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INHERITANCE OF ALLOZYME IN *SHOREA LEPROSULA* (DIPTEROCARPACEAE)

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LEE, S. L., WICKNESWARI, R., MAHANI, M. C. & ZAKRI, A. H. 2000. Inheritance of allozyme in *Shorea leprosula* (Dipterocarpaceae). Thirty-four to one hundred seeds from 14 half-sib families of *Shorea leprosula* were analysed by starch gel electrophoresis in order to verify the genetic control and mode of inheritance of allozyme loci. Of 29 enzyme systems investigated, 11 (AAT, EST, GDH, GPI, IDH, MDH, ME, PGM, SDH, TPI and UGP) exhibited high enzyme activity. To avoid uncertainties in genotyping, enzyme systems from these which were ontogenetically unstable (GDH), tissue specific (AAT), or had complex banding patterns (EST, ME and TPI) were excluded in genetic analyses. Of the remaining 6 enzyme systems (GPI, IDH, MDH, PGM, SDH and UGP), 10 codominant allozyme loci (*Gpi-2, Pgm, Idh, Mdh-1, Mdh-2, Mdh-3, Sdh-1, Sdh-2, Ugp-1* and *Ugp-2*) were intuitively postulated. Genetic analysis utilising single trees and their progenies supported the intuitive interpretation of all 10 proposed loci, with the exception of *Mdh-1*. For *Mdh-3*, since maternal heterozygous trees were unavailable, the hypothesis of genetic control must be considered as preliminary.

Key words: Starch gel electrophoresis - allozyme - mode of inheritance - Dipterocarpaceae - *Shorea leprosula* - Malaysia

LEE, S. L., WICKNESWARI, R., MAHANI, M. C. & ZAKRI, A. H. 2000. Pewarisan alozim dalam *Shorea leprosula* (Dipterocarpaceae). Sebanyak 34–100 biji masing-masing dari 14 keluarga separuh sib *Shorea leprosula* telah dianalisis menggunakan teknik elektroforesis gel kanji untuk menentukan pengawalan genetik dan mod pewarisan lokus alozim. Daripada 29 sistem enzim yang dikaji, 11 (AAT, EST, GDH, GPI, IDH, MDH, ME, PGM, SDH, TPI dan UGP) menunjukkan aktiviti enzim yang tinggi. Untuk mengelakkan ketidakpastian dalam penentuan genotip, enzim yang ontogenetik tidak stabil (GDH), tisu spesifik (AAT), atau menghasilkan corak jaluran yang kompleks (EST, ME dan TPI) telah diketepikan untuk analisis genetik. Daripada baki 6 sistem enzim (GPI, IDH, MDH, PGM, SDH dan UGP), 10 lokus alozim yang kodominan (*Gpi-2, Pgm, Idh, Mdh-1, Mdh-2, Mdh-3, Sdh-1, Sdh-2, Ugp-1* and *Ugp-2*) telah dipostulasikan. Analisis berdasarkan pokok tunggal dan progeninya menyokong postulasi kesemua lokus yang diuji, melainkan *Mdh-1*. Untuk *Mdh-3*, memandangkan ibu pokok yang heterozigot tidak diperoleh, hipotesis kawalan genetiknya masih pada peringkat awal.

Introduction

In forest trees, allozyme analyses have been widely applied in genetic studies. Such studies include analysis of genetic variation within species (Chase *et al.* 1995, Sheely & Meagher 1996), certification of parent trees and clones (Adams & Joly 1980b,Cheliak & Pitel 1984) and estimation of mating system parameters (Murawski & Bawa 1994, Doligez & Joly 1997).

Allozymes are expected to be inherited codominantly in accordance with simple Mendelian principles. However, in principle, allozymes may not show codominance due to modifier or the absence of activity ('null' alleles). Loci of related species often resemble one another in general isozyme characteristics, although species can differ in isozyme numbers, in phenotypes of similar loci, in the interaction between loci and the expression of modifier loci (Adams & Joly 1980a, El-Kassaby 1981, Harry 1983). Furthermore, some enzymes may show developmental or environmental variation that mimics Mendelian segregation (Harry 1983, Lebherz 1983, Womark 1983). It is essential, therefore, when making comparisons of isozyme variation between populations that the genetic control of the enzymes is understood. The absence of information on inheritance may lead to overestimation of heterozygosity and biased estimates of allele frequencies and mating system parameters.

Shorea leprosula Miq. (Dipterocarpaceae), commonly known as meranti tembaga, is distributed from southern Thailand (Pattani), throughout Peninsular Malaysia (except for the seasonal areas in Perlis, northwest Kedah and Langkawi Island), and Sumatra to north Borneo. It is common on well-drained or swampy sites on deep clay soil in lowland and hill dipterocarp forests below 700 m altitude (Symington 1943, Ashton 1982). In an earlier allozyme study by Gan *et al.* (1981), five enzyme systems (esterase, aspartate aminotransferase, leucine aminopeptidase, alkaline phosphatase and acid phosphatase) were investigated, but all the systems were interpreted phenotypically. Subsequently these were used by Gan *et al.* (1981) to estimate intra-population genetic variation and by Ashton *et al.* (1984) in ten *Shorea* species from Peninsular Malaysia.

Enzyme systems have been investigated in other Dipterocarpaceae (Ihara *et al.* 1986, Kitamura *et al.* 1994a, Murawski & Bawa 1994, Murawski *et al.* 1994a, b; Shiraishi *et al.* 1994), and in the majority of these cases, although the number of alleles at each locus was given and used for estimates of outcrossing or studies of population genetic structure, the genetic control of the loci was either not investigated or published. This article reports an analysis of inheritance of several enzyme systems in *S. leprosula* using half-sib families. The enzyme gene loci identified will be used in an intensive study of genetic diversity and mating system of *S. leprosula* from Malaysia.

Materials and methods

Sampling

Mature open pollinated seeds of *S. leprosula* were collected from ten and four mother trees respectively from Sungai Menyala Forest Reserve and Bangi Forest Reserve following the August 1996 fruiting season of Dipterocarpaceae in Peninsular Malaysia. Generally, seeds of *S. leprosula* are dispersed within a 30 m radius of the mother tree (Chan 1980). If mother trees were more than 50 m apart, seeds were collected from the ground beneath the maternal tree crown. When mother trees were closer together, seeds were harvested from representative branches within the crown by a tree climber or by a "shaking-catch" method. In the "shaking-catch" method, a weight attached to a nylon fishing string was shot over a branch using a catapult, and used to haul up a thicker, stronger nylon line. The ends of the line were then pulled vigorously to detach the seeds; the seeds were easily caught as they gyrated toward the ground. From each mother tree, 34–100 seeds were germinated on moistened tissue paper in the laboratory. Germinated embryos (one to seven days old with radicals about 7 mm long) were used for isozyme analysis. Leaf and inner bark tissues were also collected from each mother tree and assayed to determine their genotype. Tissue-specific effects were tested by sampling the following tissues from the same tree: (1) six-day-old germinating embryos, (2) inner bark, and (3) leaves. Since individuals of the same clone from all age classes were not available, ontogenetic effects were tested by comparing parent and progeny arrays at four developmental stages: (1) six-day-old germinating embryos, (2) six-month-old seedlings (about 0.3 m high), (3) 1–2-y-old saplings (2–3 m high), and (4) mature trees (about 30 y old).

Enzyme extraction

Embryos were homogenised in 200 µl extraction buffer (Wickneswari & Norwati 1992) whilst approximately 100 mg of inner bark or leaf tissues were homogenised in 0.4 ml extraction buffer. The crude extract was filtered through cotton and centrifuged at 6000 rpm for 5 min into an Eppendorf tube and the extract was stored at -70 °C until used for analysis.

Electrophoresis

Electrophoresis was carried out on horizontal 10–12% (w/v) starch gels (Sigma Chemical). Four buffer systems were used to resolve the enzyme systems listed in Table 1. Enzyme staining was according to Harris and Hopkinson (1976), Wendel and Weeden (1989), and Thormann and Stephan (1993) using both agar overlays and liquid stains. Scoring was done as soon as the bands appeared at an intensity that allowed them to be scored reliably.

Table 1. Summary of enzyme systems screened in *S. leprosula*. Enzyme systems with good activity that can be well resolved and scored reliably are highlighted

Enzyme system	Abbre.	E.C. No.	Buffer *	Observation
Aconitase	ACO	4.2.1.3	MC	Lacking in activity
Adenylate kinase	AK	2.7.4.3	H	Lacking in activity
Alcohol dehydrogenase	ADH	1.1.1.1	L	Lacking in activity
Aldolase	ALD	4.1.2.13	TC	Lacking in activity
Aspartate aminotransferase	AAT	2.6.1.1	L, MC	Good activity but only in leaves
Diaphorase	DIA	1.6.4.3	H	Lacking in activity
Esterases	EST	3.1.1.1	L, MC	Good activity but unscorable
Fumarase	FUM	4.2.1.2	MC	No activity
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49	MC	No activity
Glucosephosphate isomerase	**GPI**	**5.3.1.9**	**MC**	**Good activity and scorable**
Glutamate dehydrogenase	DH	1.4.1.2	TC, L	Good activity but not in seed
Glycerate dehydrogenase	GLY	1.1.1.29	H	No activity
α-Glycerophosphate dehydrogenase	αGLD	1.1.1.8	MC	No activity
Hexokinase	HK	2.7.1.1	L	No activity
Isocitrate dehydrogenase	**IDH**	**1.1.1.42**	**MC**	**Good activity and scorable**
Leucine aminopeptidase	LAP	3.4.11.1	MC	No activity
Malate dehydrogenase	**MDH**	**1.1.1.37**	**MC**	**Good activity and scorable**
Malic enzyme	ME	1.1.1.82	H, MC	Good activity but unscorable
Mannose-6-phosphate isomerase	MP	5.3.1.8	L	No activity
Menadione reductase	MR	1.6.99.2	L	Lacking in activity
Peptidase	PEP	3.4.13.11	L	Lacking in activity
Peroxidase	PER	1.11.1.7	TC	Lacking in activity
6-Phosphogluconate dehydrogenase	6PGD	1.1.1.44	MC	No activity
Phosphoglucomutase	**PGM**	**2.7.5.1**	**H**	**Good activity and scorable**
Shikimate dehydrogenase	**SDH**	**1.1.1.25**	**MC**	**Good activity and scorable**
Succinate dehydrogenase	SUDH	1.3.99.1	TC	No activity
Superoxide dismutase	SOD	1.15.1.1	MC	Lacking in activity
Triosephosphate isomerase	TPI	5.3.1.1	MC, H	Good activity but unscorable
Uridine diphosphogluconate pyrophosphatase	**UGP**	------	**MC**	**Good activity and scorable**

* Electrode and gel buffers: (H) electrode, 0.41M sodium citrate - pH 8.0 with 0.41M citric acid; gel, 5mM L-histidine pH 8.0; (TC) electrode, 0.3M boric acid - 0.1M NaOH, pH 8.6; gel, 0.1M tris - 0.0069M citric acid, pH 8.6; (MC) electrode, 0.04M citric acid - pH 6.1 with 0.068M N-3 aminoprophyl-morpholine; gel, diluted electrode buffer (20:1); (L) electrode, 0.05M lithium hydroxide - 0.19M boric acid, pH 8.5; gel, 0.065M tris - 0.01M citric acid - 10% electrode buffer, pH 8.2.

Genetic analysis

Since material from controlled crosses was not available, verification of the genetic control and the inheritance of the respective isozyme variants was performed using the method specified by Gillet and Hattemer (1989). It was based on three assumptions: (i) regular meiotic segregation during egg production, (ii) random fertilisation of the eggs by each pollen (haplo) type, and (iii) absence of differential viability selection in the offsprings prior to

the investigation. Twenty-nine to seventy-four offsprings per heterozygous tree were investigated. Goodness of fit tests were utilised in order to test the fulfillment of the quantitative tests.

For enzyme systems with either more than one zone of activity or in zones of activity with more than one allozyme, the zones/loci were designated numerically (beginning with 1) and alleles were designated alphabetically (beginning with A), both in decreasing order of relative mobility.

Results and discussion

Of the 29 enzyme systems investigated, only 6 (GPI, IDH, MDH, PGM, SDH and UGP) produced activity that could be resolved and scored reliably (Table 1). Most of the unsuccessfully developed enzyme systems exhibited weak activity (ACO, AK, ADH, ALD, DIA, MR, PEP, PER and SOD) or did not show activity at all (FUM, G6PD, GLY, αGLD, HK, LAP, MPI, 6PGD and SUDH). The enzyme extraction of higher plant species is generally tedious due to endogenous phenols, tannins, phenoloxidases and other cellular constituents such as terpenes, pectins, resins, coumarins and carotenoid that inhibit enzyme activity (Loomis 1974). This problem applies especially to Dipterocarpaceae species, which are extremely difficult to work with (Yap 1976). To date, most of the studies in genetic diversity and mating system of Dipterocarpaceae utilised not more than seven enzyme systems (Ihara *et al.* 1986, Kitamura *et al.* 1994b, Shiraishi *et al.* 1994, Murawski & Bawa 1994, Murawski *et al.* 1994a,b).

No single extraction buffer will be optimally effective in protecting all enzymes from any given tissue. Some of the additives added in the extraction buffer of this study might inhibit certain enzyme complexes. For example, diethyldithiocarbamate (DIECA) may affect copper-zinc coenzymes of superoxide dismutase, and bisulfite inhibits some dehydrogenase systems (Kelley & Adams 1977, Wendel & Weeden 1989). Furthermore, some losses in enzyme activity is inevitable during freezing. For example, in maize, freezing causes deterioration of extract quality for the products of HK and ACO loci (Wendel *et al.* 1986, 1988).

EST and TPI exhibited high enzyme activity; however, the complicated banding patterns were difficult to interpret. Due to this problem, Gan *et al.* (1981) interpreted EST phenotypically as electromophs in the study of *S. leprosula*. Murawski *et al.* (1994b) in a study of *S. megistophylla* also faced the same problem for TPI. ME seems to be polymorphic but the banding patterns were not well resolved. The putative alleles were too close to resolve and with the nature of ME which is tetrameric, the homozygote and heterozygote phenotypes could not be unambiguously differentiated. Thus, in order to avoid uncertainties in genotyping, EST, TPI and ME were excluded for further analysis.

For GDH, enzyme activity was only found in the adult, seedling and sapling stages indicating that GDH is not expressed during germination.

Therefore, expression of GDH seems to be ontogenetically unstable in *S. leprosula.* For AAT, although the gene expression was detected in all the four development stages, it was detected only in leaf tissue. This implies that the expression of AAT is tissue-specific, contrary to the study by Scandalios *et al.* (1975) on maize who reported that AAT activity could be detected in the tissues throughout the plant. Hence, GDH and AAT were omitted for further analysis.

Glucosephosphate isomerase (GPI)

In plant species investigated, GPI is a dimer encoded by two loci. The product of one locus is active in the cytoplasm, whilst the other is active in plastids (Gottlieb 1981). For *S. leprosula,* two zones of activity were observed. However, only the slower zone could be sufficiently resolved. For this indubitably dimeric zone, four alleles were present but only three of the four putative homozygous phenotypes and five of the six possible heterozygous phenotypes were observed (Figure 1). On the basis of the three-banded phenotypes observed in zymograms from putative heterozygous individuals, a dimer structure of the enzyme was confirmed. A codominant single-locus was postulated and denoted as *Gpi-2.* Qualitative and quantitative tests of a postulated single-locus mode of inheritance (Table 2) showed that each progeny possessed at least one maternal allele, and the offspring progeny array showed no significant deviation from the expected 1:1 ratio of homozygous:heterozygous offspring, thus supporting the intuitive postulate.

Phosphoglucomutase (PGM)

Studies of PGM have shown it to be monomeric, and usually encoded by two loci. The products of one locus are usually active in the chloroplast, whilst those of the other locus are usually active in the cytoplasm (Gottlieb 1981). In *S. leprosula,* only one zone of activity was observed and two alleles were present. In germinating embryos, the enzyme appears to have three (for homozygote) or five (for heterozygote) banded phenotypes which segregated as a unit, three of which (bands 'a', 'c' and 'e') might be regarded as artifact bands (Figure 1). May (1992) considered that artifactual bands may be identified by: (1) consistent variable expression among individuals on a single gel, (2) variable expression in the same individual dependent on the length of storage time, and (3) failure to fit classical electrophoretic phenotype expectations. The PGM banding patterns are consistent with criteria (1) and (3), indicating that bands 'a', 'c' and 'e' can be regarded as artifact bands. Comparison among the leaf and inner bark tissues from different developmental stages showed that artifact band 'e' was ontogenetically unstable and only present in germinating embryos, and not in the seedling, sapling or adult stages. Consequently, homozygotes were represented by two bands and heterozygotes by four bands (Figure 1).

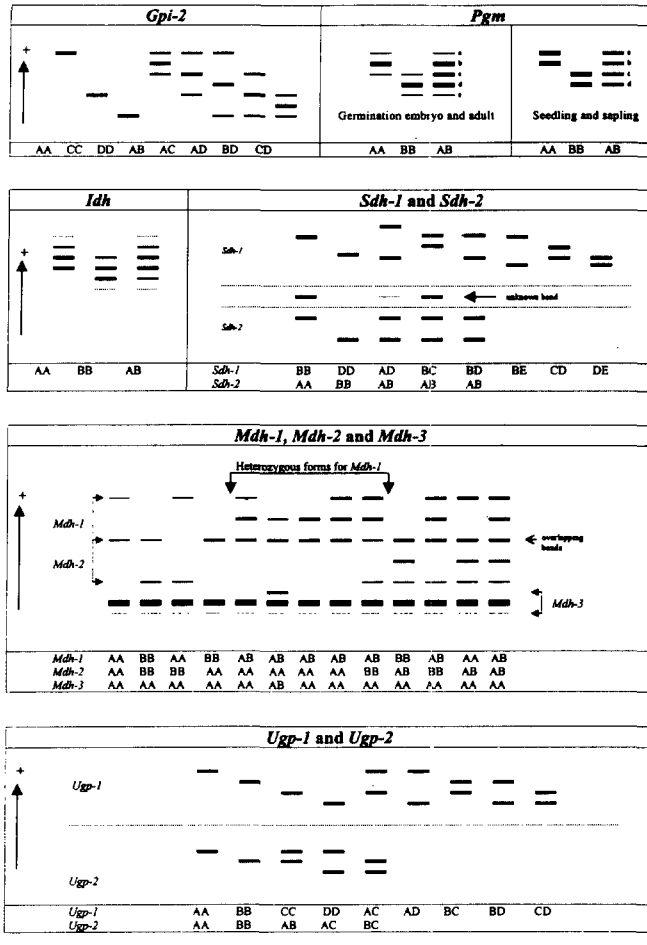

Figure 1. Observed phenotypes of the six investigated enzyme systems

A codominant single-locus was postulated, and denoted as *Pgm*. Genetic analysis of this mode of inheritance (Table 2) showed that each progeny possessed at least one maternal allele, and the offspring progeny array showed no significant deviation from the expected 1:1 ratio of homozygous:heterozygous progeny, thus supporting this postulate.

Artifact bands do not fit simple Mendelian and biochemical models, and may arise *in vivo* from post-translational enzymes modification (Goodman *et al.* 1980, Endo 1981, Harry 1983, Doebley *et al.* 1986) or *in vitro* from artifacts during sample preparation, storage or electrophoresis (Harris & Hopkinson 1976). Artifact PGM bands have been reported in *Abies balsamea* (Neale & Adams 1981), *Camellia japonica* (Wendel & Parks 1982), *Zea mays* (Stuber & Goodman 1983) and *Pteridium aquilinum* (Wolf *et al.* 1987).

Isocitrate dehydrogenase (IDH)

In most plant species studied, IDH isozymes are encoded by a single locus (Tanksley 1984, Ni *et al.* 1987). In this study, one zone of activity controlled by two alleles was observed. Each allele is represented by multiple banding patterns with four bands and six bands for homozygotes and heterozygotes respectively (Figure 1). The appearance of bands 'a' and 'f' were not consistent and were absent in some individuals. A codominant single locus was postulated and denoted as *Idh*. Inspection of family data supported this interpretation of the banding patterns. For example, when the maternal genotype was heterozygous, approximately half of the progeny were heterozygous, consistent with expectation of selfing, outcrossing or mixed mating. Also, all offspring carried at least one maternal allele (Table 2).

Malate dehydrogenase (MDH)

Interpretation of MDH zymograms in plant species is difficult due to similar migration rates and overlaps of several isozyme zones (Thormann & Stephan 1993). Hussendörfer *et al.* (1995) considered MDH to consist of two enzyme types: (1) non-decarboxylating MDH (E.C. 1.1.1.37), and (2) oxaloacetate-decarboxylating MDH (E.C. 1.1.1.38). Both MDH types that use NAD as a coenzyme can be stained using a tetrazolium salt. However, non-decarboxylating MDH can also be stained with a diazolium salt (Thormann & Stephan 1993). Using a tetrazolium salt, Hussendörfer *et al.* (1995) simplified the zymogram pattern of MDH in *Abies alba*.

In this study, only the tetrazolium method produced clear and scorable banding patterns, and three overlapping zones of activity were observed. The slowest migrating and most intensely stained zone was denoted as *Mdh-3*, whilst the fastest migrating zone was *Mdh-1* (Figure 1). All the three putative loci were variable, and allele B of locus *Mdh-1* migrated to the same position as allele A of locus *Mdh-2*. The dimeric structure of the enzyme is verified by the appearance of the intralocus hybrid bands. It is also assumed that heterozygous phenotype of *Mdh-1* can be in five different forms (Figure 1).

Qualitative tests of the hypothesis of complete codominance for *Mdh-1* supports the hypothesis (since all progeny inherited at least one maternal allele), but quantitative test of the 1:1 ratio hypothesis leads to rejection for 11 of the 14 heterozygotes families (Table 2). All the families appeared to favour heterozygotes suggesting that heterozygote individuals had an advantage.

For *Mdh-2* locus, genetic analysis supports the hypothesis of a codominant single locus (Table 2). Each progeny array possessed at least one maternal allele and the offspring showed no significant deviation from the expected 1:1 ratio of homozygous:heterozygous progenies. Heterozygous maternal trees were not available for locus *Mdh-3*, therefore genetic analysis was not carried out. Thus, the hypothesis of genetic control must be considered preliminary.

Table 2. Summary of the single tree progeny analysis for nine isozyme loci in *Shorea leprosula*. Chi-square tests support the hypothesis of codominant mode of inheritance for all the loci except *Mdh-1*.

Maternal tree			Progeny								p- value
Gpi-2 No.	Genotype	N	N_{AA}	N_{AB}	N_{AC}	N_{AD}	N_{BD}	N_{CC}	N_{CD}	N_{DD}	
MSG22	CC	35	-	-	-	-	-	26	9	-	-
MSG26	CC	34	-	-	-	-	-	19	15	-	-
MSG28	CC	30	-	-	1	-	-	20	9	-	-
MBA01	DD	36	-	-	-	-	-	-	4	32	-
MBA02	DD	38	-	-	-	-	-	-	1	37	-
MBA03	DD	38	-	-	-	7	5	-	2	24	-
MSG21	CD	40	-	-	-	-	-	16	19	5	0.100^{ns}
MSG23	CD	35	-	-	-	-	-	12	20	3	0.714^{ns}
MSG24	CD	34	-	-	-	-	-	10	21	3	1.882^{ns}
MSG25	CD	74	-	-	-	-	-	21	41	12	0.865^{ns}
MSG27	CD	40	-	-	-	-	-	19	17	4	0.900^{ns}
MSG29	CD	36	-	-	-	-	-	8	23	5	2.778^{ns}
MSG30	CD	36	-	-	-	-	-	8	17	11	0.111^{ns}
MBA04	AD	40	1	3	-	15	5	-	-	16	0.625^{ns}
Pgm No.	Genotype	N	N_{AA}	N_{AB}	N_{BB}						
MSG26	AA	33	18	15	-						-
MSG30	AA	36	26	10	-						-
MSG27	BB	40	-	23	17						-
MSG29	BB	36	-	9	27						-
MSG21	AB	40	7	16	17						1.600^{ns}
MSG22	AB	35	6	18	11						0.029^{ns}
MSG23	AB	35	1	18	16						0.029^{ns}
MSG24	AB	32	7	19	6						1.125^{ns}
MSG25	AB	74	28	44	2						2.649^{ns}
MSG28	AB	30	9	16	5						0.133^{ns}
MBA01	AB	36	12	16	8						0.444^{ns}
MBA02	AB	38	8	21	9						0.421^{ns}
MBA03	AB	32	2	12	18						2.000^{ns}
MBA04	AB	40	5	15	20						2.500^{ns}
Idh No.	Genotype	N	N_{AA}	N_{AB}	N_{BB}						
MSG28	AA	29	6	23	-						-
MSG21	BB	40	-	4	36						-
MSG22	BB	35	-	13	22						-
MSG24	BB	33	-	7	26						-
MSG25	BB	74	-	10	64						-
MSG26	BB	34	-	17	17						-
MSG23	AB	35	6	14	15						1.400^{ns}
MSG27	AB	40	7	16	17						1.600^{ns}
MSG29	AB	36	15	15	6						1.000^{ns}
MSG30	AB	35	5	19	11						0.257^{ns}
MBA01	AB	36	7	22	7						1.778^{ns}
MBA02	AB	38	10	18	10						0.105^{ns}
MBA03	AB	37	9	20	8						0.243^{ns}
MBA04	AB	37	16	14	7						1.676^{ns}

(Continued)

(Table 2 - continued)

Mdh-1 No.	Genotype	N	N_{AA}	N_{AB}	N_{BB}	
MSG21	AB	40	-	25	15	2.500^{ns}
MSG22	AB	35	-	30	5	17.857**
MSG23	AB	35	-	23	12	3.457^{ns}
MSG24	AB	34	1	23	10	4.235*
MSG25	AB	74	21	35	18	0.216^{ns}
MSG26	AB	35	1	27	7	10.314**
MSG27	AB	40	-	30	10	10.000**
MSG28	AB	30	2	26	2	16.133**
MSG29	AB	36	-	36	-	36.000**
MSG30	AB	36	2	24	10	4.000*
MBA01	AB	36	-	36	-	36.000**
MBA02	AB	38	-	38	-	38.000**
MBA03	AB	38	-	30	8	12.737**
MBA04	AB	40	-	32	8	14.400**

Mdh-2 No.	Genotype	N	N_{AA}	N_{AB}	N_{BB}	
MSG21	AA	40	30	10	-	-
MSG22	AA	35	24	11	-	-
MSG23	AA	35	25	10	-	-
MSG29	AA	36	36	-	-	-
MBA01	AA	36	36	-	-	-
MBA02	AA	38	38	-	-	-
MBA03	AA	38	38	-	-	-
MBA04	AA	40	35	5	-	-
MSG24	AB	34	12	21	1	1.882^{ns}
MSG25	AB	74	25	40	9	0.486^{ns}
MSG26	AB	34	15	18	1	0.118^{ns}
MSG27	AB	40	15	24	1	1.600^{ns}
MSG28	AB	30	14	15	1	0.000^{ns}
MSG30	AB	36	12	20	4	0.444^{ns}

Sdh-1 No.	Genotype	N	N_{AD}	N_{BB}	N_{BC}	N_{BD}	N_{BE}	N_{CD}	N_{DD}	N_{DE}		
MSG21	BB	40	-	40	-	-	-	-	-	-	-	-
MSG23	BB	31	-	22	2	-	7	-	-	-	-	-
MSG24	BB	34	-	30	-	-	4	-	-	-	-	-
MSG26	BB	35	-	33	-	1	1	-	-	-	-	-
MSG27	BB	40	-	39	-	1	-	-	-	-	-	-
MSG28	BB	30	-	27	2	-	1	-	-	-	-	-
MSG29	BB	36	-	35	-	-	1	-	-	-	-	-
MSG30	BB	36	-	33	-	3	-	-	-	-	-	-
MSG22	BD	35	-	18	-	13	1	1	1	1	-	1.458^{ns}
MSG25	BD	74	-	40	-	31	-	-	3	-	-	1.946^{ns}
MBA01	BD	36	-	10	-	17	2	-	4	3	-	0.490^{ns}
MBA02	BD	38	-	12	-	21	2	-	2	1	-	1.733^{ns}
MBA03	BD	38	2	7	-	20	4	-	5	-	-	2.667^{ns}
MBA04	BD	40	-	12	-	20	-	-	8	-	-	0.000^{ns}

(Continued)

(Table 2 - continued)

Sdh-2 No.	Genotype	N	N_{AA}	N_{AB}	N_{BB}	
MSG22	AA	33	20	13	-	-
MSG24	AA	34	30	4	-	-
MSG26	AA	35	34	1	-	-
MSG27	AA	40	38	2	-	-
MSG28	AA	30	18	12	-	-
MSG29	AA	36	30	6	-	-
MSG30	AA	36	32	4	-	-
MSG21	AB	40	2	23	15	0.900 ns
MSG23	AB	35	10	20	5	0.714 ns
MSG25	AB	74	25	37	12	0.000 ns
MBA01	AB	34	4	13	17	1.882 ns
MBA02	AB	37	3	14	20	2.189 ns
MBA03	AB	37	4	22	11	1.324 ns
MBA04	AB	40	6	24	10	1.600 ns

Ugp-1 No.	Genotype	N	N_{AA}	N_{AC}	N_{AD}	N_{BB}	N_{BC}	N_{BD}	N_{CC}	N_{CD}	N_{DD}	
MSG29	CC	36	-	-	-	-	-	-	34	2	-	-
MSG22	DD	35	-	-	10	-	-	-	-	-	25	-
MSG26	DD	35	-	-	1	-	-	12	-	6	16	-
MBA04	DD	40	-	-	-	-	-	-	-	12	28	-
MSG25	AD	74	10	3	30	-	-	3	-	4	24	1.725 ns
MSG27	AD	40	2	-	25	-	-	-	-	-	13	2.500 ns
MSG28	BD	30	-	-	-	8	1	15	-	-	6	1.035 ns
MSG21	CD	40	-	7	1	-	2	7	5	13	5	0.450 ns
MSG23	CD	35	-	2	2	-	1	2	8	13	7	0.254 ns
MSG24	CD	34	-	1	1	-	1	4	5	13	9	1.323 ns
MSG30	CD	36	-	-	-	-	-	-	15	16	5	0.444 ns
MBA01	CD	36	-	-	-	-	-	-	3	22	11	1.778 ns
MBA02	CD	38	-	-	-	-	-	-	6	18	14	0.105 ns
MBA03	CD	37	-	-	-	-	-	-	4	20	13	0.243 ns

Ugp-2 No.	Genotype	N	N_{AA}	N_{AB}	N_{AC}	N_{BB}	N_{BC}	
MSG21	AA	40	40	-	-	-	-	-
MSG23	AA	35	28	7	-	-	-	-
MSG25	AA	74	70	-	4	-	-	-
MSG27	AA	40	23	14	3	-	-	-
MSG29	AA	36	32	4	-	-	-	-
MSG30	AA	32	19	11	2	-	-	-
MBA03	AA	38	13	24	1	-	-	-
MSG24	BB	32	-	11	-	18	3	-
MSG28	BB	30	-	-	-	27	3	-
MSG22	AB	35	7	21	1	6	-	2.882 ns
MSG26	AB	35	6	18	-	11	-	0.029 ns
MBA01	AB	36	6	21	-	9	-	1.000 ns
MBA02	AB	38	14	17	1	6	-	1.243 ns
MBA04	AB	40	2	24	-	14	-	1.600 ns

ns not significant; * $p < 0.05$; ** $p < 0.01$

Shikimic dehydrogenase (SDH)

In plant species, SDH isozymes are monomeric and encoded by two loci, one in cytosol and the other in plastid (Kephart 1990). In this study, two distinct zones of SDH activity were found on the gels. The fast moving zone was denoted as *Sdh-1* and the slower zone as *Sdh-2*; five and two putative alleles were observed respectively. Two banded heterozygous individuals indicated that the functional form of SDH was monomeric (Figure 1).

Scrutiny of the progeny data supports the codominant mode of inheritance for both the zones. For example, in heterozygous maternal trees, approximately half of the progeny were heterozygous, and every progeny inherited at least one maternal allele (Table 2).

In the zone of *Sdh-2* activity, an unknown band appeared in some seeds, which could be regarded as a third allele. However, if scoring was done including this allele, family data deviated quantitatively and/or qualitatively from the hypothesis of codominant inheritance for most of the families (data not shown). Hence, this band was omitted when interpreting *Sdh-2*.

Uridine diphosphogluconate pyrophosphatase (UGP)

UGP is rarely reported in the literature. For *S. leprosula*, gels stained for UGP showed two variable zones of activity. In the faster migrating zone (Ugp-1), four putative alleles were detected, whereas in the slower zone (Ugp-2), two putative alleles were detected (Figure 1). Putative heterozygous individuals showed two-banded phenotypes in both zones, indicating that the functional structure of UGP was a monomer; inspection of the family data supported the codominant mode of inheritance for both the zones. For example, in heterozygous maternal trees, approximately half of the progenies were heterozygous, and all progeny possessed at least one maternal allele (Table 2). Hence, UGP in *S. leprosula* is controlled by two loci. Two UGP loci and a monomeric enzyme structure have been reported in *Stemonoporus oblongifolius* (Murawski & Bawa 1994).

Conclusion

Nine allozyme loci *(Gpi-2, Pgm, Idh, Mdh-2, Mdh-3, Sdh-1, Sdh-2, Ugp-1* and *Ugp-2)* were identified in *Shorea leprosula*. Each locus was defined for mode of inheritance, ontogenetic stability and non-tissue specificity that can be used subsequently for mating system and population genetic structure studies.

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