that the high fertilizer dose was responsible for low activity profiles at 72h onwards. It may be due to the presence of an inhibitor produced by the rust fungus, induced by correlation of the enzyme inhibition with the amount of rust in the tissue[5]. Similar relationship between rust and IAA oxidase activity is evident in the present studies.

In third leaf, no well evident conclusion regarding the specificity of the susceptible and resistant interaction was possible. However, more significant increase in IAA oxidase specific activity at the initial stage i.e. 72h N_0, N_{30} levels in susceptible var. and at all the three nitrogen levels in resistant var. was found. It was followed by sharp decline and subsequent recovery at later stage. There may be some host-parasite interaction at initial stage resulting in decline of auxin and protein contents and increase in IAA oxidase activity. Infection of the plant results in injury and other deformities which in turn cause high enzyme activity[6]. In present studies after the establishment of rust infection there appears some competition between host and parasite causing increase in auxin contents so the IAA oxidase activity again comes down in both the varieties. At N_{90} level, the susceptible variety showed continuous decline in IAA oxidase activity upto 96h, which might be due to participation of high nitrogen for the instant higher growth of the host by increasing the IAA concentration. However, in the resistant variety, the higher nitrogen was applicable for resistance. The N_0, N_{30} and N_{90} levels in susceptible var. showed final recovery of enzyme activity, which may proceed to rust infection by suppressing the auxins in plants. The enhanced IAA oxidase activity may be correlated with increased phenols, specially

Table 1. Summary chart of physical observation during progressive rust infection by (P. *recondita*) first and third leaves of wheat.

Wheat variety	Nitrogen levels	Duration after Inoculation						
		12h	24h	48h	72h	96h	120h	two weeks
Lal Bahadur	N_0	Wetted water streaks along veins appeared	Wetted water streaks more prominent	Visible faint pale spots occured over 20-25% leaf surface area	Visible pale spots occured over 55% leaf surface area	Observation as shown in 72h	55-60% area covered with visible pale spots	Uredosori formation at 60% area of leaf surface partial or total yellowing of leaf
	N_{30}	-do-	-do-	at 10-15% area	slight yellow patches scattered leaf surface 40-45% leaf area	-do-	scattered yellow patches and mild pale spots occured on	prominent yellowing of leaves in patches, very few uridosori formation
	N_{90}	-do-	-do-	at 30-35% area	-60-65% area appeared with visible pale spot	-do-	70-75% leaf area covered with prominent pale sport	70-75% uredosori formation with clear visible pale spots
RAJ. 3765	N_0	-do-	-do-	at 15-20% area	at 40-45% area	-do-	40-45% leaf area covered with pale spots were not much prominent as in Lalbahadur	N_0 uredosori formation leaves are looking healthy and green
	N_{30}	-do-	-do-	Not much prominent	partial yellowing and some pale spots appeared	-do-	yellow patches becomes less as compared to susceptible	-do-
	N_{90}	-do-	-do-	at 15-20%	55-60% area covered with pale spots	-do-	55-60% area covered with fainted pale spots	-do-

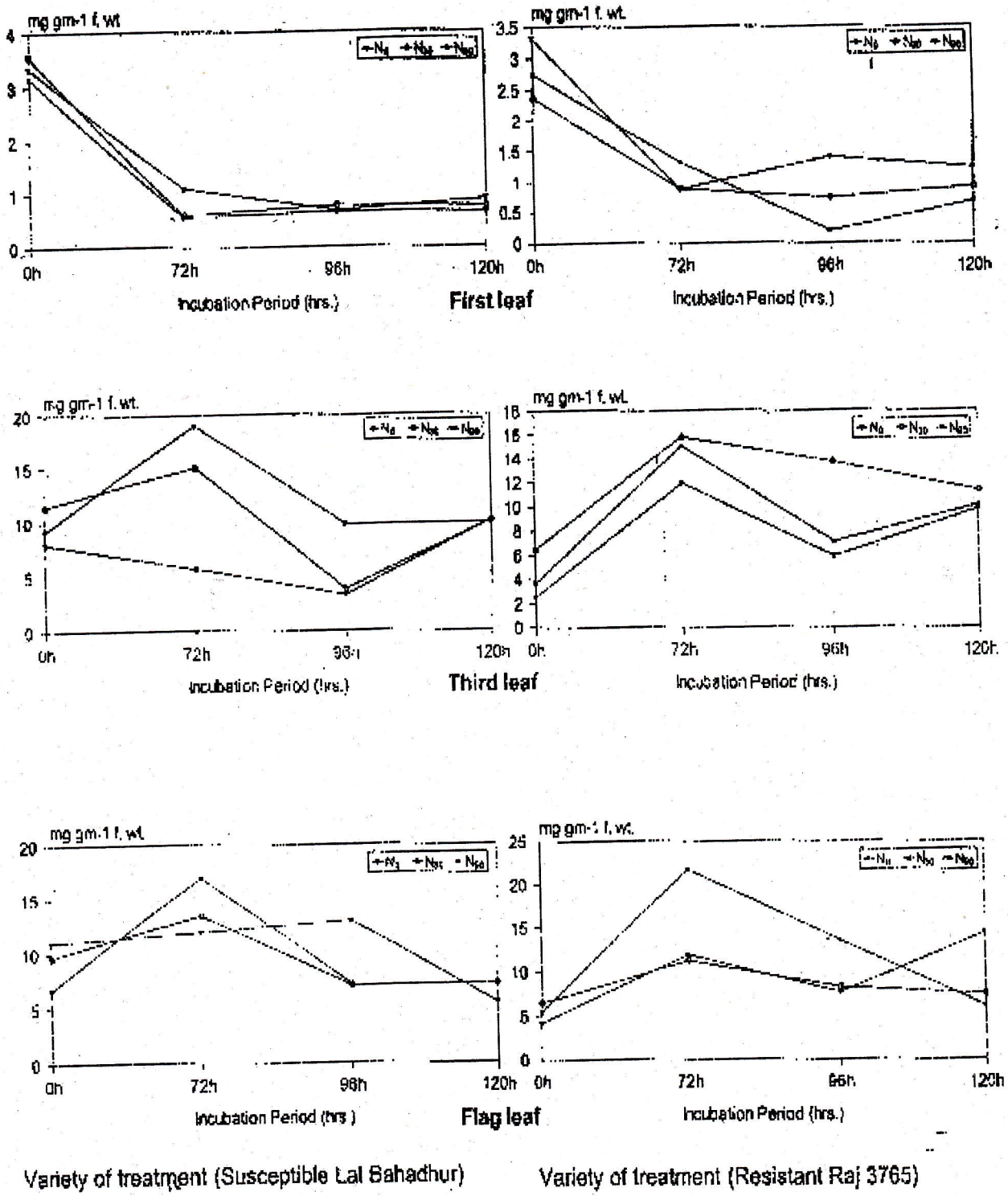

Fig. 1. IAA oxidase specific activity in first, third and flag leaf of wheat at different nitrogen levels (N_0, N_{30}, N_{90})

monophenols which might have promoted IAA-oxidase[7]. In our study of IAO at N_{30} level, associated with an overall decrease in concentration of phenols and might be due to more of monophenols. The higher nitrogen level (N_{90}) in susceptible var. showed sharp recovery of IAA oxidase activity due to increase in susceptibility towards the pathogen because the higher concentration of nitrogen or due to the accumulation of nitrogenous contents in the tissues. Enormous increase in the protein-nitrogen and protein-aminoacids observed in the host-pathogen interaction. It may also be ascribed to some extent, to be responsible for the observed increased protein content of the infected tissue. This may also be resulted into increasing IAA oxidase specific activity of tissues. As reported that the plants became more succulent in high N treatment and are more vulnerable to infection[8].

Two distinct points were observed about the rust infection physiology in terms of IAA oxidase specific activity in flag leaf. The first point remained about the general concept of progressive rust infection which was independent of the nature of the genotype in terms of increased activity upto 72h in both varieties and later on declining upto 96h in susceptible followed by recovery and upto 120h resistant variety irrespective of nitrogen levels. And the other was about the specificity to the resistant interaction where the enzymatic activity levels were more significant as compared to susceptible var. and that too particularly at higher nitrogen level (i.e. N_{90}). It seems that high dosage of nitrogen nutrition is favourable for pathogen multiplication which showed the higher enzyme activity at initial hour of inoculation which might be resulted into low auxin content by significant damage to plant and grain wt. However, the activity declined sharply in resistant var., may be due to the presence of resistant factor. The N_0 level in both the varieties of flag leaf showed increase in IAO activity upto 72h which was more significant in susceptible var. It may be due to the limiting factor at zero nitrogen level which imparts the susceptibility to host. The stage of disease development that can influence nitrogen contents of the infected tissues. Generally, in fungus-infected plants, the total nitrogen and protein contents increase [9].

It is important to note that in the later stages of disease development, the total nitrogen contents and proteins of the fungus infected plant organs usually decrease. As we come to the conclusion that in all the fertilizer treatments of the plants, the intensity of the diseases was more on flag leaf than on the younger leaf. It is evident in several studies that the disease caused by obligate parasites is accompanied by changes in concentration of hormones. But the functional significance of the changes is not known .

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