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NITROGEN AND PHENOL CONTENT IN *RHIZOCTONIA*-INFECTED TISSUES OF SEEDLING OF CAULIFLOWER

BY

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Nitrogen and phenol content, and peroxidase and polyphenoloxidase activities in healthy and diseased tissue of 'Early market' and 'Sutton Pusi' cultivars of cauliflower was carried out. Healthy tissue of the cultivars showed higher nitrogen content but lower phenol content and peroxidase activity than infected host tissues. No polyphenoloxidase activity was found in either healthy or diseased tissue of the cultivars although the pathogen showed some polyphenoloxidase activity in culture. In diseased tissue increase in peroxidase activity showed a gradual decrease in phenol content.

INTRODUCTION

Interactions of host and parasite lead to either increase or decrease in nitrogen level in infected host tissue. A decrease in nitrogen reported by a number of earlier workers has often been correlated with the increased activity of proteolytic enzymes (Porter, 1965) and associated breakdown of cell structure. Increase in phenol content, peroxidase and polyphenoloxidase activities in diseased host tissue have all been reported by Farkas and Kiraly (1962), Bateman and Daly (1967), Maxwell and Bateman (1967), Kosuge (1969), Chattopadhyay and Nandi (1976), Chattopadhyay and Samaddar (1980), Reddy and Rao (1978) and others.

The present investigation has been undertaken to study the nitrogen and phenol content and phenolases in two common cultivars of cauliflower (*Brassica oleracea* L. var. *botrytis* L), 'Early market' and 'Sutton Pusi' frequently infected by *Rhizoctonia solani* Khun causing 'damping-off' disease,

MATERIALS AND METHODS

Ten days old seedlings of 'Early market' and Sutton-Pusi' cultivars of cauliflowers grown in earthen pots in green house were inoculated with a sand-cornmeal culture of the pathogen at the rate of 2% of soil. For inoculum, the fungus was grown in Erlenmeyer flasks (250 ml) on sterilized sand-cornmeal (96% quartz sand, 4% cornmeal, water to 20% moisture, v/w) at 28°C for 8 days (Lewis, 1979). The earthen pots were then kept under humid condition at 28°C in shade for 3 days and then transferred to natural conditions. First disease symptom (damping-off) was recorded when the seedlings were 21 days old. Diseased seedlings were collected from the time of appearence of the first symptom onwards at regular intervals of 8 hours. Nitrogen and phenol content were quantitatively estimated and peroxidase and polyphenoloxidase activities were assayed.

Total nitrogen in tissue was measured colorimetrically following mainly Vogel (1961) by digesting 10 mg of dried tissue in 1 ml of concentrated H_2SO_4 in presence of a catalyst in micro-kjeldalh's flask. The digested material was bleached with 0.8 ml of 30% hydrogen peroxide (v/v). The homogeneous mixture was made to 10 ml with distilled water. An aliquot from this mixture was treated with alkaline Nessler's reagent and a mixture (1 : 1) of 10% sodium hydroxide (w/v) and sodium silicate (w/v). Intensity of the colour developed was measured by a Hilger pattern absorptiometer after 10 minutes at 430 nm using a water blank with identical treatment. Total nitrogen content was expressed as mg/100 mg dry tissue and calculated from a standard curve of nitrogen.

Soluble nitrogen in tissue was also msasured following Vogel (1961). Fresh tissue was homogenized with 10 ml distilled water, centrifuged at 5000 g for 10 minutes and the supernatant was treated with 1 ml of 50% tricholoro-acetic acid (w/v), filtered and the filtrate was made to 15ml with water. The nitrogen of the aliquot was measured following the same digestion procedure mentioned earlier

Protein nitrogen was obtained by deducting the soluble nitrogen from the total nitrogen (Lawrence *et al.*, 1959).

Total phenols were extracted following Seevers and Daly (1970). Fresh tissue (1 g) was extracted with methanol (20 ml) and centrifuged at 5000 g for 10 minutes. The supernatant was collected and the residue was washed several times with 80% methanol. The supernatant and the washings were combined.

The methanol extract was evaporated to dryness under vacum at 37°C. The residue was dissolved in distilled water (5 ml) and centrifuged 5000 g for 10 minutes. The supernatant was made 5 ml with water and assayed for total free phenols. Phenol was estimated with Folin-ciocalteu reagent following Bray and Thorpe (1954). The reaction mixture, made of 1 ml of test solution, 3 ml of 5% Na_2Co_3 (w/v) and 1 ml phenol reagent (diluted 1:10 with water), was kept in boiling water bath for 30 seconds and the intensity of the colour developed was measured at 650 nm in Photochem colorimeter using a water blank with identical treatment. Phenol content, calculated from a standard curve prepared with known concentrations of ferulic acid, was expressed as mg/100 mg dry tissue.

Peroxidase in infected host tissue and from 12 days old mycelia of the pathogen (grown on Richard's solution) was extracted and assayed following Chattopadhyay and Nandi (1976) with slight modification using buffer for extraction instead of water. Enzyme activity was measured at 430 nm using 1% hydrogen peroxide (v/v) as substrate.

Polyphenoloxidase in infected host tissue and 12 days old mycelia of the pathogen was extracted and assayed following Chattopadhyay and Nandi (1976). Activity was measured at 490 nm using catechol as substrate. Both the enzyme activities were calculated as ehange in △ O. D/min between 30-300 seconds after addition of the substrate.

RESULTS

Change in Nitrogen content :

The total nitrogen in the healthy tissues of the two cultivars differed slightly being a litttle higher in 'Early market' (3.84%) than in 'Sutton-Pusi' (3.04%). Infected tissues of both host cultivars showed considerable decrease in total, soluble and protein nitrogen. These decreased further as disease progressed and at the end of the experimental period loss in total nitrogen over respective healthy tissues were 68.02% for 'Early-market' and 63.58% for 'Sutton-Pusi' (Table 1).

Change in Phenol content :

Phenol content in healthy tissues of the cultivars were on the other hand, comparatively higher in 'Sutton-Pusi (3.52%) than in 'Early market' (2.84%)

(Table 2). In early stage of the disease, the tissues of host cultivars in the same sequence showed 1.52 and 2.1 times of phenol respectively. As the disease progressed a gradual decrease in phenol content was recorded in both cultivars. In all cases, however, the amount of phenol in infected tissue was higher than that of healthy tissue.

Table 1. *Nitrogen content* (mg/100 mg dry tissue) in healthy and infected tissues of 21 days old seedling of cultivars of cauliflower*

Host cultivars	Nitrogen content								
	Healthy tissue			Infected tissue					
	Nitrogen type	0 hr	8 hrs	16 hrs	0 hrs	Loss (%)	8 hrs	Loss (%)	16 hrs	Loss (%)
Early market'	Total	3.84	3.99	4.16	1.64	57.29	1.43	64.16	1.33	68.02
	Soluble	0.56	0.64	0.69	0.18	67.85	0.17	73.43	0.15	78.26
	Protein	3.28	3.35	3.47	1.46	55.48	1.26	62.38	1.18	65.99
'Sutton Pusi'	Total	3.04	3.22	3.46	1.55	49.01	1.38	57.14	1.26	63.58
	Soluble	0.21	0.26	0.30	0.17	19.04	0.16	38.46	0.14	53.33
	Protein	2.83	2.96	3.16	1.38	51.23	1.22	58.76	1.12	64.55

* Mean of five replicates

Table 2. *Phenol content * (mg/100mg dry tissue) in healthy and infected 21 days old seedlings of two cultivars of cauliflower*

Host cultivars	Phenol content					
	Healthy tissue			Infected tissue		
	0 hrs	8 hrs	16 hrs	0 hrs	8 hrs	16 hrs
'Early market'	2.84	2.91	2.95	6.00	5.76	5.00
'Sutton–Pusi'	3.52	3.58	3.65	5.36	4.96	4.80

* Mean of five replicates

Peroxidase and Polyphenoloxidase activities in vivo :

Peroxidase activity in both healthy and infected tissues of the 'Early market' variety was always higher than that in 'Sutton Pusi' (Fig. 1). In infected tissues of both cultivars peroxidase activity increased with increasing time. This

increased enzyme activity in diseased tissues was 4 times and 2.8 times higher than healthy tissues of 'Sutton Pusi' and 'Early market' respectively. Neither the healthy nor the infected tissues of both cultivars showed any polyphenoloxidase activity.

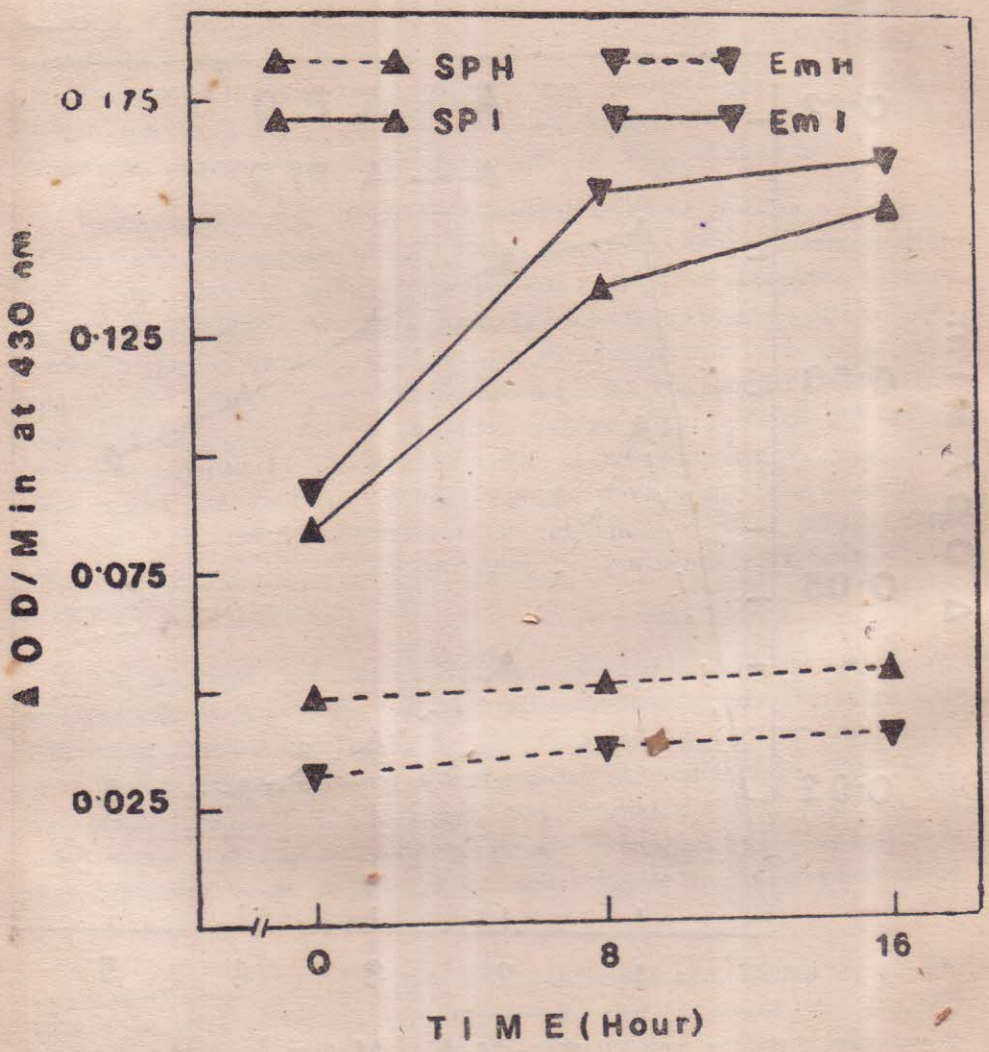

Fig. 1. Peroxidase activity in healthy and infected tissues of two cultivars of cauliflower.
Em H = Early market healthy tissue
Em I = Early market infected tissue
SP H = Sutton-Pusi healthy tissue
SP I = Sutton–Pusi infected tissue.

Peroxidase and Polyphenoloxidase activities in vitro :

Activities of these enzymes of the pathogen was studied *in vitro* to correlate their activities in infected host tissue. High peroxidase and low polyphenoloxidase activities were recorded from mycelia of the pathogen (Fig. 2).

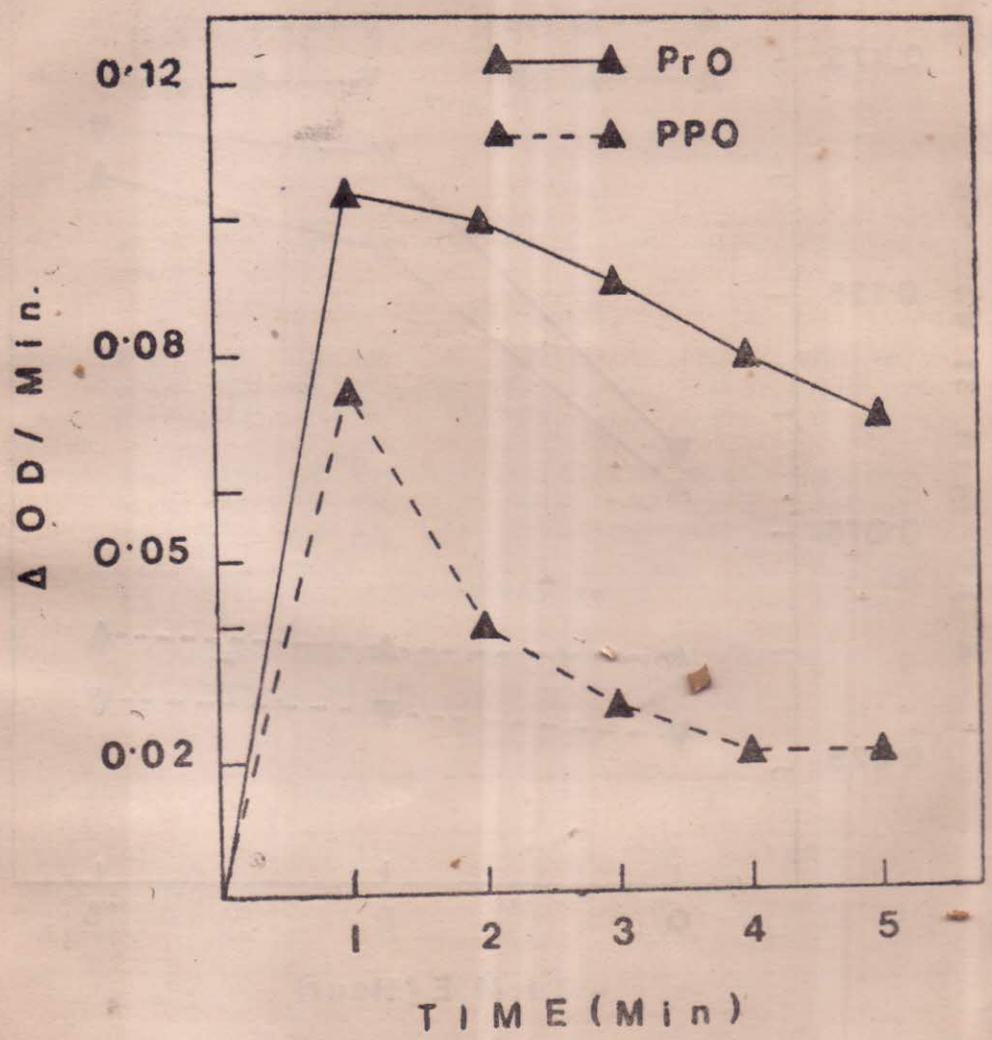

Fig. 2. Peroxidase (PO) and Polyphenoloxidase (PPO) activities of mycelial extracts of *R. solani*.

DISCUSSION

Decrease in total, soluble and protein nitrogen content in infected tissues was recorded in both host cultivars. Such decrease was reported to be associated with increased activity of proteolytic enzymes and breakdown of cell structure (Meyer *et al.* 1960 ; Porter, 1966). Lakshminarayan (1955) also recorded a decrease in nitrogen content in *Verticillium* infected succeptable cotton varieties and attributed it to the powerful oxidative amino acid deaminase. Decrease in nitrogen content in infected tissue also indicated a high nitrogen requirement by the pathogen as reported by Ali (1978) in other host-parasite interaction.

Increased amount of phenols in diseased tissue could be due to an increase in channelling of precurssors towards phenol synthesis as well as an accumulation of the intermediates of carbohydrate breakdown which were effectively used for the synthesis of phenols (Arora and Bajaj, 1978 ; Seevers and Daly, 1970). Such increase of phenol content is in accordance with Farkas and Kiraly (1962) who reported that parasitically any infection process lead to accumulation of various aromatic compounds. A post infection decrease of phenols increased succeptability as reported by Bhuller *et al.* (1972) in chillies infected with *Colletotrichum capsici* and Patil *et al.* (1962) in potato roots infected with *Verticillium alboatrum.* Such decrease might be due to formation of either peroximediate quinone (Kosuge, 1969 ; Simons and Ross, 1971) or lignin-like substance from it (Stafford, 1964 ; Brown, 1966).

The increased peroxidase activity in *Rhizoctonia* infected cauliflower seedlings was in agreements with observations in bean hypocotyls infected with *Rhizoctonia* (Bateman and Daly, 1967 ; Maxwell and Bateman, 1967) and many other host-parasite interactions (Chattopadhyay and Nandi, 1976 ; Brenneman and Black, 1979 ; and Chattopadhyay and Samaddar, 1980). Activation of latent enzymes in host or appearence of new iso-enzymes by host or both have been considered to be responsible for such increase in the enzyme activity (Kanazawa *et al.* 1965 ; Solmsy *et al.* 1967).

Absence of polyphenoloxidase activity in healthy and diseased tissue might be due to its total absence revealing thereby that peroxidase was probably responsible for oxidation of phenols in the present experiment as pointed out by Veech (1376).

Various workers emphasized a correlation between the levels of phenols and phenolases in host plant tissues and the degree of succeptability or resistance. The present experiments clearly revealed that though the two

cauliflower cultivars were succeptable to *R. solani*, the degree of succeptability was higher in 'Early market' cultivar exhibiting lower phenol content and the enzyme activity than the 'Sutton-Pusi'.

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