J Indian bot Soc Vol 75 (1996) 7-10

INTER-RELATIONSHIPS BETWEEN SPECIES OF *ALYSICARPUS* FROM ELECTROPHORETIC PATTERNS OF SEED STORAGE ALBUMINS

C.K. JOHN* AND R.J. THENGANE**
Department of Botany, University of Poona, Pune-411 007
(Accepted April 1995)

Seeds of 9 species and 2 varieties of *Alysicarpus, A. hamosus, A. monilifer, A. vaginalis, A vaginalis* var Nummularifolium, *A. bupleurifolius, A. longifolius, A. pubescens, A. regosus* var Heyneannus, *A. regosus* var *Styracifolius, A. tetragonolobus* and *A. belgaumensis* were ground and defatted. Proteins were extracted in 0.1 M phosphate buffer pH 8.00 containing 0.50 M NaCl and 1 mMPMSF. The buffer extracted proteins were dialysed against 25 mM sodium acetate buffer, pH 4.75 to precipitate the globulins and the albumin fraction was separated. Polyacrylamide gel electrophoresis (PAGE) in the native and in the presence of sodium dedecyl sulphate (SDS-PAGE) were performed. Electrophoregrams were used to calculate jaccard's similarity indices between the species and varieties and their inter-relationships discussed.

Key Words : *Alysicarpus,* albumins, seed storage proteins, PAGE.

The genus *Alysicarpus* Neck. belongs to the tribe *Desmodiae* of *Fabaceae* (Hutchinson, 1964). Many species under this genus have potential for development into forage crops and covercrops (Bailey & Bailey, 1976; Dule *et al.*, 1981). Lack of proper understanding of the inter-relationships between the species and varieties under the genus poses difficulties in attempting hybridizations. There is still controversy about the systematic position of some of its species (Baker, 1876; Prain 1897).

Recent developments in biochemical methods permit detailed studies on proteins and a direct approach to estimating gene homologies at varietal and species levels. Identification of seed protein profiles by electrophoretic techniques has indicated that they are highly stable and species specific (Johnson & Hall, 1965; Boutler *et al.*, 1967). Seed protein profiles are hardly affected by environmental conditions (Dunhill & Fowden, 1965; Lee & Ronald, 1976) and changes in chromosome structure (Johnson 1967). Since mature seed represents a conservative stage, variations due to developmental stages are excluded. Limitations of conventional methods of studying chromosome pairing in hybrids to derive conclusions on inter-relationships between species and varieties, such as chances of misleading inferences due to non homologous pairing or translocations involving chromosomes belonging to different genomes (Anfinsen, 1959), which is a major problem in long established polyploids (Sears, 1948) are not associated with this method. Hence it was decided to study the seed storage protein profiles by polyacrylamide gel electrophoresis to derive inferences on the interspecific and intervarietal relationships in the genus *Alysicarpus.*

MATERIALS AND METHODS

Seeds of 11 accessions of *Alysicarpus* representing 9 species and 2 varieties occurring in and around Pune, Maharashtra, were collected during October-December 1988. Healthy seeds were ground in a Broun grinder and defatted (Kartha & Sethi, 1957). The seed meal so prepared was extracted with 0.1 M phosphate buffer, pH 8.0 containing 0.5 M NaCl and 1 mM phenyl methyl sulfonyl flouride (PMSF), for 3 hours with continuous stirring at 7°C. It was centrifuged at 12,000 X g for 15 minutes at 4°C. The residue was re-extracted with the same buffer. The supernatants containing albumins and globulins from both the extractions were pooled together. The globulin from this fraction was precipitated by dialysis against 25 mM sodium acetate buffer pH 4.75, centrifuged at 12,000 x g for 30 minutes and separated. The supernatants were again dialysed against water and lyophylized. The lyophylized powders of albumins were suspended in 0.05 M phosphate buffer pH 7.2 and protein concentrations were estimated (Lowry *et al.*, 1951). For native polyacrylamide gel electrophoresis a 10% separating gel and a 5% stacking gel were

Received September 1993
*Present address : Division of Plant Tissue Culture, National Chemical Laboratory, Pune - 411 008.

used. The gel buffer, Tris-HCl in the separating gel was of pH 8.8 and the same in the stacking gel was of pH 6.8. The electrophoresis buffer used was Tris-Glycine pH 8.3. The samples were mixed with equal volumes of 20% glycerine and 0.005% bromophenol blue (W/V). Equal quantities of proteins were loaded per sample. Electrophoresis was then carried out at 10 mA constant current for 9-10 hours at 7°C in a cold chamber. By that time the bromophenol blue front reached the bottom of the gel. The gel was then removed and fixed in 20% trichloro acetic acid (TCA) for 30 minutes, rinsed rapidly with distilled water and stained in 0.025% coomassie brilliant blue (CBB) R 250 in 4:1:5 methanol: acetic acid water. The gel was destained in the same solvent without CBB R 250.

For SDS-Page (Weber & Osborn, 1967) to the protein samples equal volumes of 2X sample buffer containing 0.5 M Tris-HCl buffer pH 6.8 (2 ml), glycerol (2 ml), 20% SDS (1 ml), 0.05% bromophenol blue (0.4 ml) and distilled water (2.4 ml) were added, mixed thoroughly, incubated at 100°C for 5 minutes in a water bath, centrifuged at 10,000 X g for 10 minutes at room temperature and the supernatants were loaded, in equal quantities of proteins on the gel. A 12.5% separating gel and a 5% stacking gel were prepared by adding 0.1% SDS in the gel mixture before polymerization and 0.1% SDS in the electrophoresis buffer were used. The gel was run at 10 mA constant current for 10-12 hours 25±1°C. The gel was removed, fixed, stained and destained as described earlier. The experiments were repeated and were highly reproducible. The gels were photographed by placing them on a horizontal visible light illuminator, using Ilford Pan F film (50 ASA). Similarity indices between the species and varieties were calculated according to Jaccard's method (Hadocoa *et al.*, 1980).

RESULTS AND DISCUSSION

Native albumin profiles revealed 2 bands which were common to all the species and varieties studied. One more band was observed to be common to all members of Microcalycinae alone. In case of SDS-albumin profiles 4 bands of approximate molecular weight 45, 92.49, 110 and 123.75 kilodaltons were common to all species studied. The protein profiles are given as figure 1 and 2. The figures show that in general the similarity indices for the native albumins were lower and the same for albumin subunits were higher.

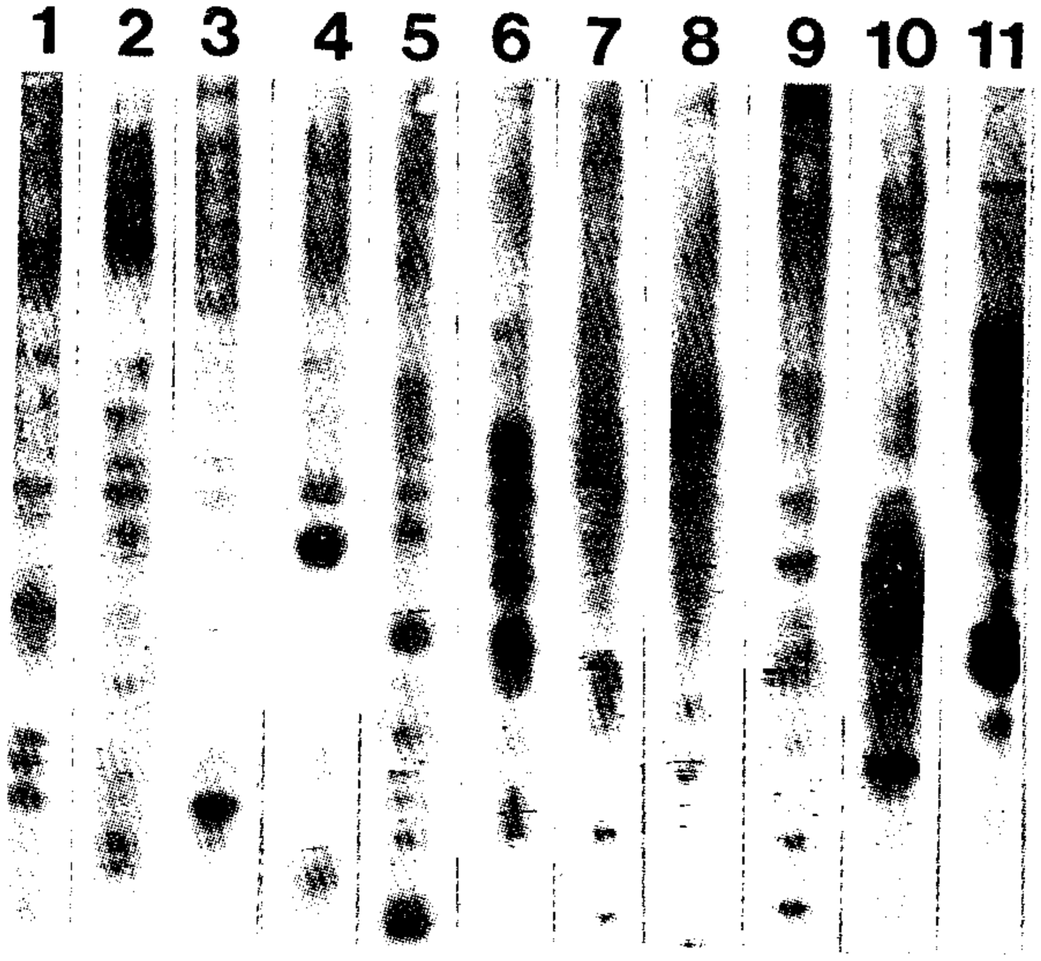

Figure 1. Native polyacrylamide gel electrophoregram of seed storage albumins in species of *Alysicarpus*
Lanes 1-11 seed storage albumin profiles of
1. *A. hamosus*, 2. *A. monilifer*, 3. *A. veginalis*, 4. *A. vaginalis* var. *Nummularifolius*, 5. *A. bupleurifolius*, 6. *A. longifolius*, 7. *A. pubescens*, 8. *A. rugosus* var. *Heyneannus*, 9. *A. rugosus* var. *Styracifolius*, 10. *A. tetragonolobus*, 11. *A. belgaumensis*.

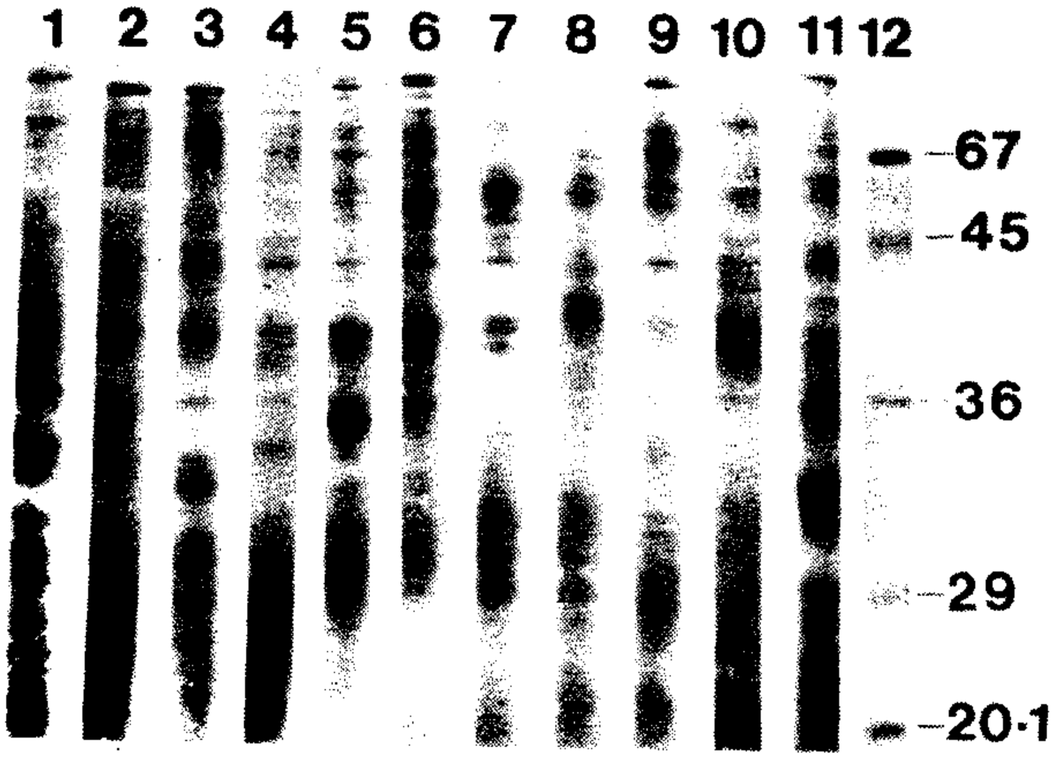

Figure 2. SDS-PAGE electrophoregram of seed storage albumins in species of *Alysicarpus*
Lanes 1-11 seed storage albumin profiles of
1. *A. hamosus*, 2. *A. monilifer*, 3. *A. vaginalis*, 4. *A. vaginalis* var. *Nummularifolius*, 5. *A. bupleurifolius*, 6. *A. longifolius*, 7. *A. pubescens*, 8. *A. rugosus* var. *Heyneannus*, 9. *A. rugosus* var. *Styracifolius*, 10. *A. tetragonolobus*, 11. *A. belgaumensis*. Lane 12 marker proteins with molecular weights Bovine serum albumin 67, Egg albumin 45, Glyceraldehyde 3 phosphate dehydrogenase 36, Carbonic anhydrase 29 and Tripsin inhibitor 20.1 Kilo daltons

The lower similarity indices for native albumins indicated the occurrence of intra-genic changes between the species and varieties. Since the migration in the native gels is mostly on the basis of net charge of the protein, the porosity and pH of the gel, it is evident that there are variations in the net charge of the proteins, because the porosity (10%) and the pH (8.8) are constant. This points towards differences in the amino acid sequences or in other words to possible changes in the triplet codons which may have taken place during the course of evolution of the species and varieties. Under denaturing conditions (with SDS) the migration of the proteins is on the basis of their size, because SDS gives a uniform negative charge to the proteins. The higher similarity indices for molecular weight classes indicate that the size of the protein subunits have not changed much inspite of changes in some amino acids.

The occurrence of common bands in the profiles of native albumin and albumin subunits of *Alysicarpus* and their varieties indicate homogeneity of the genus. In general, the species and varieties under Microcalycinae showing higher similarity indices between them than with the members of Microcalycinae or *A. belgaumensis* and *vice-versa* indicates the subgenera as natural groups. In Microcalycinae *A. monilifer, A. vaginalis* and *A. vaginalis* var Nummularifolius show closer relationships between them than *A. hamosus, A. vaginalis* and *A. vaginalis* var Nummularifolius show highest similarity index between them indicating more close relationship between them. But the similarity indices do not justify the varietal status acsribed to *A. vaginalis* var Nummularifolius. *A. bupleurifolius* showing higher similarity indices with members of Microcalycinae, especially *A. vaginalis* (18.52 and 74.07) occupies an intermediate position between the sub-genera though in morphological details it has more resemblence with members of Macrocalycinae. The two varieties under *A. rugosus, A. rugosus* var heyneannus and *A. rugosus* var Styracifolius also show closer relationships between them. But the similarity indices they show with members of Macrocalycinae and members of Microcalycinae do not point towards their varietal status.

A. belgaumensis though shows higher similarity indices with the members of Microcalycinae, it shows maximum similarity with *A. tetragonolobus* for albumin sub-units (55-56).

The similarity indices for native albumin and albumin sub-units indicate the species and varieties of *Alysicarpus* are distinct. This is in agreement without observations on karyotypic details (John *et al.*, 1986). Results of the present investigation supports Prain's (1897) classification of the genus.

REFERENCES

Anfinsen C B 1959 *The Molecular Basis of Evolution.* Johnson Wiley & Sons New York.

Bailey L H & E Z Bailey 1976 *Hortus Third.* Mac Millan New York.

Baker J G 1876 *The Flora of British India.* Reev & Co London p 172.

Boulter D, D A Thurman & E Derbyshire 1967 A disc electrophoresis study of globulin proteins of legume seeds with reference to their systematics. *New Phyotologist* **66** 27-36.

Duke J A, A E Kretschmer & C F Reed 1981 In: *Hand Book on Legumes of World Economic Importance.* (Duke *J)* Plenum Press New York.

Dunhill P M & L Fowden 1965 The amino acids of the seeds of Cucurbitaceae. *Phytochem* **4** 933-934.

Hadocoa V, V Turcoa, E hadac & E Klozoa 1980 Comparison of seed proteins of some representatives of the genus *Pisum* with the point of view of their relationships. Comparison by disc electrophoresis. *Biol Plant* **22** 7-16.

Hutchinson J 1964 *Genera of Flowering Plants Tribe Desmondiae* Clarendon Press Oxford 447-484.

John C K, S H Tulpule and R J Thengane 1986 Cytological studies in genus *Alysicarpus* Neck., *Cytologia* **51** 893-899.

Johnson B L 1967 Confirmation of genome donors of *Aegilops cylindrica. Nature* **216** 859-862.

Johnson B L & O Hall 1965 Analysis of phylogenetic affinities in *Triticinae* by protein electrophoresis. *Amer J Bot* **52** 506-513.

Kartha A R S & A S Sethi 1957 A cold percolation method for rapid gravimetric estimation of oil in small quantities of oil seeds. **27** 211-217.

Lee K W & A J Ronald 1976 Effect of environment on wheat gladein. *Nature* **216** 859-862.

Lowry O H, N N Rossenbrough, A L Farr & R J Randall 1951 Protein estimation with the folin-phenol reagent *J Biol Chem* **193** 265-275.

Prain D 1897 Some additional *Leguminosae. J Asiat Soc Bengal* **66** 384-401.

Sears E R 1948 The cytology and genetics of wheat and their relatives. *Adv Genet* **2** 239-270.

Weber K & M Osborn 1967 The reliability of molecular weight determination by sodium dodecyl sulphate polyacrylamide gel electrophoresis. *J Biol Chem* **244** 4406-4412.