

## QUALITATIVE ANALYSIS OF VARIOUS FREE AMINO ACIDS IN *PISUM SATIVUM* ROOT GALLS INCITED BY *MELOIDOGYNE INCOGNITA*

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Free amino acids of *Meloidogyne incognita* infected and healthy roots of *Pisum sativum* were identified chromatographically. Maximum number of amino acids (16) were identified in 60 days infected roots. Aspartic acid was found only in infected roots suggesting some sort of resistant response. Tryptophan was present in all the samples except 30 days old healthy roots.

**Keywords :** Free amino acids; *Meloidogyne incognita*; Paper chromatography; *Pisum sativum*.

*Pisum sativum* (L.) is commonly grown throughout the country. It is heavily infected in the sandy soil of Rajasthan by *Meloidogyne incognita* Chitwood. Study of the available literature revealed only a few reports of time bound biochemical analysis of various metabolites in pea resulting *M. incognita* infection<sup>1</sup>. The present study is undertaken to analyse the qualitative changes in various free amino acids in pea due to root-knot nematode infestation.

Surface sterilized seeds of *Pisum sativum* (cv. Bonneville) were sown singly in 15 cm earthen pots containing autoclaved river bed sand. One week old seedlings were inoculated with 1000 infective juveniles/pot. Uninoculated plants served as control. The plants were fed with full strength Long Ashton nutrient solution throughout the experimental period. Thirty, sixty and ninety days after inoculation plants were uprooted, root system was washed carefully, oven dried at 40°C and finely powdered. One gm of each sample was homogenized separately in 10ml of 80% ethanol, centrifuged and supernatant was collected. It was shaken vigorously with chloroform (1:3) in a separating funnel. The resulting upper layer was collected and concentrated. The final concentration of the extract of each sample was made to 1 ml with

50% ethanol. Only 0.1 ml of each sample was spotted to one corner of the chromatography paper (Whatman filter paper No. 1) for two dimensional chromatography. The paper was first developed in n-butanol-acetic acid-water (4:1:1 v/v) and later in phenol - water (3:1 w/v) solvent. After developing, the paper was dried under a hood and sprayed with ninhydrin reagent (300 mg of ninhydrin dissolved in 100 ml of acetone). The sprayed paper was dried at room temperature for 10—15 min and heated at 100°C for 10 min in a ventilated oven. Each amino acid spot was identified on the basis of standard chromatogram for different amino acids developed by the same procedure under the same laboratory conditions.

Sixteen amino acids were identified in 60 days old infected roots as against 14 in healthy roots of the same age (Table 1). Eighteen known and at least 10 unknown free amino acids from the healthy and *M. incognita* infected tissues have earlier been observed<sup>2</sup>. Cysteic acid and arginine were not detected in diseased as well as healthy roots. On the other hand, glycine, methionine, threonine, tyrosine, valine & proline present in all the samples. Aspartic acid was found only in infected roots. Its foliar application reduced the incidence of disease in *M. incognita* infected

**Table 1.** Qualitative detection of free amino acids in healthy and *Meloidogyne incognita* infected pea roots.

Amino acids	30 days		60 days		90 days	
	H	I	H	I	H	I
Alanine	+	-	-	+	-	-
Arginine	-	-	-	-	-	-
Aspartic acid	-	+	-	+	-	+
Cysteic acid	-	-	-	-	-	-
Glutamic acid	+	-	+	-	-	-
Glycine	+	+	+	+	+	+
Histidine	+	-	+	+	+	+
Hydroxyproline	+	-	+	+	+	+
Isoleucine	+	-	+	+	+	-
Leucine	+	-	+	+	+	-
Lysine	-	+	+	+	+	+
Methionine	+	+	+	+	+	+
Phenyl alanine	+	-	+	+	+	+
Proline	+	+	+	+	+	+
Serine	-	+	-	+	+	-
Threonine	+	+	+	+	+	+
Tyrosine	+	+	+	+	+	+
Valine	+	+	+	+	+	+
Tryptophan	-	+	+	+	+	+
Total No. of amino acids detected.	13	10	14	16	14	12

+ Present; - Absent; H = Healthy; I = Infected

pea plants<sup>3</sup>. A higher amount of aspartic acid was also found in resistant varieties of rice infected by *M. graminicola*<sup>4</sup>. Hence, it is likely that aspartic acid plays an important role in imparting resistance response to the host towards nematode. In the present study more tryptophan was found to be present in the infected roots, as the spots were more intense. Farooqui *et al.*<sup>5</sup>, also observed a higher concentration of tryptophan in root galls of tomato. The growth regulator, indole - 3- acetic acid (IAA) might be synthesized

from its precursor tryptophan and increase in IAA in the growing region of the root might lead to the formation of galls.

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