BIOLOGIA PLANTARUM 44 (3): 455-457, 2001

BRIEF COMMUNICATION

Low genetic polymorphism in natural populations of *Crotalaria longipes*

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Abstract

Natural genetic variation present in two populations of the critically endangered legume *C. longipes* was revealed by random amplified polymorphic DNA (RAPD) analysis. Out of the 30 primers used to test the intra-specific genetic polymorphism (between individuals from isolated populations) only 21 gave amplification. Eight primers produced monomorphic bands and 13 primers produced polymorphism. But the range of percent polymorphism was only 0 to 33 %. There was close similarity between individuals within and between populations. Cluster analysis based on Nei's indices did not reveal any population differentiation and individuals of both populations clustered with each other. These results point to a very low genetic polymorphism in *C. longipes* populations.

Additional key words: endangered legume, intra-specific genetic polymorphism.

Crotalaria longipes Wight & Arn. (*Fabaceae*) is an endangered plant endemic to Kolli hills, South India. It's a perennial tall shrub with showy yellow flowers. It is one of the 15 species of *Crotalaria* listed in the Red Data Books of Indian plants (Nayar and Sastry 1987). Heavy pest infestation, poor seed germination along with habitat destruction has restricted these valuable gene pools to a narrow range of distribution with small population sizes. The genus *Crotalaria* is of special interest because of the presence of pyrrolizidine alkaloids, which are known to have anti-tumor, hypotensive and mutagenic properties. With the advent of molecular biology techniques alternative DNA based methods like RFLP and RAPD has been successfully used for the detection of polymorphism in demographic studies on natural plant populations and for the estimation of genetic relatedness within populations (Weising *et al.* 1991). These molecular markers have also been used to quantify accurately the extent of genetic diversity within and between populations (Chalmers *et al.* 1992, Waugh and Powell 1992). The present study was undertaken to find out the genetic diversity in the individuals of *C. longipes* in two natural populations found at Kolli hills. Kolli hills are a group of hills on the South Western part of the Eastern Ghats in India. The altitude here ranges from 1000 to 1300 m. Vegetation is of hill category with semi-evergreen and dry deciduous type forest found in patches. Leaves of *C. longipes* were collected from 2 localities separated by around 10 - 12 km viz. Solakadu and Seekuparaipatti at Kolli hills. Solakadu population was found on roadsides at an altitude of 1000 m and Seekuparaipatti population was found on a hillside at an altitude of 1300 m.

Approximately 5 g of leaves were used for genomic DNA isolation. DNA was isolated following the CTAB method of Saghai-Maroof *et al.* (1984). Amplification of genomic DNA was done using 10 mer random oligonucleotide primers (*Operon Tech.*, Alameda, USA) of the OPA, OPB, OPC and OPD series. Polymerase chain reaction (PCR) amplification mixture of 0.025 cm^3

Received 4 September 2000, *accepted* 15 December 2000.
Abbreviations: CTAB - cetyl trimethyl ammonium bromide; PCR - polymerase chain reaction, RAPD - random amplified polymorphic DNA; RFLP - restriction fragment length polymorphism.
Acknowledgements: M. Jayanthi wishes to acknowledge Council of Scientific and Industrial Research for providing a Senior Research Fellowship and M. S. Swaminathan Research Foundation for providing the facilities to carry out the work.
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contained 50 ng of genomic DNA, 2.5 mm^3 of 10X assay buffer, 2 mm^3 of 2.5 mM $MgCl_2$, 0.5 mm^3 of 5 mM dNTP's, 15 ng of primer and 1 unit of *Taq* DNA polymerase (*Bangalore Genei*, Bangalore, India). The amplification was carried out in a DNA thermal cycler (*MJ Research*, Watertown, USA) programmed for 45 cycles. The first cyclewas programmed for 3 min at 94 °C, 1 min at 37 °C and 2 min at 72 °C, followed by 44 cycles of 1 min at 94 °C, 1 min at 37 °C and 2 min at 72 °C. An additional cycle of 15 min at 72 °C was used for primer extension. The amplified samples were electrophoresed in 1.5 % agarose gels in 1X TBE buffer. Amplification products were scored as discrete character states (present/absent) and entered into a binary data matrix. Pair-wise comparisons were calculated from the data matrix using a simple matching coefficient (Nei and Li 1979). Cluster analysis was carried out with the *UPGMA* (unweighted pair group mean average) method using the *NTSYS* statistical package.

Out of the 30 primers tested to study the genetic diversity in *C. longipes* plants belonging to two populations 21 primers gave a good amplification pattern. To ensure that the amplified bands were reproducible these primers were used in a second amplification. Only those DNA fragments, which were consistently amplified in both replicates, were considered for analysis. Eight primers gave monomorphic banding pattern and they are OPB 4, OPB 10, OPB 11, OPA 1, OPA 4, OPD 2, OPD 5 and OPC 2. A total of 158 fragments were amplified. Seventy-eight fragments were amplified in Solakadu population and 80 fragments were amplified in Seekuparaipatti population. The number of amplification products generated by each primer varied from 2 to 8. The range of percent polymorphism varied from 0 to 33 % (Table 1). Pattern of DNA bands varied with the primer used and the intensity of amplified DNA bands were of high as well as low. No RAPD primer could generate 100 % polymorphism. Position of many bands was similar between the individuals of two populations. Most of the polymorphism was because of the absence of one or two bands. Similarity matrix revealed that plants showed close similarity to each other and the similarity values ranged from 0.91 to 1.00 (Table 2). Cluster analysis produced a dendrogram and the dendrogram (Fig. 1) indicated that the two populations are not different from each other and many individuals of both populations are clustered with each other.

Table 1. Polymorphism obtained with different primers in *C. longipes* populations.

Primer	Number of polymorphic products	Polymorphism [%]
OPA 1	0	0
OPA 2	1	14
OPA 3	1	13
OPA 4	0	0
OPA 8	3	33
OPD 2	0	0
OPD 5	0	0
OPD 10	1	8
OPD 11	1	14
OPD 16	5	31
OPB 4	0	0
OPB 8	1	25
OPB 9	1	14
OPB 10	0	0
OPB 11	0	0
OPB 12	2	33
OPC 2	0	0
OPC 4	1	17
OPC 10	2	25
OPC 11	2	25
OPC 13	2	20

Table 2. Similarity matrix based on Nei's unbiased measures of genetic identity (So - Solakadu, Se - Seekuparaipatti).

	So 1	So 2	So 3	So 4	Se 5	Se 6	Se 7	Se 8
So 1	1.00							
So 2	0.99	1.00						
So 3	1.00	0.99	1.00					
So 4	0.95	0.97	0.93	1.00				
Se 5	0.99	1.00	0.97	0.98	1.00			
Se 6	0.97	0.94	0.94	0.97	0.96	1.00		
Se 7	0.95	0.96	0.93	0.96	0.97	0.97	1.00	
Se 8	0.99	0.97	1.00	0.91	0.95	0.95	0.94	1.00

The results of RAPD analysis reveal a low degree of genetic variability in *C. longipes*. Low genetic variation is reported to be one of the factors for rarity in plants (Karron 1994) and that may be due to several reasons. In case of *Meconopsis* (Sulaiman and Hasnain 1996), *Eucalyptus recurva* (Crisp 1988) and *Pedicularis furbishae* (Waller *et al.* 1987), low genetic variation is due to their reproductive strategies such as selfing and vegetative propagation, which is further reduced in small isolated populations (Schwaegerle and Schaal 1979). In

another endangered species *Cypripedium* it has been reported that there is no genetic variation among individuals of different populations. This zero genetic variation has been attributed to low flowering and fruiting rate (Bornbusch *et al.* 1994). Very low genetic diversity between populations was found when nine populations of *Vicia pisiformis,* a rare species was studied by Gustafsson and Gustafsson (1994). The low genetic variation obtained in the present study may be due to the presence of very few mature individuals. It has been observed that these populations are having only less than 20 mature individuals. Cross breeding is taking place only among these individuals and resulting in low genetic variation. Though each mature individual produces more than 200 flowers, more than 80 % are damaged due to pest infestation (Jayanthi 1999). Hence the percentage of flowers effectively becoming fruits and seeds are very low. Moreover *C. longipes* plants at Kolli hills are subjected to various threats. *C. longipes* is extensively damaged by lepidopteran pests like *Utethiesa pulchella* and *Argynus syringae.* Most of the habitats of *C. longipes* are being converted to roads and cultivable lands. Probably what we see now is only a few plants of a once existing large population. This may also be the reason for plants from sites separated by more than 10 - 12 km showing close similarity to each other. This low genetic polymorphism may be threatening to the survival of this endangered species in its natural habitat and unless urgent conservation measures are taken, this plant may be lost forever.

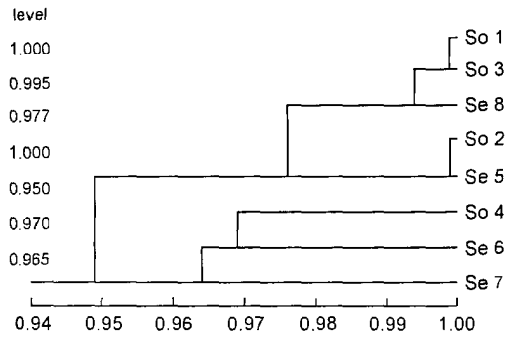

Fig. 1. Dendrogram based on Nei's genetic similarity values in *C. longipes* populations (So - Solakadu, Se - Seekuparaipatti).

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