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STUDIES ON FREE AMINO ACID POOL, AMINO ACIDS
AND TCA PRECIPITABLE PROTEINS IN *FUSARIUM*
WILT OF *LATHYRUS SATIVUS*

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The present investigation has been undertaken in order to study the effect of host-parasite interactions on the protein content of the host plant, *Lathyrus sativus*. Both the quantitative and qualitative changes of protein were noticed in *Lathyrus* plant infected with *Fusarium orthoceras* var *lathyr*. TCA precipitable protein decreased in infected issues, Alanine is absent in infected ones. Whereas serine, glycine, phenylalanine and tyrosine are present in infected ones but absent in healthy plant. gel electrophoresis studies indicate the possible utilization of protein of host plant by the pathogen itself.

INTRODUCTION

During host parasite interaction, a noticeable changes occur in the protein content of host plant. Some new enzymatic proteins are produced in the penetrated cells and some amino acids decrease or completely utilise by the pathogen while some new amino acids are also produced by them. When a hypersensitive reaction occurs there are more rapid and intense metabolic activities

involving protein metabolism (Uritani, 1971). Protein changes after infection are related to defense action. Balandina and Shvetsova (1977) have shown that the changes in pathogenic properties in clone of *V. dahliae* and *F. oxysporum* f. sp. *var infectum* are accompanied by changes in the quantity and quality of protein contents. Balsubramanian and Kalyansundaram (1978) have studied the *Fusarium* infected cotton plant and showed the disappearance of some host's protein bands and appearance of a new band to a greater degree in the compatible host pathogen combinations (*Gossypium hirsutum*/11 strain, *G. arboreum*/12 strain of *F. vasinfectum*) by polyacrylamide gel electrophoresis method. The present investigation has been undertaken in order to study the changes in protein behaviour of *Lathyrus* plant during interaction with *F. orthoceras* var. *lathyrus*.

MATERIALS AND METHODS

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. *lathyrus*. After this, 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 harvested after 24 hours, 48 hours, 72 hours and 7 days after inoculations and protein estimation were done with all of these.

Sample preparation for protein estimations were done in the following way. 3 g of plant material were ground at 4°C in a previously chilled mortar with 0.1 M tris HCl buffer (pH-8.0) containing 0.1% ascorbic acid. Then the homogenates were centrifuged at 15,000 g for 30 minutes at 4°C. After centrifugation, supernatant was decanted off and was estimated according to Lowry *et al* (1951) by folin-phenol method.

Following method of Show and Colotelo (1961) samples were prepared for free amino acids of both healthy and infected plants and the amino acids were estimated by two dimensional ascending thin layer chromatography technique of Stahl (1969). The first solvent was distilled phenol : water (4:1, V/V) and the second solvent was collidine : Lutidine : water (1:2:1, V/V). 2% ninhydrin in ethanol was used as spraying reagent. The spots were then detected by comparing with the Rf value of standard plate of known aminoacids and eluted for quantitative determinations.

RESULTS AND DISCUSSION

It has been noticed that there is appreciable alteration in free amino pool and TCA precipitable protein in the infected tissues of *Lathyrus* caused by *F. orthoceras* var. *lathyri*.

The data are given in Table 1.

Table 1. Data (mean) showing the concentration of free amino acids and TCA precipitable proteins in healthy and infected tissues of *Lathyrus sativus* with *F. orthoceras* var *lathyri*

Days after incubation	Free amino acids		TCA precipitable proteins	
	Healthy	Infected	Healthy	Infected
1	24.6 $\pm$ 1.2	26.2 $\pm$ 1.4	150.2 $\pm$ 7.0	150.4 $\pm$ 7.5
2	24.8 $\pm$ 1.4	26.9 $\pm$ 1.6	156.3 $\pm$ 7.5	128.9 $\pm$ 7.0
3	25.1 $\pm$ 1.2	27.2 $\pm$ 1.4	146.2 $\pm$ 7.6	106.3 $\pm$ 6.6
7	25.8 $\pm$ 1.4	31.4 $\pm$ 1.2	150.8 $\pm$ 7.7	94.0 $\pm$ 7.5

a) Data have been expressed in mg per g dry weight. Each value represents an average of five separate determinations from different preparations $\pm$ S. E. M.

b) Concentrations of TCA precipitable proteins were calculated by multiplying insoluble nitrogen value by 6.25.

It is evident from the data in table 1 that concentration of free amino acids pool has increased significantly in the infected tissues than the healthy ones whereas it is decreases in case of TCA precipitable protein.

The changes in concentration of individual amino acids of both healthy and infected plants of *Lathyrus* are given in Table 2.

Table 2. Data (mean) showing the concentration of free amino acids and other ninhydrine positive compounds in the healthy and the infected *Lathyrus sativus* tissues with *F. orthoceras* f. *lathyri* after 7 days of inoculation

Amino Compound	Concentrations of amino compounds	
	Healthy	Infected
Cysteine	7.2 $\pm$ 2.1	14.6 $\pm$ 2.6
Lysine	37.1 $\pm$ 1.5	56.1 $\pm$ 2.1
Leucine	27.6 $\pm$ 2.6	58.6 $\pm$ 1.8
Serine	—	31.4 $\pm$ 2.2
Glycine	—	36.4 $\pm$ 1.8
Glutamine	37.6 $\pm$ 2.8	21.4 $\pm$ 1.8
Valine	17.1 $\pm$ 3.1	19.6 $\pm$ 2.4
Methionine	36.1 $\pm$ 2.1	13.6 $\pm$ 2.2
Alanine	79.2 $\pm$ 2.4	—
Aspartic acid	112.6 $\pm$ 3.0	64.6 $\pm$ 2.6
Glutamic acid	116.1 $\pm$ 3.6	62.4 $\pm$ 3.6
Phenylalanine	—	31.2 $\pm$ 3.6
Tyrosine	—	13.1 $\pm$ 2.8
Tryptophane	26.4 $\pm$ 2.6	43.2 $\pm$ 2.8
Unknown 1	61.2 $\pm$ 4.2	46.2 $\pm$ 2.6
Unknown 2	61.2 $\pm$ 4.2	46.2 $\pm$ 2.6

a) Data have been given in $\mu\text{g/g}$ dry weight. Each value represents an average of five separate determinations from different preparation $\pm$ S. E. M.

d) Tissues from well developed and defined symptoms were taken.

c) Unidentified amino compounds,

Rf values - 1 - 0.345

2 - 0.100

The data in the above table revealed that there are ten amino acids in healthy tissues of *Lathyrus* and 13 amino acids in the infected tissues. Besides two unknown ninhydrin compounds are present in both the cases. Alanine which is present in healthy tissues is absent in infected ones. Serine, glycine, tyrosine and phenylalanine are present in infected tissues but absent in healthy ones. So these are new amino acids in the infected tissues. Moreover, the concentrations of glutamic acid, methionine, aspartic acid and glutamine decrease in the infected tissues and the concentrations of the rest increase.

The experimental data on the gel electrophoresis of protein extract from the healthy and infected tissues are given in Table 3.

Table 3. *ef value of proteins from healthy and infected Lathyrus plant*

Lathyrus plants	No of bands	ef value			
		Days after inoculation			
		1	2	3	7
Healthy	1	0.91	0.93	0.91	0.91
	2	0.83	0.88	0.85	0.83
	3	0.64	0.70	0.66	0.58
	4	0.50	0.56	...	...
	5	0.40	0.48	...	...
Infected	1	0.88	0.91	0.90	0.91
	2	0.63	0.63	0.83	0.66
	3	0.46	0.46	0.68	...
	4	0.36	0.33	0.58	...
	5	...	...	0.48	...
	6	...	...	0.33	...

The data indicate that there is considerable variation in the protein of the infected *Lathyrus* plant than the healthy one. It is also noted that upto 48 hours after infection though there is no new band in the protein pattern but still the

mobility of different bands of the protein in the infected plants have increased than the healthy one. Also there is one less band in the infected one than the healthy one from the very beginning up to 2 days. But after 3 days there are two new bands in the infected tissues than the healthy one. Of these the 4th band appears to be very rich in protein. However after 7 days only two bands are found in the infected plants which indicate that protein are possibly utilized by the pathogen. This finding confirms the earlier ones of Balasubramanian and Kalyansundaram (1978).

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