Isozyme Variations and Inheritance of Six Putative Loci on *Dryobalanops aromatica* GAERTN. f. (Dipterocarpaceae)

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Summary : Genetic variation of isozymes in *Dryobalanops aromatica* GAERTN. f. (Dipterocarpaceae) was surveyed by polyacrylamide gel electrophoresis. Open pollinated seeds were collected from each mother tree. Cotyledons and taproots were extracted as sample tissue. Six polymorphic loci were discovered in 4 enzyme systems. A total of 19 alleles were presumed in 6 putative loci. These results indicate that the techniques described herein would allow the application of genetic analysis to this species.

1 Introduction

Polymorphisms of isozyme loci have been commonly used as gene markers in order to describe the organization of genetic variation in natural plant populations (HAMRICK *et al.*, 1978; LOVELESS and HAMRICK, 1984). However, tropical tree species have been poorly studied with respect to levels and distribution of genetic variation (GAN *et al.*, 1977, 1981; HAMRICK and LOVELESS, 1986).

Data on the spatial distribution of genetic variation are primary knowledge for conservation biology. Tropical forest reserves should be established and managed so as to preserve the maximum amount of genetic variation within species. However, without data on the distribution of variation within or among populations, reasonable decisions cannot be made about the most effective ways to preserve this variation. From a more basic standpoint, studies of the distribution of genetic variation in tropical plant species might provide insights into how life history traits, such as the pollination mechanism, seed dispersal and fecundity, interact to mold the genetic structure of populations.

This study presents the preliminary results of an electrophoretic survey of *Dryobalanops aromatica* GAERTN. f. One polymorphic locus in LAP has already been observed in *D. aromatica* (SHIRAISHI *et al.*, in this volume). The detection of the polymorphisms on additional 6 putative loci of *D. aromatica* is also reported herein.

2 Materials and Methods

Open pollinated seeds were collected from branches of mother trees in 3 stands of *D. aromatica* in Brunei. Sampling was carried out in August 1991 at Bukit (Bt.) Beruang and Bt. Basong, and in July - August 1992 at Bt. Beruang and compartment 7 of the Andulau Forest Reserve. A total of 389

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seeds were investigated: 9 to 10 seeds per 8 to 10 mother trees in each stand. Cotyledons and taproots of each seed were extracted, soaked in 20% glycerol solution and stored at -30℃ for later use. The procedures of gel electrophoresis and detection of enzyme activities followed SHIRAISHI (1988).

The components of the extraction buffer were a modified form of SHIRAISHI (1988): 0.1M Tris-HCl (pH7.5), 25%(w/v) Glycerol, 1%(w/v) Tween 80, 10mM DTT, 0.4%(v/v) β-ME, and 70mg/ml PVPP.

The nomenclature of each locus and allele was as follows: loci were numbered with the highest value representing the most anodally migrating locus. Alleles were named in alphabetical order from the slowest migrating allele.

3 Results

Six putative loci in 4 enzyme systems were observed to be polymorphic. Observed phenotypes of these loci and their corresponding genotypes are shown in Fig.1. Other electrophoretic variations were detected in acid phosphatase, glutamate dehydrogenase, isocitric acid dehydrogenase, malic enzyme, phosphoglucose isomerase, phosphoglucomutase and sorbitol dehydrogenase, but genetic interpretation of the variations was uncertain. Allele frequencies of each population are shown in Table 1.

3.1 6-phosphogluconate dehydrogenase (6PGD)

Two dimeric loci were observed (*6Pgd-1* and *6Pgd-2*). We assumed 5 phenotypes with 3 alleles (*Rf*21, 23, 26) on *6Pgd-1*, however the $6Pgd\text{-}1^a/6Pgd\text{-}1^a$ homozygote was not observed. As for *6Pgd-2*, 5 phenotypes with 3 alleles (*Rf*36, 41, 43) were presumed without observing the $6Pgd\text{-}2^a/6Pgd\text{-}2^c$ heterozygote.

3.2 Alcohol dehydrogenase (ADH)

Two dimeric loci were observed (*Adh-1* and *Adh-2*). The inter-locus heterodimer banding patterns were observed between these two loci. *Adh-1* was presumed to have 3 alleles (*Rf*41, 44, 48) and also 3 alleles (*Rf*50, 51, 54) were presumed on *Adh-2*, though the following genotypes were not observed : $Adh\text{-}1^a/Adh\text{-}1^c$, $Adh\text{-}1^c$/Adh-1^c, and $Adh\text{-}2^a/Adh\text{-}2^a$.

3.3 Diaphorase (DIA)

One tetrameric locus was observed (*Dia*). Five phenotypes were detected, and 3 alleles (*Rf*38, 43, 48) were presumed. The Dia^a/Dia^b heterozygote was not observed.

3.4 Glutamic oxaloacetic transaminase (GOT)

One dimeric locus with 4 alleles (*Rf*20, 25, 30, 34) was observed (*Got*). The Got^a/Got^d heterozygote was not observed.

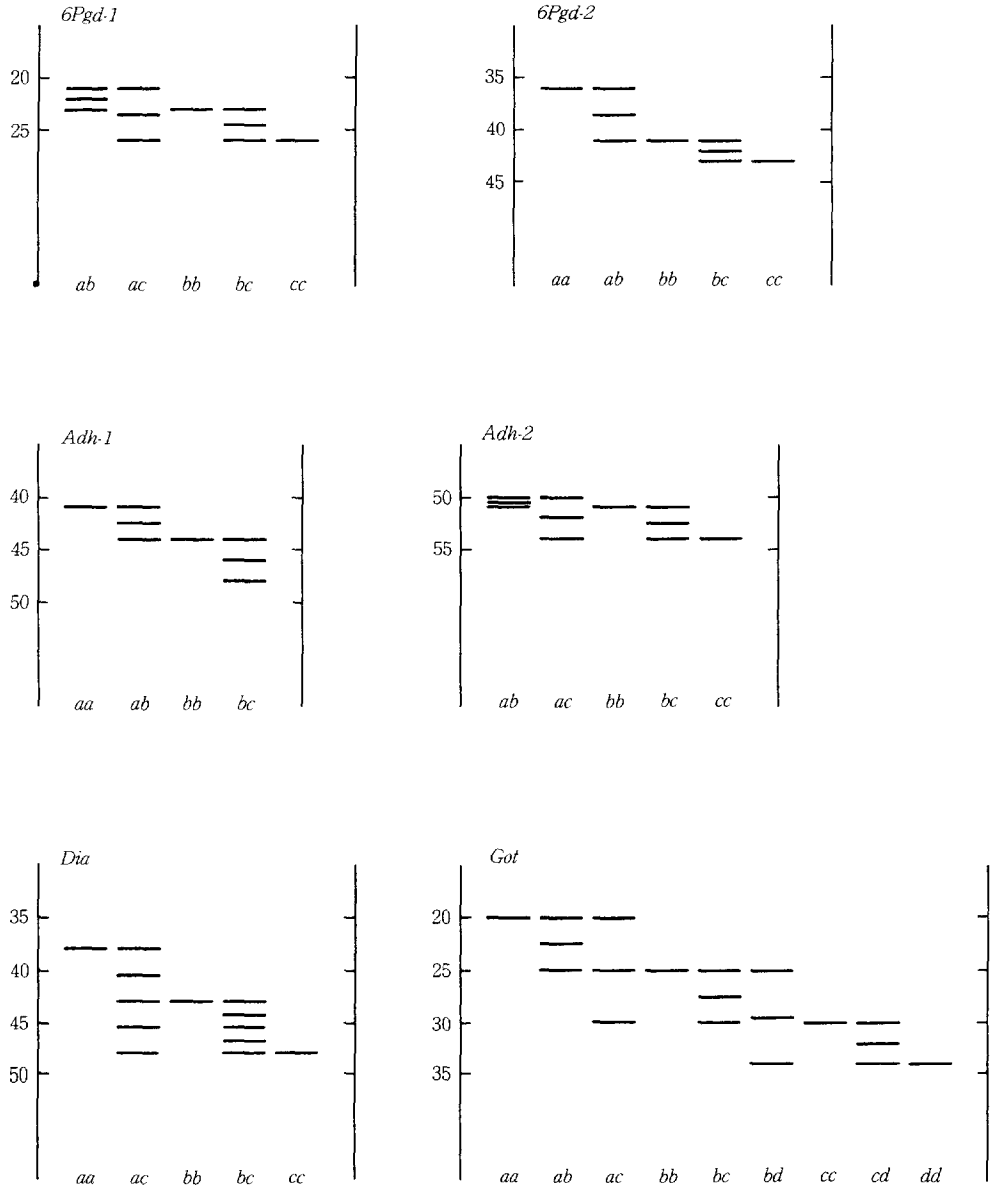

Fig.1. Observed phenotypes of 6 polymorphic loci on *D. aromatica* cotyledons and taproot.
Note : corresponding genotypes are given at the foot of each phenotype.
axis of ordinate represent Rf (relative value to the front).

Table 1. Allele frequencies of each population of *Dryobalanops aromatica.*

Population / Year		Bt.Basong ('91)	Bt.Beruang ('91)	Bt.Beruang ('92)	Andulau ('92)
Lap	*a*	0.01	0.06	0.00	0.00
	b	0.74	0.48	0.47	0.74
	c	0.09	0.06	0.10	0.04
	d	0.16	0.40	0.43	0.22
Got	*a*	0.06	0.01	0.00	0.00
	b	0.56	0.66	0.58	0.68
	c	0.38	0.31	0.37	0.28
	d	0.00	0.02	0.05	0.04
Adh-1	*a*	0.06	0.16	0.09	0.25
	b	0.93	0.83	0.88	0.74
	c	0.01	0.01	0.03	0.01
Adh-2	*a*	0.04	0.02	0.00	0.00
	b	0.11	0.38	0.25	0.33
	c	0.85	0.60	0.75	0.67
6Pg-1	*a*	0.01	0.01	0.00	0.00
	b	0.19	0.47	0.36	0.35
	c	0.80	0.52	0.64	0.65
6Pg-2	*a*	0.00	0.12	0.00	0.02
	b	0.97	0.88	1.00	0.98
	c	0.03	0.00	0.00	0.00

4 Conclusion

Six polymorphic loci in 4 enzyme systems were detected in addition to 1 locus reported by SHIRAISHI *et al.* (1989) on *D. aromatica.* These polymorphisms can be applied to studies on the genetic structures of this species.

With the accelerating destruction of tropical forests, it has become increasingly important to develop enlightened plans for the preservation of the genetic resources of tropical forest species; however, almost nothing is known about the distribution of genetic variation within tropical forests at present. Electrophoretic surveys can help this situation by describing patterns of genetic variation within and among populations or regions. Independent gene marker loci will also allow the estimation of several biologically important characteristics: gene movements within and among populations, genetic relationships of progeny, and spatial distribution.

The results of this study indicate that the techniques described herein would allow the application of genetic analysis to the dynamics of reproductive biology and increase understanding of the genetic structure of populations.

References

GAN, Y. *et al.*: Genetic variation in wild populations of rain-forest trees, Nature, **269**, 323-325 (1977)

———*et al.*: Isozyme variation in some rain forest trees, Biotropica, **13,** 20-28 (1981)

HAMRICK, J. L. and LOVELESS, M. D.: Isozyme variation in tropical trees : Procedures and preliminary results, Biotropica, **18**, 201-207 (1986)

———*et al.*: Relationships between life history characteristics and electrophoretically detectable genetic variation in plants, Ann. Rev. Ecol. Syst. **10**, 173-200 (1979)

LOVELESS M. D. and HAMRICK, J. L.: Ecological determinants of genetic structure in plant populations, Ann. Rev. Ecol. Syst., **15**, 65-95 (1984)

SHIRAISHI, S.: Inheritance of isozyme variations in Japanese Black Pine, *Pinus Thunbergii* PARL., Silvae Genet., **37**, 93-100 (1988)

フタバガキ科 *Dryobalanops aromatica* GAERTN. f.の6推定遺伝子座におけるアイソザイム多型

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摘　　要

ブルネイ国におけるフタバガキ科*Dryobalanops aromatica* GAERTN. f.のアイソザイム変異をポリアクリルアミドゲル電気泳動法により探索した。天然林の母樹から自然受粉種子を母樹別に採取し，子葉と主根から泳動用サンプルを抽出した。その結果，4酵素種の6推定遺伝子座で多型が観察された。これら6遺伝子座で合計19対立遺伝子が探索された。ここで得られた多型遺伝子座を標識遺伝子として利用することにより*D. aromatica*個体群の遺伝的なアプローチが可能となった。

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