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CHANGES IN POLYPHENOLOXIDASE AND PEROXIDASE IN *LATHYRUS SATIVUS* INFECTED BY *FUSARIUM ORTHOCERAS* APP. WOLLEN VAR. *LATHYRI* BHIDE AND UPPAL

BY

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Polyphenoloxidase (PPO) and Petroxidase (PO) activities were detected in the *Lathyrus sativus* tissues infected with *Fusarium orthoceras* and also in the mycelial extracts of *F. orthoceras* grown in culture medium with two different carbon sources viz. 1 5% sucrose ano 1.5% glucose. In the culture filtrates. there is no PPO activity upto 48 hours after inoculation and there is no PO activity in the culture filtrates of *F. orthoceras* f. *lathyri.* However the enzyme activity is less in the infected tissues than in the healthy ones.

INTRODUCTION

Polyphenoloxidase are capable of oxidizing Phenolic compounds to quinones or semiequinones and there by improve the defensive mechanisms in plants (Farkas and Kiraly, 1962). According to Rubin and Artsikhovakaya (1963) in hypersensitive reactions a major part is played by Polyphenoloxidase system. Peroxidase enzyme has a role in the oxidation of other metabolites in the respiration of higher plants. The literature thus so far has been obfained on the nature, origin, and occurances of these enzymes in infected and healthy plant is conflicting. (Hancock *et al* (1964), Kuc' (1964), Robb *et al* (1964), Tomiyama and Stalman (1964), Stalman *et al* (1966), Weber *et al* (1967), Hislop and Stalman (1971), Stalman and Demorest (1973), Beckman *et al* (1979) Majumdaf and Roychaudhury (1977), Maitra and Samajpati (1979).

The present investigation has been undertaken to provide some information about the activities of PPO and PO enzymes in the wilt disease of *Lathyrus* with *F. orthoceras.*

MATERIALS AND METHODS

Pathogen and cuiture

A virulent isolates of *F. orthoceras* f. *lathyri* was used in the study. A single spore culture of this pathogen was obtained from infected *Lathyrus* plants collected from the Agricultural field at the Baruipur Farm, Calcutta, West Bengal.

Plants and Infection

Lathyrus plant of 30 days old were uprooted from the pots carefully without causing any damage to roots and then after washing with distilled water were placed for 2-3 hours in conidial suspension of *F. orthoceras* var. *lathyri*. After thie seedlings were removed, washed and again placed on the sandy pot and then potted plants were covered with moist polythene tent. Some potted plants were kept as control which were also uprooted but treated with distilled water and after 2-3 hours, plants were again potted just like inoculated ones. All the potted plants were supplied with Knop's solution and were placed under natural light. Different sets of plants both normal and inoculated were hervested after 24 hsurs, 48 hours, 72 hours, and 7 days after inoculations and stored in a polythene bags at −20°C untill extraction.

Preperation of Mycelial Extract :

The fungus was grown on Richard's medium in conical flasks containing 1.5% sucrose and 1.5% glucose. The inoculated flasks were incubated at 27±0.05°C for 8—10 days. After the incubation period mycelia were separated by filtration, washed twice with deionised water and extracts were prepared by grinding the the mycelia in 0.1 *M* Sodium Phosphate buffer at pH 7.1 (2 ml/g fresh weight) in a clean mortar. Mycelia were filtered and the filtrates were centrifuged at 5,000 g for 20 minutes at 4°C. The supernatant clear fluid was used for enzyme assays.

Preparation of tissue Extract :

Enzyme extracts were prepared by grinding tissues in 0.1 *M* Sodium Phosphate buffer at pH 7.1 (2 ml/g fresh weight) in a clean mortar. These tissues were filtered and the filtrates were centrifuged at 5,000 g for 20 minutes at 4°C. The supernatant fluid was used for enzyme assays. (Maxwell and Bateman, 1967).

Enzyme Assays

Phenoloxidase and Peroxidase enzyme assays were carried out in a spectronic 20 colorimeter at 27±05°C. When the compounds were transferred into a colorimeter tube, it was then rapidly inverted several times and placed in the colorimeter. The needle was adjusted to the nill point and readings were taken every 30 seconds for 5 minutes.

To determine the phenoloxidase activity, the reaction mixture contained 2.0 ml enzyme extract, 1.0 ml 0.2 *M* Sodium Phosphate buffer at pH 6.5 and 1.0 ml 10^{-3} M Catechol brought to a final volume of 6.0 ml with distilled water. The activity of PPO was expressed as the change in absorbance per 1.0 ml of extract per minute at 495 nm.

Reaction mixture for peroxidase (PO) assay consisted of 1.0 ml. Tissue extract diluted 1 : 1000 with distilled water, 1.0 ml sodium citrate phosphate buffer at pH 4.1, 0.4 ml 0.1% H_2O_2 and 1.0 ml 0.1 *M* P—Phenylenediamine brought to a final volume of 6.0 ml with distilled water. The pH of the reaction mixture was 5.8. The activity was recorded as the change in absorbance 0.001 ml extract per minute at 485 nm.

The data represented here are the average of five separate determinations.

RESULTS AND DISCUSSION

The experimental data obtained during these investigations have been given in the following tables.

TABLE 1. Polyphenoloxidase aclivity in the mycelial extracts of *F. orthoceras* f. *lathyri* and the eztracts of healthy and infeceed *Lathyrus* tissues.

Days after inoculation	Specific activity[a]			
	Mycelial extract		Lathyrus tissue	
	with 1.5% Sucrose	with 1.5% Glucose	Healthy	Infected[b]
1	—	—	0.80±0.02	0.64±0.04
2	—	—	0.92±0.02	0.68±0.02
3	0.10±0.02	0.12±0.02	0.88±0.06	0.84±0.08
7	0.14±0.04	0.14±0.02	0.84±0.04	0.80±0.14

a Specific activity has been expressed as change in absorbance per minute per 1.0 at 495 nm. Each value represents an average of five separate determination from different preparation ± S. E. M.

b Tissues from well developed and defined symptoms were were taken.

From the data in Table 1 it reveals that there is no polyphenelooxidase activities in the culture filtrates upto 48 hourse after inoculation. Then it starts gradually and increase slightly at 7 days efter inoculation. However the enzyme activity is less in the infected tissues than the healthy ones though in both the cases enzyme activities are noticed with in 24 hours after inoculation.

The data in Table 2 reveal that there is no peroxidase (PO) activity in the culture filtrates of *F. orthceras f. lathyri*.

However the enzyme activity is found in both the healthy and infected tissues of *Lathyrus* though the activity is higher in infected tissues than healthy ones. The mechanism of activation of phenoloxidase is not completely known. However, as in several instances phenoloxidase seems to be adaptive (Farkas and Kivaly 1962) it is contended that the increased enzyme activity might

TABLE 2. Peroxidase (PO) activity in the mycelial extract of *F. orthoceras* f. *lathyri* and in the healthy and infected *Lathyrus* tissues

Days after Inoculation	Specific activity[a]			
	Mycelial extract		Lathyrus tissues	
	with 1.5% Surcose	with 1.5% Glucose	Healthy	Infected[b]
1	—	—	0.14±0.00	0.21±0.04
2	—	—	0.15±0.02	0.24±0.02
3	—	—	0.21±0.02	0.25±0.02
7	—	—	0.11±0.04	0.15±0.04

a Specific activity has been expressed as change in absorbance/minute/0.001 ml at 485 nm. Each value represents an average of five separate determination from different preparations ± S. E. M.

b Tissues from well developed and different symptoms were taken.

be due to the enhanced phenol contents of the infected leaves which is believed due to ths activity of pathogenic β—glucosidase in releasing phenolic substance from their bound froms. Though the PPO and PO activities have received serious consideration as terminal oxidase yet their role remain uncertain until now. But their participation in hypersensitive reactions are almost conclusive. Further research works are needed to elucidate the inhibition mechanism of fungal PPO and PO by the susceptible varieties of the host.

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