forced to establish states in each of the three adaptational subsystems, which still may develop evolutionary flexibility when control ceases; genetic diversity and its maintenance or conservation is an indispensable prerequisite to this.

To conclude this paper, a more general adaptational characteristic of plants shall be addressed that refers to low associations among variable modifying and adaptive conditions. This characteristic concerns the individual sessility of plants as opposed to the individual motility of most animals. Motility can be considered to have evolved as a capacity to respond to unfavourable conditions by evasion. In essence, this ability puts animals into the position to reduce both the temporal and spatial variability of their adaptive environments and thus to generally avoid the implied considerable adaptational challenges. In contrast, the sessility of plants forces them to face these challenges by repeatedly evolving mechanisms of altering the effects of their adaptive environments in ways such as demonstrated in the above relation (*iii*). This requires maintenance of genetic diversity that is not needed in animals. As a consequence of this situation, it is conceivable that environmental changes that are global in the sense that they cannot be escaped may create adaptational problems solvable by plants but not by animals.

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# Isozyme Variation of Natural Populations of Sal *(Shorea robusta)* in the Terai Region, Nepal

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**Abstract**

Genetic variation of sal *(Shorea robusta)* was investigated in three natural populations in the Terai region, Nepal, using 12 loci from 8 isozyme systems. The mean number of alleles per locus was 2.16 and 58.3% of loci were polymorphic (95% criterion for polymorphism). The mean observed and expected heterozygosities ranged from 0.105 to 0.129 with an average of 0.117, and from 0.130 to 0.158 with an average of 0.143, respectively. Only 4.7% of the total genetic diversity was due to differentiation among the populations and the mean value of genetic distance was 0.018. The results indicated that the majority of species' genetic variation was found within the studied populations and there was high genetic similarity among three natural populations of *S. robusta*. The sharing of one gene pool among the studied populations suggests a lack of barriers to gene flow.

*Key words:* gene flow, genetic differentiation, isozymes, populations, *Shorea robusta*.

## Introduction

Knowledge of the distribution of genetic variation within and between the populations is of substantial benefit in the conservation of plant genetic resources (HEDRICK, 1985; BROWN et al., 1990; ADAMS et al., 1992). Isozyme variation in species, and within and among the populations has been extensively studied in many woody plants (HAMRICK and GODT, 1990; HAMRICK et al., 1992; MUONA, 1990; YANG et al., 1997). There are different conclusive evidences which show significant or no correlations between isozyme variation distribution and quantitative traits (KNOWLES and MITTON, 1980; KNOWLES and

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GRANT, 1981; MITTON, 1983; MITTON and GRANT, 1984; STRAUSS, 1987; BUSH and SMOUSE, 1992; WANG, 1996); however the role of the isozymes as genetic markers is of great importance in plant breeding and for the conservation of genetic resources. Isozymes have proven to be the most efficient and inexpensive method for the study of genetic variation in tree species when compared to other biochemical or molecular techniques, or the use of morphological characteristics (YEH, 1989).

Sal *(Shorea robusta* GAERTN. f., family *Dipterocarpaceae)* is the most important tree species in Nepal. The species belongs to the *Dipterocarpaceae* family, which is prevalent in tropical and subtropical Asia where it forms large forests in the northern states of India and in the neighboring countries (JOSHI, 1980). The total area of *S. robusta* forests is more than 10 million hectares (e.g. TEWARI, 1995). In the southern plains of Nepal *S. robusta* forests cover most of the lowest fragile foothills of the Himalayas. Its maximum altitude in Nepal is about 1200 m (SHRESTHA, 1989). In Nepal the natural forests have been subject to selection felling encroachment, uncontrolled grazing and annual forest fires, and the forest area has declined rapidly. At present the annual loss of forest cover is about 1.3%, the total area of mixed sal forests in Nepal is roughly 1.4 million hectares, of which 240 000 ha are in national parks and protected reserves.

Characteristics to *S. robusta* is its utmost commercial importance as timber tree, which has led to extensive utilization. Unfortunately, until now, only phenotypic traits of *S. robusta* populations have been studied (MAITHANI et al., 1989; JACKSON, 1994; TEWARI, 1995; RAUTIAINEN and SUOHEIMO, 1997), little information exists on the intra-specific variation of *S. robusta* and its adaptation to prevailing environmental conditions at different altitudes. This information is essential, for example, for the development of silvicultural guidelines for the sustainable forest management in the Terai region. Moreover, knowledge on the genetic structure is important for the development of conservation and utilization programmes of the species' gene resources in Nepal.

The aim of this study was to compare the genetic variability among different natural populations of *S. robusta* in the Terai region, Nepal, using isozyme analysis, so as to obtain genetic parameters to be used as indicators for sustainability of management and conservation.

## Materials and Method

*Plant materials*

The fruits were collected from three natural populations in Makwanpur and Bara districts *(Table 1). S. robusta* fruits are typical for the dipterocarp family: indehiscent, 1 g to 2 g of weight. The fruits are also recalcitrant, loosing the viability normally in about two weeks (CHAMPION, 1928). These fruits (each containing two seeds of which normally only one is visible) were collected in June 1998. The fruits were sampled randomly from at least 15 open-pollinated trees in each population of *S. robusta*; samples from each tree were kept separate and transported to the laboratory in Helsinki, Finland, within three days of sampling.

*Isozyme electrophoresis and detection*

In Helsinki, Finland, the fruit samples were germinated in wet horticultural peat at room temperature (20 °C to 25 °C). The entire germinated fruit was homogenized in an extraction buffer (CHELIAK and PITEL, 1984; IBRAHIM, 1996). 138 to 147 fruits were electrophoretically assayed from each sampled population.

Three buffer systems were used to separate 8 isozyme systems in 12% starch gel electrophoresis (WENDEL and WEEDEN, 1989; KEPHART, 1990) *(Table 2).* One isozyme locus was revealed for each of the isozyme systems G6PD, ME, MR, SOD, while two loci were revealed for each of DIA, MDH, 6PGD and PGI. The loci and the alleles within locus were numbered from anode to cathode. Twelve loci were scored: DIA1, DIA2, G6PD, MDH1, MDH2, ME, MR, 6PGD1, 6PGD2, PGI1, PGI2, SOD.

*Data analysis*

Genetic parameters such as mean number of alleles per locus, percentage of polymorphic loci (by convention, loci were considered polymorphic if the frequency of the most common allele did not exceed 0.95), observed and expected heterozygosities, were calculated for the three *S. robusta* populations. The genetic differentiation among the populations were estimated by partitioning the total gene diversity ($H_T$), the gene diversity within populations ($H_S$) and among populations ($D_{ST}$). The degree of genetic differentiation ($G_{ST}$) was calculated as $D_{ST}/H_T$ (NEI, 1973, 1978). The genetic distances (D) were calculated according to NEI (1978). The Biosys-1 computer program was used in the present study (SWOFFORD and SELANDER, 1981).

## Results

*Genetic diversity*

Of 12 loci from 8 isozyme systems investigated, DIA1, DIA2, G6PD, ME, MR, SOD were monomeric and MDH1, MDH2, 6PGD1, 6PGD2, PGI1, PGI2 were dimeric. The mean percentage of polymorphic loci was 58.3%, ranging from 50.0% in Chu-

*Table 1.* – Origin of three natural populations of *S. robusta* used in the study.

| Locality | Latitude | Longitude | Altitude (m) | Mean annual rainfall (mm) | Mean annual temperature (°C) |
|---|---|---|---|---|---|
| Churia | 27°25′ N | 85°03′ E | 550 | 2100 | 24 |
| Manahari | 27°35′ N | 84°48′ E | 300 | 2200 | 22 |
| Parsa | 27°15′ N | 84°59′ E | 100 | 1800 | 20 |

*Table 2.* – Buffer systems for isozyme analysed.

| Buffer system | Usage | Composition | pH | Isozyme |
|---|---|---|---|---|
| TC 7.0 | Electrode | 0.135 M Tris | pH7.0 | DIA (E.C.1.6.4.3) |
| | | 0.043 M Citric Acid | | G6PD(E.C.1.1.1.49) |
| | Gel | 1:14 | pH7.0 | SOD (E.C.1.15.1.1) |
| TC 7.8 | Electrode | 0.135 M Tris | pH7.8 | MDH (E.C.1.1.1.37) |
| | | 0.039 M Citric Acid | | ME (E.C.1.1.1.40) |
| | Gel | 1:14 | pH7.8 | MR (E.C.1.6.99.2) |
| HC 6.5 | Electrode | 0.065 M L-histidine | pH6.5 | 6PGD(E.C.1.1.1.44) |
| | | 0.007 M Citric Acid | | PGI (E.C.5.3.1.9) |
| | Gel | 1:3 | pH6.5 | |

*Table 3.* – Sample size, number of alleles per locus, percentage of loci polymorphic, observed and expected heterozygosities per population in *S. robusta.*

| Population | Sample size | Mean no. of alleles per locus | Percentage of loci polymorphic*) | Mean heterozygosity | |
|---|---|---|---|---|---|
| | | | | observed | expected |
| Parsa | 147 | 2.16 (0.3) | 58.3 | 0.117(0.023) | 0.142(0.029) |
| Manahari | 138 | 2.25 (0.4) | 66.7 | 0.129(0.035) | 0.158(0.047) |
| Churia | 142 | 2.08 (0.3) | 50.0 | 0.105(0.016) | 0.130(0.030) |
| Mean | 143 | 2.16 | 58.3 | 0.117 | 0.143 |

*) 0.95 criterion

ria to 58.3% in Parsa and 66.7% in Manahari. Within the populations the mean allelic diversity per locus ranged from 2.08 to 2.25 with a mean of 2.16 alleles per locus *(Table 3).* The observed heterozygosity ($H_o$) in the population samples ranged from 0.105 to 0.129, whereas the range for expected heterozygosity ($H_e$) was from 0.130 to 0.158. The observed heterozygosities were slightly lower than those expected. The grand mean over three populations was 0.117 for $H_o$ and 0.143 for $H_e$, respectively.

*Genetic differentiation*

The values of genetic differentiation are shown in *table 4.* The total gene diversity and the gene diversity within populations were 0.150 and 0.143, respectively. The relative degree of genetic differentiation was as low as 0.047. The genetic distances and geographical distances are shown in *table 5.* The average genetic distance was 0.018, showing a very low level of differentiation among the studied populations. The maximum genetic distance, 0.025, was observed between the Churia population and the Parsa population, while the minimum, 0.012, occurred between the Churia and Manahari populations. There was no correlation between genetic distance and geographical distance in the present study.

## Discussion

The present study showed that the genetic variability within populations of *S. robusta* was higher than in other plant species summarized by HAMRICK and GODT (1990) and HAMRICK et al. (1992): for example, the percentage of polymorphic loci per population was 58.3% at 0.95 polymorphic criteria, which was 13.2 percentage points higher than that in the average of

*Table 4.* – The total gene diversity ($H_T$), the gene diversity within population ($H_S$), the gene diversity among populations ($D_{ST}$) and the degree of genetic differentiation ($G_{ST}$) in *S. robusta.*

| Locus | $H_T$ | $H_S$ | $D_{ST}$ | $G_{ST}$ |
|---|---|---|---|---|
| DIA1 | 0.179 | 0.169 | 0.010 | 0.056 |
| DIA2 | 0.149 | 0.144 | 0.005 | 0.034 |
| G6PD | 0.211 | 0.197 | 0.014 | 0.066 |
| MDH1 | 0.183 | 0.175 | 0.008 | 0.044 |
| MDH2 | 0.117 | 0.111 | 0.006 | 0.051 |
| ME | 0.099 | 0.095 | 0.004 | 0.040 |
| MR | 0.066 | 0.064 | 0.002 | 0.030 |
| 6PGD1 | 0.203 | 0.191 | 0.012 | 0.059 |
| 6PGD2 | 0.176 | 0.167 | 0.009 | 0.051 |
| PGI1 | 0.116 | 0.111 | 0.005 | 0.043 |
| PGI2 | 0.165 | 0.159 | 0.006 | 0.036 |
| SOD | 0.135 | 0.128 | 0.007 | 0.052 |
| Mean | 0.150 | 0.143 | 0.007 | 0.047 |

*Table 5.* – Genetic distance (above the diagonal) and geographical distances (km, below the diagonal) among three populations of *S. robusta.*

| Population | Churia | Manahari | Parsa |
|---|---|---|---|
| Churia | ----- | ***0.012*** | ***0.025*** |
| Manahari | 30 | ----- | ***0.018*** |
| Parsa | 20 | 35 | ----- |

angiosperm woody plants in general (45.1%), and 5.3 percentage points higher than that in outcrossing wind-pollinated woody plants in general (53.0%). The mean number of alleles per locus per population was 2.16, which was higher than the average of 1.44 in dicots, 1.68 in angiosperm woody plants, 1.79 in woody plants and 1.84 in outcrossing windpollinated woody plants. The mean expected heterozygosity per population (0.143) in this study was consistent with the averages of both angiosperm (0.143) and outcrossing wind-pollinated woody plants (0.154). However, it was lower than the average of 17 tropical tree species (0.217) (MORAN, 1992; HOUSE and BELL, 1994; IBRAHIM, 1996; MARTINS-CORDER and LOPES, 1997).

Most dipterocarps have been found to be predominantly outbreeders, with high within-population variability as discussed by SOERIANEGARA and LEMMENS 1994, which, however, is reference to the humid tropics. In the present study, the total genetic diversity was 0.150 in the material, and a partitioning of the genetic diversity revealed that there was a great amount of diversity residing within a given population (0.143). The overall proportion of inter-population gene differentiation, 4.7%, indicated the majority of the variation resides within populations. This study thus suggests that natural populations of *S. robusta* in the Terai region, Nepal, come close to approximating a single panmictic unit. Strong winds and storms during the season of pollen dispersal (cf. TEWARI, 1995) may favor such an intensive gene flow. The results are very similar to previous conclusions according to which most of the genetic diversity resides within rather than among populations in many tropical tree species, for example, in *Azadirachta indica* (KUNDU, 1998), *Eucalyptus urophylla* (HOUSE and BELL, 1994), *Faidherbia albida* (IBRAHIM, 1996), *Taiwania cryptomerioides* (LIN et al., 1993). Recent studies on tropical tree species by DNA markers also support such conclusion (GRATTAPAGLIA et al., 1997).

Since isozymes were analysed on the basis of germinating fruits, the excess of homozygotes observed here may have been caused by inbreeding, a fact well known in many other studies (e.g. KEPHART, 1990; MUONA, 1990; BUSH and SMOUSE, 1992), it may be due to partial selfing, for example, in *Eucalyptus regnans,* higher level of inbreeding found in natural stands compared with a seed orchard was explained by the spatial genetic structure (MORAN et al., 1989; MUONA, 1990). In addition, the small samples sizes investigated may also contribute to inbreeding. In the present study, the results suggest some inbreeding, but because of the high outcrossing rates this is likely to represent mating between close relatives rather than selfing.

A comparison between geographic distance and NEI's genetic distance in *S. robusta* populations did not indicate significant correlation. Such a situation has been also reported for other tropical woody species in the previous studies (BELLETTI and LANTERI, 1995).

Furthermore, these results have two additional implications for practical operations. First, if the genetic resources of *S. robusta* are to be conserved, the sharing of one gene pool among the studied populations and a lack of barriers to gene flow in *S. robusta* populations must be considered; Second, the majority of species' genetic variation within the studied populations and high genetic similarity among three natural populations of *S. robusta* must be taken into consideration when breeding programmes are established.

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