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QUALITATIVE ESTIMATION OF FREE AMINO ACIDS FROM THE ROOT NODULES OF *TRIBULUS TERRESTRIS* L.

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Abstract

Free amino acids in the root nodules of *Tribulus terrestris* L. were qualitatively analysed by means of micro-chromatography using polyamide micro-plates. Altogether, 22 free amino acids were identified, glutamic acid, glutamine, aspartic acid and aspargine being the major amino acids. The pattern of distribution of amino acids in these nodules resembled with *Myrica gale* as well as leguminous nodules. Their resemblence with the amino acids of leguminous nodules strengthens the rhizobail nature of the *Tribulus* endophyte. The common occurrence of most of the free amino acids in the nodules of *T. terrestris* on one hand and leguminous nodules on the other may also suggest a chemotaxonomic link between these two major groups of nodulated angiosperms.

Introduction

The observations of Sabet (1946); Mostafa & Mahmoud (1951) and Athar & Mahmood (1972) provide some evidence of nitrogen fixation by nodulated Zygophyllaceous plants. All these workers have concluded that the possible endophyte in root nodules of Zygophyllaceae is a *Rhizobium* species.

The identity and relative abundance of free amino acids in nitrogen fixing root nodules are of particular significance in connection with the biochemical mechanism of nitrogen fixation. Nitrogen metabolism of nodulated plants thus acquires a special interest. Observations on the amino acids in leguminous root nodules have been made by Hunt (1951); Zelitch, Wilson & Burris (1952); Sen & Burma (1953); Pfenning (1956); Butler & Bathurst (1958); Weichsel (1961); Pate, Walker & Wallace (1965); Pliskova (1967) and Risch (1971, 1972). Similarly the amino acids in non-leguminous root nodules have been studied by Miettinen & Virtanen (1952, 1953-a,b); Virtanen & Miettinen (1953); Leaf, Gardner & Bond (1958, 1959); and Wheeler & Bond (1970).

The present studies describe the pattern of free amino acids in the root nodules of *Tribulus terrestris.*

Materials and Methods

Nodules of *T. terrestris* were collected from the plants growing at Karachi University Campus under natural conditions of growth. Nodules were collected from different

plants and they were of different sizes. Extraction of free amino acids from the nodules and their subsequent detection was carried out by dansylation (Gray & Hartley, 1963) with dansyl chloride (Dans-Cl, 1-dimethylamino-naphthalene-5-sulfonyl chloride). The extraction procedure was adopted as described by Neuhoff (1973). To extract the amino acids, 241 mg of fresh nodules of *T. terrestris* were homogenized for 2 minutes in 4.28 ml of 0.05 M $NaHCO_3$ buffer (pH 10.2). The extract was centrifuged for 30 minutes at 15,000 rpm in capillary centrifuge and the clear supernatant obtained was mixed with an equal volume of acetone. The mixture was kept for 60 minutes at -20°C to precipitate any proteins. This was centrifuged again for 30 minutes at 15,000 rpm in capillary centrifuge to discard any precipitate formed. After centrifugation 4 µl of supernatant was removed in a reaction tube and treated with 4 µl of dansyl chloride solution. The mixture was then incubated for 30 minutes at 37°C for dansylation and dried under vacuum. The dry residue was taken up in 5 µl of acetone/acetic acid 3:2, v/v (Woods & Wang, 1967) and was shaken to dissolve the contents for chromatography.

Micro-polyamide sheets were used because the micro-polyamide layers are more homogeneous and give a sharper resolution of dansyl derivatives (neuhoff, Briel & Maelicke, 1971). Polyamide sheets (Schleicher & Schul TLC Ready-Plastic Sheets F 17,00 Micro-Polyamide, Dassel, Germany) were used for microchromatography. The sheets coated on both sides with polyamide (layer thickness 25 µm) were cut to 3 x 3 cm microplates with a pair of well sharpened large scissors. An aliquot (0.2-0.5 µl) was applied on a microplate using a very fine capillary pipette specially prepared for micro-chromatography. The point of application was at one corner of the microplate, 3-4 mm from the edges. The diameter of application point was approximately 0.5 mm and it never exceeded 1 mm.

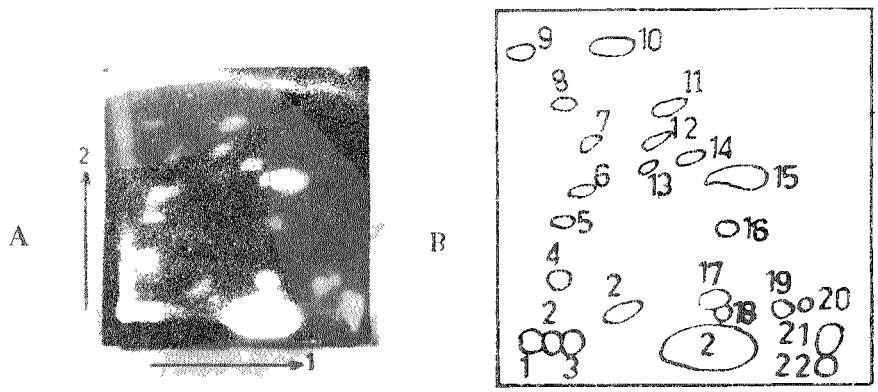

Fig. 1 Two dimensional thin-layer chromatography on 3x3 cm polyamide microplate Solvent system 1st dimension----water/formic acid 100:3 (v/v)
2nd dimension----benzene/acetic acid 9:1 (v/v)

a. chromatogram of dansylated amino acids from root nodules of *Tribulus terrestris*

b. copy showing localization of spots. 1 origin 2. dans-OH 3. dans-cystine/cysteine 4. dans-tryptophan 5. $dans_2$ ornithine 6. $dans_2$-lysine 7. dans-phenylalanine 8. $dans_2$-histidine 9. $dans_2$-tyrosine 10. dans-leucine/isoleucine 11. dans-proline 12. dans-valine 13. dans-methionine 14 dans-GABA 15. dans-alanine 16. dans-glycine 17. dans-glutamic acid 18. dans-asparric acid 19. dans-glutamine 20. dans-serine 21. dans-asparagine 22. dans-arginine.

Two dimesional chromatographic development was carried out in 50 ml beakers using formic acid/water 1.5:50 v/v (Neuhoff, Briel & Maelicke, 1971) as solvent system for the first development and benzene/acetic acid, 9:1 (Woods & Wang, 1967) as solvent system for the second development. For evaluation of the micro-chromatograms, the dansyl spots were marked under UV light with a sharp soft lead pencil. The dans. amino acids were identified by comparing the R_f values with those of the standard dans. amino acds. This technique has been used for the first time in the qualitative analysis of amino acids from the nodules.

Results

The results are tabulated in Table 1 and Fig. 1 shows chromatogram of dansylated amino acids from root nodules of *T. terrestris*. Only those spots were marked which gave yellow to yellow-orange fluorescence under UV light. Altogether, 22 amino acids were detected. Visual inspection of chromatograms suggested that glutamic acid, glutamine, asparatic acid and asparagine were the major amino acids. Along with these major amino acids, 18 other amino acids were also identified (Table 1).

Table 1. Amino acids identified from root nodules of *T. terrestris*.

Composition	Amino Acids
Major Amino Acids	Glutamic acid, Glutamine, Aspartic acid, Asparagine.
Other Amino Acids	Cystine, Cysteine, Tryptophan, Serine, Proline, Glycine, Alanine, Valine, Methionine, Leucine, Isoleucine, Tyrosine, Phenylalanine, γ -Amino butyric Acid, Ornithine, Lysine, Histidine, Arginine.

Discussion

Wheeler & Bond (1970) examined the amino acids of 9 species of non-legumes and reported 21 amino acids from them. Citrulline was not found in any of them except *Alnus* species. They established two patterns of distribution of free amino acids in non-legumes viz., *Alnus glutinosa* pattern and *Myrica gale* pattern. In nodules of *A. glutinosa*, the major amino acids detected were citrulline, glutamine, glutamic acid and aspartic acid while in *M. gale* nodules major amino acids were asparagine, aspartic acid, glutamine and glutamic acid. The latter pattern appeared in most of the plants examined by them. The pattern of distribution of amino acids in *T. terrestris* nodules is more closely related with *M. gale* than the other non-legumes described by Wheeler & Bond (1970). This can be said on the basis that citrulline was not detected and asparagine, aspartic acid, glutamine and glutamic acid were more frequent. of the 18 other amino acids detected (Table 1), 15 of these were reported from non-legume nodules, while 3 of them viz., cystine, cysteine and tryptophan observed in the *T. terrestris* have not been found by Wheeler & Bond (1970).

Leaf, Gardner & Bond (1959) have reported that free amino acid composition of many legumes resembles some what to that of *M. gale* nodules. However, glutamine which is present in *M. gale* nodules may or may not be present in the legume nodules. Butler & Bathurst (1958) analysed the amino acids of 10 legumes and glutamine was observed only in one of them, whereas Hunt (1951) and Virtanen & Miettinen (1953) mentioned the presence of glutamine in various legume nodules. Sen & Burma (1953) did not find glutamine in the study of the amino acids in nodules of 4 legume species. Glutamine was consistently observed in the present studies.

Hunt (1951) analysed the amino acid component of 5 species of legumes belonging to different cross-inoculation groups. It is interesting to note that citrulline was absent from the nodules of all the 5 species. Sen & Burma (1953), Butler & Bathurst (1958), and Risch (1971, 1972) also reported absence of citrulline from different legume species, while most of the amino acids found in these species and in the species described by Hunt (1951) were present in *T. terrestris*. The common occurrence of free amino acids in *T. terrestris* nodules and the nodules of legumes strengthens the observations of Sabet (1946), Mostafa & Mahmoud (1951), and Athar & Mahmood (1972) regarding the rhizobial nature of the endophyte. Leaf, Gardner & Bond (1958) working with *Alnus* nodules, have suggested that the fractions of total nitrogen examined by them were derived from two sources namely the endophyte and the protoplasm of the nodule cells. The bulk of soluble nitrogen was derived from the latter source, since it is the protoplasm of the nodule cells where the greater part of the fixed nitrogen is further metabolized and prepared for transport. This conclusion partly explains the common occurrence of most of the major amino acids and majority of other amino acids in the two non-legumes *T. terrestris* and *M. gale* on one hand and between *T. terrestris* and legumes on the other which share a common endophyte in their nodules. The amino acid pattern of *T. terrestris* nodules may also suggest a chemotaxonomic link between legumes and non-legumes, the two major groups of Angiosperms.

Sen & Burma (1953) have stressed the point that detection of amino acids is correlated with the age of the nodular tissue. As mentioned earlier, the nodules used in this study were collected from plants growing in natural habitat and the nodules were of different age and of different sizes. The analysis of amino acids from the plants cultured in controlled conditions and the sampling of nodules at definite intervals would provide a more comprehensive picture of the pattern of amino acids. Further work on the qualitative and quantitative estimation of free amino acids in Zygophyllaceae is being carried out with particular reference to the seasonal variations and the age of the nodules.

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