Plant Pathology Bulletin 14:221-226, 2005

Changes in the Nitrogen Fractions and Amino Acid Metabolism of Turmeric (*Curcuma longa* L.) Roots Infected with *Fusarium solani*

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Accepted publication: September, 25, 2005

ABSTRACT

Reddy, M. N., Sridevi, N. V., and Charitha Devi, M. 2005. Changes in the nitrogen fractions and amino acid metabolism of turmeric (*Curcuma longa* L.) roots infected with *Fusarium solani*. Plant Pathol. Bull. 14:221-226.

Fusarium solani infection greatly influenced the total proteins, free amino acids and nitrogen fraction levels of turmeric roots as compared to the corresponding healthy tissues. Both qualitative and quantitative changes in free and protein bound amino acids were observed. Infection resulted in marked increase in total, protein, soluble and amino nitrogen in the infected roots.

Key words : *Fusarium solani*, Turmeric (*Curcuma longa* L.) roots, nitrogen fractions, total proteins, amino acids.

INTRODUCTION

Turmeric (*Curcuma longa* L.) belonging to the family Zingiberaceae, is a tropical rhizomatous crop cultivated for its under ground rhizomes. It is best known as a condiment, though the plant has uses in all the social and religious life of the people in South - East Asia. It is known to India from ancient times and has been used in innumerable ways, not only for fvouring of food and other dietary preparations but also in indigenous medicines. India remains the largest producer of this crop accounting to about 80% of the world production [3,8], but 92% of the total production is consumed within the country.

Root rot caused by *Fusarium solani* (Mart.) Sacc., is one of the destructive diseases of turmeric (*Curcuma longa* L.) [14]. Depending on the intensity of the disease, death of whole plant occurs and the formation of rhizome is ceased. In the plants where rhizome formation occurs, the disease spreads to the rhizomes also resulting in partial to total rotting of the rhizome.

Though the root rot is gaining importance as one of the widespread and destructive pathogens of turmeric, very little attention has been paid on the etiology and epidemiology of the disease and pathology and physiology of host-pathogen interaction. Hence, the present study was under taken to understand some of the biochemical characteristics of the host - pathogen complex.

MATERIALS AND METHODS

Preparation of *Fusarium solani* inoculum

Healthy seeds of turmeric variety 'Mydukur' were used in the study. The seeds were surface sterilized, washed and sown in sterilized soil contained in seed pans. The seeds germinated and seedlings and emerged in about 10-15 days. Ten day old cultures of *F. solani*, grown in 3% oatmeal - sand medium (97 g of sand + 3 g of oat meal w/w) was thoroughly mixed with sterilized soil at 10% level (90 g sterilized soil + 10 g inoculum w/w). This

inoculum - soil mixture was then distributed in clay pots (12" in diameter) and kept undisturbed for two days. After this period, 15 days old seedlings grown in seed pans were lifted carefully without causing visible damage to the root system, surface sterilized the root system with 0.1% mercuric chloride and then transplanted into the pots containing inoculum - soil mixture.

Sampling of turmeric roots

The progress of disease on the turmeric roots of transplanted seedlings was followed periodically. Samples of roots with lesions were collected for analysis at the following four stages of lesion development and decay of roots.

Stage: 1 The early stage of infection (15 days after inoculation): This was characterized by the appearance of water-soaked lesions on the roots, especially at the tips.

Stage: 2 The intermediate stage of infection (25 days after inoculation): Root lesions extended up further and turned brown or dark brown in colour. Yellowing of young leaves was initiated.

Stage: 3 Late stage of infection (35 days after inoculation): This was characterized by increase in the intensity of rotting, blackening and decay of roots with the lesions extending further upwards without vascular browning. The yellowing of leaves still further uniformly.

Stage: 4 Very late stage of infection (45 days after inoculation): This was characterized by the destruction of entire root system, total yellowing of all leaves, wilting and death of the plants.

For control, roots of healthy seedlings were collected for analysis at periods corresponding to each of the stages of the infested plants.

Estimation of nitrogen fractions

Total nitrogen of turmeric was estimated according to the method of Markham [12] and protein nitrogen by that of Thimann and Loos [23]. Soluble nitrogen fraction was then calculated by subtracting protein nitrogen from total nitrogen. Amino nitrogen in the ethanol extract was determined by the ninhydrin method of Moore and Stein [13].

Amino acid analysis

The separation of free amino acids in the ethanol extract was carried out by two - dimensional paper chromatography using 2-butanol - formic acid -water (75 : 13: 12 v/v/v) and buffer saturated phenol as the first and second solvent systems, respectively [24]. The dried chromatograms were sprayed with 0.2% ninhydrin in n-butanol and the identity of the substances was confirmed by co-chromatography and also by comparing with the R_F values from a known mixture of amino acids.

The elution and quantitative estimation of the individual amino acids was carried out according to the method of Giri *et al.* [4]. The eluates were read at 540 nm, except for proline and asparagine which were read at 410 nm and 390 nm, respectively. The amount of each amino acid was then determined by reference to standard curves prepared using authentic samples. Total proteins were extracted and estimated according to the method of Lowry *et al.* [9].

RESULTS

Various nitrogen fractions (total, protein, soluble and amino nitrogen) in healthy and *F. solani* - infected turmeric roots were estimated at different stages of disease development and the results are summarized (Table 1). All the nitrogen fractions (total, protein, soluble and amino nitrogen) showed a marked increase at various stages of disease development, as compared to the corresponding healthy controls. The roots of inoculated plants contained higher concentration of total N_2 than the healthy plant and the rate of increase was also high. The increased rates were 68.5%, 80.4% , 80.7% and 72.2%, respectively, at stages 1, 2, 3 and 4 as compared with healthy ones. The roots of infected plants, on the other hand showed a significant increase in the quantity of protein N_2. The increased rates were 68.6%, 130.8%, 186.7%, 213.5% respectively as compared with healthy ones. There was no significant alteration in the soluble N_2 contents of the roots as a consequence of infection.

Infection resulted in higher quantities of amino N2 at all stages of disease development. It is higher by 43.3%, 37.6%, 37.2% and 63.7% respectively as compared to healthy ones. Infection also resulted in marked increase in total proteins as compared to healthy (Table 2).

Qualitative and quantitative changes in the free amino acid pools of both infected and healthy root tissues are presented in the Table 3. Altogether sixteen ninhydrin positive compounds were detected and identified. In healthy plants, with the exception of glutamic acid, serine and threonine which tended to diminish at all stages, the other amino acids - aspartic acid, tyrosine and valine showed a gradual increase with advancing age. Excepting glutamic acid, serine and threonine, which were decreased gradually in their quantities, all other amino acids increased in their content (Table 3).

Table 1 : Changes in nitrogen fractions (mg/g fr. wt) in healthy and *Fusarium solani* - infected turmeric roots at various stages of disease development

Stages[2]	Total nitrogen[1]			Protein nitrogen[1]			Soluble nitrogen[1]			Amino nitrogen[1]		
	Healthy	Infected	Percentage over control	Healthy	Infected	Percentage over control	Healthy	Infected	Percentage over control	Healthy	Infected	Percentage over control
1.	2.89 ±0.08[3]	4.86 ±0.03	+68.52[4]	1.50 ±0.05[3]	2.52 ±0.01[3]	+68.69[4]	1.39 ±0.04[3]	2.33 ±0.01[3]	+68.33[4]	106.60 ±1.67[3]	153.30 ±17.2	+43.35[4]
2.	3.33 ±0.05	6.01 ±0.04	+80.47	1.45 ±0.01	3.36 ±0.02	+130.80	1.87 +0.04	2.65 ±0.02	+41.31	104.10 ±2.21	143.30 ±6.03	+37.61
3.	3.92 ±0.02	7.08 ±0.10	+80.72	1.40 ±0.01	4.03 ±0.05	+186.70	2.48 ±0.03	3.05 ±0.05	+22.73	100.80 ±3.64	138.30 ±7.27	+37.26
4.	4.33 ±0.04	7.46 ±0.08	+72.26	1.38 ±0.01	4.32 ±0.05	+21.35	2.95 ±0.03	3.14 ±0.04	+6.32	55.80 ±19.60	80.80 ±20.70	+63.74

[1] Each value is an average triplicate samples.
[2] Stages 1, 2, 3, 4 represent 15, 25, 35,45 days after inoculation, respectively.
[3] ± Standard error.
[4] + Increase over control.

Table 2 : Changes in the content of total protein (mg/g fr. wt.) in healthy and *F. solani* - infected turmeric roots at various stages of disease development

Stages[2]	Total proteins[1]		
	Healthy	Infected	Percentage over control
1	1.05 ± 0.0333	1.08 ± 0.0578[3]	+5.67[4]
2	0.83 ± 0.0667	1.23 ± 0.0882	+50.36
3	0.70 ± 0.0333	1.42 ± 0.0667	+97.23
4	0.49 ± 0.0667	1.51 ± 0.0677	+191.00

[1] Each value is an average triplicate samples.
[2] Stages 1, 2, 3, 4 represent 15, 25, 35,45 days after inoculation, respectively.
[3] ± Standard error.
[4] + Increase over control.

Table 3 :Effect of *F. solani* - infection on free amino acid content (mg/g fr.wet) of healthy and infected roots at various stages of disease development

S.No.	Amino acids	Stage I[1]		Stage II[1]		Stage III[1]		Stage IV[1]	
		H[2]	I	H[2]	I	H[2]	I	H[2]	I
1.	Aspartic acid	0.01[3]	0.01	0.03[3]	0.04	0.03[3]	0.03	0.04[3]	0.05
2.	Glutamic acid	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.01
3.	Serine	0.02	0.01	0.01	0.01	0.11	0.01	0.01	0.01
4.	Asparagine	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
5.	Threonine	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
6.	Glycine	0.01	0.01	0.02	0.02	0.01	0.01	0.02	0.02
7.	Alanine	0.05	0.06	0.06	0.06	0.06	0.07	0.06	0.07
8.	Arginine	0.01	0.02	0.02	0.02	0.01	0.01	0.01	0.02
9.	γ-aminobutyric acid	0.08	0.09	0.10	0.12	0.11	0.13	0.13	0.15
10.	Valine	0.02	0.02	0.02	0.03	0.03	0.03	0.03	0.04
11.	Histidine	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
12.	Phenylalanine	0.07	0.07	0.08	0.08	0.10	0.12	0.12	0.13
13.	Proline	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.03
14.	Leucine / Isoleucin	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.03
15.	Glutamine	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
16.	Tyrosine	0.04	0.05	0.05	0.06	0.06	0.06	0.06	0.07
	Total (mg)	0.45	0.05	0.52	0.56	0.65	0.59	0.60	0.68

[1] Stages I, II, III, IV represent 15, 25, 35, 45 days after inoculation.
[2] H = Healthy; I = Infected.
[3] Each value is an average of 3 replicate samples.

DISCUSSION

Fusarium solani infection of turmeric roots resulted in marked increase in all the nitrogen fractions including total nitrogen, protein nitrogen, soluble nitrogen and amino nitrogen. Increase in protein nitrogen may be ascribed to increased protein synthesis and soluble nitrogen to increased synthesis or hydrolysis of proteins or both. There are several reports of higher amino nitrogen content in different host-pathogen interactions [1,2,6]. The accumulation of amino nitrogen in wheat was reported to be due to release of free amino acids from proteins of plant cells by the action of proteolytic enzymes of the pathogen [10].

In the present investigation a higher amount of total proteins was observed in infected tissues as compared to the healthy tissues. It is possible that this increase of proteins in infected tissues may be due to the accumulation of nitrogenous constituents in the tissues as a result of changes in nitrogen metabolism of infected plants. Increased protein content in infected tissues can also be attributed to increased catabolic reactions or decrease in proteolytic degradation or both, as reported by Tayal *et al.* [22]. Changes in protein metabolism in plant tissues during the disease development have been well reviewed [11,18,22,25]. A possible explanation for protein synthesis is offered by the increased ribosomal content upon infection resulting in an increased capacity of the host plant for protein synthesis.

Fungal infections are known to alter the free amino acid composition of host tissues [19,26]. Some of them are related with the host resistance [19] while others are associated with symptom expression [5].

Fusaium solani infection resulted in a marked influence on the free amino acid pool of root tissues. Some of the amino acids - glutamic acid, serine and threonine declined at different stages, while others showed an increasing trend, ranging from limited to a considerable extent. The striking diminution of glutamic acid and threonine was also reported by Shaw and Colotelo [19] and Rudolph [17].

Singh and Prasad [21] observed decreased amount of certain amino acids as well as accumulation of new amino acids in the infected parts of peach fruit with *Rhizopus stolanifera*. They concluded that the cause for accumulation was due to the accumulation of proteases of host origin. The decrease of amino acids in the infected tissues may be due to their utilization by the pathogen or due to their utilization in the synthesis of proteins during host-parasite interactions [20].

The increase in amino acids and or the synthesis of new amino acids may be due to an interference of host metabolism by the pathogen or due to host pathogen interaction as suggested by Webster [27]. Quantitative increase in certain amino acids in the host tissues by infection may either be due to *de novo* synthesis by the host [15] or proteolysis of certain host tissue proteins, since diseased tissues often showed higher proteolytic activity than healthy tissues [7,16].

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摘 要

Reddy, M. N.[1,2], Sridevi, N. V.[1], and Charitha Devi, M.[1] 2005. *Fusarium solani* 感染薑黃根後對氮含量及胺基酸代謝的影響. 植病會刊 14:221-226. ([1] Department of Applied Microbiology, Sri Padmavati Mahila Visvavidyalayam, Tirupati-517502, Andhra Pradesh, India; [2] 聯絡作者，E-mail: mopuri-nr@yahoo.com)

薑黃根部遭受 Fusarium solani 感染後會顯著地影響總蛋白、游離胺基酸和各種氮素含量。無論是游離的胺基酸和蛋白質的質與量均會改變。受害的根部在總氮、蛋白氮、可溶性氮及胺基氮等均明顯地增加。

關鍵詞：*Fusarium solani*、薑黃根、氮、總蛋白、胺基酸