Asian J. Exp. Sci., Vol. 23, No. 1, 2009; 249-252

Phenols with their Oxidizing Enzymes in Defence against Black Spot of Rose (*Rosa centifolia*)

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Abstract : Total phenols with their oxidizing enzymes, peroxidase and polyphenol oxidase in diseased leaves of Rose (*Rosa centifolia*) caused by *Alternaria tenuis* were measured at different progression periods of infection.There was an increase in the activity of peroxidase and polyphenol oxidase in host plants in response to infection, which was found to be coupled with an increased phenol content in disease leaf tissue as compared to the healthy tissues. This clearly reveals that the elicitation of the enzymes in response to infection is directly correlated with the resistance of the plant.

Key words : Peroxidase, Polyphenol oxidase, Total phenol, *Rosa centifolia*, *Alternaria tenuis.*

Introduction

Rose (*Rosa centifolia*) belonging to the family Rosaceae is an important floricultural plant and enjoys a high demand of premium price due to its glorious and beautiful flowering pattern and also having the value for preparation of good quality perfumes. Black spot disease of rose (*Rosa centifolia*) caused by *Alternaria tenuis* which was recorded a new from India by Khatun *et al*., (2008) is a major problem posing a serious threat to floriculture. The disease causes browning / blackening of the leaves resulting in enormous loss to the total photosynthates of the plant followed by reduced yield of the plant. Increased activity of polyphenol oxidase and peroxidase in response to infection by the pathogen has been reported by many workers (Vidyasekaran, 1988; Karthikeyan and Bhaskaran, 1992; Chatterjee and Banik, 1993; Ojha *et al* , 2005) and is considered to play an active role in contributing to disease resistance in certain plant host-pathogen interaction following infections. Higher levels of total phenols following infection with the pathogens have been reported by previous workers (Majumdar and Pathak, 1989; Jyosthna *et al*., 2004; Madhavi *et al*., 2005; Anand *et al*., 2007) where phenols may play an important role as post-infectional factors in the disease resistance. Changes in phenol and phenoloxidizing enzyme activities in black spot affected rose leaves were determined in the present communication with a view to correlate the resistance of the host through elicitation of its defence system.

Materials and Methods

The young healthy rose plants in the Departmental garden, were inoculated with the causal pathogen. Following inoculation, symptoms appear on the leaves. After 7 days interval of inoculation, total phenols and peroxidase and polyphenol oxidase activities were measured.

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Extraction and assay of Peroxidase

To extract the enzyme, 100 mg of each of infected and healthy leaf tissues were ground separately with a pinch of neutral sand in 20 ml. cold distilled water in a mortar at 0°C. The extract was obtained by filtering off the debris with a clean cloth and centrifuging at 3000 rpm for 15 minutes in a refrigerated centrifuge. The supernatants were recovered and kept in a tube in an ice bath until assayed.

Peroxidase activity was estimated following the method of Mahadevan and Sridhar (1982). 5 ml of freshly prepared pyrogallol reagent (prepared by mixing 10 ml of 0.5 M pyrogallol solution and 12.5 ml of 0.66 M phosphate buffer and the volume made to 100 ml. with distilled water) and 1.5 ml of the enzyme extract were mixed in a spectrophotometer tube and the mixture was immediately adjusted to zero absorbance of a spectrophotometer. 0.5 ml of 1% H_2O_2 solution was added to it and the content was mixed by inverting the tube. The reaction was initiated by the addition of H_2O_2. Enzyme activity was recorded as the change in absorbance per minute (ΔA / mint/δ) at 430 nm immediately after the addition of substrate. Similarly, control of non-enzymatic oxidation was maintained by heating the extract at 100°C where the activity was always measured zero indicating its complete inactivation by the heat treatment.

Extraction and assay of polyphenol oxidase

100 mg of healthy and infected leaf tissues were homogenized separately with a pinch of sand in 6 ml. Phosphate buffer of 0.1 M at pH 7.0 at 0°C. The extract was filtered with a clean cloth, centrifuged at 3000 rpm for 15 minutes and stored in an ice-bath until used. Polyphenol oxidase activity was measured by the method of Sadasivam and Manickam (1996). 2 ml of enzyme extract and 3 ml. of distilled water were mixed together in a spectrophotometer tube and adjusted to zero absorbance of a spectrophotometer. 1 ml of catechol solution (0.4 mg / ml) was added to the above mixture and the reactants were quickly mixed. The enzyme activity was measured as the change in absorbance per minute (ΔA / mint.) at 490 nm immediately after the addition of catechol solution which initiated the reaction. Control in similar manner was maintained by heating at 100°C which always showed zero absorbance.

Extraction and estimation of total phenol

1 gm of healthy and infected leaf tissues were cut into pieces of 1-2 cm. They were kept in 5-10 ml. 80% ethyl alcohol immediately and allowed to boil for 5-10 mint. in a hot water bath. The extract was cooled in a pan of cold water. The tissues were crushed thoroughly in a mortar and pestle for 5-10 minutes, then passed through a double-layered cloth. The ground tissue was extracted in boiling 80% alcohol, it was cooled and passed through Whatman's No. 1 filter paper.

Total phenol was estimated by the method of Mahadevan and Sridhar (1982), 1ml. of alcoholic extract was pipetted in a graduated tubes. Then 1 ml. of folin-ciacalteau reagent was added followed by 2 ml. of 10% Na_2CO_3 solution. The tube was shaken and heated in boiling water bath for 1-2 minutes. The tube was cooled under running tap water. The blue solution was diluted to 25ml with distilled water and absorbance of it was measured at 650 nm in spectrophotometer. A control containing all the reagent except plant extract should be used to adjust the absorbance at zero.

Results and Discussion

The results revealed that the activity of both the phenol oxidizing peroxidase and polyphenol oxidase was higher in infected leaf tissues than in uninfected ones and that it increased considerably with the increase in progression of infection (Table 1). Phenol

Table 1 : Peroxidase and Polyphenol oxidase activity in healthy and Alternaria infected leaves of Rosa centifolia at different periods of infection*

Days after Infection	Peroxidase activity g^{-1} min^{-1}		Polyphenol oxidase g^{-1} min^{-1}	
	Healthy	Infected	Healthy	Infected
0 days	0.040 ± 0.000	0.042 ± 0.002	0.036 ± 0.002	0.038 ± 0.002
7 days	0.062 ± 0.002	0.222 ± 0.007	0.048 ± 0.002	0.248 ± 0.014
14 days	0.052 ± 0.002	0.282 ± 0.002	0.054 ± 0.002	0.328 ± 0.012
21 days	0.046 ± 0.002	0.330 ± 0.012	0.056 ± 0.002	0.460 ± 0.018

For Peroxidase: CD at 5% = 0.073, For Polyphenol oxidase : CD at 5% = 0.103, SEM = ± 0.025, SEM = ± 0.036
** Data are mean values of five replicates*

Table 2 : Total Phenol in healthy and Alternaria infected leaves of Rosa centifolia at different periods of infection*

Days after infection	Healthy Total Phenol (at 650 nm)	Infected Total Phenol (at 650 nm)
0 days	0.522 ± 0.009	0.550 ± 0.016
7 days	0.536 ± 0.007	0.850 ± 0.000
14 days	0.542 ± 0.004	0.984 ± 0.002
21 days	0.532 ± 0.010	1.152 ± 0.142

Total Phenol : CD at 5% = 0.174; SEM = + 0.063
** Data are mean values of five replicates*

content was found to be increased simultaneously in diseased leaf tissues in comparison to the healthy tissues with increase in the period of infection (Table 2).

Increase in the activity of peroxidase and polyphenol oxidase in host tissues in response to infection by the pathogen has been reported in many cases (Yedidia *et al*., 1999; Dutta and Chatterjee, 2000; Jose *et al*., 2001).

Increased peroxidase activity upon infection might be required for an additional deposition of lignin around the lesion court induced by pathogen. Peroxidase is a key enzyme in the biosynthesis of lignin and other oxidized phenols (Bruce and West, 1989). Peroxidase and polyphenol oxidase mediate the oxidation of phenols and oxidized phenols are highly toxic to the pathogen (Sequeira, 1983). PO and PPO catalyse the oxidation of phenolic compounds through a PPO-PO-H_2O_2 system (Srivastava, 1987). A number of studies have found a correlation between PPO and the resistance response (Velazhahan and Vidhyasekaran, 1994). PO itself was also found to inhibit the spore germination and mycelial growth of certain fungi (Joseph *et al*., 1998). Peroxidase may be rapidly involved in the peroxidation of substrate molecule, leading to the accumulation of highly toxic compounds (i.e., phenolic compounds), which may contribute to resistance via their antifungal potential (Ward, 1986).

The role of phenol oxidases in resistance is based on the observations that the activity of these enzymes is increased in infected tissues and that the oxidized phenols i.e., quinones are more reactive and more toxic to microorganisms compared to their non-oxidized form (Batsa, 2004).

Total phenols increased in infected plant than the healthy ones and it is well known that phenolic compounds are fungitoxic. Moreover, they increase the physical and mechanical strength of the host cell wall and thus inhibit fungal invasion. Therefore, from the present observation, the greater activity of PO and PPO, along with higher amount of total phenols enhancing host resistance is in compliance with the previous report.

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