J. Phytol. Res. 9 (2): 169-172, 1996

BIOCHEMICAL QUANTITATION OF VARIOUS CELLULAR METABOLITES OF *PISUM SATIVUM* L. ROOT GALLS INCITED BY *MELOIDOGYNE INCOGNITA* CHITWOOD

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The present investigation deals with the biochemical quantitation of carbohydrates, total proteins, free amino acids, proline and ascorbic acid in healthy and *Meloidogyne incognita* infected *Pisum sativum* L. root galls collected after 30, 60 & 90 days after inoculation. Infected roots showed an increase in total and reducing sugar content, whereas starch and non reducing sugars decreased. The protein content of infected roots of all ages exceeded that of healthy roots. More amount of free amino acids was estimated in galled roots as compared to the healthy roots. The infection of pea plants was associated with a large accumulation of free proline. In 60 days infected roots, 693.51 per cent increase in proline was estimated. The higher occurrence of ascorbic acid in infected roots suggested some sort of resistant response by the host plant.

Keywords: Ascorbic acid; Carbohydrates; Free amino acids; *Meloidogyne incognita*; *Pisum sativum*; Total proteins.

Introduction

The pea (*Pisum Sativum* L.) occupies a position of considerable importance in our agricultural economy. Importance of pea as a pulse and a vegetable crop in human diet needs no emphasis. It is heavily infected by *Meloidogyne incognita* in the sandy soil of North-East Rajasthan where it is grown extensively. The development of disease syndrome is dependent on biochemical reactions taking place between substances secreted by the pathogen and those already present or produced by the host as a response to the infection. Perusal of the literature revealed only a few reports of time bound study of biochemical quantitation in a pea resulting *M. incognita* infection[1]. The present study was undertaken to have a better understanding of host parasite interaction.

Materials and Methods

Surface sterilized seeds of *Pisum sativum* L. (CV Bonneville) were sown singly in 15 cm earthen pots containing autoclaved river-bed sand. The seeds were previously treated with the specific strain of *Rhizobium*. One week old seedlings were inoculated with 1000 juveniles of *M. incognita* by pipetting and pouring the larval suspension through three holes around the roots. Uninoculated plants served as control. The seedlings were fed throughout the experimental period with full strength long ashton nutrient solution. Thirty, 60 and ninety days after inoculation, plants were uprooted and the root system was washed carefully and used for estimation either as fresh or oven dried at 40°C and finely powdered. The modified methods used for various quantitative estimations are shown in Table 1.

Observations

Sugars and Starch:- The total sugar content in the infected as well as healthy roots increased with the advancing age of the plant. Infected roots showed a higher sugar content over corresponding healthy roots. Its magnitude was more pronounced 30 days after inoculation resulting 63.46 per cent increase (Table 2).

The reducing and non-reducing sugars showed increasing trends in diseased and healthy roots. Infected roots contained more reducing sugars than their healthy counterparts. However, non-reducing sugars decreased in infected roots by 9.28 per

Table 1. Methods For Biochemical Quantitation.

METABOLITES	METHOD EMPLOYED	REAGENT	ABSORBANCE READ AT
Sugars and Starch	Dubois *et al.*[2]	5% Phenol and conc. H_2SO_4	490
Total Proteins	Lowry et al.[3]	Folin-Ciocalteau reagent	750
Total Free Amino acids	Lee and Takahashi[4]	Ninhydrin reagent	570
Proline	Bates et al.[5]	Acid Ninhydrin reagent	520
Ascorbic acid	Ranganna[6]	2, 6-Dichlorophenol indophenol	546

Table 2. Amount of various carbohydrates in healthy and *Meloidogyne incognita* infected pea roots (mg/100 mg) (Each value is mean of three replicates).

Constituents	30 days old			60 days			90 days old		
	H	In	% I/D over H	H	In	% I/D over H	H	In	% I/D over H
Reducing Sugars	1.60	2.60	+ 62.50	2.40	5.60	+ 133.30	2.60	6.20	+ 138.40
Non-reducing Sugars	3.42	5.60	+ 63.70	7.22	6.55	- 9.20	8.26	6.93	- 16.10
Total sugars	5.20	8.50	+ 63.40	10.00	12.50	+ 25.00	11.30	13.50	+ 19.40
Starch	3.05	2.62	- 14.10	4.07	2.47	- 39.30	4.22	4.80	+ 13.70

H = Healthy; In = Infected ; I = Increase; D = Decrease

cent and 16.11 per cent over healthy ones after 60 and 90 days of inoculation, respectively (Table 2).

Thirty and 60 days after inoculation starch content got reduced in infected roots over healthy ones. However, 90 days after inoculation. starch content increased by 13.74 per cent over healthy roots (Table 2).

Proteins:- Protein content of infected roots of all ages exceeded that of healthy roots of corresponding ages (Table 3). The increase in protein content was more pronounced in 30 days old infected roots than 60 and 90 days old infected ones. In healthy as well as infected roots, the amount of proteins increased initially upto 60 days after inoculation, thereafter, it decreased.

Total Free Amino acids:- Quantitatively free amino acids in galled roots increased over healthy roots (Table 3). Maximum increase was observed in 90 days old roots (86.66 per cent). In healthy as well infected roots, the amount of free amino acids increased upto 60 days and decreased afterwards.

Proline:- The amount of free proline showed trends like total free amino acids. In 60 days infected roots, proline showed 693.51 per cent increase over its healthy counter parts. Proline constituted 22.5 per cent and 24.09 per cent of total free amino acids in 60 and 90 days infected roots, respectively (Table 3).

Ascorbic acid:- Ascorbic acid content was higher in infected roots than the healthy roots of corresponding ages. Ninety days infected

Table 3. Amount of various metabolites in healthy and *Meloidogyne incognita* infected pea roots (Mean of three replicates).

Metabolites	Days after Inoculation	Healthy roots	Infected roots	% Increase over healthy roots
Total Proteins (mg/g)	30	81.50	97.50	19.63
	60	97.50	100.00	2.56
	90	93.50	96.50	3.20
Total Free Amino acids (µg/g)	30	1600	1700	6.25
	60	3100	4700	51.61
	90	1500	2800	86.66
Proline (µg/g)	30	74.99	108.32	44.44
	60	133.32	1057.91	693.51
	90	108.32	674.73	522.90
Ascorbic acid (mg/g)	30	0.32	0.38	18.75
	60	0.38	0.40	5.26
	90	0.38	0.56	47.36

roots showed 47.36 per cent increase over healthy roots of the same age (Table 3).

Discussion

Carbohydrates:- Quantitatively infected roots of all ages showed an increase in total sugar content whereas starch was decreased except in 90 days old roots. Increase in total sugar might be attributed to the hydrolysis of starch which was decreased. Hydrolysis of starch was probably brought about by the hydrolytic enzymes secreted by the nematode. Increased starch content in 90 days infected roots might be due to the death and inactivation of the parasite. Similar observations were also recorded by Sharma and Sethi[7] in cowpea infected by *M. incognita*. Decreased starch content in nematode infected hosts were also reported by other workers[8-9].

Reducing sugars were observed to increase in infected roots as compared to healthy roots, while non-reducing sugars decreased in infected roots. Non-reducing sugars, such as sucrose, were hydrolysed by invertase into reducing sugars, such as glucose and fructose. These simple forms of sugars were easily assimilated by the pathogen. The localization of invertase in the oesophagus and intestine of the nematode parasite and in and around giant cells suggested the possibility of its secretion by the nematode into the host tissue, thus, resulting into changed carbohydrate metabolism during the course of host-parasite interaction[10].

Total Proteins:- In 30 days infected roots 19.63 per cent increase in protein content was observed over healthy roots of the same age. In older roots the increase was less pronounced. It seemed that the new proteins for the benefit of the nematode were synthesized under its influence when young and active, Similar results were also obtained in egg plant[9], cowpea[11-12] and okra[13]. The rate of intermediary metabolism, especially in pathway leading to synthesis of proteins, was accelerated in galls[14].

Total Free Amino acids:- Increased amount of amino acids was observed in galled roots as compared to healthy ones of the same age. It was suggested that the nematode broke down the proteins faster than they could be absorbed

by the nematode[15]. A higher protease activity was observed by Tayal and Agrawal[16] working on *M. incognita* infected *Solanum melongena* leading to break down of proteins into easily assimilable forms of amino acids. However, the origin of free amino acids could not be attributed solely to protein degradation. Experiments on the synthesis of amino acids from ^{14}C showed that the labelled carbon was rapidly assimilated into amino acids in excised roots and that the label did not accumulate in the parasite[14].

Proline :- In the present study upto 694 per cent increase in proline was observed in infected roots. An increase of 720 per cent in tomato[17], 1900 per cent in bur marigold[18] and 238 per cent in okra[19] was earlier observed. Its concentration increased with increasing density of inoculum of nematode and varied with time, the highest being at the time of egg production[20]. Thus, the increased proline content was a nematode response in the susceptible plants.

Ascorbic acid :- More amount of ascorbic acid was found in galled roots as compared to healthy ones. The development of cyamide-resistant respiration was conditioned by the presence of ascorbic acid in the cells. Thus ascorbic acid could be considered as a factor of primary importance in the biological defense mechanism of plants and animals[21]. Arrigoni *et al.*[22] demonstrated that a decrease in ascorbic acid in plants induced a reduction in their resistance of root-knot nematode. Conversely, susceptible cultivars irrigated with water solution of ascorbic acid reacted similarly to resistant cultivars. In pea plants, the higher occurrence of ascorbic acid indicated a limited resistance response.

References

1. Sharma R K and Tiagi B 1986, *Biol. Bull. India* **8** 48.
2. Dubois N, Gilles G A, Hamilton J K, Rebero P A and Smith F 1956, *Ann. chem.* **28** 350
3. Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951, *J. Biol. chem.* **193** 265
4. Lee Y P and Takahashi T 1966, *Ann. Biochem.* **14** 17
5. Bates L S, Waldren R P and Theare I D 1973, *Plant Soil* **39** 203
6. Ranganna S 1977, *Manual of analysis of fruit and vegetable products* Tata-McGraw Hill Publ. Co., New Delhi
7. Sharma N K and Selhi C L 1976, *Indian J. Nematol.* **6** 171
8. Owens R G and Novotny H M 1960, *Phytopathology* **50** Abstr. 650
9. Singh I, Sharma J and Sharma R 1978, *India J. Nematol.* **8** 122
10. Roy T K 1979, *Indian J. Exptl. Biol.* **17** 1357
11. Kannan S and Chandraguru T 1981, *Second Nematol. symp. Coimbatore, India* (Abstr.) 110
12. Ganguly A K, Raman R and Dasgupta D R 1991, *Indian J. Nematol.* **21** 113
13. Basu S P S and Sukul N C 1983, *Indian J. Nematol.* **13** 66
14. Owens R G and Rubinstein J H 1966, Contrib. *Boyce Thompson Inst.* 22 199
15. Muge S G 1956, *Doklady Akad. Nauk. USSR* **108** 164
16. Tayal M S and Agrawal M L 1982, *Indian J. Nematol.* **12** 379
17. Owens R G and Specht H N 1966, *Contrib. Boyce Thompson Inst.* **23** 181
18. Epstein E and Cohn E 1971, *Nematologica* **3** 334
19. Sharma W and Trivedi P C 1996, *Indian J. Nematol.* **26** 152
20. Meon S, Fisher J M and Wallace H R 1978, *Plant Pathol.* **12** 251
21. Arrigoni O 1979, *Root-Knot nematodes (Meloidogyne Species) - Systematics, Biology and Control* Ed. Lamberti F and Taylor C E, Academic Press, New York. 457
22. Arrigoni O, Zacheo G, Arrigoni - Liso R, Bleve-Zacheo T and Lambetti F 1979, *Root-knot nematodes (Meloidogyne species) - Systematics, Biology and control* Ed. Lamberti F and Taylor C E, Academic Press, New York. 469