

Short Communication

Isozyme diversity in *Cassia auriculata* L.

R. Siva^{1,2*} and K.V. Krishnamurthy²

¹The Institutes for Applied Research, Ben Gurion University of the Negev, Beer Sheva, Israel.

²Department of Plant Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.

Accepted 16 June, 2005

***Cassia auriculata* is considered to be one of the important dye yielding and medicinal plants in India. In the present study seeds from fourteen different localities were collected all over India and nine enzymes were screened by native polyacrylamide gel electrophoresis (PAGE) technique and thirty-four putative loci were totally detected. Cluster and factor analyses indicated that there are two major distinct groups or clusters, and thus, seeds collected from a few different localities are enough to capture the genetic variation held by this species. Also isozyme analysis is a reliable, efficient and effective marker technology for determining genetic variations in *C. auriculata*.**

Key words: Genetic diversity, isozyme, *Cassia auriculata*, dye.

INTRODUCTION

Genetic variation is the fundamental component of adaptation and thus, of stability of forest ecosystems. This is particularly important when the long-term stability of forest ecosystems is increasingly threatened by environmental stress and mismanagement. During the past 20 years, enzyme electrophoresis has been used to describe the population genetic structure of over 700 plant taxa (Hamrick and Godt, 1989). This information has contributed greatly to an understanding of the evolutionary history of individual species and related group of species (Hauffler, 1987). These studies have shown that angiosperms have high level of genetic variations. Variation is the basic resource to be explored for genetic improvement in any species and hence play a key role in plant improvement programmes (Hedegart, 1976; Zobel and Talbert, 1984; Tiwari, 1992). Many researchers have studied the genetic variability in inter- and intra-populations on natural ecosystems for the purposes of gene pool conservation (Amaral, 2001; Lakshmikumaran et al, 2001). Morphological characteristic might themselves be insufficient to distinguish between pairs of closely related species,

geographical races, or ecotypes, because not all-genetic differentiation results in morphological differentiation. Thus, a genetic characterization of natural resources is an essential step for a better understanding of genetic resources for the implementation of in situ and ex situ conservation activities (NBPGR, 2000). So adequate knowledge about the plants is necessary for planning sustainable development of any region like India, where the flora is rich in diversity and endemism (Siva, 2003). Thus, the present study is aimed in at exploring in a preliminary way the genetic diversity of *Cassia auriculata* seeds collected from fourteen different locations of India.

Cassia auriculata Linn., belonging to the family Caesalpiniaceae, is commonly known as Tanner's cassia. It is a small bush, which grows wild in South India with flowers and pods throughout the year (Matthew, 1983). It is a cross-pollinated taxon and pollination is by lepidopteran insects. Its seeds are dispersed when the legume fruit splits open. It thrives on dry stony hills and on black soils, along roadsides, in degraded forests, and on wastelands. It is the source of yellow coloured dye, obtained from its flowers and seeds (Chandramouli, 1995). It also possesses medicinal properties: the bark is astringent, leaves and fruits anthelmintic, seeds used in eye troubles and root employed in skin diseases. It has been used for the treatment of ulcers, leprosy and liver disease (Kumar et al, 2002). The dried flower bud

*Corresponding authors Email: rsiva77in@rediffmail.com.

powder is used as a substitute for tea in the case of diabetic patients and it is also supposed to improve the complexion in women. It has been widely used in Ayurvedic medicine as "Avarai Panchaga Choornam" and the main constituent of Kalpa herbal tea has come under extensive study in the light of its anti-diabetic effects (Pari and Latha, 2002).

Although this plant possesses so many medicinal aspects, it is mainly used in south India for dyes (Siva, 2003). The quality of seed, its vitality and performance (production of dyes) depends on many factors, of which the genotypic structure and realized mating system are the most important one. Thus, the main objective of this study is to evaluate whether the seeds collected from fourteen different localities shows any genetic variations.

MATERIALS AND METHODS

Plant material

Seeds of *C. auriculata* procured from fourteen different geographic localities in India were studied. Seeds separated from fruits that were randomly collected from different individuals of the same population were sun dried and stored. Thus each collection of seeds represents the total gene pool of each of the population studied. The details on sampling procedure for collection of materials and analysis of isozyme data are as follows. At least 50 individual plants in each of the localities (forming a coherent population) were randomly chosen for collection of seeds. The randomly chosen individual plants were almost of the same age. Similar pooling methodology has been followed by several investigators in the past (Bayer, 1988; Cosner and Crawford, 1990; Miller et al., 1998; Tomimatsu and Ohara, 2003).

Enzyme electrophoresis

Soaked seeds were homogenized in two volumes of cold (4°C) extraction buffer containing 0.1 M Tris-HCl, pH 7.2, 5% Sucrose, 0.5% (w/v) PVP, 10 mM β-mercaptoethanol. The samples were then centrifuged at 15,000 rpm for 15 min at 4°C, supernatant was collected in a separate vial. Each sample was applied to a native discontinuous polyacrylamide gel (6% stacking, 8% separating gel) and the runs were performed on a mini gel apparatus in Tris-Glycine (pH 8.3) buffer. Nine enzyme systems were examined in this study. They are alcohol dehydrogenase (ADH, E.C.1.1.1.1), acid phosphatase (ACP, E.C.3.1.3.2), acetyl esterase (AEST, E.C. 3.1.1.6), malate dehydrogenase (MDH, E.C.1.1.1.37), glutamate dehydrogenase (GDH, E.C.1.4.1.2), esterase (EST, E.C. 3.1.1.1), polyphenol oxidase (PPO, E.C.1.10.3.2), peroxidase (PRX, E.C.1.11.1.7) and catalase (CAT, E.C. 1.1.1.48)

Gel scoring and data analysis

The banding patterns observed after enzyme electrophoresis were compared among the different locations. Different patterns occurring in each zone of activity were scored as discrete variables using "1" to indicate the presence and "0" to indicate the absence of a unique pattern. A dendrogram depicting the degree of relationships among the taxa were produced on the basis of the hierarchical cluster analysis performed by "STATISCA" software (Uma Shaanker and Ganeshiah, 1997). The total percentage of shared loci and band frequency were calculated. The Percentage

of shared loci was calculated by number of loci present in all fourteen locations and band frequency was calculated by the number of alleles present in each localities.

RESULTS

Thirty-four putative loci in the nine different enzyme assays were resolved with sufficient consistency and clarity. Of these, EST yielded the highest number of bands (eight each) while GDH has a single band. High levels of genetic diversity were detected in the fourteen different localities from where materials were collected. EST – 4 was found only in material of localities L9 and L11. ACP – 5 was detected only in L1 and L2. ACP – 6 was found only in L3 and L4. Likewise, PPO – 2 was detected only in L10 and L11. Out of 34 bands, two bands were found in all fourteen localities. They are EST – 1 and PRX – 1. Of the 34 putative loci, L9 showed the maximum number of loci with 25 and the band frequency was 0.73. Of all localities L13 consisted of the least number of loci with 14 and with an allele frequency of only 0.41. All populations of *C. auriculata* are monomorphic with reference to the GDH-1

Cluster analysis was performed using isozyme data and it produced stable and consistent patterns (Figure 1). Two main clusters were observed. One cluster consisted of seven different localities (L1 to L7). This cluster is further divided into two subgroups, the first subgroup containing two localities (L1 and L2), thus demonstrating that populations from these two localities are closely related, and the second subgroup containing five localities. Of the latter, L3 and L7 are closely related, while L4 and L5 are closely related. The second cluster is further divided into two subgroups consisting of L8, L9 and L14 as one group, in which L8 and L9 are closely related, and L10 to L13 as another group. In the latter, L12 and L13 showed a close relation, while L11 was found as a separate group.

DISCUSSION

The result contained in this study identifies the degree of genetic diversity based on isozyme data in *C. auriculata*. This is perhaps, as far as the author is aware of, the first attempt to study genetic variation in dye-yielding plants. Information on the levels and distribution of genetic diversity of any plant species may contribute to the knowledge of their evolutionary history and potential, and is critical to their conservation and management (Schaal et al., 1991; Hamrick and Godt, 1996). High levels of genetic variation would bestow the population or species more flexibility to fit a variable environment, or to occupy new ecological sites and new habits (Huenneke, 1991).

The present investigations, as also the previous ones, have demonstrated that the use of isozyme technique for studying genetic diversity (Amaral, 2001). Although, a

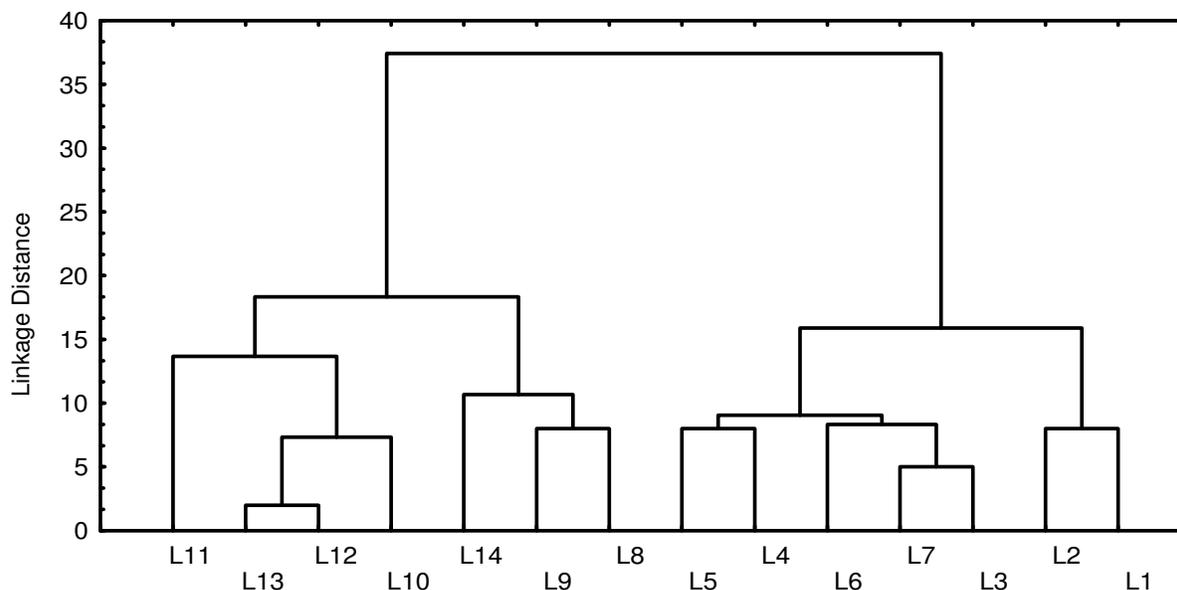


Figure 1. Dendrogram of the clustering of various localities of *C. auricular* L. L1– Keeranur, L2 – Kajamalai, L3 – Musiri, L4 – Udayapatti, L5 – Tiruchirappalli, L6 – Pulangudi, L7 – Surioor, L8 – Sivakasi, L9 – Paramakudi, L10 – Madurai, L11 – Thuvarangkuruchi, L12 – Pune, L13– Pondicherry, and L14 – Hyderabad.

wide array of DNA-based molecular procedures introduced in the last two decades allow genetic diversity to be estimated with greater precision, isozyme studies still have numerous advantages like wider applicability, low cost and speed of estimation (Pasquet, 2000; Benzie et al., 2000).

In conclusion it can be stated that the availability of isozyme loci has substantially increased our knowledge of the genetics of plant populations. These results have important implications for the conservation strategy

REFERENCES

- Amaral W (2001). Characterization, evaluation and conservation of forest genetic resources: The potential and limitation of new biotechnology tools. In: Uma Shaanker R, Ganeshiah KN, Bawa KS (Eds.). *Forest Genetic Resources: Status, Threats and Conservation Strategies*, Oxford and IBH Publishing Co. Pvt. Ltd, New Delhi. pp. 115-125.
- Bayer R (1988). Patterns of isozyme variation in Western North American *Antennaria* (Asteraceae: Iruleae). Sexual species of Dioicae. *Syst. Bot.* 13: 525-537.
- Benzie JAH, Ballment E, Edyvane K (2000). Allozyme as Genetic identification markers of *sargassum* spp. (Pheophyta) from the great barrier Reef, Australia. *Botanica Marina* 43: 169-179.
- Cosner MB, Crawford DJ (1990). Allozyme variation in *Coreopsis* (Compositae). *Syst. Bot.* 15: 256-265.
- Chandramouli KV (1995). *Sources of Natural Dyes in India*. A compendium with regional names, PPST Foundation, Adayar, Madras Tamil Nadu, India.
- Hamrick JL, Godt MJ (1996). Conservation genetics of endemic plant species. In: Avise Hamrick JL (Eds.), *Conservation Genetics*. Chapman and Hall, New York. pp. 284 - 304.
- Hamrick JL, Godt MJW (1989). Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (Eds.). *Plant Population Genetics, Breeding and Genetic Resources*. Sinauer Associates, Sunderland. pp. 43-63.
- Haufler CH (1987). Electrophoresis is modifying our concepts of evolution in homosporous pteridophytes. *Am. J. Bot.* 74: 953-966.
- Hedegart T (1976). Tropical trees variation, breeding and conservation. In: Burley J, Styles BT (Eds.). *Linnean Society Symposium Series No 2*. Oxford University. pp. 109 –121.
- Holsinger KE, Gottlieb LD (1991). *Genetics and Conservation of rare plants*, Oxford University Press, New York, pp.195 – 208.
- Huenneke LF (1991). Ecological implications of genetic variation in plant populations. In: Falk DA, Holsinger KE (Eds.), *Genetics and Conservation of Rare plants*. Oxford University Press. New York. pp. 31-44.
- Kumar RS, Ponmozhi M, Nalini M (2002). Effect of *Cassia auriculata* leaf extract on lipids in rats with alcoholic liver injury. *Asia Pacific J. Clin. Nutr.* 11(2): 157-163.
- Lakshmikumaran M, Srivastava PS, Singh A (2001). Application of molecular technologies for genome analysis and assessment of genetic diversity in forest tree species. In: Uma Shaanker R, Ganeshiah KN, Bawa KS (Eds.). *Forest Genetic Resources: Status, Threats and Conservation Strategies*, Oxford and IBH Publishing Co. Pvt. Ltd, New Delhi. pp. 153-181.
- Matthew KM (1983). *The Flora of the Tamil Nadu Carnatic*, The Diocesan Press, Madras, TamilNadu, India.
- Miller CJ, Strauss SH, Conkle MT, Westfall RD (1988). Allozyme differentiation and biosystematics of the Californian closed – cone pines (*Pinus* subsect. Oocarpae). *Syst. Bot.* 13: 351-370.
- NBPGR (2000). *Manual on Exploration and Collection of Plant Genetic Resources and related Indigenous Knowledge*, National Bureau of Plant Genetic Resources, New Delhi, India.
- Pari L, Latha M (2002). Effect of *Cassia auriculata* flowers on blood sugar levels, serum and tissue lipids in streptozotocin diabetic rats. *Singapore Med. J.* 43(12): 617-621.
- Pasquet RS (2000). Allozyme diversity of cultivated cowpea *Vigna unguiculata* (L.) Walp. *Theor Appl. Genet* 110: 211-219.
- Schaal BA, Veverich WJ, Rogstad SH (1991). A Comparison of methods for assessing genetic variations in plant conservation of rare plants. In: Falk A, Holsinger KE (Eds.), *Genetics and Conservation of Rare plants*. Oxford University Press New York.

- pp. 123 - 134.
- Siva R (2003). Assessment of Genetic Variations in Some dye yielding plants using Isozyme data. Ph.D. thesis submitted to the Bharathidasan University, Tiruchirappalli, India.
- Tiwari DN (1992). A monograph on Teak, International Book Distributors, Dehradun.
- Tomimatsu H, Ohara M (2003). Genetic diversity and local population structure of fragmented population of *Trillium camschatcense* (Tilliaceae). *Biol. Conser.* 109: 249-258.
- Uma Shaanker R, Ganeshiah KN (1997). Mapping genetic diversity of *Phyllanthus emblica*: Forest gene banks as a new approach *in situ* conservation of genetic resources. *Current Science.* 73: 163-167.
- Zobel B, Talbert J (1984). Applied forest tree improvement. John Wiley and Sons. pp. 505.