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Genetic diversity in some perennial plant species with-in short distances

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Abstract: *Distinct morphophysiological variations observed for over 2 years with-in short distances among four perennial plants indicated genetic diversity among the lines growing at three places. The isozyme and SDS polyacrylamide gel banding patterns as genetic markers were used to investigate four perennial species, namely, Dalbergia sissoo Roxb., Delonix regia (Boj.) Refin., Cassia fistula L. and Calotropis procera R. Br. Plant materials collected from three locations (Agra, Gwalior and Lucknow) differing in climo-edaphic variables were examined for 4 enzyme systems, viz., esterase, polyphenol oxidase, peroxidase and superoxide dismutase (EST, PPO, PRX and SOD). Among the four isozymes SOD and PRX revealed best discriminating power. Protein banding patterns as well as zymogram revealed that Dalbergia sissoo growing at Gwalior was closer to that of Agra; Delonix regia depicted highest similarity between Lucknow and Agra and Calotropis procera of Lucknow location was more closer to Gwalior than Agra. The results confirm genetic diversity in the species as a means of adaptation to differing climo-edaphic variables.*

Key words: *Cassia fistula, Calotropis procera, Dalbergia sissoo, Delonix regia, Genetic variability, Isozyme*

Introduction

The use of electrophoresis to estimate certain genetic characteristics of plant populations as well as perennial tree species has become widespread since many years (Bendiab *et al.,* 1993). Many plants have been characterized by isozyme variations. Large morphophysiological variations were observed in four perennial species, namely, *Dalbergia sissoo* Roxb., *Delonix regia* (Boj.) Refin., *Cassia fistula* L. and *Calotropis procera* R. Br., growing at short distances. Variation in isozyme patterns in these species has been studied to provide evidence whether they possess genetic differences in morphophysiological variations due to climo-edaphic conditions.

Single gene markers, particularly, allozyme/isozyme banding patterns have been used to measure the genetic diversity of the species. It is known that the isozyme pattern (zymogram) is genetically controlled and constant for a tissue and development stage. Apart from being the popular research tool for cultivar identification, electrophoresis differentiated in general protein and specific isozyme and has led to increased understanding about the changes occurred during course of adaptation of the species when grown/ established at different locations. Isozyme and protein characterization is gaining importance as a supplement to morphological characterization because of its speed, repeatability, stability and relative independence of environmental conditions (Tanksley and Orton, 1983; Hamrick and Godt, 1997). The technique assumes more relevance to the species in question as they are growing at a very short distance and depicting large morphophysiological variations. Therefore, the present study was aimed to investigate whether genetic differences are responsible for the observed large morphophysiological variations during 2 years within short distances. The species are *Dalbergia sissoo* Roxb., *Delonix regia* (Boj.) Refin., *Cassia fistula* L. and *Calotropis procera* R. Br., and the locations are Agra, Gwalior and Lucknow, differing in climo-edaphic variables.

Materials and Methods

Plant material and enzyme extraction : Plant samples were collected in the month of September 2004 from all four species growing under natural conditions. Fully expanded and fresh plant leaf materials collected from the three places of the four species, namely, *Dalbergia sissoo* Roxb., *Delonix regia* (Boj.) Refin., *Cassia fistula,* L. and *Calotropis procera* R. Br., were homogenized in three fold volume of cold extraction buffer composed of 50 mM Tris-HCl, pH 7.5, 10 % sucrose, 1.0 mM EDTA, 1 % Triton X-100 (v/v), 1 % polyvinyl pyrrolidone (w/v) and 1 mM 2-mercaptoethanol (added freshly). The mixture was centrifuged at 10,000 rpm for 15 min. The supernatant obtained was used as enzyme source and stored at 4°C. For SDS gel the supernatant was mixed with sample buffer in 1:1 ratio and before loading on 12 % SDS gel sample was boiled for 5 min.

Gel electrophoresis : The analysis of four isozymes *viz.*, peroxidase (PRX, E.C. 1.11.1.17), esterase (EST, E.C. 3.1.1.2), polyphenol oxidase (PPO, E.C. 1.14.18.1) and superoxide dismutase (SOD, E.C. 1.15.1.1) was carried out using native polyacrylamide gel (10 %) electrophoresis method (Laemmli, 1970) with discontinuous buffer system, conducted at low temperature to resolve isozyme. About 150 µg of protein sample mixed with 5 µl of bromophenol blue (tracking dye) was loaded into the well from cathodal end. The gel was run at 100 V till dye

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Table – 1: Morpho-physiological variations depicted by *D. sissoo, D. regia, C. fistula* and *C. procera* at Agra, Lucknow and Gwalior

Species	Morphophysiological variations at three locations		
	Agra	Lucknow	Gwalior
Dalbergia sissoo	Branches drooping, flat pods 8–10 cm, 2-5 seeded, flowering March- August	Branches not drooping, flowering April-September and on to October, Pods 8-10 cm,2-5 seeded	Branches not drooping, flowering April to July and again in Oct./Nov. pods 4-7 cm, 1-2 seeded
Delonix regia	Flowering April to August, flowers up to 10 cm across	Flowering March to December, flowers up to 10 cm across	Flowering April to August, flowers up to 8 cm across
Cassia fistula	Flowering March to December, inflorescence like inverted pyramidal shape, pods maturing in summer	Flowering March to October, inflorescence inverted pyramidal, pods maturing by next April	Flowering April to July, inflorescence oval bunch shape, pods full grown by December
Calotropis procera	Leaves ovate, large, flowering throughout the year but more profusely and fruiting during March to June	Leaves large, ovate, flowering throughout the year but more profusely and fruiting during March-June	Leaves obovate, flowering middle April to November; few buds up to 2 mm across, visible on bigger plants, remaining dormant during winter up to next April

crossed the stacking gel and then at 200 V till the dye was ½-1 cm away from bottom.

Staining and zymogram construction : Gel was detached from the glass plates and placed in distilled water to avoid exposure to air and a piece of gel was cut at the right hand corner to mark the side. Gel was incubated in staining solution (substrate), the zones where the enzymes located in the gel were visualized due to the appearance of colored reaction product. The gels were stained as described by Veech (1969) for peroxidase and as described by Wendel and Weeden (1989) for esterase,

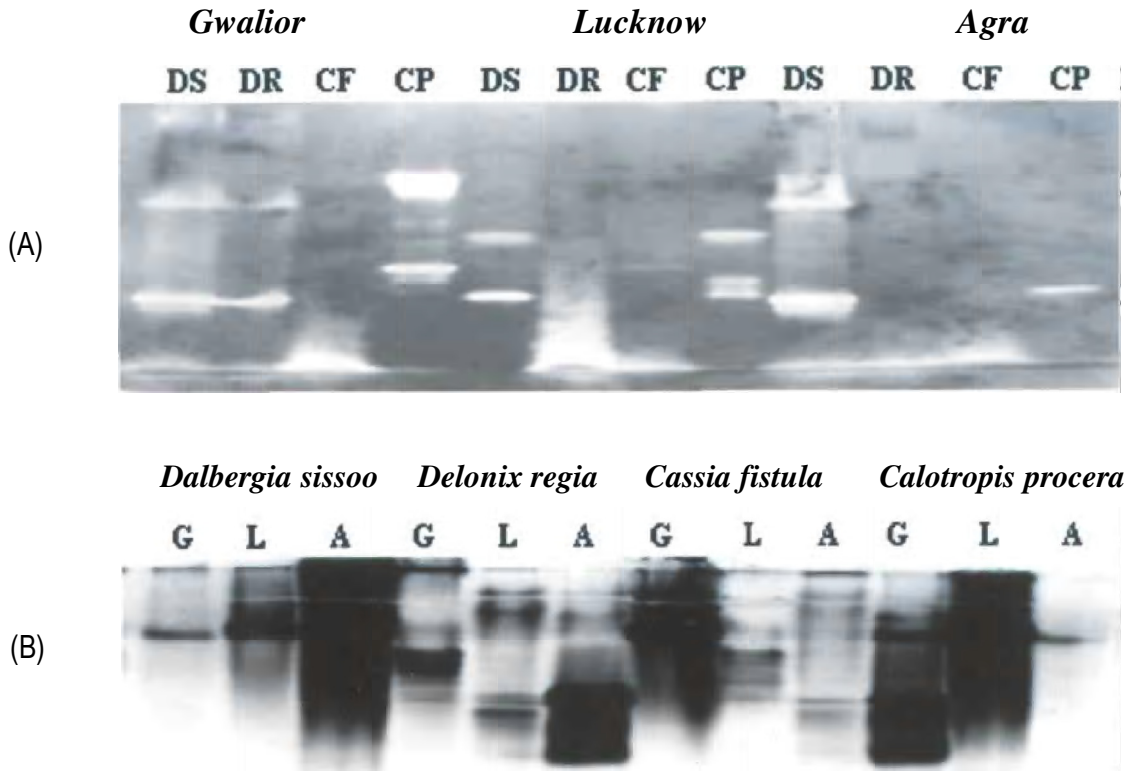

Fig. 1: (A) Polyacrylamide gel showing SOD patterns in four tree species. (DS=*Dalbergia sissoo*, DR=*Delonix regia*, CF=*Cassia fistula* and CP=*Calotropis procera*). **(B)** Peroxidase zymogram in four tree species collected from 3 different regions of the country. (G=Gwalior, L=Lucknow, A=Agra)

superoxide dismutase and polyphenol oxidase. The zymogram of gels was prepared by measuring the distance of each band from the point of separating gel and relative mobility (R_m) of each band was calculated as the ratio of distance traveled by the band to the tracking dye. Bands were numbered on the basis of increasing R_m values. Loci and alleles were subsequently numbered and lettered respectively. The isozyme patterns were defined by taking into account the number and position of bands.

The site and associated characteristics : Agra is semi-arid with Pleistocene alluvium overlain with alluvium of river Yamuna, Lucknow is also semi-arid with Pleistocene alluvium, while Gwalior is dry deciduous with red residual soils from folded shales of Vindhyan (Precambrian) series. The mean annual rainfall of Agra, Gwalior and Lucknow observed are: 631, 1015, and 799 mm respectively. Average maximum and minimum temperature of these places reported similar and are 32 and 18 ^{0}C.

Results and Discussion

Consulting available regional floras (Duthie, 1960; Haines, 1961; Maheshwari, 1963; Oommachan, 1977), morphophysiological variations belonging to four perennial species is presented in Table 1. In total 11 loci were generated by four isozymes, namely, esterase (EST, E.C.3.1.1.2), polyphenol oxidase (PPO, E.C.1.14.18.1), peroxidase (PRX, E.C. 1.11.1.17) and superoxide dismutase (SOD, E.C. 1.15.1.1). Superoxide dismutase and peroxidase were more informative as they produced more loci with higher number of alleles in comparison to other two isozymes (Fig. 1A and B). Zymogram indicated maximum number of peroxidase isozyme bands produced by *Delonix regia* and minimum by *D. sissoo* (Fig. 2). In case of *D. sissoo* three distinct loci each having two alleles were observed and of these three loci, locus 1 and 3 were more informative and indicated that *D. sissoo* growing at Gwalior was more closer to that of Agra. These two have shared 58 % alleles similarity with respect to all four isozymes (Table 2). However, Lucknow material showed similar level of homology with Agra and Gwalior materials. In case of *Delonix regia* the variability among the three locations was more than 60 % and highest similarity obtained between Lucknow and Agra was 40 %. This indicated that adaptation led to maximum variation in alleles of this species. More than 70 % similarity in total alleles was observed in Gwalior and Lucknow materials of *Calotropis procera*. Agra materials was as similar to Lucknow as it was to Gwalior. *Calotropis procera* of Gwalior was distinct as it possessed three additional alleles which were otherwise absent in both Lucknow and Agra material. Nevertheless, Lucknow material was more closer to Gwalior than Agra. Maximum numbers of alleles were observed with peroxidase in case of *Cassia, Calotropis* and *Delonix*. The Lucknow *Delonix* yielded some unique alleles, which were absent in both Agra and Gwalior materials. SDS PAGE produced 8-12 bands of different molecular weight and corroborated the observations obtained with isozyme analysis.

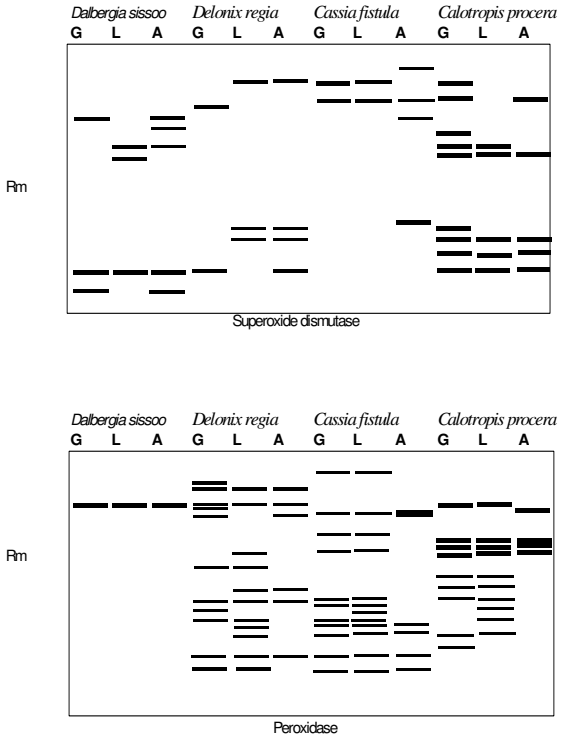

Fig. 2: Zymogram of SOD and PRX of four tree species collected from the three locations (G=Gwalior, L=Lucknow, A=Agra), Rm=Relative mobility

Table - 2: Percentage of similar alleles in terms of relative mobility of four isozyme (PPO, SOD, PRX and EST) shared by species growing at the three locations

Locations	The species			
	Dalbergia sissoo	***Delonix regia***	***Cassia fistula***	***Calotropis procera***
G-L	41	36	70	68
L-A	47	40	40	38
G-A	58	28	40	41

(G=Gwalior, A=Agra, L=Lucknow)

The isozyme patterns as observed clearly pinpointed that the plants growing at different locations possessed similar alleles in terms of relative mobility (Rm) and that speculate the level of closeness. Though molecular markers would be better in identifying the differences, nevertheless, the mosaic isozyme patterns as observed in case of *Dalbergia sissoo, Delonix regia, Cassia fistula* and *Calotropis procera* were found to correspond closely to mosaic patterns of the habitat at three different places.

The study conclusively proves that in the four perennial plant species, there is genetic diversity in morphophysiological characters within short distances due to varying climo-edaphic factors. It appears that genotypes are formed as a sum total effect of the total environment (Baker, 1965). This genetic variability in perennial plant species must be occurring as a rule

to cope up with the varying environment, of course, within their genetic plasticity.

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