

SYSTEMATIC RELATIONSHIP AMONG THREE SPECIES OF *DESMODIUM* DESV. NOM. CONS. THROUGH QUALITATIVE ANALYSIS OF FREE AMINO ACIDS

MD. KHURSHEED ALAM

Biosystematics and Molecular Biology Laboratory, Department of Botany, Patna University, Patna- 5, India.

Qualitative study of free amino acids has been carried out on three species of *Desmodium* viz. *D. gangeticum*, *D. latifolium* and *D. triflorum*, to establish relationship among them. Study revealed that all three spp. contained species specific amino acids. *D. gangeticum* had four amino acids viz. *L-hydroxyproline*, *glutamic acid*, *DL-methionine* and *L-tyrosine*. *D. triflorum* was characterised by the presence of *L-cysteine*, *L-leucine*, *nor-leucine* and *D. latifolium* showed the presence of *arginine*, *DL-threonine*, *L-proline* amino acids. The study was conducted both on leaves and seeds, and is helpful in differentiating these three species.

Keywords : Chromatogram; Isolation value; Methionine; Non-Protein amino acids; Paired affinity; Taxonomic marker.

Introduction

Desmodium Desv. nom. cons. a genus of sub-family Faboideae has pantropical distribution¹ with South east Asia as main centre of diversity². It has 450 species world wide³ and about 67 species found in India⁴. The species of the genus has great economic potential⁵ and possessed taxonomic ambiguity¹. Amino acids are the organic compounds which contain at least one carboxylic-COOH and an amino-NH₂ group. The amino group is attached to the alpha carbon atom. The plant amino acids are conveniently divided into two groups, the protein and non-protein amino acids. The protein amino acids are generally recognised to be twenty in number and are found in acid hydrolysates of plant and animal proteins. They also occur in the free amino acid pool of plant tissue at concentration varying between 20 and 200µg fresh weight. In general glutamic and aspartic acids are tend to be present in larger amount than the others, since they represent a storage form of nitrogen. There are a number of studies which point out the utility of free amino acids in taxonomic evaluations⁶. In the present study variations have been noted at interspecific level in the quality of free amino acids. Systematic relationships among three species of *Desmodium* viz. *D. gangeticum*, *D. latifolium* and *D. triflorum* has been established through isolation value, paired affinity and group affinity values⁷.

Material and Method

Free amino acid study of leaves and seeds has been carried out on three species of *Desmodium* viz. *D. gangeticum*

(L.) DC., *D. latifolium* DC. *D. triflorum*(L.)DC. Morphologically *D. gangeticum* and *D. latifolium* were shrubs with unifoliate leaves and panicle inflorescence, whereas, *D. triflorum* was creeper with trifoliate leaves and solitary flower. All three species has got characteristic half indented pods.

For qualitative analysis of free amino acid, method of Ranjan and Lalorya was followed⁸. Fresh leaves and seeds were taken. They were separately mixed with ethyl alcohol in a ratio of 1: 3 and grinded in mortar and paste. After repeated centrifugation at 7000 rpm for 30 minutes, supernatant was collected and used as mother solution on which chromatography was conducted. The chromatograms were allowed to run in the first solvent BAW; n-Butanol, glacial acetic acid and distilled water (90:10:29) and then in second solvent containing purified phenol and distilled water in the ratio of 75:25. Amino acids were developed on chromatogram by spraying 0.2 % Ninhydrin solution in water saturated n-butanol. Rf values were calculated for every spot. The amino acids were identified by comparing the Rf of spot contained with those of Rf values of the standard known amino acid. The paired affinity, group affinity and isolation value were calculated by using standard statistical formulae⁷.

Results and Discussion

The number of amino acids detected in leaves of *D. latifolium* was 9 and in *D. triflorum* and *D. gangeticum* it was 12 and 11, respectively. It was remarkable to note that DL-Serine and Unclassified VI were the only amino

Table 1. Distribution of Free amino acids in three species of *Desmodium*.

Sl.No.	Amino acids	<i>D.gangeticum</i>		<i>D. latifolium</i>		<i>D. triflorum</i>	
		Leaf	Seed	Leaf	Seed	Leaf	Seed
1	L-Histidine	+	-	-	+	+	+
2	DL-Serine	+	+	+	+	+	-
3	L-hydroxy proline	+	-	-	-	-	-
4	Glutamic acid	+	-	-	-	-	-
5	DL-Aspartic acid	+	+	+	-	-	+
6	DL-1-Amino-n- butyric acid	+	+	-	+	+	+
7	L-Tyrosine	+	+	-	-	+	-
8	DL-Methionine	+	+	-	-	+	-
9	DL-B-phenylalanine	+	+	-	+	+	+
10	Arginine	-	-	+	-	-	-
11	DL-Threonine	-	-	+	+	-	-
12	L-Proline	-	+	+	-	-	+
13	L-Cysteine	-	-	-	+	+	+
14	L-Leucine	-	+	-	+	+	-
15	DL-nor-keycube	-	-	-	-	+	+
16	Unclassified -I	-	+	-	-	+	+
17	Unclassified -II	-	-	+	-	-	-
18	Unclassified -III	-	-	+	+	-	-
19	Unclassified- IV	-	+	+	-	+	-
20	Unclassified -V	+	-	-	-	+	+
21	Unclassified- VI	+	-	+	+	+	-

acids commonly present in the leaves of three species (Table 1). Three aminoacids, viz. DL-serine, Unclassified IV and VI, have been found to be present in *D. latifolium* and *D. triflorum*. In case of leaves, hydroxyproline, glutamic acid and methionine were specific to *D. gangeticum*; arginine, threonine, proline, unclassified II and III to *D. latifolium*; and cysteine, leucine, nor-leucine and unclassified I to V to *D. triflorum*. The highest paired affinity was observed between *D. gangeticum* and *D. triflorum* (69.56), followed by *D. latifolium* and *D. triflorum* (47.61) and minimum between *D. latifolium* and *D. gangeticum* (30). The group affinity value was highest in the leaves of *D. triflorum* (217.12), followed by *D. gangeticum* (199.56) and *D. latifolium* (177.61). The number of unique amino acids was highest in *D. latifolium* (5), followed by *D. triflorum* (4) and *D. gangeticum* (3). Isolation value was also highest in *D. latifolium* (23.80). It was minimum in *D. gangeticum* (14.28) and intermediate in *D. triflorum* (19.04).

The number of amino acid in seeds of three species have been found to be highest in *D. gangeticum* (10) and lowest in *D. latifolium* (9) and *D. triflorum* (9) (Table 1). DL-1 amino-n-butyric acid and DL-B-phenylalanine were present commonly in the seeds of the three species of *Desmodium*. Four amino acids have been found to be commonly present in the seeds of *D. latifolium* and *D. triflorum* viz. histidine, DL-1-amino-n-butyric acid, DL-B-phenylalanine and cysteine. In case of seeds, tyrosine, methionine, DL-1-amino-n-butyric acid and unclassified IV were specific to *D. gangeticum*. *D. latifolium* was characterised by the presence of threonine, unclassified III and IV while the *D. triflorum* by the presence of nor-leucine and unclassified V. Among the primary products of the plant, studies on the amino acid provide valuable information about the phylogeny and systematic relationship. Changes in the amino acid within the protein, reflect change in the base sequence of the DNA. specific units of DNA are coded for each amino acid. Thus, amount of genetic divergence can be estimated from the amino acid sequence of an organism. Evidence shows the sequence of amino acid in the genera of the same

family are more or less similar to each other than to other families and the morphological characters of closely related families resemble more closely than less related families⁹.

Thus, qualitative study of free amino acid of leaves and seeds may be used as an additional parameter in better understanding of genetic variations occurring among the species. Such variations can be utilised for delimitation of taxa and in establishing systematic relationship among them. Further, this kind of study could lead to detection of specific amino acid in particular species of a genus which can be used as a reliable taxonomic marker.

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References

1. Polhill RM 1976, Genisteeae (Adans.) Benth, and related tribes (leguminosae). *Bot. Syst.* 1 143-368.
2. Ohashi H 1995, An enumeration of Chinese *Desmodium* and its allied genera (leguminosae). *J. Jap. Bot.* 70(2) 111-117.
3. Lewis 2005, Legumes of the Ward. Royal Botanical Gardens, Kew.
4. Sanjappa 1982, A new name for an Indian *Desmodium* Desv. (Fabaceae). *Bull. Bot. Surv. India* 22 229.
5. Kapoor 1992, Leguminous seeds potential industrial sources for gum, fat and protein. *J. Sci. Ind. Res. (India)* 51 415-424.
6. Chennaveeraiah M 1983, Biosystematics; Presidential address, 70th session, *Ind. Sci. Cong. Asso.* Pt. 11; 1-22.
7. Sokal RR and Sneath PHA 1963, Principles of numerical taxonomy. W.H. Freeman and Co. San Francisco.
8. Ranjan and Laloriya 1990, Seed protein of the family Rosaceae, characterisation studies and homologies. *J. Ind. Bot. Soc.* 69 321-324.
9. Fair brother DE 1983, Evidence from nucleic acid and protein chemistry in particular serology in angiosperm classification. *Nord. J. Bot.* 3 35-41.