E-2.3.2 Genetic Diversity of Wild Leguminous and Gramineous Plants in Tropical Forest

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Abstract

High species diversity in the tropical forest ecosystem is based on high level of speciation. Speciation occurs as a result of the accumulation of genetic variation within species, i.e., intraspecific variation. However, few information is available for the intraspecific variation of wild plants in tropical forest ecosystem. Therefore, we analyzed isozyme variation of some leguminous plants (*Vigna* sp.) collected in tropical ecosystem to examine whether the level of isozyme variation of plants in tropical ecosystem is higher than that of the same or closely related species collected in subtropical ecosystem (the Nansei Archipelago). As a result, we obtained data showing that the level of isozyme variation was not significantly different between tropical and subtropical ecosystem.

Species diversity in tropical forest ecosystem become possible based on the difference of life strategy. Nitrogen fixation of leguminous plants is an important factor concerning with their life strategy. Therefore, we tried to estimate the variability of nitrogen fixing ability of leguminous tree species in Pasoh by natural ^{15}N abundance method. Low $\delta^{15}N$ value of non-leguminous seedlings (average = -0.07) indicated low $\delta^{15}N$ value of soil surface in Pasoh. It is difficult to estimate nitrogen fixation under this condition. $\delta^{15}N$ value of tall leguminous tree was 5.19. This may indicate higher $\delta^{15}N$ value in lower soil layer, and suggest the possibility of estimating nitrogen fixing ability of tall leguminous trees in Pasoh.

Key Words Leguminous Plants, Intraspecific Variation, Isozyme, Interspecific Variation, Nitrogen Fixing Ability

1. Introduction

Tropical rain forest in Southeast Asia is believed to be the ecosystem with the highest species diversity in the world. In tropical forest ecosystem, high level of speciation occurred. For example, 386 species in 12 genera are described for Dipterocarpaceae within the territory of Malaysia. Speciation occurs as a result of the accumulation of genetic variation within species, i.e., intraspecific variation. However, few information is available for the intraspecific variation of wild plants in tropical forest ecosystem. Therefore, we analyzed isozyme variation of some leguminous plants (*Vigna* sp.) collected in tropical ecosystem to examine whether the level of isozyme variation of plants in tropical ecosystem is higher than that of the same or closely related species collected in subtropical ecosystem (the Nansei Archipelago).

Leguminous tree species which occupy an important ecological niche in tropical forest ecosystem are generally considered to have an ability of fixing atmospheric nitrogen. Variation in the ability of atmospheric nitrogen fixation of leguminous tree species may play an important role in natural distribution of the species and may affect the diversity and speciation of the species. However, the information on nitrogen fixing ability of leguminous tree species in tropical forest ecosystem is very limited. Therefore, we tried to estimate the ability of atmospheric nitrogen fixation of leguminous tree species in Pasoh Reserved Forest by means of natural ^{15}N abundance method.

2. Research Method

2-(1) Isozyme analysis

A total of 42 samples consisting of 1 sample of *V.minima*, 7 of *V.reflexo-pilosa* and 34 of *V.trinervia* were used for isozyme analysis. These wild leguminous plants were found on roadside, edge of plantation, and edge of tropical forest. For reference, 50 samples of *V.riukiuensis* and 17 of *V.reflexo-pilosa* were also analyzed. Half cotyledon (two to three days after watering) was homogenized with 100 μl of 0.02M Tris-HCl (pH8.0) containing 5% sucrose. After centrifugation, 10 μl of crude extract was directly placed on the well of 7.5% polyacrylamide gel. Electrophoresis was conducted for two hours at 220 V under 4°C condition. Six enzyme systems were analyzed, i.e., SDH, LAP, ACP, EST, 6PGDH and GOT.

2-(2) Natural ^{15}N abundance method

Leaves were sampled from eight seedlings of leguminous and non-leguminous trees in Pasoh Reserved Forest. Several plant samples were also used as reference. Leaves were oven dried at 70°C for 2 days, weighed and powdered by milling. The 300 mg of leaf sample was digested in 3 ml of heated concentrated sulfuric acid with occasional addition of 3 to 4 ml of 30% hydrogen peroxide. Digested sample was steam distilled after addition of 10N sodium hydroxide for alkalization. For the analysis of nitrogen contents, the released ammonia was collected into a 3ml of 4% boric acid, and the amount was determined by titration. For the analysis of ^{15}N natural abundance, the ammonia was collected into a 1.5ml of 1N sulfuric acid solution up to 60ml and its volume was reduced to 1ml on a hot plate. The concentrate was reacted with sodium hydromite solution in a Rittenberg tube and evolved nitrogen gas was purified by circulation of the gas in a vacuum line and introduced into mass spectrometry (Finigan MAT 251) and its ^{15}N contents was determined[1)]. The ^{15}N natural abundance of sample ($\delta^{15}N$) was expressed as a per mil deviation from that of atmospheric N_2 as follows;

$$\delta^{15}N = (Rsample - Rair)/(Rair) \times 1000\ ‰$$

3. Results and Discussion

3-(1) Intraspecific variation in isozymes

Phenotype frequency of 6 enzymes in *V.trinervia* and *V.reflexo-pilosa* collected in Malaysia was shown in Tables 1 and 2, respectively. No polymorphism was observed in 6PGDH and GOT for both species. For SDH, two banding patterns were recognized in *V.trinervia* and no polymorphism was observed in *V.reflexo-pilosa*. Other three enzyme systems (LAP, ACP, EST) were polymorphic in both species. Phenotype frequency of 2 enzymes (SDH,LAP) in *V.riukiuensis* and *V.reflexo-pilosa* collected from the Nansei Archipelago was shown in Tables 3 and 4, respectively. For SDH, *V.riukiuensis* showed no polymorphism, while *V.reflexo-pilosa* had two isozymes. For LAP, both species showed 3 types of isozyme phenotypes.

To compare the level of isozyme variation between *Vigna* species in Malaysia and the Nansei Archipelago, proportion of polymorphic enzymes (Pe), and average number of polymorphic phenotypes per enzyme (Np) were calculated (Table 5). About half of the enzymes examined showed polymorphism in most of the species collected from Malaysia and the Nansei Archipelago. In Malaysia, average number of isozymes per enzyme (Np) was 2.00 and 1.50 for *V.trinervia* and *V.reflexo-pilosa*, respectively. In the Nansei Archipelago, Np value was 2.00 and 2.50 for *V.riukiuensis* and *V.reflexo-pilosa*, respectively. Relatively higher Np value observed in *Vigna* species in the Nansei Archipelago seemed to be attributable to larger number of samples examined, and difference of intraspecific isozyme variation between *Vigna* species from Malaysia and the Nansei Archipelago seems not to be significant.

3-(2) Ability of Nitrogen fixation

Natural ^{15}N abundance ($\delta^{15}N$) of eight leguminous tree seedlings ranged from -1.74 to 3.14 (average = 0.22), while that of eight non-leguminous tree seedlings ranged from -3.34 to 1.69 (average = -0.07)(Table 6). Low $\delta^{15}N$ value of non-leguminous seedlings indicated low $\delta^{15}N$ value of soil surface in Pasoh. $\delta^{15}N$ value of soil surface in Pasoh has not been studied. In Japan, $\delta^{15}N$ of upland fields ranged from +1.5 to +8.1, while that of forest ranged from -4 to -3. Therefore, it is reasonable that the $\delta^{15}N$ in Pasoh is low. If this is the case, it is considered to be difficult to estimate nitrogen fixation of legume seedlings in Pasoh. $\delta^{15}N$ value of non-leguminous weed in oil palm plantation near Pasoh was -0.17, while in rubber plantation in Pera State was +2.022. These low $\delta^{15}N$ values of non-leguminous weeds growing on plantation soil may indicate that surface soil in Malaysia has low $\delta^{15}N$ values in general.

On the other hand, $\delta^{15}N$ value of tall leguminous tree in Pasoh was 5.19, which was the highest $\delta^{15}N$ value among the samples analyzed. This may indicate higher $\delta^{15}N$ value in lower soil layer of Pasoh, and therefore suggest the possibility of estimating nitrogen fixing ability of tall leguminous trees in Pasoh Forest by natural ^{15}N abundance method.

4. Reference

1) Yoneyama,T (1987) N_2 fixation and natural ^{15}N abundance of leguminous plant and *Azolla*. *Bull Natl. Inst. Agrobiol. Resour. Japan, 3, 59-87.*

Table 1. Phenotype frequency of 6 enzymes of *V.trinervia* collected in Malaysia

n	6PGDH	GOT	SDH		LAP			ACP		EST		
	a	a	a	b	a	b	c	a	b	a	b	c
23	1.00	1.00	0.41	0.59	0.55	0.09	0.36	0.47	0.53	0.47	0.21	0.32

Table 2. Phenotype frequency of 6 enzymes of *V.reflexo-pilosa* collected in Malaysia

n	6PGDH	GOT	SDH	LAP		ACP		EST	
	b	b	c	d	e	b	c	d	e
5	1.00	1.00	1.00	0.40	0.60	0.40	0.60	0.40	0.60

Table 3. Phenotype frequency of 2 enzymes of *V. riukiuensis* collected on the Nansei Archipelago

Origin	n	SDH	LAP		
		A	A	B	C
Iriomote	23	1.00	0.26	0.48	0.26
Yonaguni	24	1.00	0.58	0.42	0.00
Ishigaki	1	1.00	1.00	0.00	0.00
Hateruma	1	1.00	1.00	0.00	0.00
Total	49	1.00	0.45	0.43	0.12

Table 4. Phenotype frequency of 2 enzymes of *V. reflexo-pilosa* collected on the Nansei Archipelago

Origin	n	SDH		LAP		
		A	B	A	B	C
Iriomote	5	1.00	0.00	0.00	1.00	0.00
Yonaguni	11	1.00	0.00	0.73	0.18	0.09
Okinoerabu	1	0.00	1.00	1.00	0.00	0.00
Total	17	0.94	0.06	0.53	0.41	0.06

Table 5. Proportion of polymorphic enzymes (Pe), and average number of phenotypes per enzyme (Np) in 3 *Vigna* species collected in Malaysia and the Nansei Archipelago

Origin	Species	n	Pe (%)	Np
Malaysia	*V.trinervia*	23	66.70	2.00
Malaysia	*V.reflexo-pilosa*	5	50.00	1.50
Nansei Archipelago	"	17	100.00	2.50
Iriomote	"	5	0.00	1.00
Yonaguni	"	11	50.00	2.00
Nansei Archipelago	*V.riukiuensis*	50	50.00	2.00
Iriomote	"	23	50.00	2.00
Yonaguni	"	24	50.00	1.50

Table 6. Natural abundance of ^{15}N in leaves

Species	N%	$\delta^{15}N$
Non-legume tree seedling in Pasoh		
Dalbergia pinnata	1.83	-1.27
Eratiospermum tapos	1.45	-1.93
Nephelium hamulatum	1.39	-0.05
Phyllagathis rotundifolia	0.80	-3.34
Psychotria angulata	2.82	1.331
Psycotria angulata	3.28	1.694
Pterisanthes polita	2.12	-0.68
Species A (unidentified)	1.61	0.934
Species A (unidentified)	1.49	0.799
Thottea dependens	1.98	1.732
Average		**-0.07**
Legume tree seedling in Pasoh		
Abrus precaforus	0.86	1.409
Abrus pulchellus	2.18	0.744
Archidendron clypearia	1.70	0.716
Bauhinia audex	1.73	-0.71
Bauhinia audex	1.79	0.045
Bauhinia audex	1.59	0.277
Millettia atropurpurea	2.21	3.14
Peltophorum sp.	1.98	-0.56
Peltophorum sp.	2.84	-1.74
Peltophorum sp.	1.77	-0.86
Pithecellobium angulatum	2.14	-1.26
Species B (unidentified)	1.92	-1.56
Species B (unidentified)	0.98	0.945
Species B (unidentified)	0.99	2.528
Average		**0.221**
Legume tall tree in Pasoh		
Parkia speciosa	1.74	5.192
Non-legume weed near Pasoh		
Melastoma melabathrium	1.68	-0.17
Legume weed near Pasoh		
Calopogonium mucunoides	1.88	-1.88
Centrocema pubescens	2.49	-3.21
Mimosa invisa	2.67	-0.62
Mimosa pudica	2.27	-1.37
Pueraria phaseoloides	3.27	-3.40
Vigna trinervia	2.12	-4.13
Average		**-2.43**
Malaysia (Pera, Sunghai)		
Paspalum conjugatum	0.69	2.022
Vigna trinervia	3.78	1.72
Okinawa (Nago)		
Ipomoea pes-caprae	1.65	4.036
Vigna marina	2.46	-2.59