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ROLE OF ALLOPURINOL IN THE ACTIVATION OF DEFENCE RELATED ENZYMES IN WHEAT

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Present study focused on effect of allopurinol on defence related enzymes peroxidase, catalase, lipid peroxidation and superoxide dismutase in rust susceptible wheat var. 'Agra local'. All the enzyme activity was greatly influenced by the allopurinol treatment.

Keywords : Allopurinol; Catalase; Lipid peroxidation; Peroxidase; Superoxide dismutase; Wheat var. 'Agra local'.

Introduction

In India wheat is the main food crop next to rice but suffers from many diseases caused by fungi. Among these, rust of wheat is very severe[1]. The maximum loss due to rust is reported in wheat[2]. The reduction or prevention of crop losses caused by infectious plant diseases assumed increasing importance.

Indirectly enzymes play an active role in plant disease controlling. Shewry and Lucas[3] reported role of the defence related enzymes in disease resistance. According to Jebakumar *et al.*[4] induction of peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase and pathogenesis related proteins activities are one of the important aspects of the defence mechanisms of plants against the invading pathogens. Veeramohan and Ramaswamy[5] reported an increase in the activity of oxidative enzymes such as polyphenol oxidase and peroxidase in chilli leaves infected with *Alternaria solani*. The enzyme participating in the synthesis of phenolic compounds, polyphenol oxidase and peroxidase are known to play important role in the host parasite relationships[6].

Allopurinol (4-hydroxypyrazolo [3,4-d] pyrimidine) a purine analogue inhibitor of xanthine oxidase enzyme attained prime importance in inhibition of leaf rust caused by *Puccinia recondita*[7]. Currently it is used to increase wheat seed dormancy in field[8] and to manage number of plant diseases[9]. Marte and Montalbini[10] have also tried its efficacy on biotrophic growth of some powdery mildew fungi in their specific hosts. In addition, plant growth regulatory activity of allopurinol[8], its effects on photosynthate partitioning in yam bean[11], and on the development of rust disease on bean[9] has been reported.

From the foregoing literature survey it is very clear that allopurinol plays a major role in developing defence mechanism or disease resistance by changing physiological metabolism. In the present investigation allopurinol was used, in different concentrations (25, 50, 75 100 µM) on rust susceptible wheat var. 'Agra local', to know their effect on development of defence mechanism through pre-treatment and physiological attributes which are (one or the other way) involved in defence mechanism against pathogen. These physiological attributes include defence related enzymes peroxidase, catalase, lipid peroxidation and superoxide dismutase, which are studied by giving pretreatment. The results obtained are discussed in relation to defence mechanism and development of disease resistance in light of recent literature. Hopefully the study will provide an incite to develop a chemical manipulation strategy against rust.

Material and Methods

The seeds of rust susceptible wheat *(Triticum aestivum* L.) var. 'Agra local' were obtained from Regional Wheat Rust Research Station, Mahabaleshwar, Dist. Satara (Maharashtra). They were surface sterilized with 0.1% $HgCl_2$ for 1 min and washed repeatedly with distilled water and dried at room temperature. Surface sterilized seeds (20) were kept in sterilized petridishes (9 cm in diameter) over Whatman No. 1 filter paper at room temperature. The filter paper was moistened with 10 ml glass distilled water in control and allopurinol at 25, 50, 75 and 100 µM concentrations in respective petridishes. The seeds were allowed to germinate at room temperature (28 to 30°C).

Fig.1.

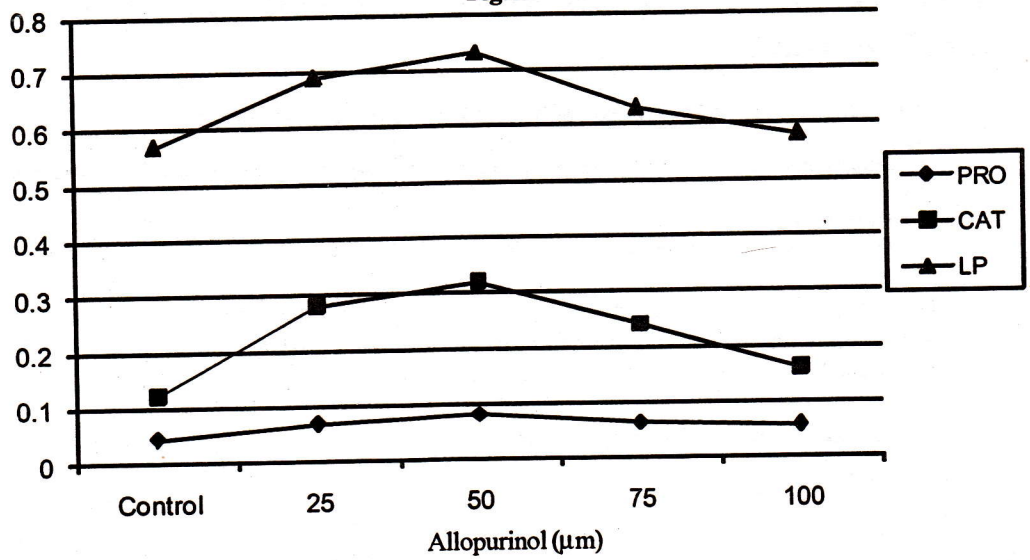

Fig.2.

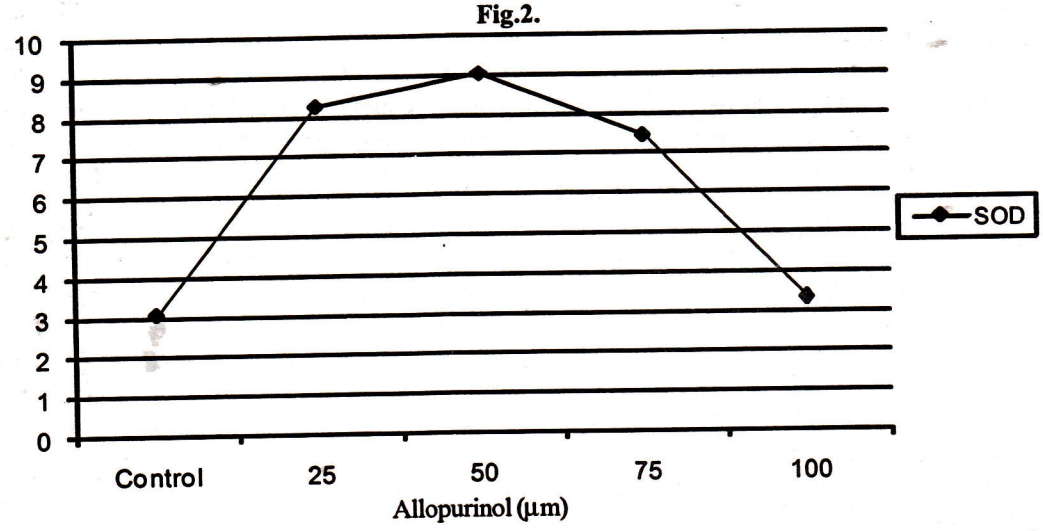

Fig.1-2. Effect of Allopurinol on enzyme peroxidase (PRO), catalase (CAT), lipid perodixation (LP) and superoxide dismutase (SOD) in wheat var. 'Agra local' after 72 h of germination.

The activity of various enzymes was determined, *viz.* peroxidase studied by using the method of Maehly[12], catalase by Sadasivam and Manickam[13], lipid peroxidation by Carkmak and Hort[14] and superoxide dismutase by Giannopolitis and Ries[15], in 72 h old seedlings.

Results and Discussion

The peroxidase activity increased by allopurinol at 50 µM concentration over control. The other concentrations also showed stimulatory effect over control (Fig. 1). The catalase activity exhibited marked stimulation over control. Though 75 and 100 µM allopurinol exhibited reduction in catalase activity, the reduction was not gone down below the control values (Fig. 1). All the concentrations of allopurinol exhibited increase in level of lipid peroxidation (Fig. 1) and superoxide dismutase (Fig. 2) activity. The highest activity was observed in 50 µM allopurinol. Though the higher concentrations of allopurinol (75 and 100 µM) showed reduction in the activity, this reduction was not below the level of control.

Enzyme peroxidase plays pivotal role in lignin synthesis and auxin catabolism[16] which indicates role of peroxidase in plant growth and development. In the present

investigation increase in peroxidase activity was recorded due to allopurinol treatment. The increase in the peroxidase activity may contribute to decrease the intensity of oxidative stress in allopurinol treated seedlings.

Catalases belong to groups of enzymes involved in regulating the cellular levels of active oxygen species. Catalase plays an important role in protective mechanism against oxidative stress and is a sink for H_2O_2 and is indispensable for stress defence[17]. The stimulation of catalase activity in lower concentrations of allopurinol can increase the resistance of seeds to oxidative stress and cause growth stimulation of the seedlings and may ultimately help in induction of defence mechanism in plants.

Malondialdehyde, decomposition product of lipid peroxidation present in host cell membranes is often found to increase during pathogenesis. Lipid peroxidation is a widely used stress indicator and it has been shown to mediate photoperoxidative damage and destruction of chlorophyll[18], causes drought induced increase membrane permeability[19]. Recently, Chaudhuri and Chaudhuri[20] have recorded a high degree of membrane damage in sensitive jute cultivars, due to increased rate of lipid perodixation in response to salinity stress.

In pants, the role of superoxide dismutase during environmental adversity such as drought, chilling, hypoxia, temperature, high light intensity and pathogenic injury have been correlated with superoxide dismutase activity[21]. Cross-tolerance to man-made oxidants (herbicides) and natural environmental stresses have also been reported[22]. In the present investigation the higher rates of lipid peroxidation and superoxide dismutase suggest an enhanced production of activated oxygen species due to allopurinol and at higher concentration, the ability of the plant metabolic machinery to scavenge these radicals is apparently diminished. The present study has provided basic incite to undertake further studies of chemical manipulation of wheat rust management.

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