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EFFECT OF SALICYLIC ACID ON SOME BIOCHEMICAL ASPECTS IN RUST SUSCEPTIBLE WHEAT VAR. 'AGRA LOCAL'

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A pot experiment was conducted to study the influence of foliar application of various concentrations of salicylic acid (25,50,75 and 100μM) on polyphenols, nitrogen and defence related enzymes viz. peroxidase and phenylalanine ammonia-lyase in highly rust susceptible wheat var. 'Agra local'. Salicylic acid (50μM) application exhibited increase in polyphenol content while decreased the nitrogen content. The activity of peroxidase and phenylalanine ammonia-lyase was also stimulated by 50 μM salicylic acid application. None of the other concentrations of salicylic acid reduced the enzyme activity below the level of control value. Perusal of the data indicates that foliar application of salicylic acid would help in the development of disease resistance in wheat against rust.

Keywords : Disease resistance; Salicylic acid; Wheat rust.

Introduction

Studies during last several years have established that salicylic acid (SA) plays an essential role in plant disease resistance[1]. In many plant species, elevated level of SA was found associated with resistance of the infected plant to the invading pathogen[2]. Polyphenols represent a secondary product of plant metabolism, accumulate in all infected plants[3]. Several reasons have been ascribed for accumulation of polyphenols in diseased plants, the major one is a defence mechanism adopted by the host plant[4]. There is an extensive literature on the effect of nitrogen on plant diseases because of their role in mediating diseases resistance. A high nitrogen concentration increases the severity of infection by obligate parasites, such as rust and smuts, but has the opposite effect on diseases caused by facultative parasites such as *Alternaria, Fusarium* and most bacterial diseases.

The enzymes are frequently involved in explaining disease resistance have well established[5]. Jebakumar *et al.*[6] concluded that induction of peroxidase (POD), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL) and pathogenesis related protein activities are one of the important aspects of the defence mechanism of plants against the invading pathogens.

Salicylic acid is known for its antipathogenesity, has been employed in the present investigation. This chemical was used in different concentration to study their effect on highly rust susceptible wheat var. 'Agra local' particularly to know their effect on development of defence mechanism through physiological attributes which are one or the other way involve in defence mechanism against pathogen.

Material and Methods

The surface sterilized seeds of wheat (*Triticum aestivum* L.) var. 'Agra local' were sown in an earthen pots containing soil mixed with farm yard manure (FYM) in the proportion of 3:1 and sterilized by treating with 5% formalin to kill the soil borne fungal pathogens. Wheat (8 plants/pot) were raised in natural light (1600 $\mu Em^{-2}s^{-1}$) with temperature 30±2°C and 19±2°C day - night and kept free from diseases and pests.

The aqueous solution of different concentrations of SA (25,50,75 and 100 μM) was used for foliar application. Fifteen days old plants were sprayed with respective concentrations for 5 times at an interval of 8 days upto drain-out point at 10 a.m. using manually operated air pneumatic spray pump. The control plants were sprayed with equal amount of distilled water. The plants were analysed for defence related enzymes peroxidase and phenylalanine ammonia-lyase and organic constituents such as polyphenol and nitrogen.

Polyphenols were estimated by the method of Folin and Denis[7] and nitrogen by Hawk *et al.*[8] The peroxidase activity was determined by the method described by Maehly[9] and PAL by Mahadevan and Shridhar[10].

Results and Discussion

The total polyphenol and nitrogen content analysed from leaf tissue of wheat var. 'Agra local' under the influence of salicylic acid is given in Table-1. It is very clear from the table that polyphenol content was stimulated by all the concentrations of SA but the maximum stimulation was observed in 50μM treatment. 75 and 100μM treatment exhibited inhibitory trend but the inhibition was not below the level of control.

Table 1 : Effect of foliar application of different concentrations of salicylic acid on total polyphenol and nitrogen content in the leaves of highly rust susceptible wheat var. 'Agra local'.

Treatments	Polyphenol (%)	Nitrogen (%)
Control	0.44	0.047
25 μM	0.61	0.025
50 μM	0.87	0.021
75 μM	0.72	0.028
100 μM	0.53	0.030

The values are mean of three determinations.

It is well known that the phenolic level is higher in diseased plants than in healthy ones[11]. According to Bhaskar *et al.*[12] potassium application exhibits positive correlation with phenol content. It indicates that the application of potassium increase the synthesis of phenols, inturn phenols increase the crop resistance to disease by inhibiting pathogen growth. Biswas[13] also reported accumulation of phenols inhibit pathogen growth. As such phenolics have been associated extensively with the chemical defence of plants against microbes, insects and other herbivores[14]. Similarly foliar spray of aqueous solution of acetylsalicylic acid induce resistance in barley, potato and sugar beet against various biotic and abiotic stress[15].

The nitrogen content was greatly affected by SA and there is large reduction in total nitrogen content of SA applied plants than that of non-applied one. The maximum inhibition was noted at 50μM as compared to other concentrations. In the obligate parasitic fungal diseases viz. rust, mildews, clubrots the high nitrogen increases the susceptibility of the plants to disease[16]. Since SA suppresses nitrogen content, it can safely be recommended for chemical manipulation of rust disease. According to Gour *et al.*[16] experiments with the stem rust disease of wheat (*Puccinia graminis f.sp. tritici*) infection type of the host had never changed by nitrogen treatments, however the number of infection sites usually increase with increasing nitrogen. Similar results were observed by Last[17] in powdery mildew of wheat.

The POD and PAL activity studied under the influence of different concentrations of SA exhibited marked stimulation over control (Fig. 1). The POD and PAL activity increased with increasing concentration of SA upto 75μM, while 100 μM showed reduction in enzyme activity but this reduction was not below the level of control.

The lignification is now being recognized as an important defence mechanism of plants against microbial attack[18]. In some plant-pathogen interactions, increased levels of POD were shown to be closely linked to induced lignification as a mechanism of disease resistance[19]. POD is also postulated to be involved in the cross-linking of phenolics of (glyco) proteins in the plant cell wall[20], which in turn has been proposed to be a factor in resistance of plants to pathogens[21]. Many plant tissues exhibit an increase in their POD activity in response to infection with viruses, bacteria and fungi[22]. Increased levels of POD were also reported by Veeramohan and Ramaswamy[23] in chilli leaves inoculated with *Alternaria solani* and in wheat infected with *Puccinia recondita* by Jonson and Cuningham[24]. Stimulation of POD activity after 72 h of inoculation of *Fusarium oxysporium* in tomato was shown by Paz *et al*[25]. Similar results were also observed by Caruso *et al*[26].

Several reports suggest that PAL is a key regulatory enzyme in the synthesis of SA and establishment of systemic acquired resistance (SAR). Mauch-Mani and Slusarenko[27] showed that in *Arabidopsis*, PAL activity is essential for accumulation of SA and expression of the hypersensetive response (HR). Several studies of PAL promoter activity in transgenic plants demonstrated activity in vascular tissues, where PAL is thought to provide the precursors for lignin deposition in the xylem[28,29]. According to Nicholson and Hammerschmidt[30] PAL and PPO activities are the markers of biochemical resistance in wheat to *Neovossia indica*.

Systemic acquired resistance (SAR) involves salicylic acid as a primary signal, which is the only plant-derived substance that can also be applied exogenously. In this respect Padmaja and Jayraman[31] studied effect of SA (2mM and 3mM) on peroxidase and PAL in cotton. Their study revealed that treatment with SA increase POD and PAL activities, however maximum of these enzyme activities were in case of *Gossypium barbedense* treated with 3mM SA at 16 h intervals.

The stimulation of POD and PAL due to salicylic acid in highly susceptible wheat var. 'Agra local' to rust infection possibly bring conversion of phenolic compounds to a toxic substances like quinone to minimize disease intensity. The reduction in nitrogen and stimulation of polyphenol content due to foliar application of SA in the present investigation is indicative of the fact that it could be used in formulation of chemical control strategy for rust disease.

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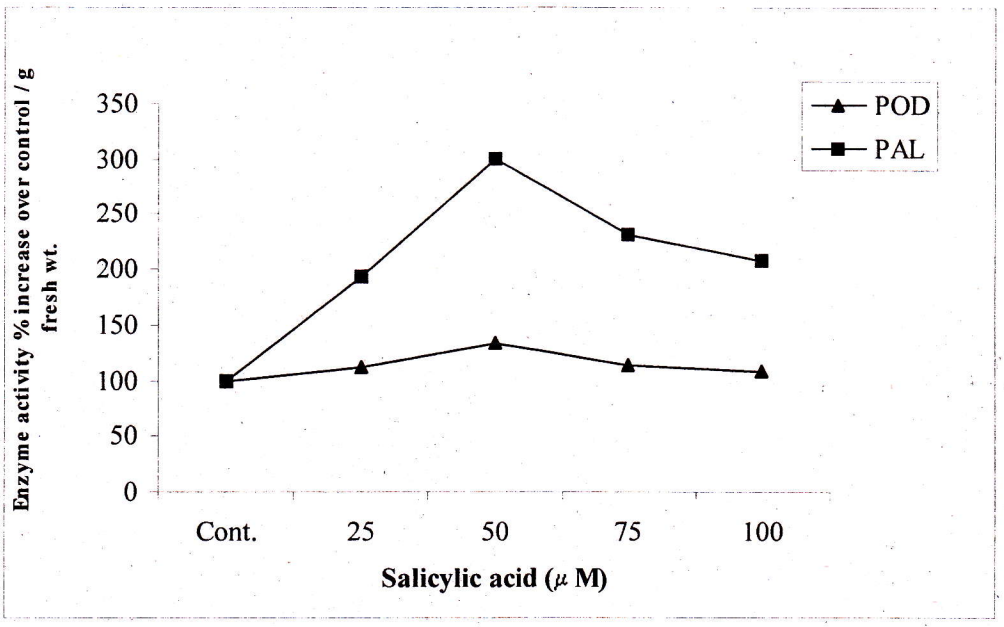

Fig. 1. Effect of foliar application of different concentrations of salicylic acid on enzyme peroxidase (POD) and phenylalanine ammonia-lyase (PAL) in highly rust susceptible wheat var. 'Agra local'.

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