Journal of Forest Science
Vol. 24, No. 1, pp. 27~34, April 2008

Evaluation of Genetic Differentiation of *Albizia lucida* Populations from Eastern Region of the Indian Sub-continent by ISSR Markers

Subhashree Aparajita and G. R. Rout*

Plant Biotechnology Division, Regional Plant Resource Centre, Bhubaneswar-15 Orissa

ABSTRACT : Level and distribution of genetic diversity in seven populations of *Albizia lucida* Benth. in eastern region of the Indian sub-continent were estimated using ISSR markers. Relatively higher level of genetic diversity within populations was observed in seven populations of *A. lucida* (mean of 0.38). From the result of AMOVA, majority of genetic diversity was allocated within populations (96.2%) resulting in a moderate degree of population differentiation. The observed distribution pattern of I-SSR variant among the populations was coincided with the typical pattern of long-lived woody tree species. Genetic relationships among the populations, reconstructed by UPGMA method, revealed two genetic groups. The population of Anugul and Bargarh turned out to be the most closely related despite a distance location between them. These formations will be of great value in the development of conservation plans for species exhibiting high levels of genetic differentiation due to fragmentation, such as indication of conservation unit size, which populations should be chosen as priority in conservation plans and which samples should be introduced in areas with a low number of individuals of *A. lucida*.

Keywords : *Albizia lucida*, Genetic variation, Random amplified polymorphic DNA, Inter simple sequence repeat

INTRODUCTION

Information on the magnitude and structure of genetic variations within a species is an integral part of evolutionary biology, planting program and conservation of plant genetic resources. Population genetic theory predicts that the decrease in the genetic diversity limits a species ability to keep pace with the changing selection pressure (Young and Merriam, 1992). Plant species, especially the perennials such as trees, rely on the available genetic diversity for stability and survival under the ever-changing environments (National Research Council, 1991). Conservation of plant genetic diversity has recently generated a lot of interest in the tropics as a result of many years of mismanagement, adverse environment as well as socioconomic changes. Traditionally, provenance and progeny tests coupled with biometrical analysis of phenotypic traits were the standard methods for describing and quantifying genetic variation in forest tree species (National Research Council, 1991). However, this approach requires the establishment of expensive field trails and the traits are to be measured under strong environmental effects. Moreover this approach is particularly slow when the aim is to rapidly estimate the patterns and distribution of genetic variation for monitoring of genetic resource.

Molecular markers are now considered the best tools for the detection of genetic variation within and among populations of a species. Inference based on surveys of variability in molecular markers are now routinely used as a basis for management recommendations under assumptions that maximizing marker variability will provide remnant populations with the greatest evolutionary potential, and at the same time, minimize the negative consequences of inbreeding (e.g. Avise and Hamrick, 1996; Haig, 1998; Knapp and Rice, 1998). Likewise, the degree of differentiation in marker genes has been suggested as a measure for guiding decisions on population conservation (e.g. Mortiz et al., 1995) and sources for translocation or resto-

* Corresponding author: (E-mail) grrout@rediffmail.com

ration projects. Molecular marker techniques have provided valuable information about the genetic structure of natural plant populations (Hamrick 1990; Powell et al., 1995, 1996a, b; Ouborg et al., 1999).

Albizia lucida Benth. belonging to sub-family Mimosacae of family Leguminosae is a medium to large evergreen tree. It has thin flat shinning pods, which ripen between march-April. The leaves are pale green when young and grey-green at maturity with 1-2 pairs of pinnae only. Rachis with a gland below, leaflets elliptical- oblong and acuminate. Flowers sessile with shot calyx lobes and corolla exserted to about twice the length of calyx. *Albizia lucida* is native to the Sub-Himalayan region and is commonly found along roadside and in mixed deciduous forest of Indian sub-continent. The tree provides good quality fodder and wood, which is used in construction work, furniture making and also for fuel. It also serves as an avenue tree and is often planted to give shade along road side and also in tea and coffee plantations. Despite its economical and ecological value, little is known about the genetic variability and population structure of *Albizia lucida*. The species is found to be growing naturally in the most economically developing and polluted regions of India, social and economic pressure have started devasting the natural strands of *A. lucida* forest.

Molecular characterization of the variability may help to manage and preserve genetic resources for long-term survival of species and for further applications such as domestication and use in breeding programmes.

In this study we used ISSR to characterize the genetic variability and its partitioning within and between seven populations along the Eastern region of the Indian subontinent. The main goal of this work is to establish priority areas for conservation of *A. lucida* populations, based on identifying the distribution of the observed genetic variation.

2. Materials and Methods

2.1. Study sites and plant sampling

Young and nonsenescence leaves were collected from 121 individual trees, representing seven populations that were located within the native range of *A. lucida* in eastern regions of the Indian sub-continent. The location of *A. lucida* population sampled in this study along with the eco-geographical conditions has been shown in Fig. 1 & Table 1. For ISSR analysis, the number of samples per population varied from 9 to 32 according to population size and the availability of the following conditions: leaves

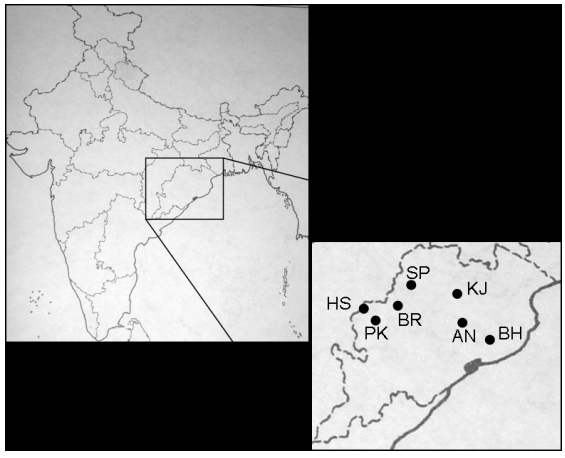

Fig. 1. Location of *A. lucida* population in the eastern region of Indian subcontinent.

Table 1. The ecological and geographical parameters of *Albizia lucida* Populations sampled.

Population	Nature of strand	N	Latitude	Longitude	Altitude	Tn	Rn
Harisankar (HS)	Natural forest	32	20°40'	83°16'	1552	25.0-30.5	100-200
Paikamala (PK)	Natural forest	16	20°42'	83°18'	656	25.0-30.5	100-200
Bargarh (BG)	Road sie	18	21°22'	83°46'	642	27.0-32.0	60-100
Sambalpur (SP)	Roadside	15	21°30'	83°59'	648	27.0-32.0	60-100
Anugul (AN)	Roadside	10	21°02'	85°26'	656	22.0-29.0	80-120
Bhubaneswar (BH)	Reserve forest	9	20°08'	85°58'	220	25.0-29.5	200-400
Keonjhar(KJ)	Natural forest	21	21°43'	85°36'	1440	10.0-22.5	200-400

N-Sample size of each population; Tn-Annual Temperature in ℃; Rn-Annual rainfall in mm.

were sampled from trees at least 50 m apart, >6 m height and with a diameter at breast height >3 cm in order to decrease the risk of genetic uniformity. Collected leaves were stored at -20℃ until DNA extraction. Total DNA was extracted from the pooled samples by the modified CTAB method (Doyle & Doyle, 1987). DNA quantification was performed by visualizing under UV light, after electrophoresis on 0.8% (w/v) agarose gel comparing it against a known amount of uncut λ-DNA. The resuspended DNA was then diluted in sterile distilled water to 5 ng/μl concentration for use in amplification reactions.

2.2. PCR amplifications and electrophoresis

Polymerase chain reactions (PCR) was carried out in a final volume of 25 μl containing 20 ng template DNA, 100 μM of each deoxyribonucleotide triphosphate, 20 ng of I-SSR primer 1.5 mM $MgCl_2$, 1x Taq buffer (10 mM Tris-HCl [pH-9.0], 50 mM KCl, 0.01% gelatin), and 0.5 U Taq DNA polymerase (M/S Bangalore Genei, Bangalore, India). Amplification was performed in a PTC-100 thermal cycler (MJ Research, Watertown, MA, USA) programmed for a preliminary 2 min denaturation step at 94℃, followed by 40 cycles of denaturation at 94℃ for 20 sec., annealing at required temperature for 30 sec. depending on the sequence of I-SSR primer and extension at 72℃ for 1 min, finally at 72℃ for 10 min amplification. The details of primers are presented in Table II. Amplification products were separated alongside a molecular weight marker (1.0 Kb plus ladder, M/S Bangalore Genei, Bangalore, India) by 2% agarose gel electrophoresis in 1x TAE (Tris Acetate EDTA) buffer stained with ethidium bromide and visualized under UV light. Gel photographs were scanned through Gel Doc System (Gel Doc. 2000, BioRad, California, USA) and the amplification product sizes were evaluated using the software Quantity one (BioRad, California, USA).

2.3. Data analysis

ISSR reactions were performed twice for each primer-individual DNA sample combination and only reproducible bands were included in the study. Furthermore, gel images from the 121 individuals were carefully and independently scored by two persons. Data was coded on the binary basis (1-presence of band; 0-absenceof band) and analyzed with POPGENE software Ver. 1.31 (Yeh et al., 1999). The following parameters of genetic variations were assessed for each population: gene diversity (Ho) (Nei, 1973), Shannon's information index (Io), observed number of alleles (Ao) and effective number of alleles (Ae). Genetic divergence between populations was further investigated using Nei's (1978) unbiased genetic distance (GD) and genetic identities (GID). In addition the heterozygosity was calculated for two levels: the average diversity within population (Hs) and the diversity in overall populations (H_T). The proportion of the total genetic differentiation found among population (G_{ST}) was obtained according to Nei (1987) to estimate the gene flow (Nm) among the populations. Nei's (1978) unbiased genetic distances were calculated for all population pairs and used to construct a Phylogenetic tree (UPGMA). The genetic distance matrix was also used to perform a hierarchical analysis of molecular variance (AMOVA; Excofficer et al., 1992) essentially as described by Huff et al. (1993) using the Arlequin software package (2.0; Schneider et al., 2000). The correlation between genetic and geographical distances was analyzed using the Mantel test option implemented in the Arlequin package. Support for clusters was determined by bootstrap analysis (i.e., repeated sampling with replacement) of 1000 permutated datasets that were generated using WinBoot software (Yap and Nelson, 1996).

3. RESULTS

ISSR analysis of 121 individuals using 10 ISSR primers provided a total of 102 markers, 92 (90.19%) of which were polymorphic. An example of the amplification products obtained is shown in Fig. 2a, b. The primers differed in their ability to detect polymorphism within populations. The detailed of the primer used and the band amplified by them has been given in Table 2. The average over all population of polymorphic bands amplified per primer

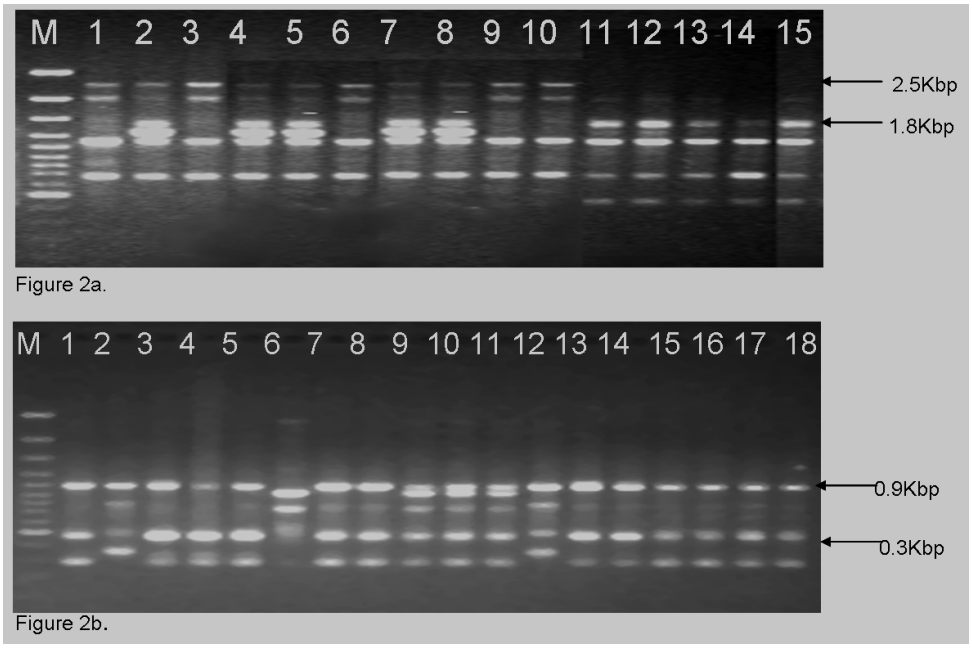

Fig. 2a. ISSR banding patterns of Sambalpur (SP) population generated by ISSR primer IG-23[(GA)8C]. M- Low range molecular weight marker. 1-15 are the individuals from Sambalpur region. Arrow indicated some of the polymorphic bands scored.

Fig. 2b. ISSR banding patterns of Bargarh (BG) population generated by ISSR primer IG-12 [(AC)8T]. M- Low range molecular weight marker. 1-18 are the individuals from Bargargh region. Arrow indicated some of the polymorphic bands scored.

Table 2. ISSR primers used for PCR amplification of *A. lucida*.

Primer	Primer sequence	*Tm	Total No. of bands	No. of Polymorphic bands
IG-1	AGGGCTGGAGGAGGGC	56	11	09
IG-2	AGAGGTGGGCAGGTGG	54	09	09
IG-3	GAGGGTGGAGGATCT	48	08	07
IG-10	(AG)8T	50	09	09
IG-11	(AG)8C	52	10	09
IG-12	(AC)8T	50	11	09
IG-13	(AC)8G	52	07	06
IG-14	(GA)8A	50	12	11
IG-15	(GA)8T	50	13	12
IG-23	(GA)8C	52	12	11
Total	------	--	102	92

·Tm-Annealing temperature of Primer.

varied from 13 (IG-15) to 07 (IG-13) and from 11 amplicons (IG-15 in HS population) to 04 amplicons (IG-03 in BH population) for individual populations. In individual populations, Nei's gene diversities (Ho) and Shannon's indicies of Diversity (Io) varied from 0.079 to 0.224 and from 0.106 to 0.323 respectively. For the whole set of populations, the Ho and Io values were 0.291 and 0.386 respectively, and the number of alleles per locus (Ao) and the effective number of alleles per locus (Ae) equaled 1.708 and 1.421 respectively. Across populations, the average Nei's gene diversity (Ho) and Shannon's index of diversity (Io) were 0.162 and 0.231, respectively. The highest levels of diversity were detected in populations HS (Harisankar) and the lowest diversity in population BH (Bhubaneswar),

Table 3A. Genetic Variability within population of *Albizia lucida* detected by ISSR analyses.

Population	Ao	Ae	Ho	Io	P(%)
HS	1.397	1.232	0.224	0.323	55.72
PK	1.322	1.231	0.170	0.243	38.29
BG	1.305	1.124	0.176	0.259	44.55
SP	1.341	1.150	0.182	0.260	40.13
AN	1.359	1.152	0.182	0.261	41.96
BH	1.092	1.036	0.079	0.106	14.27
KJ	1.267	1.205	0.123	0.166	32.79
Mean	1.326	1.161	0.162	0.231	38.24
Total population	1.708	1.421	0.291	0.386	90.19

Ao-Observed mean number of allele frequency, Ae-mean effective number of allele frequency, Ho-mean Nei's genetic diversity, Io-mean Shannon's informative index, % P-percentage of polymorphic loci

Table 3B. Nei's Analysis of gene diversity in sub divided populations of *Albizia lucida*.

Molecular marker	Variation component			Gene flow Nm (G_{ST})
	H_T	H_S	G_{ST}	
ISSR	0.36 +/- 0.023	0.18 +/- 0.013	0.320	0.871

H_T-Total gene variability; H_S-Gene diversity within populations; G_{ST}-Genetic Differentiation; Nm-Gene flows.

Table 4. Analysis of Molecular variance (AMOVA) for 121 individuals in seven populations of *Albizia lucida* using ISSR markers.

Source of variation	Degree of freedom	% total variance	p-value
Among Population	6	3.8	<0.001
Within Population	114	96.2	<0.001

with both Ho and Io showing the same tendency (Table 3A).

When all populations were compared, a high level of genetic differentiation was detected (G_{ST} = 0.320) indicating that genetic differentiation among the studied population was substantial. The total gene diversity was high in *Albizia lucida* (H_T = 0.31), but 19% of this was within population variation (H_S = 0.19) (Table 3B). Estimated gene flow (Nm) for *A. lucida* population was 0.871 indicating that the genetic exchange between populations is not high. The hierarchical analysis conducted using AMOVA revealed that 3.8% of total variance was attributable to differences between populations and 96.2% to variation between individuals within populations (Table 4).

The UPGMA cluster analyses based on Nei's (1978) unbiased genetic distances was performed to further show the genetic relationships among populations (Fig. 3). The

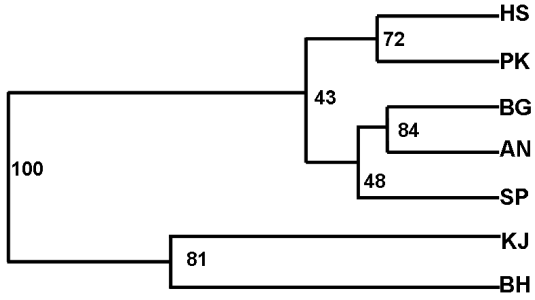

Fig. 3. Dendogram showing the clustering pattern of seven population of *A. lucida*.

dendogram grouped the populations into two clusters. First cluster consisting of Population BH (Bhubaneswar) and KJ (Keonjhar) while the second cluster consisting of the rest five populations (HS, PK, BH, SP and AN). A mantel test with 1000 random permutations revealed a positive correlation between pair wise genetic distance values and geographical distance (r = 0.73 at P < 0.001).

4. DISCUSSION

The proportion of polymorphic loci amplified in *A. lucida* was 90.19%, which is similar to figures reported for another tropical tree such as *Euterpe edulis* (Cardoso et al., 2000), *Caesalpinia echinata* Lam. (Cardoso et al., 1998) and *Populus tremuloides* (Yeh et al., 1995). In constast a lower percentage of polymorphic loci was observed in *Gliricidia sepium* (Chalmers et al., 1992) and *Theobroma cacao* (Russell et al., 1993) using RAPDs , *Gyptostrobus penslis* (Li and Xia, 2005) by ISSR markers and in *Moringa oleifera* using AFLPs (Muluvi et al., 1999). The results showed high levels of genetic variation in *A. lucida*. Despite this, the proportion of polymorphic loci for the population BH was quite low (14.27%). Nevertheless, at the species level the results are consistent with data from other tree species, in which high genetic variation has been related to life history and ecological characteristics such as a wide geographic range, primarily outcrossing and seed dispersal mechanisms (Hamrick and Loveless, 1989).

Genetic relationship among the populations was constructed by UPGMA method. The observed genetic relations among the seven population grouped into two groups, the Population (HS and PK) and (BG and AN) showed a high probability of sharing the same ancestors, where the 72% and 84% confidence limit was estimated from the replicated analyses with 1000 pseudo-replicate data set prepared by bootstrapping. In general, if natural population originated from same hypothetical ancestors and underwent stable evolution, geographically close population should show the closest genetic relationship (Hong et al., 2004). However the overall genetic relationships among the seven population contucted by UPGMA method coincide with geographical distance but not fully. A shortest genetic distance was observed between AN and BG (0.091), but geographic distance between them is relatively far. The discrepancy between geographical distribution and genetic relationships among the population AN and BG suggests that they might have undergone the random changes in genetic composition due to some kinds of disturbances (i.e., logging, natural fire, artificial fire for preparing ground for cultivation and afforestastion etc). These random changes might result in random genetic drift induced by drastic changes in population size and transplantation from other populations for reforestations. These results indicates that genetic distance is not solely dependent on geographical distance, although the dendrogram shows that in most cases, clustering based on genetic distances reflect geographical relationships. For example, the cluster formed by HS and PK population is located on the same mountain ridge complex. Population of Keonjhar (KJ) and Bhubaneswar (BH) form a single cluster which may reflect their relative geographical isolation from the other populations. The amount of genetic differentiation present among populations was considerable G_{ST} = 0.320 despite the relatively short geographical distances between the examined populations. The divergence among the *A. lucida* populations may be explained partly by limited gene flow Nm = 0.871. Mountainous regions and habitat fragmentation may be the significant gene flow barriers for *A. lucida*.

The overall genetic differentiation between populations was highlighted by AMOVA, which showed that only 3.8% of the total variation was attributed to between population variations and 96.8% to within population variation. Such results are similar to expectations for woody, long lived and predominantly outcrossed species, which are expected to maintain most variation within populations (Hamrick, 1989). Distribution range and population size have been identified as major correlates of within population genetic variation in tropical tree species, with restricted populations showing significantly less variation than those with broader distribution (Travis et al., 1996). The relatively low levels of genetic variation observed in the smaller populations BH (Bhubaneswar) where as on the other hand, Anugul (AN) population, which is also represented by a small sample size, showed higher values of genetic diversity. This result indicates that levels of genetic diversity do not depend solely on sample size but on several factors, including founder effects and genetic bottlenecks (Suyama et al., 2000).

The extensive genetic differentiation detected among *A. lucida* populations has a number of implications for the management and conservation of this species. From a conservation view point, each population should be managed and maintained separately because they possess partially unique genetic characteristics. In such a case, the loss of any single population may lead to loss of genetic variation for the species as a whole (Shrestha et al., 2002). The data suggests that conservation measurements should focus on the conservation of different populations *in* or *ex situ*. In order to secure genetic sources, an obvious approach is to establish seed orchards of genetically diverse population that would promote a high level of genetic diversity among plantations.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the help of the Department of Biotechnology, Ministry of Science and Technology, Government of India for financial assistance under grant No. BT/PR/3750/AGR/16/309/2003-04.

REFERENCES

Avise, J. C., Hamrick, J. L., 1996. Conservation genetics. Case histories from nature. Chapman and Hall, New York.

Cardoso, S. R. S., Eloy, N. B., Provan, J., Cardoso, M. A., Ferreira, P. C. G., 2000. Genetic di.erentiation of Euterpe edulis Mart. populations estimated by AFLP analysis. Mol. Ecol. 9: 1753-1760.

Cardoso, M. A., Provan, J., Powell, W., Ferreira, P. C. G., Oliveira, D., 1998. High genetic differentiation among remnant populations of the endangered *Caesalpinia echinata* Lam. (Leguminosae-Caesalpinioideae). Molecular Ecology 7, 601-608.

Chalmers, K. J., Waugh, R., Sprent, J. I., Simons, A. J., Powell, W., 1992. Detection of genetic variation between and within populations of *Gliricidia sepium* and *G. maculata* using RAPD markers. Heredity 69, 465-472.

Doyle, J. J., Doyle, J. L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull. 19, 11-15.

Excoffier, L., Smouse, P., Quattro, J., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131, 479-491.

Haig, S. M., 1998. Molecular contributions to conservation. Ecology 79, 413-425.

Hamrick, J. L., 1990. Isozymes and the analysis of genetic structure in plant populations. In: Isozymes in Plant Biology (eds Soltis ED, Soltis OS), 87-105. Chapman & Hall, London.

Hong, Y. P., Kwon, H. Y., Kim, K. S., Hong, K. N., Kim., Y. Y., 2004. Discordance between geographical distribution and genetic relationship among Population of Japanese Red Pine in Korea revealed by analysis of I-SSR markers. Silvae Genetica 53(3), 89-92.

Huff, D. R., Peakall, R., Smouse, P. E., 1993. RAPD variation within and among natural populations of outcrossing bu.alograss [Buchloe dactyloides (Nutt.) Engelm.]. Theor. Appl. Genet. 86, 927-934.

Hamrick, J. L., 1989. Isozymes and the analysis of genetic structure in plant populations. In: Soltis D. E. and Soltis P. S. (eds), Plant Biology. Dioscorides Press, Portland, Oregon, pp. 87-105.

Hamrick, J. L, Loveless, M. D., 1989. The genetic structure of tropical tree populations: association with reproductive biology. In: The Evolutionary Ecology of Plants (eds Bock J, Linhart YB), pp. 130-146. Westview Press, Boulder, CO.

Fagen, L., Nianhe, X., 2005. Population structure and genetic diversity of an endandered species *Glyptostrobus pensilis* (Cupressaceae). Bot. Bull. Acad. 46, 155-162.

Knapp, E. E., Rice, K. J., 1998. Comparison of isozymes and quantitative traits for evaluating patterns of genetic variation in purple needlegrass (*Nasella pulchra*). Conservation Biology 12, 1031-1041.

Moritz, C., Lavery, S., Slade, R., 1995. Using allele frequency and phylogeny to define units of conservation and management. American Fish Society Symposium 17, 249-262.

Muluvi, G. M., Spren, J. I., Soranzo, N. 1999. Amplified fragment length polymorphism (AFLP) analysis of genetic variation in *Moringa oleifera* Lam. Molecular Ecology 8, 463-470.

National Research Council 1991. Managing global genetic resources. Forest Trees National Academy Press, Washington D. C. pp 228.

Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York. Obayashi K., Tsumura Y., Ihara-Ujino T., Niiyama K., Tanouchi H. and Suyama Y. et al., 2002. Genetic diversity and outcrossing rate between undisturbed and selectively logged forests of Shorea curtisii (Dipterocarpaceae) using microsatellite DNA analysis. Int. J. Plant Sci. 163, 151-158.

Nei, M., 1978. Estimation of average heterozigosity and genetic distances from a small number of individual. Genetics 89, 583-590.

Nei, M., 1973. Analysis of gene diversity in Subdivided populations. Proc. Nat. Acad. Sci. USA 70, 3321-3323.

Ouborg, N. J., Piquot, Y., van Groenendael, J. M., 1999. Population genetics, molecular markers and the study of dispersal in plants. Journal of Ecology 87, 551-568.

Powell, W., Machray, G. C., Provan, J., 1996a Polymorphism revealed by simple sequence repeats. Trends in Plant Science 1, 215-222.

Powell, W., Morgante, M., Andre, C., 1996b. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding 2, 225-238.

Powell, W., Orozco-Castillo, C., Chalmers, K., Provan, J., Waugh, R., 1995. Polymerase chain reaction-based assays for the charac-

terization of plant genetic resources. Electrophoresis 16, 1726-1730.

Russell, J. R., Hosein, F., Johnson, E., Waugh, R., Powell, W., 1993. Genetic differentiation of cocoa (*Theobroma cacao* L.) populations revealed by RQAPD analysis. Molecular Ecology 2, 89-97.

Suyama, Y., Obayashi, K., and Hayashi, I., 2000. Clonal structure in a dwarf bamboo (Sasa senanesis) populations inferred from amplified fragment length polymorphism (AFLP) fingerprints. Mol. Ecol. 9, 901-906.

Schneider, S., Excoffier, L., Kueffer, J-M., Roessli, D., 1997. *ARLEQUIN*, Version 1.1: A software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Shrestha, M. K., Goldhirsh, A. G., and Ward, D., 2002. Population genetic structure and the conservation of insolated populations of Acacia raddiana in the Negev Desert. Biol. Conserv. 108, 119-127.

Travis, S. E., Maschinski, J., Keim, P., 1996. An analysis of genetic variation in Astralagus cremnophylax var. cremnophylax; a critically endangered plant, using AFLP markers. Mol. Ecol. 5, 735-745.

Yap, I., Nelson, R. J., 1996. A program for performing bootstrap analysis of binary data to determine the confidence limit of UPGMA based dendrograms. IRRI Discussion Paper series 14. International Rice Research Institute.

Yeh, F. C., Chong, D. K. S., Yang, R. C., 1995. RAPD variation within and among natural populations of trembling aspen (*Populus tremuloides* Michx.) from Alberta. Journal of Heredity 86, 454-460.

Yeh, F. C., Yang, R. C., Boyle, T., 1999. POPGENE. Microsoft Windows-Based Freeware for Population Genetic Analysis. Release 1.31. University of Alberta, Edmonton.

Young, A., Merriam, H. G., 1992. The effect of forest fragmentation on genetic variation in an *Acer saccharium*. (March sugar maple) population. Paper to: International symposium on population genetics and gene conservation of forest trees. IUFRO working parties S2.04-05, S2.04-01 and S2.02.00. Carcaus Maubuuisson, France. 24-28 August 1992.