Comparative Karyological and Biochemical Studies of *Albizzia procera* Benth. and *A. chinensis* (Orb.) Merr.

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Summary The somatic chromosome number and karyomorphological details were investigated in two tree legumes: *Albizzia procera* and *A. chinensis*. Somatic chromosome number was 26 in both the species with common symmetric karyotype formula $A_2 B_{14} C_{10}$. Karyological similarities indicate a close relationship between these two taxa. Significant biochemical difference of soluble seed protein content was recorded. Analysis of the soluble seed protein profile through SDS-PAGE (12%) technique clearly exhibited differences in their protein bands between the two species. The characteristic common protein banding pattern both in number (6) and their molecular weights (kDa) also reflected homology between the taxa. The present study on seed protein analysis was found to be useful in demarcation of genetic diversity between the two species of *Albizzia* which otherwise showed very close in their karyomorphological detail.

The genus *Albizzia* of Mimosaceae is especially prominent in the Indian subcontinent. In recent years the species of *Albizzia* have emerged as an useful multipurpose plantation trees of various economic importance (Mohiuddin 1997). Among them, *A. procera* and *A. chinensis* are very close in morphological and phenological point of view though they differed mainly in stipule character and variation in bark texture with wood specific gravity of 0.64 and 0.40 respectively (Deb 1982, Bhat 1997). The species are also known to have wide adaptability in a variety of soil climate, their seeding performance and seed production may vary according to soil types and seedling time (Bhardwaj *et al.* 1994). This versatility of the species in terms of both wide adaptation and multipurpose use has necessitated cytological and biochemical studies in genome characterization and genetic improvement programme of the taxon. Therefore, in the present context, an effort has been made to characterize and resolve the extent of similarities and variation between *A. procera* and *A. chinensis* using comparative karyological analysis and soluble seed protein profile by SDS-PAGE technique.

Materials and methods

Cytological study

Somatic chromosomes of the two species of *Albizzia* were studied from root tip cells. Root tips of 4–5 mm in size were collected from 3–4 days old seedlings. Root tips were pretreated with saturated para-dichlorobenzene (pDB) solution for 4.5 h at 15°C. This was followed by fixation in 1 : 3 acetic ethanol for overnight. Root tips were hydrolysed with 5 N HCl for 10–12 min at 15°C and washed thoroughly with distilled water. Finally root tips were treated with 45% acetic acid for 5–8 min and subsequently stained with 2% aceto-orcein for overnight and squashed in 45% acetic acid. Five metaphase cells with well spread and condensed chromosomes from each species were

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randomly selected for measurement and comparison between the species. Comparative karyotype analysis was carried out with following chromosome measurements. These included mean length of the chromosomal complements, relative chromosome length and centromeric indices. Relative length was determined by dividing the length of the chromosome by the length of the total haploid genome times 100 percent. The terminology used for chromosome morphology and classification was based on Levan *et al.* (1964).

Biochemical study

Mature dry seeds of *Albizzia procera* and *A. chinensis* were used for buffer soluble protein extraction. Seeds were crushed in 0.1 M Tris-HCl buffer pH 6.8 containing 1% (w/v) SDS, 1% PVP, 4.0% (v/v) 2-mercaptoethanol, 0.25 M sucrose, 0.1% ascorbic acid and 1 mM EDTA at 4°C. The extract was centrifuged at 12000 rpm for 1 h and the supernatant was used for total soluble seed protein content by Lowry's method (Lowry *et al.* 1951). The same extract was also used for analysis of soluble seed protein profile using modified sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing condition in 12% acrylamide slab gel (Weber *et al.* 1972). Protein bands were identified after complete gel run by staining with coomassie brilliant blue R and molecular weights of respective bands were determined by the method of Weber and Osborn (1975) using Pharmacia low molecular weight standard. Molecular markers included phosphorylase b, 94 kilodalton (kDa); albumin, 67 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 30 kDa; trypsin inhibitor, 20.1 kDa and α -lactalbumin, 14.4 kDa.

Results and discussion

The somatic chromosomes of *A. procera* and *A. chinensis* were analysed. The number of somatic chromosomes were found to be $2n=26$ in both the species (Figs. 1, 2). The size of chromosomes in general did not markedly differ and mostly represented by median to small which also corroborated with previous reports (Mehra and Sareen 1969, Khosla and Sareen 1978). On the other hand karyomorphological data averaging measurements of chromosomal complements from 5 well-spread metaphases in both the species showed variation in size and position of centromeres in their minute details. The chromosomal complements were found to have one pair of relatively long chromosomes (2.85 ± 0.43 – $3.08 \pm 0.79 \mu\text{m}$) with secondary constrictions, 7 pairs of chromosomes (1.46 ± 0.18 – $2.83 \pm 0.58 \mu\text{m}$) with nearly median primary constrictions and 5 pairs of chromosomes (1.65 ± 0.23 – $2.38 \pm 0.47 \mu\text{m}$) with submedian primary constrictions. Therefore, the different chromosome types recorded to be common between the 2 species of *Albizzia* were categorised into three karyomorphological types:

Type A—Chromosomes with submedian primary constriction and bearing secondary constriction.

Type B—Chromosomes with nearly median primary constriction.

Type C—Chromosomes with submedian primary constriction.

The karyotypes of *A. procera* and *A. chinensis* exhibit a gross similarity in their nature of chromosomal complements, number of chromosome bearing secondary constriction, total haploid chromosome length and TF% with common karyotype formula (Table 1). However, in spite of the same somatic chromosome number ($2n=26$) and karyotype formula, the minute detail of the karyomorphology and composite idiogram comparing relative length and centromeric position between chromosomes of the 2 species differed at species levels (Fig. 3). From cytological point of view *Albizzia* spp. are not primitive with common basic number $x=13$ although, morphologically they are considered primitive amongst legume (Bir and Sidhu 1967). The small size of the chromosome of this taxon are also in accordance with the characteristics of the other woody members of the flowering plants (Mehra 1972). The range of TF% values (38.86, 39.07) recorded in the present species indi-

Figs. 1–3. 1) Somatic metaphase plate of *A. procera* Benth. Showing $2n=26$ chromosomes. 2) Somatic metaphase plate of *A. chinensis* (Orb.) Merr. Showing $2n=26$ chromosomes. 3) Composite idiogram comparing relative length, position of centromere, and secondary constriction between chromosomes of diploid cells in metaphases from *A. chinensis* (Open bars) and *A. procera* (Solid bars).

Table 1. Karyomorphological details of *A. procera* and *A. chinensis*

Species	Somatic chromosome number ($2n$)	Total haploid chromosome length (μm) (Mean $\pm$ SE)	Karyotype formula	TF%
<i>A. procera</i> Benth.	26	26.94 ± 1.75	$A_2 B_{14} C_{10}$	39.07
<i>A. chinensis</i> (Orb.) Merr.	26	$28.46 \pm 2.65^*$	$A_2 B_{14} C_{10}$	38.86

* Not significant $p > 0.05$ ($t = 1.52$).

cate that the karyotype is symmetrical in nature.

Quantitative and qualitative analysis of total soluble seed protein content clearly revealed variation between the 2 species. Mean percentage of protein content by their fresh weight was much higher in *A. procera* than *A. chinensis* and the difference was statistically highly significant (Table

Table 2. Comparative analysis of soluble seed protein profiles between *A. procera* and *A. chinensis*

Species	Seed protein percentage (mg/g.fr.wt) mean $\pm$ SE	total no. of protein bands	Range of mol. wt. (kDa)	Mol. wt. of common protein band (kDa)	Mol. wt. of dissimilar protein band (kDa)
<i>A. procera</i> Benth.	12.56 $\pm$ 0.23	13	13.6–>94	>94, 66, 39, 27.5, 21.8, 17.5	55, 34, 30.5, 25, 20.8, 16
<i>A. chinensis</i> (Orb.) Merr.	9.64 $\pm$ 0.15*	14	12–>94	>94, 66, 39, 27.5, 21.8, 17.5	79, 58, 48, 45.8, 41, 35.6, 15, 12

* Highly significant $p < 0.05$ ($t = 12.73$).

2). Analysis of soluble seed protein profile through SDS-PAGE was successfully adopted and used as an additional tool to establish the relationship and differences between the 2 species which by their karyomorphological details (Table 1) revealed gross similarities. Seed protein profile of *A. procera* revealed 13 distinct protein bands with molecular weight ranging 13.6 kDa–>94 kDa while that of *A. chinensis* showed 14 distinct protein bands with molecular weight ranging 12 kDa–>94 kDa (Table 2). Six protein bands with molecular weight of >94, 66, 39, 27.5, 21.8, 17.5 kDa were found to be common in both the species (Fig. 4). These similarities in polypeptide bands indicate the degree of homology between the two species of *Albizzia* along with their common karyotypic formula. Significance of SDS-PAGE technique in characterization and identification of species or varieties have also been demonstrated in many other members (Vairinhos and Murray 1983, Renganayaki *et al.* 1987, Das and Mallick 1990, Mohan *et al.* 1993). On the other hand distinct variation in 7–8 protein bands was recorded in terms of their molecular weights and staining intensities (Fig. 4, Table 2). These variations in polypeptide bands along with significant 't' value of seed protein content could be an important parameter in characterization and identification of species in these tree legumes. The heterogeneous macromolecular nature of seeds also suggest that genetic background played a role in determining species demarcation. However, more extensive analysis of such parameter is necessary in other members of *Albizzia* before establishing macromolecular heterogeneity or homogeneity in these versatile taxon of tree legumes.

Fig. 4. Coomassie blue stained SDS-PAGE profiles of the seed proteins from *A. procera* (lane A and B) and *A. chinensis* (lane D and E) along with Pharmacia low molecular markers (lane C). From top to bottom: phosphorylase b, 94 kilodalton (kDa); albumin, 67 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 30 kDa; trypsin inhibitor, 20.1 kDa and a-lactalbumin, 14.4 kDa. Relative molecular masses in kDa common in both the species as shown by numerical values adjacent to the gel profiles (right).

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