International Journal of Engineering Applied Sciences and Technology, 2018
Vol. 3, Issue 3, ISSN No. 2455-2143, Pages 32-36
Published Online July 2018 in IJEAST (http://www.ijeast.com)

Characterization of aromatic amino acids in Brady rhizobium induced root nodules of Trigonella foenum-graecum, Cyamopsis Tetragonoloba and Canavalia Gladiate

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Abstract: **The objective of this study is to characterization of aromatic amino acids in root nodules Bradirhizobium induced root nodules of Trigonella foenum-graecum, Cyamopsis tetragonoloba and Canavalia gladiate under pot trial. The amino acid profile was detected by using HPLC reverse phase technique. The total aromatic amino acid such as phenylalanine, tryptophan and tyrosine profile were compared between effective and ineffective nodules of Trigonella foenum-graecum, Cyamopsis tetragonoloba and Canavalia gladiate. Tyrosine was found as predominant aromatic amino acid in all legume roots.**

Keywords: **Rhizobium bacteria, effective and ineffective nodules**.

I. INTRODUCTION

The aromatic amino acids (AAA) phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp) are central molecules in plant metabolism. They are key components in protein synthesis in plants, and they also serve as precursors of secondary metabolites for plant growth (Tzin and Galili, 2010). Besides their function as building blocks of proteins, the three AAA serve as precursors for a variety of plant hormones, such as auxin and salicylate (Tzin and Galili, 2010). These important properties account for the major motivation to characterise the AAA in root nodules of Bradyrhizobium induced root nodules of Trigonella foenum-graecum, Cyamopsis tetragonoloba and Canavalia gladiate.

This study address the call from Fujihara, Terakado and Nishibori (2006), to establish effective symbiosis with legumes, Rhizobia requires different amino acid. *R*hizobium supplies some of these amino acids and the remaining are supplied by the host plant (Randhawa and Hassani, 2002). Symbiotic nitrogen fixation between a host legume and rhizobia is fuelled by the supply of a dicarboxylic acid by the plant to the microsymbiont (bacteroid) and in return the microsymbiont reduces N_2 to ammonia. Reid et al, (1996) have shown that the fixed nitrogen is exported as a mixture of ammonium and alanine. While we have shown the bacterial partner secretes alanine it has long been speculated that nodule metabolism might be regulated by the supply of amino acids to the bacteroid by the plant. The rhizobia bacteria *(Rhizobium, Bradyrhizobium, Azorhizobium, Mesorhizobium* and *Sinorhizobium)* induce the formation of specialized organs, called nodules, on the roots of legume plants and a non-legume plant *Parasponia.* These bacteria enter the nodule cells and get converted into an endo-symbiotic form, called bacteroids, which fixes nitrogen. Prell et al, (2009) proposed that metabolic precursors of branched-chain amino acid biosynthesis could become limited due to bacteroid metabolism and therefore bacteroids have to be supplied with these amino acids by the plant host.

Rhizobia require the availability of 20 amino acids for the establishment of effective symbiosis with legumes. Some of these amino acids are synthesized by rhizobium, whereas the remaining are supplied by the host plant (Randhawa and Hassani, 2002). Sarma and Emerich (2005) suggested that these compounds may/may not be supplied by the plant, but play a pivotal role in the growth and development of bacteroid. Symbiotic nitrogen-fixation and enrichment of the soil with nitrogen could be increased through the inoculation of the seeds with specific Rhizobium spp strains. The processes of root nodulation and nitrogen fixation do not solely depend on biological factors (Halverson and Stacey, 1986). They also depend on a number of abiotic components such as soil profile and certain synthetic chemicals including biocides, fertilizers, insecticides and fungicides (Lenssen, 2015). Balasubramanian and Sadasivam, (1989) noted that Bradyrhizobia introduction to the soil led to significant increase in nodulation and nodule dry weight. Singleton and Stockinger (1983) reported that 75% of effective nodules accounted for 95% of maximum N accumulation in soybeans. Similarly, Bulyaba, (2015) reported that up to 280 kg N ha-1 could be fixed symbiotically, accounting for about 70% of the total plant nitrogen requirement in soybeans. This ability to fix large sources of nitrogen depends on soybean's efficiency for symbiosis between plant roots and Bradyrhizobium japonicum. Aromatic amino acid profile of Rhizobium spp induced legume plants were not studied. The objective of this study is to characterization of aromatic amino acids in root nodules Bradyrhizobium induced root nodules of Trigonella foenum-graecum, Cyamopsis tetragonoloba and Canavalia gladiate under pot trial conditions.

II. MATERIALS AND METHODS

A pot trial was carried out using Sandy Clay Loam from Guntur, Andhra Pradesh region. Soil was representative for the widely spread clay soils in Sofia region. The studied soil had the following agrochemical properties: ph. 6.97, Nitrogen 94.6 Mg, Phosphorus 1.5, Potassium 6.1, Ferrous 3.31, Zinc 0.50, Manganese 6.41 and bulk density 1.31 and organic carbon percentage 0.2. In each pot with volumetric capacity 10 kg of air dry soil four plants of fenugreek were cultivated in four replications. The moisture was maintained at two levels – 60% and 40% water holding capacity (WHC) of the soil.

- Pot culture experiment

1. Inoculum preparation

About 100 ml of broth was taken in a 250 ml Erlenmeyer flask and 1 ml of pure suspension culture containing 6 ×10-7 cells was inoculated. It was kept on a rotary shaker to produce heavily turbid suspension and incubated at 28 ± 2°C for 4 to 6 days. Then the broth cultures (containing 6 ×10-7 cells ml-1) (both effective and ineffective) were mixed with sterilized lignite carrier for seed inoculation.

2. Seed inoculation

Prior to sowing, the seeds of Trigonella foenum-graecum, Cyamopsis Tetragonoloba and Canavalia gladiate were mixed with rhizobial cluture-carrier material and made air dry. These inoculated seeds were sown in pots which had already been prepared.

3. Preparation of earthen pots

Soil from fallow plots was mixed well, sieved and filled in earthenware pots at the rate of 10 kg per pot. The pots were watered to the level of 50 per cent moisture holding capacity of the soil and sterilized in a large horizontal autoclave at 20 lbs pressure for 2 h. They were then allowed to incubate in a pot culture house for 4 days and the soil in each pot was loosened and mixed well with the help of a stout glass rod. In order to compare the effective and ineffective root nodules, the root nodulating tropical legumes, Trigonella foenum-graecum, Cyamopsis tetragonoloba and Canavalia gladiate which produces both effective and ineffective nodules were selected. The seeds of Trigonella foenum-graecum , Cyamopsis tetragonoloba and Canavalia gladiate and Bradyrhizobium strains S24 (effective) and S24A06 (ineffective) were obtained from Tamil Nadu Agricultural University (TNAU), Coimbatore. The *Bradyrhizobium* inoculated seeds were sown in the pot and watered well. Trigonella foenum-graecum, Cyamopsis tetragonoloba and Canavalia gladiate were harvested at full maturity stage 50 days after sowing. Amino acid profile of nodules was studied by Geigenberger et al, (1996) method using HPLC reverse phase technique (Model- SHIMADZU, JAPAN)

III. RESULTS

This section explores analysis amino acids found in root nodules of 3 legume species. In the analysis, 14 amino acids i.e. major amino acids such as Asparagine, Glutamate, Histidine, Alanine, Serine, Tyrosine, Aspartate, Arginine, Phenylalanine and Glutamine. Minor amino acid such as Lysine, Valine, Tryptophan and Methionine The composition of amino acid both effect and ineffective nodules was summarised in table 1.

Table 1: Amino acid profile

Amino acids (µg/mg)	Trigonella foenum-graecum		Cyamopsis tetragonoloba		Canavalia gladiate	
	Effective	Ineffective	Effective	Ineffective	Effective	Ineffective
Alanine	7.571	3.001	5.761	2.998	1.763	6.615
Arginine	5.01	3.124	4.969	2.474	3.312	3.149
Asparagine	40.12	8.31	35.51	9.41	8.56	39.01
Aspartate	5.841	4.54	6.341	4.523	3.549	4.673
Glutamate	18.86	10.556	16.25	12.323	9.002	14.653
Glutamine	4.678	4.508	6.435	5.431	2.128	3.756
Histidine	8.55	5.606	9.111	6.221	4.76	7.35
Lysine	3.59	3.42	4.62	4.53	1.31	2.23
Methionine	0.44	0.54	0.66	0.38	0.43	0.61
Serine	7.371	7.542	8.542	9.479	5.134	6.263
Tryptophan	0.671	1.634	0.497	2.731	2.978	0.532
Tyrosine	7.055	3.264	8.763	4.316	2.345	6.54
Phenylalanine	4.76	2.784	5.45	3.567	1.639	3.128
Valine	2.671	3.981	4.682	5.741	4.56	1.562
Total	**117.188**	**62.81**	**117.591**	**74.124**	**51.47**	**100.071**

A.Trigonella foenum-graecum

AAA account for 14.63 of total amino acid composition. Tyrosine was found dominant in AAA group of both roots of *Trigonella foenum-graecum* induced with effective and ineffective strains. Effective stains of Brady rhizobium produces (12.48 µg/mg) AAA 2 times higher than ineffective strains (7.68 µg/mg) does. The percentage of AAA is 14.632 of total protein in effective root whereas 4.825 % in ineffective nodules (see table 2)

Table 2: Amino acid profile Trigonella foenum-graecum

Amino acids (µg/mg)	Root nodules	
	Effective	Ineffective
Phenylalanine	4.76	2.784
Tryptophan	0.671	1.634
Tyrosine	7.055	3.264
Total	12.486	7.682
Percentage / to	14.632	4.825

Cyamopsis tetragonoloba

AAA account for 14.63 % of total amino acid composition. Tyrosine was found dominant in AAA group of both roots of *Trigonella foenum-graecum* induced with effective and ineffective strains. Effective stains of Bradyrhizobium produces (14.71 µg/mg) AAA 2 times higher than ineffective strains (10.61 µg/mg) does. The percentage of AAA is 17.29 of total protein in effective root whereas 7.86% in ineffective nodules (See table 3)

Canavalia gladiate

AAA account for 14.63 of total amino acid composition. Tyrosine was found dominant in AAA group of both roots of *Trigonella foenum-graecum* induced with effective and ineffective strains. Effective stains of Brady rhizobium produces (6.9 µg/mg) AAA 2 times higher than ineffective strains (10.2 µg/mg) does. The percentage of AAA is 3.583 of total protein in effective root whereas 10.207 % in ineffective nodules (See table 4)

Table3: Amino acid profile Cyamopsis Tetragonoloba

Amino acids (µg/mg)	Root nodules	
	Effective	Ineffective
Phenylalanine	5.45	3.567
Tryptophan	0.497	2.731
Tyrosine	8.763	4.316
Percentage	17.29	7.86

Table 4: Amino acid profile *Canavalia gladiate*

Amino acids (µg/mg)	Root nodules	
	Effective	Ineffective
Phenylalanine	3.128	1.639
Tryptophan	0.532	2.978
Tyrosine	6.54	2.345
Percentage	3.583341	10.20724

IV. DISCUSSION AND CONCLUSION

The aromatic amino acids (AAA), phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp) are central molecules in plant metabolism. The aromatic amino acids profiles of root nodules of Trigonella foenum-graecum, Cyamopsis tetragonoloba and Canavalia gladiate induced by Bradyrhizobium studied. This study showed that the quantity of AAA in effective nodules is significantly more ineffective nodules of Trigonella foenum-graecum. The AAA quantity of effective nodules is double than ineffective nodules of Cyamopsis tetragonoloba. The AAA quality in effective nodules is lower that ineffective nodules. Tyrosine is found to be predominant aromatic amino acid in root nodules of Trigonella foenum-graecum, Cyamopsis tetragonoloba and Canavalia gladiate.

V. REFERENCES

- Balasubramanian, T. and Sadasivam, S. (1989) 'Changes in carbohydrate and nitrogenous components and amylase activities during germination of grain amaranth', Plant Foods for Human Nutrition, 39(4), pp. 325–330. doi: 10.1007/BF01092069.
- Bulyaba, R. (2015) Influence of Bradyrhizobium inoculation and fungicide treatment on development and yield of selected pulse crops Nutritional composition of pulse legume leaves and the impact of leaf removal on yield by Rosemary Bulyaba A thesis submitted to the graduate.
- Fujihara, S., Terakado, J. and Nishibori, N. (2006) 'Accumulation of an aromatic amine, β-phenethylamine, in root nodules of adzuki bean Vigna angularis', Plant and Soil, 280(1–2), pp. 229–237. doi: 10.1007/s11104-005-3096-4.
- Geigenberger, P. et al. (1996) 'Phloem-specific expression of pyrophosphatase inhibits long-distance transport of carbohydrates and amino acids in tobacco plants', Plant, Cell and Environment, 19(1), pp. 43–55. doi: 10.1111/j.1365-3040.1996.tb00225.x.
- Halverson, L. J. and Stacey, G. (1986) 'Signal exchange in plant-microbe inter-

actions.', Microbiological reviews, 50(2), pp. 193–225.

- Lenssen, A. (2015) Influence of Bradyrhizobium inoculation and fungicide treatment on development and yield of selected pulse crops Nutritional composition of pulse legume leaves and the impact of leaf removal on yield by Rosemary Bulyaba A thesis submitted to the graduate.

- Prell, J. et al. (2009) 'Legumes regulate Rhizobium bacteroid development and persistence by the supply of branched-chain amino acids', Proceedings of the National Academy of Sciences, 106(30), pp. 12477–12482. doi: 10.1073/pnas.0903653106.

- Randhawa, G. S. and Hassani, R. (2002) 'Role of rhizobial biosynthetic pathways of amino acids, nucleotide bases and vitamins in symbiosis', Indian Journal of Experimental Biology, pp. 755–764.

- Reid, C. J., Walshaw, D. L. and Poole, P. S. (1996) 'Aspartate transport by the Dct system in Rhizobium leguminosarum negatively affects nitrogen-regulated operons', Microbiology, 142(9), pp. 2603–2612. doi: 10.1099/00221287-142-9-2603.

- Sarma, A. D. and Emerich, D. W. (2005) 'Global protein expression pattern of Bradyrhizobium japonicum bacteroids: A prelude to functional proteomics', Proteomics, 5(16), pp. 4170–4184. doi: 10.1002/pmic.200401296.

- Singleton, P. W. and Stockinger, K. R. (1983) 'Compensation against Ineffective Nodulation in Soybean1', Crop Science, 23(1), p. 69. doi: 10.2135/cropsci1983.0011183X002300010019x.

- Tzin, V. and Galili, G. (2010) 'The Biosynthetic Pathways for Shikimate and Aromatic Amino Acids in Arabidopsis thaliana', Arabidopsis Book, 8, p. e0132. doi: 10.1199/tab.0132.