ASSESSMENT OF GENETIC VARIATION IN RUBIA CORDIFOLIA L. USING ISOZYME DATA

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ABSTRACT

Isozyme can provide effective information on the genetic variations at inter- and intra- populations level. The results reported in this paper concern the genetic variations in *Rubia cordifolia* L., which is a dye yielding plant. Seeds were collected from five different climatic conditions. Polyacrylamide gel electrophoresis study of eleven enzymes revealed 40 putative alleles. Among the 40 putative alleles, 21 were found in all populations. Allelic frequency and polymorphic loci were determined for each population and the data were further subjected to Cluster analysis. The results revealed greater genetic variations at lower altitude populations than at higher.

KEYWORDS: : Alleles, allelic frequency, Isozymes, *R. cordifolia,* polymorphic loci, dendrogram, genetic variation.

I. NTRODUCTION

Rubia cordifolia L., commonly known as Indian madder or Bengal madder, belongs to the family Rubiaceae. It is a tropical wild plant, growing in hills above 800 meters as thickets. It is a rugose vine with quadrate stem, profusely branched and grows up to 10 meters height. The flowers in dichasial cymes are white to greenish color with peak flowering during November to January. Fruit is a drupe. Indian madder is a source of natural madder dye, which is obtained from roots. The coloring matter present is a mixture of purpurine and munjistin [1]. Genetic diversity is an ubiquitous property of all species

in nature. The distribution and organization of genetic variation within populations of a species are the consequences of its evolution. As an important tool to assess genetic diversity, isozyme variation in many plants and their wild relatives has been extensively surveyed in relation to their geographic distribution [2-6]. Many researchers have also studied the genetic variability at inter- and intra- population levels for the purpose of gene pool conservation [7, 8]. In this paper, we have studied genetic diversity in *R. cordifolia* using isozyme markers in order to exploit the results for future improvement of the taxon for better dye content.

II. MATERIALS AND METHODS

A. Plant Material

R.cordifolia L. seeds were collected from different climatic localities of Tamil Nadu-India; Sirumalai (L1-800MSL), Palni hills (L2-1900MSL), Kolli hills (L3-1100MSL), Pacchai malai (L4-850MSL) and Shervaroy hills (L5-1700MSL).

B. Preparation of sample

500 mg of air-dried seeds were soaked in water to activate germination. Then the seeds were homogenized using chilled pestle and mortar with 1ml of extraction buffer containing 1.57g of Tris.Hcl, 100ml of distilled water, 5% sucrose and 14mM mercaptoethanol. pH adjusted to7.2. Samples were centrifuged at 4 ^{0}C for 15,000 rpm for 15 minutes. The clean supernatants were collected in separate vials.

C. Preparation for electrophoresis

Polyacrylamide gel electrophoresis (PAGE) was done using Acrylamide and Bis-acrylamide in the presence of Ammonium per Sulphate (APS) and TEMED. 10% gel showed the better resolution. 40µl of sample was loaded along with bromophenol blue to each well. PAGE was run at 60 V for 4-6 hours with electrode buffer containing, 1.5g of Tris.Hcl and 7.2g of glycine dissolved in 500 ml of distilled water and pH adjusted to 8.3.

D. Gel staining

Poly acrylamide gels were stained for following eleven enzymes; Acetyl esterase (AEST, E.C. 3.1.1.6) [9], -Amylase (-AMY, E.C.3.2.1.1) [10], Catalase (CAT, E.C. 1.11.1.6) [10], Esterase (EST, E.C. 3.1.1.1) [11]. Glutamate dehydrogenase (GDH, E.C. 1.4.1.2) [10], NAD diaphorase (DIA, E.C. 1.6.2.2) [10], Peroxidase (PRX, E.C. 1.11.1.7) [11], Polyphenol oxidase (PPO, E.C. 1.10.3.2) [11], Ribulose bisphosphate carboxylase (RBC, E.C. 4.1.1.39) [12], Superoxide dismutase (SOD, E.C. 1.15.1.1) [13], Xanthine dehydrogenase (XDH, E.C. 1.2.1.37) [14].

E. Data analysis

Gel images were captured with Lab Works GDS-8000 Bioimaging system gel documentation unit. Data were reported only for those loci which exhibited consistent activity and clearly interpretable banding patterns in the gel. Presence of a band was scored as 1.000 and absence

of a band was scored as 0.000 [15]. Allelic frequencies and polymorphic percentages were calculated for each population. For gene diversity statistics, "statistica" software package were used. Squared Euclidean Distance (SED) computation was used for all variables of the five samples and finally dendrogram was drawn by Ward's method.

III. RESULTS

Eleven enzymes, presumably coding 40 alleles were scored. The number of alleles per enzyme are as follows, EST-8, DIA-6, RBP-4, AEST-4, PRX-4, PPO-4, XDH-3, -AMY-2, GDH-2, SOD-2 and CAT-1. Data on loci shared for each population and allele frequencies for all populations are given in Table-1. If more than one isozymes are expressed for an enzyme they were designated by the numeral one onwards.

Among the 40 putative alleles, Sirumalai population (L1) consisted of the maximum number of 36 alleles and their total allelic frequency was 0.90, followed by Pacchai malai (L4) with 35 alleles and their total allelic frequency was 0.87, Kolli hills (L3) with 31 alleles and their total allelic frequency was 0.77 and Palni hills (L2) and Shervaroy hills (L5) with 29 alleles and their total allelic frequency was 0.72 (see Table-1).

Cluster analysis revealed two main clusters: the first cluster consisted of three populations (L2, L5 and L3) and the second one consisted of two populations (L1 and L4). In the first cluster, two subclusters were observed. In this subcluster, L2 and L5 were closely related than L3. The second cluster had no subclusters and L1 and L4 showed close relation (see figure -1).

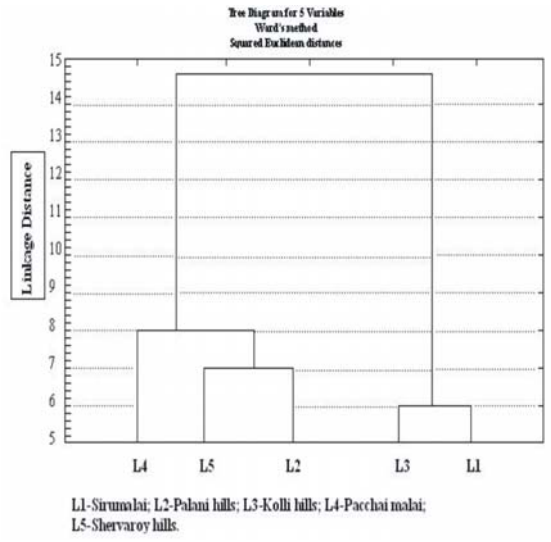

Fig. 1 Dendrogram of the clustering of various samples of R. conlifolia

TABLE 1. Allele scoring and frequency

		L1	L2	L3	L4	L5	Polymorphic Loci (%)
AEST	1	1.000	1.000	1.000	1.000	1.000	100
	2	1.000	1.000	1.000	0.000	1.000	80
	3	1.000	1.000	1.000	1.000	1.000	100
	4	1.000	0.000	1.000	1.000	0.000	60
Allelic Frequency		(1.00)	(0.75)	(1.00)	(0.75)	(0.75)	
AMY	1	1.000	1.000	1.000	1.000	1.000	100
	2	1.000	1.000	1.000	0.000	1.000	80
		(1.00)	(1.00)	(1.00)	(0.50)	(1.00)	
CAT	1	1.000	1.000	1.000	1.000	1.000	100
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	
DIA	1	1.000	1.000	1.000	1.000	0.000	80
	2	1.000	1.000	1.000	1.000	1.000	100
	3	0.000	0.000	0.000	1.000	0.000	20
	4	1.000	1.000	1.000	1.000	1.000	100
	5	1.000	1.000	1.000	1.000	0.000	80
	6	1.000	1.000	1.000	1.000	1.000	100
		(0.83)	(0.83)	(0.66)	(1.00)	(0.66)	
EST	1	1.000	1.000	1.000	1.000	1.000	100
	2	1.000	1.000	0.000	1.000	1.000	80
	3	1.000	0.000	1.000	1.000	0.000	60
	4	1.000	1.000	0.000	1.000	1.000	80
	5	1.000	0.000	1.000	1.000	1.000	80
	6	1.000	0.000	1.000	1.000	0.000	60
	7	1.000	0.000	0.000	0.000	0.000	20
	8	1.000	0.000	0.000	0.000	1.000	40
		(1.00)	(0.37)	(0.50)	(0.75)	(0.62)	
GDH	1	1.000	1.000	1.000	1.000	1.000	100
	2	1.000	1.000	1.000	0.000	0.000	60
		(1.00)	(1.00)	(1.00)	(0.50)	(0.50)	
PPO	1	1.000	1.000	1.000	1.000	1.000	100
	2	1.000	0.000	0.000	1.000	0.000	40
	3	1.000	1.000	1.000	1.000	1.000	100
	4	0.000	0.000	0.000	1.000	0.000	20
		(0.75)	(0.50)	(0.50)	(1.00)	(0.50)	
PRX	1	1.000	1.000	1.000	1.000	1.000	100
	2	1.000	1.000	1.000	1.000	1.000	100
	3	1.000	1.000	1.000	1.000	1.000	100
	4	0.000	1.000	0.000	1.000	0.000	40
		(0.75)	(1.00)	(0.75)	(1.00)	(0.75)	
RBC	1	1.000	1.000	1.000	1.000	1.000	100
	2	1.000	1.000	1.000	1.000	1.000	100
	3	1.000	1.000	1.000	1.000	1.000	100
	4	1.000	1.000	1.000	1.000	1.000	100
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	
SOD	1	1.000	1.000	1.000	1.000	1.000	100
	2	1.000	0.000	0.000	1.000	1.000	60
		(1.00)	(1.00)	(0.50)	(1.00)	(1.00)	
XDH	1	1.000	1.000	1.000	1.000	1.000	100
	2	0.000	0.000	1.000	1.000	1.000	60
	3	1.000	1.000	1.000	1.000	1.000	100
		(0.66)	(0.66)	(1.00)	(1.00)	(1.00)	
Total allelic Frequency	0.90	0.72	0.77	0.87	0.72		

IV. DISCUSSION

We found that the differentiation of *R. cordifolia* populations were associated with large allelic frequency differentiation at many isozyme loci. EST-7 was found

only in population of Sirumalai (L1). Likewise DIA-3 and PPO-4 were found only in Pacchai malai (L4). Out of 40 alleles 21 alleles were found in all populations. They were AEST-1 and 3, -AMY-1, CAT-1, DIA-2, 4 and 6, EST-1, GDH-1, PPO-1 and 3, PRX-1, 2 and 3, RBC-1, 2, 3 and 4, SOD-1 and XDH-1 and 3. Almost all enzymes are polymorphic, but Catalase only is monomorphic. Our results indicate that the variation between populations depends on the climatic conditions. The populations which were collected from above 1000MSL coming under one category (L2-1900MSL, L5-1700MSL and L3- 1100MSL) and rest of the two collected from below 1000MSL coming under another group (L1-800MSL and L4- 850MSL). Hence, lower altitude populations showed grater genetic diversity than higher altitude populations.

REFERENCES

[1] Sharma, O P., 1993, Plant Taxonomy Tata Mac Graw-Hill Publishing Company Limited, New Delhi.

[2] Brown, Jain, S K., 1978, Isozymes, plant population genetic organization and genetic conservation. Theor. Appl. Genet, (52), pp. 145-157.

[3] Brown, Jain, S K., 1979, Reproductive system and pattern of genetic variation in two Limnanthes species. Theor. Appl. Genet, (54), pp. 181-190.

[4] Nevo, B A., Ben-Shalomi, B., 1984, The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. In: Mani GS (ed) Evolutionary Dynamics of Genetic Diversity. Lecture notes in biomathematics. springer, New York, (53), pp. 13-213.

[5] Allard, R W., 1996, Genetic basis of the evolution of adaptedness in plants. Euphytica, (92), pp. 1-11.

[6] Allard, R W., Garcia, P., Saenz de Miera, L E, Perez de la Vega, M, 1993, Evolution of multilocus genetic organization in Avena hirtula and Avena barbata. Genetics (135), pp. 1125-1139.

[7] Amaral, W., 2001, Characterization, evaluation and conservation of forest genetic resourses: The potential and limitation of new biotechnology tools. In: Uma Shaanker R, Ganeshaiah KN, Bawa KS (eds) Forest Genetic Resources: Status, Threats and Conservation Strategies, Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi, pp. 115- 125.

[8] Lakshmikumaran, M., Srivastava, P S., Singh, A., 2001). Application of molecular technologies for genome analysis and assessment of genetic diversity in forest tree species. In: Uma shaanker, R, Ganeshaiah KN, Bawa KS (eds) Forest Genetic Resources: Status, Threats and Conservation Strategies. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi, pp. 153-181.

[9] Nash, K., Davis, J E., 1975, A text manual of Plant Enzymology and Histoenzymology. Kalyani Publishers, New Delhi, India.

[10] Valleojos, C E., 1983, Enzymes actively staining In: Tanksley SD, Orton TJ (eds) Isozymes in Plant Genetics and Breeding Part –A. Elsevier Science Publishers, Amesterdam, pp. 257- 287.

[11] Sadasivam, S., Manickam, A., 1996, Biochemical methods. II Edition. New age International Publishers, New Delhi, pp. 140-141.

[12] Wendal, E O., Weeden, N F., 1990, Visualization and interpretation of plant isozymes. In: Soltis DE, Soltis PS (eds) Isozymes in Plant Biology. Chapman and Hall, London, pp. 5-45.

[13] Beauchamp, C., Fridorich, I., 1971, Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal. Biochem. (44), pp. 276-277.

[14] Brewer, G J, 1970, An Introduction to Isozyme techniques. Academic Press, New York, London, pp. 186.

[15] Godt, M J W., Hamrick, J L., 1993, Genetic diversity and population structure in Tradescantia hirsuticaulis (Commelinaeceae). Amer. J. Bot, (80), pp. 956-966.

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