# Characterization of *Sesbania* Accessions Based on Isozyme System

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A set of 40 accessions of *Sesbania* belonging to 13 *Sesbania* species were characterized by fractionating 6 isozyme systems on polyacrylamide gel electrophoresis (PAGE). The isozyme systems studied were: acid phosphatase, amylase, catalase, glutamate dehydrogenase, peroxidase and malate dehydrogenase. These isozyme systems produced 193 polymorphic bands. A dendrogram was obtained using Jaccards similarity co-efficient and Unweighted Paired Group Method with Arithmetic Average (UPGMA) clustering method. Accessions were grouped into 11 clusters. Four accessions from *S. rostrata* species grouped together in the same cluster. Similar pattern of grouping was observed for *S. aculeata* accessions. An accession from *S. macrantha* species was uniquely isolated from rest of the clusters. However, there was no strict one to one relationship between botanical origin of accessions and their location in the dendrogram. The diversity analysis based on isozyme system may be used to identify diverse parents for inter and intraspecific hybridization in *Sesbania.*

**Key words: *Sesbania*, Isozyme, Cluster analysis, Genetic diversity, UPGMA**

The genus *Sesbania* is one of the important genera of legumes with multifarious benefits for mankind. It is an organic rich biofertilizer due to its heavy root nodulation. *Sesbania* species have also been used as indigenous folk and Ayurvedic, Unani and Siddha systems of medicines. Besides, it has many other uses like ruminant fodder, firewood, wood product, human food (Veasey *et al.*, 1999) and fibre as substitute for jute (Data and Bachi, 1991). With the alarming depletion of natural resources, continuing energy crisis, growing ecological concern from intensive cultivation and use of chemical fertilizers, pesticide and other inputs led to a renewal interest in the activities of research and utilization of *Sesbania* in the recent years, particularly in tropics and sub-tropics (Qadir *et al.*, 2002).

Considering the immense importance of the *Sesbania* species, systematic breeding efforts are required to improve the plant type and economic traits of the crop. Breeding programmes are contingent on germplasm resources. Accordingly, detailed genetic characterization of the *Sesbania* species is desired both for high efficiency of breeding strategy and for commercial seed production. Collection that has not been systematically characterized may contain many duplicates (Steiner and Poklemba, 1994).

Morphological characters have been generally used to study genetic diversity (Tanksley, 1983), but these characters are influenced by environmental factors. Protein markers including seed storage protein and isozyme are among the first group of molecular markers (Cooke, 1984) exploited for genetic diversity assessment. These markers are easy to screen, relatively simple to perform and the cost per sample is low. Isozymic markers have not been completely explored for the study of genetic diversity in the *Sesbania* species. Few reports are available on electrophoretic protein profile and isozymic characterization of *Sesbania.* Sarswati *et al.* (1993) analysed seed protein profile of 17 *Sesbania* species using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The authors reported species-specific protein profiles for 15 of the 17 species. Recently, 22 accessions belonging to seven species of *Sesbania* were evaluated for isozyme electrophoretic patterns (Veasey *et al.*, 2002). They used eight isozymes: acid phosphatase (ACP), isocitrate dehydrogenase (IDH), phosphoglucomutase (PGM), malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), glutamate oxaloacetate transaminase (GOT), peroxidase (PRX) and catalase (CAT) and classified the accessions in eight groups corresponding to the seven species and separate group for an accession of *S. exasperata.*

This study was intended to assess genetic diversity among 40 *Sesbania* accessions using six polymorphic isozyme systems *viz.*, acid phosphatase, amylase, catalase, glutamate dehydrogenase, peroxidase and malate dehydrogenase.

## Materials and Methods

### *Plant Material*

A set of 40 accessions representing a cross-section of variability among *Sesbania* species were obtained from National Bureau of Plant Genetic Resources (NBPGR),

New Delhi. These include 16 indigenous and 24 exotic collections belonging to 13 species from four different countries (Table 1). These accessions were coded: T1, T2 ....T40 to have brief text in their description.

Seeds of the accessions were imbibed on moist filter paper in petridishes at a constant temperature of 30± 2°C in an incubation.

### *Isozyme Assay*

The following six isozyme systems were studied: acid phosphatase (E.C.3.1.3.2), amylase (E.C.3.2.1.1), catalase (E.C.1.11.1.6), glutamate dehydrogenase (E.C.1.4.1.2), peroxidase (E.C.1.11.1.7) and malate dehydogenase (E.C. 1.1.1.3.7).

### *Extraction of Enzymes from the Seeds*

About 500 mg of 24 h imbibed seeds were used to prepare the enzyme extracts in 1.5 ml of appropriate buffer containing 5mM 2-mercaptoethanol and 5 mM EDTA using hand grinding method in chilled pestle and mortar by keeping in ice bucket. Crude extract was centrifuged at 10,000 rpm for 30 minutes at 4°C temperature. The supernatant was collected, stored in refrigerator at 4°C and used the same day for enzyme studies. Samples for catalase, peroxidase, glutamate dehydrogenase and malate dehydrogenase were extracted in 0.05 M sodium phosphate buffer (pH 7.4). Amylase and acid phosphatase were extracted in 0.05 M acetate buffer (pH 5.0). Enzymes were fractionated on polyacrylamide gels (8% running and 2.5% stacking gel) in anionic system using vertical electrophoretic apparatus. Tris buffer (pH 8.3) was used as an electrode buffer. The electrophoresis was allowed to begin with

**Table 1. Details of 40 accessions of *Sesbania***

| Coded Acc. No. | National Identity (IC/EC) | Species | Source |
|---|---|---|---|
| T1 | EC 331970 | *S. rostrata* | IRRI, Philippines |
| T2 | EC 331973 | *S. rostrata* | IRRI, Philippines |
| T3 | IC 277784 | *S. rostrata* | PAU, Ludhiana (Punjab), India |
| T4 | EC 223312 | *S. rostrata* | IRRI, Philippines |
| T5 | EC 178342 | *S. rostrata* | IRRI, Philippines |
| T6 | EC 218472 | *S. rostrata* | IRRI, Philippines |
| T7 | EC 213472-A | *S. rostrata* | IRRI, Philippines |
| T8 | EC 213473 | *S. sesban* | IRRI, Philippines |
| T9 | IC 277791 | *Ses.PDCSR-1* | PDCSR, Modipurum (UP), India |
| T10 | IC 27777 | *S. aculeata* | PAU, Ludhiana (Punjab), India |
| T11 | IC 277786 | *S. aculeata* | PAU, Ludhiana (Punjab), India |
| T12 | IC 277782 | *S. aculeata* | PAU, Ludhiana (Punjab), India |
| T13 | IC 277775 | *S. aculeata* | PAU, Ludhiana (Punjab), India |
| T14 | EC 509439 | *S. sesban* | Brazil |
| T15 | EC 509434 | *S. exasperata* | Brazil |
| T16 | EC 509442 | *S. virgata* | Brazil |
| T17 | IC Ses H-9 | *S. aculeata* | Haryana, India |
| T18 | IC Ses. H-1 | *S. aculeata* | Haryana, India |
| T19 | IC Ses. H-6 | *S. aculeata* | Haryana, India |
| T20 | IC 277789 | *S. aculeata* | PAU, Ludhiana (Punjab), India |
| T21 | EC 493668 | *S. simplicuscula* | Australia |
| T22 | EC 493666 | *S. simplicuscula* | Australia |
| T23 | EC 493664 | *S. exaltala* | Australia |
| T24 | EC 493681 | *S. bispinosa* | Australia |
| T25 | EC 493687 | *S. exasperata* | Australia |
| T26 | EC 493676 | *S. leptocarpa* | Australia |
| T27 | EC 493695 | *S. campylocarpa* | Australia |
| T28 | EC 493688 | *S. exasperata* | Australia |
| T29 | EC 493667 | *S. simplicinscula* | Australia |
| T30 | EC 493662 | *S. exaltala* | Australia |
| T31 | EC 493700 | *S. cannabina* | Australia |
| T32 | EC 493701 | *S. macrantha* | Australia |
| T33 | EC 493702 | *S. macrantha* | Australia |
| T34 | EC 493706 | *S. speciosa* | Australia |
| T35 | IC Pant-1 | *S. aculeata* | Uttranchal, India |
| T36 | IC Pant-3 | *S. aculeata* | Uttranchal, India |
| T37 | IC NBPGR3 | *S. aculeata* | Najafgarh, New Delhi, India |
| T38 | IC Ses.PDCSR2 | *S. aculeata* | Modipurum (UP), India |
| T39 | IC NIC-4149 | *S. aculeata* | India |
| T40 | IC NIC-4223 | *S. aculeata* | India |

15 mA current and supplied till tracking dye entered the gel and subsequently the current was increased to 40 mA for the plates till the marker dye recorded.3.5 mm from the other end of the plate. The enzymes acid phosphatase, amylase, GDH and MDH were detected using the staining methods of Stoddart (1971), Pearse (1972), Malik and Singh (1980) and Soltis *et al.* (1983), respectively. The methods of Robinson (1966) were used to detect catalase and peroxidase enzymes. Binary data matrix was generated taking '1' as presence and '0' as absence of a band. The genetic similarity for all possible pairwise combinations among 40 accessions were estimated using Jaccard's similarity index (1908). The distance measures were computed as complement of Jaccard's similarity index. Accessions were grouped by unweighted paired group method with arithmetic average (UPGMA) using the distance matrix (Romesburg, 1984).

**Results and Discussion**

Abundance of polymorphism for isozymic bands from six isozymic systems was present in *Sesbania* accessions. A total of 193 polymorphic bands were generated from 6 isozymic systems, namely acid phosphatase, amylase, catalase, GDH, peroxidase and MDH. Isozyme markers-based dendrogram grouped *Sesbania* accessions into 11 clusters (Fig. 1). The groups are presented in Table 2. Cluster 1 consisted of 7 accessions, and all were from Australia. Cluster 2 was the largest cluster containing 9 accessions: 3 from Brazil and 6 from India. Cluster 3 contained 6 accessions all from Philippines except one from Australia. Four accessions were included in cluster 4, three were from Australia and one was indigenous. Cluster 5 contained only two accessions, one from Australia and another from India. Three accessions were included in the cluster 6, two accessions were indigenous and one from Philippines. Cluster 7 and 8 contained only one accession each and both were from India. Cluster 9 consisted of 4 accessions, all were from India. The clusters 10 and 11 contained two and one accession, respectively and all were from Australia.

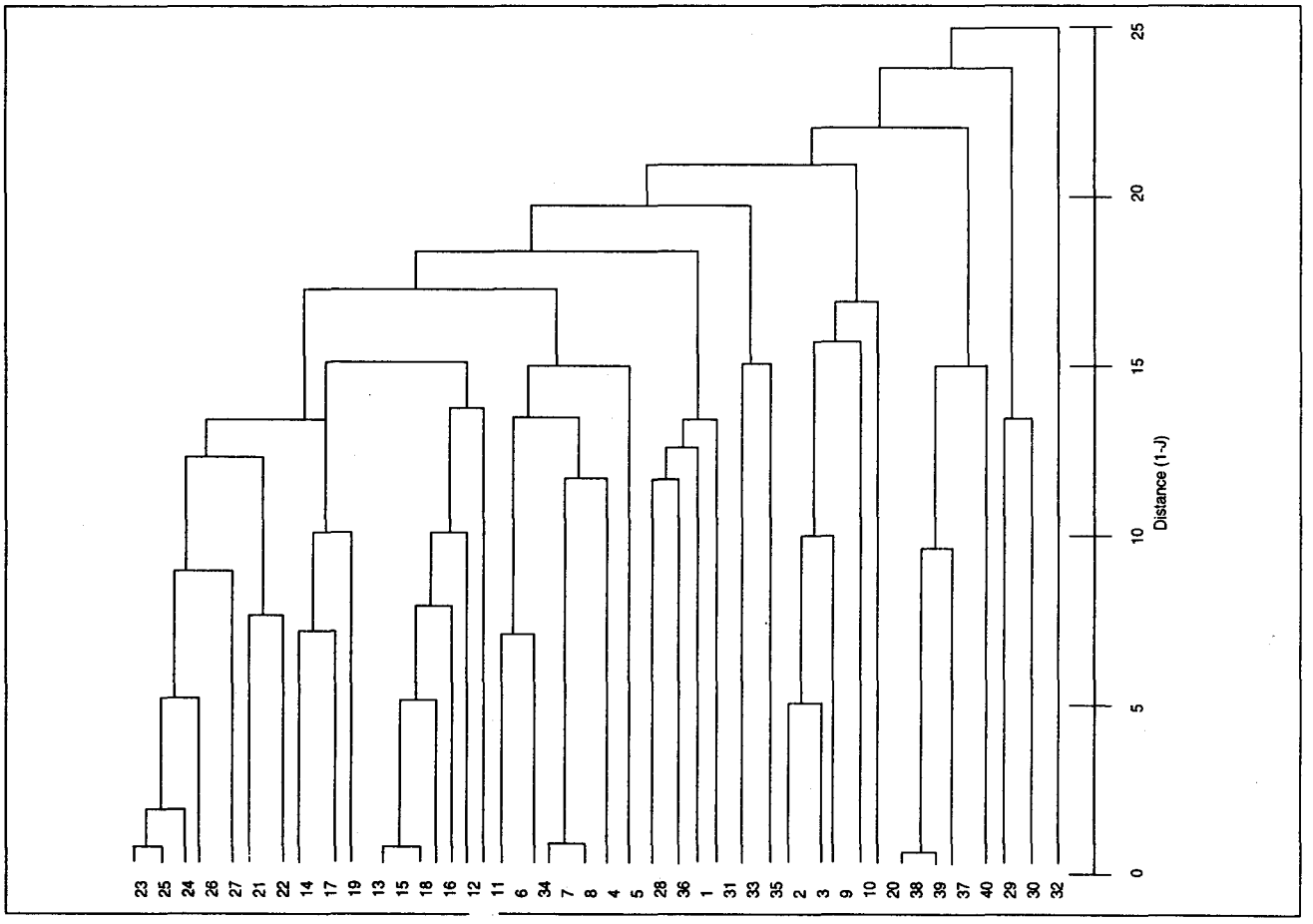


*Fig. 1: Dendrogram from the cluster analysis with presence/absence of band (193 bands) from six isozymatic systems, using Jaccard index (J) and UPGMA method*

Interestingly, four accessions from *S. rostrata* species grouped together in the same cluster (Cluster number 3). More or less, similar pattern of grouping was observed for *S. aculeata* accessions. However, overall grouping of the accessions did not exhibit one to one relation between botanical origin of accessions and their location in the dendrogram. For example, accessions of *S. exaltala*, *S. bispinosa*, *S. exasperata*, S. *leptocarpa*, *S. campylocarpa*, and *S. simpliciuscula* were not separated in the dendogram and were present together in cluster number 1. Similar results have also been reported by Samec *et al.* (1998) in pea and Veasey *et al.* (2002) in *Sesbania*. To the best knowledge of the authors, there is only one report on isozymic characterization in *Sesbania* by Veasey *et al.* (2002). The accession T32 from *S. macrantha* species was uniquely isolated from rest of the clusters and located in cluster number 11. The dendrogram produced from isozymic profiling revealed larger interspecific variation than intraspecific variation. The diverse accessions selected from isozymic based dendrogram may be used to exploit potential parents for inter and intraspecific crossing in *Sesbania*. Similar to grouping pattern in isozymic-based dendrogram, the accessions of *S. rostrata* and the accessions belonging to *S. aculeata* also grouped in their respective clusters in the dendrograms produced from morphological data (Poonam, 2005). However, there were some differences in dendrogram pattern generated from morphological and isozymic data. This may be due to several reasons, such as lack of some of the important morphological/ taxonomic characters related to botanical origin of accessions; isozymic profiles from six isozymic systems

**Table 2. Composition of isozyme based clusters and the geographical origin of *Sesbania* accessions**

| Cluster | No. of Accessions | Coded Acc. | Species | Accession | Origin/Source |
|---|---|---|---|---|---|
| 1 | 7 | T 23 | *S. exaltala* | EC 493664 | Australia |
| | | T 25 | *S. exasperata* | EC 493687 | Australia |
| | | T 24 | *S. bispinosa* | EC 493681 | Australia |
| | | T 26 | *S. leptocarpa* | EC 493676 | Australia |
| | | T 27 | *S. campylocarpa* | EC 493695 | Australia |
| | | T 21 | *S. simpliciuscula* | EC 493668 | Australia |
| | | T 22 | *S. simpliciuscula* | EC 493666 | Australia |
| 2 | 9 | T 14 | *S. sesban* | EC 509439 | Brazil |
| | | T 17 | *S. aculeata* | IC Ses H-9 | Haryana (India) |
| | | T 19 | *S. aculeata* | IC Ses H-6 | Haryana (India) |
| | | T 13 | *S. aculeata* | IC 277775 | PAU, Ludhiana (India) |
| | | T 15 | *S. exasperata* | EC 509434 | Brazil |
| | | T 18 | *S. aculeata* | IC Ses H-1 | Haryana (India) |
| | | T 16 | *S.virgata* | EC 509442 | Brazil |
| | | T 12 | *S. aculeata* | IC 277782 | PAU, Ludhiana (India) |
| | | T 11 | *S. aculeata* | IC 277786 | PAU, Ludhiana (India) |
| 3 | 6 | T6 | *S. rostrata* | EC 218472 | IRRI, Philippines |
| | | T34 | *S. speciosa* | EC 493706 | Australia |
| | | T7 | *S. rostrata* | EC 213472-A | IRRI, Philippines |
| | | T8 | *S. sesban* | EC 213473 | IRRI, Philippines |
| | | T4 | *S. rostrata* | EC 223312 | IRRI, Philippines |
| | | T5 | *S. rostrata* | EC 178342 | IRRI ,Philippines |
| 4 | 4 | T28 | *S. exasperata* | EC 493688 | Australia |
| | | T36 | *S. aculeata* | IC Pant-3 | Uttranchal (India) |
| | | T1 | *S. rostrata* | EC 331970 | Australia |
| | | T31 | *S. cannabina* | EC 493700 | Australia |
| 5 | 2 | T33 | *S. macrantha* | EC 493702 | Australia |
| | | T35 | *S. acuelata* | IC Pant-1 | Uttranchal (India) |
| 6 | 3 | T2 | *S. rostrata* | EC 331973 | IRRI, Philippines |
| | | T3 | *S. rostrata* | IC 277784 | PAU, Ludhiana |
| | | T9 | *S. aculeata* | IC 277791 | Modipurum (U.P.) |
| 7 | 1 | T10 | *S. aculeata* | IC 277777 | PAU, Ludhiana |
| 8 | 1 | T20 | *S. aculeata* | IC 277789 | PAU, Ludhiana |
| 9 | 4 | T38 | *S. aculeata* | IC PDCSR2 | Modipurum (U.P.) |
| | | T39 | *S. aculeata* | IC NIC-4149 | India |
| | | T37 | *S. aculeata* | IC NBPGR-3 | Najafgarh, New Delhi |
| | | T40 | *S. aculeata* | IC NIC-4223 | India |
| 10 | 2 | T29 | *S. simpliciuscula* | EC 492667 | Australia |
| | | T30 | *S. exaltala* | EC 493662 | Australia |
| 11 | 1 | T32 | *S. macrantha* | EC 493701 | Australia |

may have covered only part of whole of the genome; genotype x environment interaction for morphological characters, and variables are standardized (mean zero and variance one) while Euclidean distances among accessions are calculated from agromorphological data. This made all variables (15 agromorphological characters) equally important in determining these distances. Thus, standardization has the effect of minimizing group differences, because if groups are separated well by $X_i$ variable, then the variance of $X_i$ will be large (Manly, 1994). Therefore, inbuilt mechanism of the computation procedure adopted for field data may be one of the factors for observed discrepancy between clustering patterns from field observations via-a-vis isozymic electrophoresis data. This requires a further detailed study for its confirmation. Despite the increasing significance of DNA markers in cultivar identification and phylogenetic studies, the use of isozymes in combination with DNA markers and morphological traits may be still very useful and important (Havey and Muehlbauer, 1989; Chase *et al.*, 1991; Gepts, 1995; Torres *et al.*, 1994; Hoey *et al.*, 1996; Samec *et al.*, 1998). The study established the utility of isozymes for assessing genetic diversity and in grouping of *Sesbania* accessions. However, further investigations using DNA based molecular markers are required to establish the phylogenetic relationship of botanical origin of *Sesbania* accessions.

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