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HPTLC-Based Analysis of Root-Knot Nematode-Induced Changes in L-Cysteine and L-Methionine in Pigeon Pea

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ABSTRACT

Root knot nematode infection causes significant change in biology and physiology of host plants. In this context, an experiment was conducted to assess change in free amino acids in pigeon pea roots infected by root knot nematode. In HPTLC analysis it was found that L-glutamic acid was seen in all treatments of resistant variety, however L-ornithine and L-tyrosine was seen in susceptible variety. L-methionine and L-cysteine were absent in resistant healthy varieties but inoculation of nematode these two amino acids were appeared. The resistant variety (UPAS-120) infected sample showed a considerable rise in the L-methionine. The amount of the amino acid L-proline was increased most in the roots of the susceptible pigeon pea varieties (IPA-14-7 and CO-6). This method was found highly effective, and useful for the analysis of amino acids for better understanding of nematode-plant interactions.

Keywords: Amino acids, HPTLC, Root knot nematode, Pigeon pea

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INTRODUCTION

In tropical and subtropical regions, pigeon peas are a significant grain legume crop. One of its most important advantages in the agricultural ecosystem is its function in fixing nitrogen in the soil. According to estimates from the food and agriculture organization (FAO), India generated 59% of the world's 5.6 Mt of pigeon pea production (FAO, 2019). Compared to other legumes, pigeon peas is a long duration crop, therefore susceptible to a variety of diseases and pests. Large numbers of plant parasitic nematode species have been associated with pigeon peas globally (Sharma et al., 1994). Root-knot nematodes (Meloidogyne spp.) are stationary endoparasites that parasitize almost all species of higher plants. The morphology and physiology of the host were dramatically changed by the root knot nematode (Williamson and Gleason, 2003). In india Meloidogyne incognita cause average yield loss of 23% in pulse crop (Kumar et al., 2020). Severe yield loss up to 19 to 22% in chickpea, 17-23% in urdbean and 14 to 29% in mungbean has been reported by Kumar et al. (2020); Sasser (1989). Infection with Meloidogyne spp. can reduce production by 9-19% in pigeon pea cultivars (Khan et al., 2004).

The majority of research attempts to develop nematode-resistant crop genotypes have focused on detecting morphological, anatomical, and anti-nutritional plant characteristics. However, altering the nutritional makeup of host plants may play a key role - in their defense mechanism. The most significant hurdle to developing and deploying insect-resistant crop plants has been a lack of understanding of the biochemical pathways underlying nematode-plant interactions. This is mostly due to the predominant focus of biochemical profiling on host plants, whereas mapping the changes in biochemical profile/nutritional requirements of target nematode in response to eating on resistant/susceptible host plants has received minimal attention. Imbalances in nutritional content may also be used to manage nematode pests. To map the biochemical inconsistency in host plants and target nematode species, a uniform, repeatable biochemical protocol/method is required. Efforts are being made to determine the role of various amino acids found in host plants and their accessibility to target nematode species in terms of growth and development, which requires an accurate and reliable quantitative analysis approach. Amino acid analysis with high performance liquid chromatography (HPLC) is dependent on several steps, including the collection and processing of test samples for hydrolysis, the methodology of sample hydrolysis, the derivation procedure of hydrolyzed samples, the gradient mixture of different eluents and flow time, and the detector, which allows for better separation of different amino acids.

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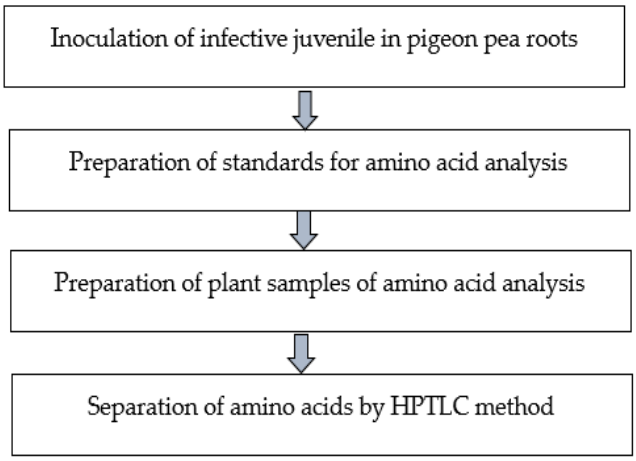


Fig. 1. Workflow for separation of amino acids from roots

Separation of amino acids and amides by HPTLC in roots

Preparation of test samples for analysis of amino acids and amides

Root samples of each treatment were taken and ground separately with 20 ml ethyl alcohol (80%). Such macerates had been boiled in hot water bath for 5 to 10 minutes after maceration. The alcoholic root extract was filtered through a double layered cheese cloth, in order to remove the floating plant debris. Then, the extracts were centrifuged for 10 minutes at 5000 rpm. The supernatant liquid in the test tubes were carefully poured into separate watch glass, and pellets were discarded. Through holding them at room temperature, the supernatant liquid was allowed to evaporate to complete dryness in hot air oven at room temperature. The watch glass finally obtained a syrupy substance which was slightly yellowish in colour. The syrupy substance was dissolved in 1 ml of 10% isopropyl alcohol and transferred to a separate glass tube.

Preparation standard amino acids and amides

The standard maps were prepared using twenty amino acids. The amino acids were L-threonine, L-lysine, l-histidine, L-arginine, L-glycine, L-alanine, L-proline, L-tryptophan, L-tyrosine, L-methionine, L-valine, L-leucine, L-isoleucine, L-phenyl alanine, L-aspartic acid, L-glutamic acid, L-cysteine, L-ornithine, L-hydroxyproline. The standard solutions of each amino acid were made by dissolving 0.5 mg of each in 1 ml of 10% isopropyl alcohol solution. It was not very easy to dissolve the amino acids namely, L-phenylalanine, L-tryptophan and L-glutamic acid. A few drops of 100 per cent hypo-chloric acid have subsequently been added to dissolve them.

Instrumentation and chromatographic condition

HPTLC was performed on 20 × 10 cm aluminium backed plates coated with silica gel 60F 254 (Merck, Germany). 8 µL of standard solution and 10 µL of sample solution were applied to the plates as bands 6 mm wide and 10 mm from bottom edge of the same chromatographic plate by use of a Camag (Switzerland) Linomat V sample applicator equipped with a 100 µL Hamilton (USA) Syringe. Ascending development was performed at room temperature with n-butanol: acetic acid: water (160:40:40) as mobile phase. After development, the plates were air dried and then dipped in ninhydrin solution (0.25%) and the plates were dried in hot air oven. Quantitative analyses of the compounds were done by scanning the plates at 254 nm with a Camag TLC scanner with WINCAT software.

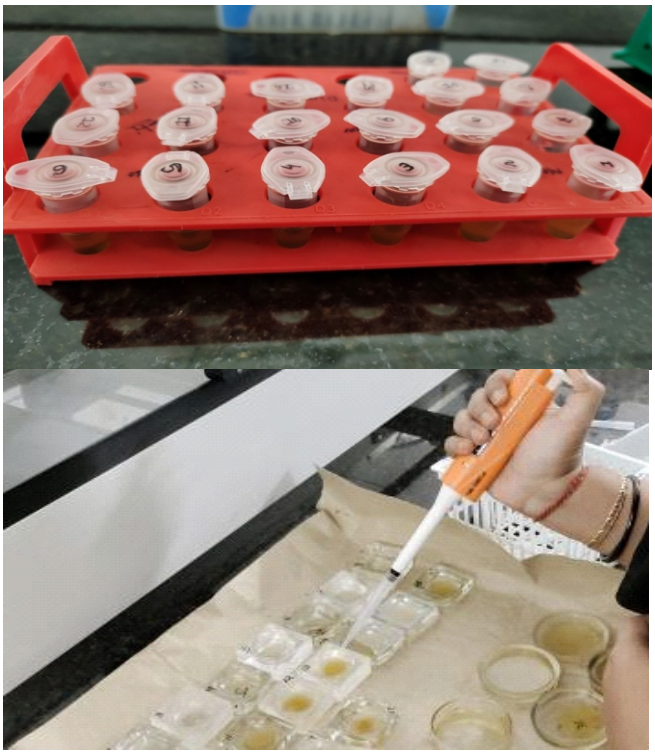


Fig. 2. Preparation of plant sample

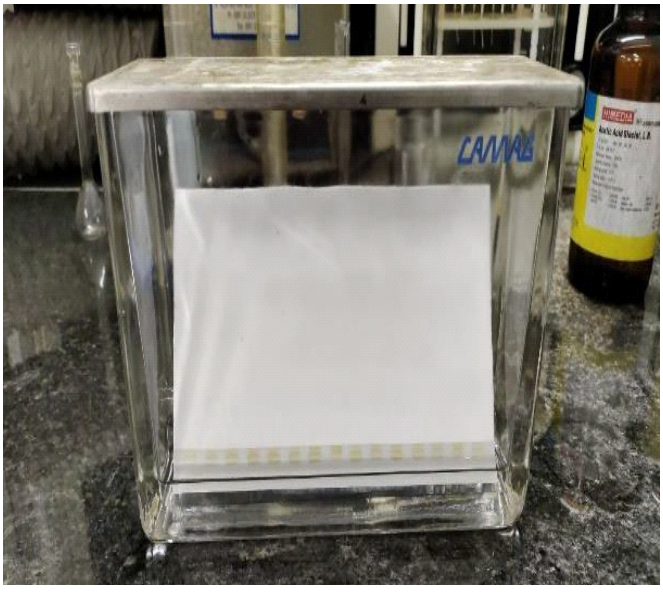


Fig. 3. Loading of sample

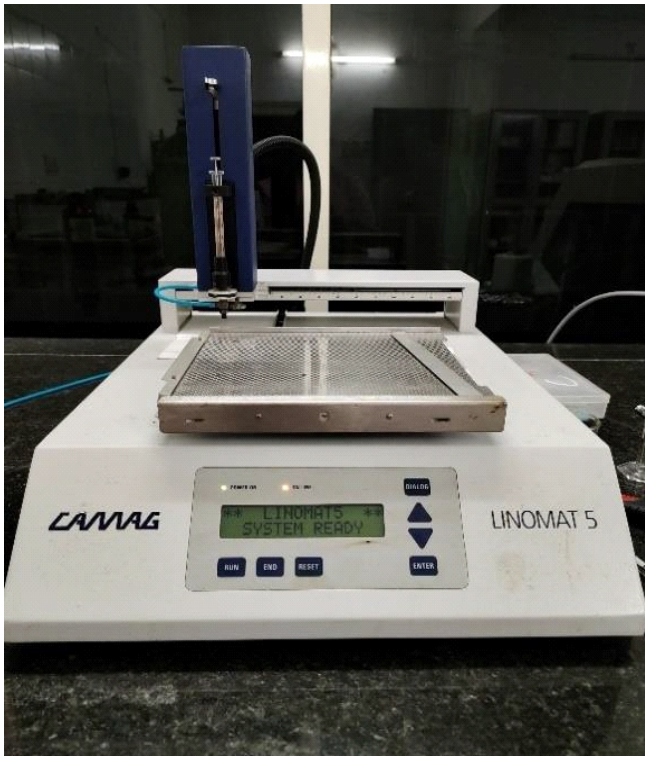


Fig. 4. Analysis using HPTLC

RESULTS AND DISCUSSION

Result presented in Table-1 revealed that nine amino acids viz. L- Glycine, L-hydroxyproline, L-proline, L-serine, L-threonine, L- alanine, L-aspartic acid, L-isoleucine and L-phenylalanine were found in all treatments of UPAS-120, IPA-14-7 and CO-6. L-glutamic acid was seen in all treatments of UPAS-120 however L-ornithine and L- tyrosine was seen in IPA-14-7 and CO-6. L-lysine and L-cysteine were absent in resistant healthy varieties but increase in inoculum level of nematode in treatments T1, T2, T3 and T4 these two amino acids were appeared. L-tyrosine also absent in uninoculated resistant var. but reappeared after inoculation of 1000J2. In two susceptible healthy pigeon pea varieties (IPA-14-7 and CO-6) two amino acids like L-valine and L-methionine were absent but detected after nematode infection in treatments T1, T2, T3, and T4. In treatment T4 where 2000 J2 nematode was inoculated the amino acid L-histidine was getting depleted in the all varieties. There was decrease in L-arginine content in all inoculated treatments of all varieties over check. Maximum decrease was seen in susceptible varieties than resistant varieties. The resistant variety (UPAS-120) infected sample showed a considerable rise in the sulphur-containing amino acid L-methionine. L-methionine is frequently used in the trans-methylation process to create methylated molecules like thiamine, methylated phenols, and lignin. It also plays a significant part in the development of resistance reactions. The amount of the amino acid L-proline increased most in the roots of the susceptible pigeon pea varieties (IPA-14-7 and CO-6) and least in the roots of the resistant UPAS-120 after nematode inoculation. The stress condition of the nematode-induced host plants may be related to L-proline accumulation in infected samples. Less L-proline was accumulated in T1 of all varieties of pigeon pea plants when 500J2 infected roots compared to their control. A free proline generated in the leaves and transported to the site of nematode infection provides the energy needed by the high metabolic activity in roots linked to large cells, gall formation, and egg production. The decrease in L-tryptophan was observed in resistant UPAS-120, susceptible IPA-14-7 and highly susceptible CO-6 after inoculation of nematode M. incognita which could be concerned as the precursors of IAA in the galling activities and thus imparts susceptibility to the host plants.

Table 1. Separation of amino acids in pigeon pea roots infected by root knot nematode

Sample	(R)					(S)						(HS)				
Amino Acid	T1	T2	T3	T4	T5	T1	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
L-Lysine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Histidine	+	+	-	-	+	+	-	-	-	-	+	-	-	-	-	+
L-Ornithine	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
L-Arginine	+	+	+	-	+	+	-	-	-	-	+	-	-	-	-	+
L-Glycine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Glutamic Acid	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	+
L-Hydroxyproline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Proline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Serine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Threonine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Cysteine	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	-
L-Alanine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Aspartic Acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

L-Methionine	-	-	-	+	-	-	+	+	+	+	-	+	+	+	+	-
L-Valine	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-
L-Tyrosine	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+
L-Isoleucine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L- Leucine	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
L-Phenylalanine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Tryptophan	+	+	-	-	+	+	+	-	-	-	+	-	-	-	-	+

Note: R = Resistant, S= Susceptible and HS= Highly Susceptible; + =Present, - =Absent

Result revealed that nine amino acids viz. L- Glycine, L-hydroxyproline, L-proline, L-serine, L-threonine, L- alanine, L-aspartic acid, L-isoleucine and L-phenylalanine were found in all treatments of UPAS-120, IPA-15-1, IPA-14-7 and CO-6. L-glutamic acid was seen in all treatments of UPAS-120 and IPA-15-1 however L-ornithine and L- tyrosine was seen in IPA-14-7 and CO-6. L-lysine and L-cysteine were absent in resistant healthy varieties but increase in inoculum level of nematode in treatments T1, T2, T3 and T4 these two amino acids were appeared. L- tyrosine also absent in uninoculated resistant var. but reappeared after inoculation of 1000J2 of UPAS-120, IPA-15-1, IPA-14-7 and CO-6. Results obtained in this investigation along with the information available in literature indicate increase in amino acid content during post infection period, which is considered to be disease related (Epstein and Cohn, 1971; Howell and Krusberg, 1966; Mohanty and Das, 1976). Increase in the concentration of tryptophan in infected roots compared with healthy roots may have role in gall production, as a precursor of IAA. Such a phenomenon suggests possible interconversion of one amino acid to another (Steward and Browell, 1962) . The appearance of L-proline in nematode inoculated samples is considered to be important. This amino acid is always associated with stress condition. Appearance of L-methionine in only nematode inoculated samples seems to have some role in symbiotic nitrogen fixation and plant nematode interaction. It is also interesting to note that L-glutamic acid identified in healthy tissue was missing in infected roots. An increase in certain amino acid with concomitant decrease in another is known in some host infected with nematode (Mohanty and Das, 1976). It is also interesting to note that L-serine, L-histidine and L-aspartic acid identified in healthy roots were not detected in inoculated samples. Also, it was investigated that amino acid content of two brinjal varieties viz. Pusa Purple Long (susceptible) and Ghatikia White (resistant) inoculated with root-knot nematode, M. incognita and revealed that five amino acids like L-cysteine, L-serine, L-tryptophan, L-leucine and L-isoleucine were found to be common in both the varieties (Mohanty et al., 1995). Higher concentration of various amino acids and amides were detected in each variety upon nematode inoculation except L- tryptophan.

CONCLUSION

Infection by Meloidogyne incognita induced notable changes in the amino acid profiles of resistant (UPAS-120), susceptible (IPA-14-7), and highly susceptible (CO-6) pigeon pea varieties. The presence or absence of specific amino acids and alterations in their levels indicated differential biochemical responses associated with nematode infection and host resistance. The comparatively lower accumulation of L-proline in the resistant variety, together with a considerable increase in L-methionine in infected UPAS-120, suggests their potential involvement in the host defense response. Appearance of L-methionine in only nematode inoculated samples seems to have some role in symbiotic nitrogen fixation and plant nematode interaction. In contrast, greater L-proline accumulation in susceptible varieties may reflect increased physiological stress and metabolic alterations associated with gall formation and nematode development. These findings provide insights into the biochemical responses of pigeon pea to root-knot nematode infection and may contribute to identifying potential biochemical markers associated with resistance; however, further quantitative and molecular studies are required to validate their roles in nematode resistance.

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