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Characterization of aromatic amino acids in Bradyrhizobium induced root nodules of Trigonella foenum-graecum, Cyamopsis Tetragonoloba and Canavalia Gladiata

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Abstract: 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 gladiata* 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 gladiata*. 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 gladiata*.

This study address the call from Fujihara, Terakado and Nishibori (2006), to establish effective symbiosis with legumes, *Rhizobia* requires different amino acid. *Rhizobium* 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 gladiata* 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 $28 \pm 2^\circ\text{C}$ 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 gladiata* were mixed with rhizobial culture-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 gladiata* which produces both effective and ineffective nodules were selected. The seeds of *Trigonella foenum-graecum*, *Cyamopsis tetragonoloba* and *Canavalia gladiata* 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 gladiata* 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 gladiata	
	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 strains of Bradyrhizobium 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 strains 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 gladiata

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 strains 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 gladiata*

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 metabo-

lism. The aromatic amino acids profiles of root nodules of *Trigonella foenum-graecum*, *Cyamopsis tetragonoloba* and *Canavalia gladiata* 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 than ineffective nodules. Tyrosine is found to be predominant aromatic amino acid in root nodules of *Trigonella foenum-graecum*, *Cyamopsis tetragonoloba* and *Canavalia gladiata*.

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