# FREE AMINO ACID CHANGES IN THE ROOTS OF BRINJAL INFECTED WITH ROOT KNOT NEMATODE, *Meloidogyne incognita*.

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**ABSTRACT**

Chromatographic analysis for free amino acids of brinjal (var. Pusa purple long, Kantimundi and Ghatikia white) infected with root knot nematode *Meloidogyne incognita* under pot culture conditions revealed the presence of 6 free amino acids such as L-cysteine, L-serine, L-tryptophan, L-leucine ,L-isoleucine and L-tyrosine both in healthy and nematode inoculated samples of susceptible and resistant varieties. The rate of decrease of tryptophan was less in resistant varieties as compared to susceptible variety, Pusa purple long. The decrease of phenylalanine and tyrosine were more pronounced in resistant varieties during post infection period. Quantitative increased in most of the amino acids, observed in nematode infected samples except L-tryptophan, L- tyrosine, L-phenylalanine and L-methionine which were decreased to various extent as a result of nematode infection.

**Key words**: Free amino acids, Brinjal, *Meloidogyne incognita.*

Brinjal, commonly known as egg plant is one of the most widely grown vegetable crops in India as well as in Odisha. Besides its nutritional value, it is well known for its medicinal value and is useful against liver complaints, toothache, diabetes etc. In addition to pest and diseases, plant parasitic nematode have also become limiting factor in successful cultivation of brinjal. Amongst, the nematode, root knot nematode, *Meloidogyne incognita* is considered as most serious nematode pest of brinjal and causes significant yield loss. Though the nematicides are effective against nematodes, yet they are hazardous for health, soil and environment. Plant resistance is one of the eco-friendly options for the management of nematode disease. For this development of authentic resistant varieties, it is utmost essential to study the biochemical and molecular reactions of plant nematode interaction. With a view to establish a link between mechanism of resistant and biochemistry and physiology of pathogenesis the present investigation was undertaken to find out the changes in amino acids in three varieties (one susceptible and two resistant) of brinjal following *M. incognita* infection.

## MATERIALS AND METHODS

In order to understand the basis of nematode resistance relating to various amino acids three brinjal varieties namely Pusa purple long (susceptible), Kantimundi and Ghatikia white (resistant) were selected for this study. Stock cultures of the root- knot nematodes, *Meloidogyne incognita* for the present study was obtained from a single egg mass progeny, maintained and multiplied on susceptible brinjal variety, Pusa Purple long. Earthen pots of 15cm dia were surface sterilized with formaldehyde solution (1.0%) and filled with autoclaved soil @1 kg/ pot. Surface sterilized seed of above three brinjal varieties were sown in pots. One month after germination these were thinned keeping one seedling per pot. The second stage juveniles of *M. incognita* were inoculated @ 1000 $j_2 \pm 20$ per kg soil. Side by side for each variety a healthy un-inoculated check sets were also maintained. After 45 days of nematode inoculation the plants were uprooted and the roots were thoroughly washed with tap water and double distilled water. Then the roots were surface sterilized in 0.1% Mercuric chloride solution followed by repeated washing with distilled water. The washed roots were blotted and weighed. Five gram of root samples of each treatment were taken, ground separately with 80% ethyl alcohol in a sterile mortar and pestle till the roots were macerated to pulp. These materials after grinding were boiled for 5-10 minutes in a hot water bath and filtered through a double layered fine cheese cloth. The alcoholic root extracts were centrifused at 5000 rpm for 10 minutes. The supernatants carefully decanted into separate watch glasses and allowed to evaporate till a slight yellowish colour syrupy material obtained. This syrupy material was dissolved in 1 ml of 10% isopropyl alcohol and transferred to separate glass vials representing for a single lot and stored in the freezer compartment of refrigerator. The above solutions were used for analysis of amino acid by two dimensional ascending paper chromatographic techniques (Smith 1960) and subsequently by using TLC scanner (densiometer). After measuring the density of various amino acids and amides from developed chromatograms, their concentrations were calculated with the help of standard graph of known concentration. The result were expressed in terms of milligram of amino acid per gram fresh root.

## RESULT AND DISCUSSION

A perusal of the Table-1 and 2 revealed that, 6 amino acids viz. L-cysteine, L-serine, L-tryptophan, L- leucine, L-isoleucine and L- tyrosine were found to be common in healthy and infected samples of both susceptible and resistant varieties. However, 3 aminoacids like L- glutamic acid, L- arginine and L- asparagine identified in susceptible variety were not detected in both the resistant varieties. Similarly L-phenylalanine, L-valine and L-glycine were identified only in resistant samples. The intensity of ninhydrine positive spots along with the result of quantitative study clearly indicates the increase in various amino acids in both susceptible and resistant plant during post infection period except L-tryptophan, L-phenylalanine and L-tyrosine. Similar observations were also recorded earlier by many workers. (Mohanty and Pradhan,1990;Mishra and Mohanty 2007; Tripathy and Mohanty;2008. Mohanta and Mohanty, 2013). The increase in various amino acids in infected plant may be due to

progressive hydrolysis of plant proteins by the proteolyitic enzymes secreted by root- knot nematode, *Meloidogyne incognita* during feeding. The mechanism of giant cell formation is now known to centre around the auxin activity in the galled tissue. In the present study the reduction of free tryptophan as infection progressed might be concerned in the galling activity as a precursor of IAA. The decrease in L-tryptophan content of resistant varieties were less as compared to susceptible check which clearly indicates the restricted release of IAA in the resistant varieties.

The quantitative decrease of aromatic amino acid L- phenylalanine and L- tyrosine in the nematode infected samples appears to be intresting and significant. These two amino acids are known to have significant role in imparting resistant reaction through lignifications. Aromatic deamination of both the amino acids play key role for providing the phenyl propane skeleton necessary for biosynthesis of aromatic compounds (Mote, Dasgupta and Ganguly,1990). The accumulation of L-proline in infected samples of all the varieties is in accordance with the findings of earlier workers. ( Lewis and Mc Clure, 1975; Mishra and Mohanty 2007; Mohanta and Mohanty, 2013). Ratio of proline and hydroxyl proline play vital role in imparting susceptible and resistant reaction. Epstein (1972) has observed a unique therapeutic quality of L-Proline, which is produced in *Longidorous* infected plants as a defence mechanism against infection. The reduction of L-Methionine in the inoculated sample of two resistant varieties and detection only in susceptible inoculated samples appears to be interesting. L-Methionine is widely involved in the trans-methylation reaction in the synthesis of methylated compounds like thiamine, methylated phenol, lignin etc. and play vital role in imparting resistant reaction. It is also interesting to note that, during post infection period some of the amino acids appeared and on the other hand some other disappeared and increase in certain amino acids led to concomitant decrease in some other amino acids. The phenomenon is known to occur in many host infected with the nematode (Epstein and Cohn, 1971; Sahu and Mohanty, 1987) which suggests possible interconversion of one amino acid to another.

It can be concluded from the study that the resistant reaction of brinjal varieties Kantimundi and Ghatikia white to *M. incognita* was triggered off by nematode infection and root knot nematode induced and altered the amino acid metabolism of host, thus resulting in rapid and enhanced synthesis of resistant imparting biomolecules.

Table- 1. Qualitative determination of free amino acids in brinjal roots infected by root knot nematode, *Meloidogyne incognita*.

| Sl. No. | Name of the Amino acids | Rf value in % | Intensity | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Pusa Purple Long | | Kantimundi | | Ghatikia white | |
| | | | Healthy | Inoculated | Healthy | Inoculated | Healthy | Inoculated |
| 1 | L- Cysteine | 6 | + | ++ | ++ | +++ | ++ | +++ |
| 2 | L-Serine | 18 | ++ | +++ | + | + | + | ++ |
| 3 | L-Tryptophan | 51 | +++ | + | ++ | + | ++ | + |
| 4 | L-Leucine | 72 | ++ | ++ | ++ | +++ | + | ++ |
| 5 | L-isoleucine | 76 | + | ++ | ++ | +++ | ++ | +++ |
| 6 | L-Glutamic acid | 24 | ++ | +++ | - | - | - | - |
| 7 | L-Alanine | 33 | ++ | - | + | ++ | ++ | +++ |
| 8 | L-Threonine | 27 | ++ | - | ++ | +++ | + | ++ |
| 9 | L- Phenylalanine | 61 | - | - | +++ | ++ | +++ | ++ |
| 10 | L-Valine | 64 | - | - | ++ | +++ | ++ | +++ |
| 11 | L- Arginine | 13 | ++ | +++ | - | - | - | - |
| 12 | L- Lysine | 10 | ++ | - | ++ | +++ | ++ | +++ |
| 13 | L- Tyrosine | 42 | +++ | ++ | +++ | ++ | ++ | + |
| 14 | L- Methionine | 56 | - | + | ++ | + | +++ | ++ |
| 15 | L-Proline | 36 | - | +++ | - | ++ | - | ++ |
| 16 | L-Asparagine | 12 | - | + | - | - | - | - |
| 17 | L- Histidine | 9 | - | ++ | + | ++ | + | ++ |
| 18 | L- Glycine | 21 | - | - | + | ++ | + | ++ |

N.B (+) means Lightly developed chromatogram, (++) means Moderately developed chromatogram, (+++) means Intensely developed chromatogram, (-) means Amino acid absent

**Table 2**- Quantitative estimation of amino acids and amides in mg/g fresh weight of brinjal root infected by root knot nematode, *Meloidogyne incognita*.

| SL. No | Name of the Amino acids and amides | Pusa Purple Long | | | Kantimundi | | | Ghatikia White | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Healthy | Inoculated | % age increased (+) or decreased (-) | Healthy | Inoculated | % age increased (+) or decreased (-) | Healthy | Inoculated | % age increased (+) or decreased (-) |
| 1 | L- Cysteine | 0.048 | 0.061 | (+) 27.08 | 0.068 | 0.079 | (+) 16.18 | 0.067 | 0.079 | (+) 17.91 |
| 2 | L-Serine | 0.073 | 0.081 | (+) 10.96 | 0.051 | 0.059 | (+) 15.69 | 0.057 | 0.071 | (+) 24.57 |
| 3 | L-Tryptophan | 0.058 | 0.042 | (-) 27.58 | 0.063 | 0.058 | (-) 7.94 | 0.045 | 0.042 | (-) 6.66 |
| 4 | L-Leucine | 0.054 | 0.056 | (+) 3.70 | 0.064 | 0.071 | (+) 10.94 | 0.043 | 0.049 | (+) 13.95 |
| 5 | L-isoleucine | 0.049 | 0.053 | (+) 8.16 | 0.067 | 0.073 | (+) 8.95 | 0.072 | 0.078 | (+) 8.33 |
| 6 | L-Glutamic acid | 0.070 | 0.075 | (+) 7.14 | - | - | - | - | - | - |
| 7 | L-Alanine | 0.065 | - | - | 0.044 | 0.050 | (+) 13.63 | 0.052 | 0.063 | (+)21.15 |
| 8 | L-Threonine | 0.056 | - | - | 0.066 | 0.079 | (+) 19.70 | 0.040 | 0.049 | (+) 22.50 |
| 9 | L- Phenylalanine | - | - | - | 0.065 | 0.058 | (-) 10.77 | 0.073 | 0.065 | (-) 10.95 |
| 10 | L-Valine | - | - | - | 0.056 | 0.072 | (+) 28.57 | 0.047 | 0.060 | (+) 27.65 |
| 11 | L- Arginine | 0.068 | 0.076 | (+) 11.76 | - | - | - | - | - | - |
| 12 | L- Lysine | 0.065 | | - | 0.055 | 0.059 | (+) 7.27 | 0.068 | 0.076 | (+) 11.76 |
| 13 | L- Tyrosine | 0.074 | 0.073 | (-) 1.35 | 0.070 | 0.068 | (-) 2.90 | 0.067 | 0.064 | (-) 4.48 |
| 14 | L- Methionine | - | 0.045 | - | 0.048 | 0.045 | (-) 6.25 | 0.063 | 0.058 | (-) 7.94 |
| 15 | L-Proline | - | 0.081 | - | - | 0.067 | - | - | 0.049 | - |
| 16 | L-Asparagine | - | 0.032 | - | - | - | - | - | - | - |
| 17 | L- Histidine | - | 0.069 | - | 0.046 | 0.052 | (+) 13.04 | 0.039 | 0.043 | (+) 10.25 |
| 18 | L- Glycine | - | - | - | 0.031 | 0.042 | (+) 35.48 | 0.028 | 0.035 | (+) 25.00 |

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