Journal of Applied Botany and Food Quality 83, 148 - 150 (2010)

Department of Botany, University of Karachi, Karachi-75270, Pakistan.

Comparative study of free amino acids from root nodules of four tree legume species

Qadri, R., Mahmood, A., Athar, M., Mahmood, N.

(Received December 2, 2009)

Summary

Free amino acid composition in nitrogen fixing root nodules of the leguminosae (Fabaceae) *Albizia lebbeck* (L.) Benth., *Pithecellobium dulce* (Robx.) Benth., *Samanea saman* (Jacq.) Merr. of subfamily Mimosoideae and *Dalbergia sissoo* Roxb. ex DC of subfamily Papilio-noideae was determined. In all, 14 amino acids were detected. On the basis of their contribution to the total amino acid composition of nodules, seven of them, namely aspartic acid, glutamic acid, serine, tyrosine, alanine, leucine and valine were designated as major amino acids. They were present in all four species. They together formed the bulk of the total amino acid nitrogen (TAAN). Seven amino acids, i.e. histidine, lysine, cystine, glycine, isoleucine, phenylalanine and arginine were sporadically distributed in nodules of all the four legume species. They were present in small amounts and were designated as minor amino acids. The TAAN in members of Mimosoideae was found higher than the Papilionoideae. Minor amino acids were not detected in nodules of Papilionoideae. The pattern of free amino acids in the root nodules of these four legume trees was compared with non-leguminous and actinorhizal nodules.

Abbreviations: h: hour; µL: microlitre; ml: milliliter; TAAN: total amino acid nitrogen

Introduction

Useful information regarding the mechanism of symbiotic nitrogen fixation by legumes may be obtained from a detailed investigation of amino acid composition of infected regions of root nodules. This would require extensive qualitative and quantitative surveys involving different taxonomic groups of legumes. Depending on host species, root nodules are of determinate ureide exporting type found in tribe Phaseoleae or of the indeterminate amide exporting type found in tribe Viceae (SPRENT, 1981). Literature survey of amino acid composition of root nodules of legumes indicates that mainly herbaceous legumes have been examined in the past (BLOM et al., 1981; BUTLER and BATHURST, 1958; HUNT, 1951; PEITER et al., 2004; SEN and BURMA, 1953; SNAPP and VANCE, 1986). Indigenous tree legumes deserve greater attention because they play an important role in the overall nitrogen increment of Pakistani soils (MAHMOOD, 1999; QADRI and MAHMOOD, 2009). In the present study a comparative account of free amino acid composition of four species of tree legumes is presented.

Materials and methods

Free amino acids were analyzed from root nodules of four tree legume species commonly found in Pakistan. These species included *Albizia lebbeck* (L.) Benth. *Pithecellobium dulce* (Robx.) Benth., *Samanea saman* (Jacq.) Merr. (Mimosoideae) and *Dalbergia sissoo* Roxb. ex DC (Papilionoideae). Plants of these species were grown from seeds in 13 cm diameter pots containing vermiculite, watered with nitrogen-free Hoagland solution and maintained under an 18-h photoperiod for 12 weeks, in the greenhouse of Department of Botany, University of Reading, England. Nodulation in these species was induced by inoculating the seeds with effective rhizobial strains (SOMASEGARAN and HOBEN, 1994). In most cases nodules were drawn from at least five plants of each species and the bulked nodules (fresh weight usually 6-12 g) were washed, then frozen in a dry ice-alcohol mixture and stored at -20°C until required for analysis. For the analysis, a sample of 5 g fresh weight drawn from the bulked nodules of each species was ground up in 50 ml of 80 % ethyl alcohol and centrifuged at 15,000 x g for 20 minutes. The supernatant was evaporated to dryness at 50°C in a rotary evaporator, added 5 ml of water and evaporated to dryness again. The residue was re-suspended in 10 ml of sodium acetate buffer solution (pH 2.2). 200 µL of each sample was diluted to 1 ml with acetate buffer and diluted 5 times. One ml of a solution of norleucine at a concentration of 1.0 µmole/ml was substituted for 1 ml of the sodium citrate buffer at this stage as an internal standard. Aliquots were analyzed for amino acid composition in EQBA Strumentazone Automatic Amino Acid Analyzer 13A29. Flow rate of buffer in column was maintained at 25 ml/h. Amino acid concentration was expressed in nmoles nitrogen/ml.

Results and discussion

In all, 14 amino acids were detected from root nodules of four the legume species (Tab. 1). Seven of them, namely alanine, aspartic acid, glutamic acid, leucine, serine, tyrosine and valine were present in nodular extracts of all the four species. These amino acids were designated as major amino acids. Out of 14 amino acids, 12 were found in the nodules of *A. lebbeck*, 11 in *P. dulce*, 10 in *S. saman* and 7 in *D. sissoo* (Tab. 1).

Other amino acids detected were arginine, cystine, glycine, histidine, isoleucine, lysine and phenylalanine. These were distributed sporadically in the nodules of *A. lebbeck, P. dulce*, and *S. saman*; hence were labeled as minor amino acids. They were not detected from *D. sissoo* nodules. The TAAN in terms of nmoles nitrogen/ml (rounded) was found highest in the nodules of *A. lebbeck* (2808 nmoles/ml) followed by *P. dulce* (1397 nmoles/ml), *S. saman* (1236 nmoles/ml) and *D. sissoo* (783 nmoles/ml).

Preliminary results of the analytical studies indicated that major amino acids account for 86.5 % of TAAN in *A. lebbeck*, 45 % in *P. dulce*, 41 % in *S. saman* and 28 % in *D. sissoo* nodules. A closer look at the amino acid contents of nodules indicates that aspartic acid, glutamic acid and serine were the main transport vehicle of fixed nitrogen in *A. lebbeck, P. dulce, S. saman* and *D. sissoo* nodules (Tab. 1). These three amino acids together accounted for 67.5 % of the TAAN in terms of nmoles nitrogen/ml.

Amongst minor amino acids, histidine was not detected from *A. lebbeck, P. dulce*, and *S. saman* nodules. Phenylalanine was detected only from nodules of *A. lebbeck* and lysine was detected from nodules of *P. dulce* and *S. saman*. Arginine was detected only from *S. saman*, glycine was detected from *A. lebbeck* and *P. dulce* nodules, cystine was detected only from *A. lebbeck* nodules. Isoleucine was detected from nodules of *A. lebbeck* and *P. dulce* (Tab. 1). The contribution of minor amino acids to the TAAN was 13 % in *A. lebbeck*, 6.0 % in *P. dulce*, 0.04 % in *S. saman* and 0 %

Tab. 1: Amount of free amino acids obtained from root nodules of four species of tree legumes expressed in terms of nmoles nitrogen / ml.

Plant Species	Amount of major Amino Acids *							Total Nitrogen from major Amino acids	Amount of minor Amino Acids **							Total Nitrogen from minor Amino Acids	Total Amino Acid Nitrogen TAAN ***
	Aspartic Acid	Glutamic Acid	Serine	Tyrosine	Alanine	Leucine	Valine		Histidine	Lysine	Cystine	Glycine	Isoleucine	Phenyl alanine	Arginine		
A. lebbeck	810	726	355	187	97	173	77	2426	101	Not detected	26.42	23	54	172	Not detected	376	2802
P. dulce	247.	136	398	184	23	149	95	1231	11	16	Not detected	6	134	Not detected	Not detected	166	1397
S. saman	270	317	366	18	109	43	18	1141	25	20	Not detected	Not detected	Not detected	Not detected	50	96	1236
D. sissoo	123	152	198	105	145	49	11	783	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Zero	783

* Major Amino Acids include Aspartic Acid, Glutamic Acid, Serine, Tyrosine, Alanine, Leucine, Valine
** Other Amino Acids include Histidine, Lysine, Cystine, Glycine, Isoleucine, Phenyl alanine, Arginine
*** Total amino acid nitrogen (TAAN)

in *D. sissoo* nodules (Tab. 1). SEN and BURMA (1953) estimated the quantities of aspartic acid and glutamic acid from root nodules of four legume species. They found that amount of glutamic acid was always higher than the aspartic acid. In the present study the amount of aspartic acid was found higher than the glutamic acid in *A. lebbeck* and *P. dulce* nodules, while amount of aspartic acid was less than glutamic acid in *S. saman* and *D. sissoo* nodules.

Citrulline has been described as one of the dominant amino acids detected from root nodules of some non-legumes (BLOM et al., 1981; LEAF et al., 1958; MIETTIENEN and VIRTANEN, 1952; WHEELER and BOND, 1970). BECKING (1983) analyzed the amino acid content of *Parasponia parviflora* and did not find citrulline; but indicated high levels of glutamine, aspartate and amides. This would suggest that *Parasponia* nodules are amide exporters similar to legumes of temperate origin (WEBSTER et al., 1995). Synthesis and transport of these amino acids in nodules of *Parasponia andersonii* were confirmed by BAKER et al. (1996). Citrulline was not detected in any of the four species investigated in the present study. Our results support HUNT (1951) who stated that citrulline is generally absent from root nodules of legumes. HUNT (1951) identified 24 ninhydrin sensitive compounds from nodules of five legume species. Fourteen amino acids obtained in the present study were also listed by HUNT (1951). LEAF et al. (1959) have reported that free amino acid composition of many legumes resembles somewhat to that of *Myrica gale* nodules. However glutamine which is present in *M. gale* nodules may or may not be present in legume nodules (EGERAAT, 1972). Such nodules are referred to as amide exporters (SCHUBERT, 2007; SPRENT, 1980). Asparagine and serine were detected as major amino acids in indeterminate nodules of *Vicia faba* (PEITER et al., 2004). Serine was also detected as one of the predominant amino acids in present studies. BUTLER and BATHURST (1958) analyzed the amino acids of 10 legumes and glutamine was observed only in one of them. SEN and BURMA (1953) also did not find glutamine in four legumes species studied by them. Glutamine was not detected in all the four species in the present study. However, HUNT (1951) and MIETTIENEN and VIRTANEN (1952) have mentioned the presence of glutamine in various legume nodules. Glutamine was also consistently found in *Tribulus terrestris* root nodules (ATHAR and MAHMOOD, 1980). SPRENT (1981) has analyzed the export of nitrogenous products in the tribes Viceae and Phaseoleae of the subfamily Papilionoideae. Tribe Viceae is considered temperate in origin while Phaseoleae is tropical or subtropical in origin (POLHILL and RAVEN, 1981). The nitrogenous products were exported principally in the form of amino acids and amides in tribe Viceae while in the form of ureides (allantion and allantoic acid) in tribe Phaseoleae. This conclusion has been generalized by PARSONS and SUNLEY (2001) and extended to legumes of temperate and tropical origin. According to them the primary nitrogenous products synthesized in nodules are transferred to the host cells in the form of amides (glutamine and asparagine) in legumes of temperate origin and ureides (allantion and allantoic acid) in legumes of tropical origin. In the present investigations, TAAN detected in root nodules of members of Mimosoideae was found higher than those of Papilionoideae. Moreover the amino acid composition of members of Papilionoideae was solely based on major amino acids since minor amino acids were absent.

In conclusion, it may be said that available data on amino acid profile in nodulated legumes varies with legume species. In addition the existing data is mostly confined to herbaceous legumes. There is a need for extensive surveys of amino acid composition of leguminous plants with particular reference to legume trees.

Acknowledgements

The first author expresses her sincere gratitude to the British Council for providing technical assistance under the ODA Linkage Program to work at the University of Reading, England. We are thankful to Dr. J. Barnett for providing research facilities at the School of Plant Science, University of Reading, England. Thanks are also extended to the Amino Acid Analyzer Unit, Early Gate Laboratory, Department of Agriculture, University of Reading, England, for Providing Laboratory Research Facilities.

References

ATHAR, M., MAHMOOD, A., 1980: Qualitative estimation of free amino acids from the root nodules of *Tribulus terrestris*. Pakistan J. Bot. 12, 91-96.

BAKER, A., DODD, C.D., PARSONS, R., 1996: Identification of amino compounds synthesized and translocated to symbiotic *Parasponia*. Plant, Cell and Environment. 19, 1249-1260.

BECKING, J.H., 1983: The *Parasponia parviflora-Rhizobium* symbiosis. Host specificity, growth and nitrogen fixation under various conditions. Plant Soil. 75, 309-342.

BLOM, J., ROELOFSEN, W., AKKERMANS, A.D.L., 1981: Nitrogen assimilation

in alder. New Phytol. 89, 321-326.

BUTLER, G.W., BATHURST, N.O., 1958: Free and bound amino acids in legume root nodules: Bound γ-aminobutyric acid in the genus *Trifolium*. Australian J. Biol. Sci. 11, 529-537.

EGERAAT, A.W.S.M. van, 1972: Pea root nodules exudates and their effect upon root nodule bacteria. PhD thesis, Agriculture University Wageningen, The Netherlands.

HUNT, G.E., 1951: A comparative chromatographic survey of the amino acids in five species of legume root nodules. Amer. J. Exp. Bot. 38, 452-457.

LEAF, G., GARDDNER, I.C., BOND, G., 1958: Observations on the composition and metabolism of the nitrogen fixing root nodules of *Alnus*. J. Exp. Bot. 9, 320-331.

LEAF, G., GARDNER, I.C., BOND, G., 1959: Observations on the composition and metabolism of the nitrogen fixing root nodules of *Myrica*. Biochem. J. 72, 662-667.

MAHMOOD, A., 1999: A comparison of nitrogen concentration between wild and cultivated legumes of Sindh. Pakistan J. Bot. 31, 183-192.

MIETTIENEN, J.K., VIRTANEN, A.I., 1952: The free amino acids in the leaves, roots and root nodules of the alder. Physiol. Plant. 5, 540-547.

PARSONS, R., SUNLEY, R.J., 2001: Nitrogen nutrition and the role of root-shoot nitrogen signaling particularly in symbiotic system. J. Exp. Bot. 52, 435-443.

PEITER, E., YAN, F., SCHUBERT, S., 2004: Amino acids export from infected cells of *Vicia faba* root nodules: Evidence for an apoplastic step in the infected zone. Physiol. Plant. 122, 107-114.

POLHILL, R.M., RAVEN, P.H., 1981: Advances in Legume Systematics, Part 1 and 2. Royal Botanic Garden, Kew.

QADRI, R., MAHMOOD, A., 2009: A comparative account of symbiosis in nitrogen-fixing tree legumes: Symbiosis in *Albizia*, *Pithecellobium*, *Samanea* and *Dalbergia*. VDM Verlag, Germany.

SCHUBERT, S., 2007: The apoplast of indeterminate legume nodules: compartment for transport of amino acids, amides and sugars? In: Sattelmacher, B., Horst, W.J. (eds.), The apoplast of higher plants: Compartment of storage, transport and reactions, 445-454. Springer, New York.

SEN, S.P., BURMA, D.P., 1953: A study with paper chromatography of the amino acids in legume nodules. Bot Gaz. 115, 1185-190

SNAPP, S.S., VANCE, C.P., 1986: Asparagine biosynthesis in alfalfa (*Medicago sativa* L.) root nodules. Plant Physiol. 82, 390-395.

SOMASEGARAN, P., and HOBEN, H.J., 1994: Handbook for Rhizobia: Methods in Legume-*Rhizobium* Technology. Springer-Verlag, New York.

SPRENT, J.I., 1980: Root nodule anatomy, type of export product and evolutionary origin in some Leguminosae. Plant Cell Environ. 3, 35-43.

SPRENT, J.I., 1981: Functional evolution in some Papilionoid root nodules. In: Polhill, R.M., Raven, P.H. (eds.), Advances in legume systematics, 671-676. Part-II, Royal Botanic Gardens, Kew.

WEBSTER, G., DAVEY, M.R., COCKING, E.C., 1995: With rhizobia: a neglected non-legume nitrogen fixing symbiosis. Ag Biotech, News Info. 7, 119-124.

WHEELR, C.T., BOND, G., 1970: The amino acids of non-legume root nodules. Phytochem. 9, 703-708.

Address of the authors:

Prof. Dr. Raiha Qadri, Department of Botany, University of Karachi, Karachi-75270, Pakistan.

Prof. Dr. A. Mahmood, Department of Botany, University of Karachi, Karachi-75270, Pakistan.

Dr. Mohammad Athar, California Department of Food and Agriculture, 3288 Meadowview Road, Sacramento, CA 95832, USA. (Corresponding Author's E-Mail: atariq@cdfa.ca.gov)

Nadeem Mahmood, Department of Computer Science, University of Karachi, Karachi-75270, Pakistan.