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Genetic Differences between the Tolerant and the Sensitive Trees in an Air Polluted *Prunus sargentii* Stand[1]

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大氣汚染 被害地域의 산벚나무 林分內 耐性 및 感受性 個體의 遺傳的 差異[1]

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ABSTRACT

The estimates of genetic diversity based on 7 polymorphic loci coding for 6 isozymes were compared between the tolerant and the sensitive trees in an air polluted *Prunus sargentii* stand located on Ulsan industrial area. Although we could not observe statistically significant differences in allele and genotype frequencies between the two groups except the gene locus *Got-2*, the unique alleles and genotypes were observed in the tolerant group. All the genetic parameters such as genetic multiplicity, genetic diversity, and heterozygosity revealed that a greater amount of genetic variations existed in the tolerant group.

Key words : air pollution, adaptation, genetic diversity, isozyme, Prunus sargentii

요 약

대기오염의 피해가 심각한 것으로 추정되는 울산 지역 산벚나무 임분으로부터 외형적으로 피해정도가 크지 않은(내성) 개체와 피해가 큰(감수성) 개체를 선발하여 6개 동위효소, 7개 다형적 유전자좌에서의 유전변이를 조사하였다. *Got-2* 유전자좌를 제외한 모든 유전자좌에서 내성그룹 및 감수성그룹간 대립유전자빈도 및 유전자형빈도는 통계적으로 유의한 차이가 없는 것으로 나타났으나, 감수성그룹에서는 발견할 수 없었던 대립유전자 및 유전자형이 내성그룹내 개체에서 발견되었다. 유전적 다양도를 추정하기 위한 모든 통계치(유전적 다수도, 유전적 다양도 및 이형접합도)로부터 내성 개체가 감수성 개체에 비해서 다양한 유전변이를 보유하고 있는 것으로 나타났다.

INTRODUCTION

Air pollution has been implicated in large-scale forest declines in North America and Europe (Scholz et al., 1989; Giannini, 1991). Detrimental effects of air pollution on forests have been recognized for nearly 100 years. Especially, in the past decade, it became obvious that forest tree species are subject to damage caused by air pollution. Namely, there is direct injury of foliage by pollutants such as SO_2, NO_x and photooxidating agents including ozone, and by soil acidification as a result of the accumulation of acidifying substances from the atmosphere and heavy metals. Other probable source of pollutants, such as various organic pollutants, ammonia, viral or bacterial epidemics, are not yet sufficiently verified (Scholz

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et al., 1989; Giannini, 1991).

Environmental stress can affect the genetic architecture of populations in different ways; changes in the frequency distribution of alleles and genotypes due to viability and/or fertility selection (Müller-Starck, 1985; Bergmann and Scholz, 1987; Ruetz and Bergmann, 1989), loss of genetic multiplicity and the level of genetic variation at the individual or the population level (i.e., heterozygosities, genetic diversities; refer to Müller-Starck, 1985, 1989; Bergmann and Scholz, 1989; Geburek et al., 1987; Oleksyn et al., 1994). Meanwhile, there have been some reports that the genetic structures of populations are not related to the effects of the environmental stress (Geburek et al., 1986; Liu et al., 1990).

Prunus spp. is an important component of many street trees in urban areas and valued for pollutant resistant species (Harlow et al., 1996). Visible symptom due to air pollution does not always indicate the reduction in leaf photosynthesis or plant growth. However, many researchers report that air pollutants may be sufficient to alter the physiology of *Prunus* spp. such as enzyme activity, stomatal aperture, photosynthetic apparatus and water use efficiency (Marquis, 1990; Samuelson, 1994).

The objectives of the present study were to detect genetic differences between the tolerant and the sensitive trees to air pollutants in *Prunus sargentii* Rehder and to test the hypothesis that tolerant individuals to air pollution have more genetic variation than sensitive ones at allozyme level. The research strategy was to compare the genetic structures between the groups of the relatively healthy (putative tolerant) and the heavily damaged (putative sensitive) trees in a forest stand located in highly polluted area, Ulsan industrial area.

MATERIALS AND METHODS

1. *Study area*

This study site is located on the east side of the Ulsan industrial area. Monthly average precipitation in this area varies from 186.9mm (May) to 6.3mm (December). The average maximum temperature ranges from 28.7℃ (July) to 2.0℃ (Jan.) (Ministry of Agriculture, Forestry and Fisheries, 1997). This area is also close to a chemical manufacturing factory (Table 1). This facility produces many kinds of chemicals, and is a source of SO_2. The flat topography and low elevation of the area also contribute to concentrating the SO_2 emissions (Table 1). Understory of the stand is mainly occupied by *Rosa* and *Miscanthus* spp. Although the source of pollution has not been identified yet, high temperature in the summer, high light intensity and high SO_2 concentrations could result in the production of secondary pollutants such as O_3 and PAN (Reich, 1987).

2. *Selected trees*

The putative tolerant (30 trees) and the sensitive (26 trees) individuals were sampled based on the degrees of chlorosis and necrosis (Table 1; Umbach and Davis, 1984). Chlorosis and necrosis for the putative tolerant individuals were smaller than those for the putative sensitive ones (Table 1).

Table 1. Characteristics of site condition and *Prunus sargentii* sampled in this study.

	Tolerant trees	Sensitive trees
Location	Yeocheon-dong, Namgu, Ulsan, Kyungnam	
Distance from industrial facilities (m)	500 or so	
Slope (°)	2	
Elevation (m)	100	
Soil texture	sandy loam	
Tree age (year)	18.7	
Chlorosis	1	4-5
Necrosis	1	4-5

1 : 1-20%; 2 : 21-40%; 3 : 41-60%; 4 : 61-80%; 5 : 81-100%

3. *Isozyme analysis*

Very young leaves were collected from each individual tree for isozyme analysis because they yielded the best results in exploratory studies of enzyme electrophoresis. Collected leaves were placed on ice and returned to the laboratory and stored at 4℃ until analyzed. In a preliminary experiment, 12 enzyme systems were analyzed with a subset of samples. Of these six enzyme systems, which revealed consistent and clear resolution of isozymes in the preliminary experiment, were assayed by starch gel electrophoresis using two buffer systems (for more details for electrophoresis refer to Kim et al., 1993) : Gluta-mate dehydrogenase (GDH, E.C.1.4.1.2), glutamate-oxaloacetate transaminase (GOT, E.C.2.6.1.1), isocitrate dehydrogenase (IDH, E.C.1.1.1.42), leucine aminopeptidase (LAP, E.C.3.4.11.1), malate dehydrogenase (MDH, E.C.1.1.1.37), 6-phosphogluconic dehydrogenase (6PGDH, E.C.1.1.1.44).

Because of the lack of full-sib progenies, genetic interpretations of isozyme gel or zymogram were deduced based on the known subunit structures and cellular compartmentalization of the enzymes (Weeden and Wendel, 1989). Loci and alleles were designated according to the mobility of the enzyme. Namely, they were encoded from the fastest to the slowest one. The genotypes at the 7 putative polymorphic loci analyzed here were assigned to each tree and the allele frequencies in each group (tolerant and sensitive) were calculated.

4. *Genetic parameters*

The allelic and genotypic structures at each isozyme locus were determined for the tolerant and the sensitive groups and compared by the log likelihood ratio test (*G*-test) of heterogeneity in contingency tables. Intrapopulational variation was measured by the genetic multiplicities [the number of alleles (A_L) and of genotypes per locus (G_L), the proportion of polymorphism (P)], the observed (H_o) and expected heterozygosities (H_e), and the genetic diversities (v and v_{gam}; refer to Bergmann et al., 1990). Finally, we calculated latent genetic potential (LP), which is the difference between total number of alleles and effective number of alleles summed over all loci (Bergmann et al., 1990). Computations were performed using GSED program (Gillet, 1994) and BIOSYS-1 program (Swofford and Selander, 1989).

RESULTS

1. *Differences between the genetic structures of the tolerant and the sensitive groups*

The distribution of allele and genotype frequencies did not show any statistically significant differences between the tolerant (T) and the sensitive (S) groups with the exception of *Got-2* in allele frequencies (Table 2). Consequently, the actual numbers and frequencies of alleles and genotypes were not quite different in both groups. On the other hand, there was no S group specific allele which was not present in the T group. On the contrary, there were 3 T group specific alleles which were observed in heterozygote individuals. In the case of genotype distributions, 1 S group specific and 4 T group specific genotypes were observed (Table 2).

As described above, marked difference in the distribution of allele frequencies between two groups was observed only in the case of the locus *Got-2* (4 alleles in the T group vs. 3 alleles in the S). The allele frequencies of $Got\text{-}2_b$ and $Got\text{-}2_c$ were higher in the T group, while that of $Got\text{-}2_d$ was higher in the S group. Though there was no significant difference in the distribution of genotype frequencies, the heterozygote $Got\text{-}2_b2_c$ was about three times as frequent in the T group as in the S group and the heterozygote $Got\text{-}2_a2_b$ was observed only in the T group, while the homozygote $Got\text{-}2_d2_d$ was about six times as frequent in the S group as in the T group (Table 2).

2. *Genetic diversities and heterozygosities in the tolerant and the sensitive groups*

The observed single locus heterozygosities ranged from 0.000 to 0.391 within the S group, and from 0.067 to 0.500 within the T group (Table 3). The expected values ranged from 0.000 to 0.594 within the S group, and from 0.066 to 0.682 within the T group. The gene

Table 2. Comparisons of the genetic structures at 7 polymorphic loci and the frequencies of alleles and genotypes between the tolerant (T) and the sensitive (S) groups of *Prunus sargentii* in an air-polluted stand.

locus	Genotype	Allele	Frequency S group (No. of trees)	Frequency T group (No. of trees)	*G*-test
Gdh	*ab*		.038 (1)	.167 (5)	2.63 n.s.
	bb		.962 (25)	.833 (25)	
		a	.019	.083	2.49 n.s.
		b	.981	.917	
Got-2	*ab*		-	.083 (2)	9.71 n.s.
	bb		.435 (10)	.375 (9)	
	bc		.087 (2)	.292 (7)	
	bd		.130 (3)	.125 (3)	
	cc		.087 (2)	.083 (2)	
	dd		.261 (6)	.042 (1)	
		a	-	.042	9.91 (p.≤05)
		b	.543	.625	
		c	.130	.229	
		d	.326	.104	
Idh	*ab*		-	.033 (1)	2.00 n.s.
	bb		.043 (1)	.100 (3)	
	bc		.348 (8)	.200 (6)	
	cc		.565 (13)	.633 (19)	
	cd		.043 (1)	.033 (1)	
		a	-	.017	1.21 n.s.
		b	.217	.200	
		c	.761	.767	
		d	.022	.017	
Mdh-1	*aa*		.038 (1)	-	1.82 n.s.
	ab		.385 (10)	.333 (10)	
	bb		.577 (15)	.667 (20)	
		a	.231	.167	0.72 n.s.
		b	.769	.833	
6Pgdh-1	*ab*		-	.067 (2)	2.56 n.s.
	bb		1.000 (26)	.933 (28)	
		a	-	.033	2.53 n.s.
		b	1.000	.967	
6Pgdh-2	*aa*		.040 (1)	.033 (1)	6.65 n.s.
	ab		.040 (1)	.100 (3)	
	ac		.080 (2)	.033 (1)	
	bb		.480 (12)	.233 (7)	
	bc		.080 (2)	.267 (8)	
	cc		.280 (7)	.333 (10)	
		a	.100	.100	1.86 n.s.
		b	.540	.417	
		c	.360	.483	
Lap-2	*aa*		.150 (3)	.238 (5)	8.70 n.s.
	ab		-	.190 (4)	
	ac		.050 (1)	.048 (1)	
	bb		.150 (3)	.190 (4)	
	bc		.200 (4)	.048 (1)	
	cc		.450 (9)	.286 (6)	
		a	.175	.357	5.53 n.s.
		b	.250	.310	
		c	.575	.333	

* n.s. : non significant at 5% level, At some loci, some trees were missed for scoring the genotypes due to the poor resolution.

Table 3. Heterozygosities and genetic diversities for the tolerant (T) and the sensitive (S) groups of *Prunus sargentii* in an air-polluted stand.

Gene locus	Heterozygosity H_o T	H_o S	H_e T	H_e S	Genetic diversity(v) T	S
Gdh	0.167	0.038	0.155	0.038	1.180	1.039
Got-2	0.500	0.217	0.556	0.594	2.194	2.388
Idh	0.300	0.391	0.378	0.382	1.592	1.596
Mdh-1	0.333	0.385	0.282	0.362	1.385	1.550
6Pgdh-1	0.067	0.000	0.066	0.000	1.069	1.000
6Pgdh-2	0.400	0.200	0.593	0.580	2.397	2.319
Lap-2	0.286	0.250	0.682	0.591	2.990	2.360

loci *Gdh*, *6Pgdh-1*, *6Pgdh-2*, and *Lap-2* clearly revealed higher levels of observed (H_o) and expected (H_e) heterozygosities in the T group, while the gene loci *Idh* and *Mdh-1* showed higher levels of heterozygosity in the S group. Interestingly, in the case of *Got-2*, H_o was clearly higher, but H_e was slightly lower in the T group. One of the plausable explanations for this result may be the difference in the calculating process for those two values. The H_o is calculated directly from the observed genotype frequencies, whereas the magnitude of H_e, like that of genetic diversity v, is a function of the proportion of polymorphic loci, the number of alleles per polymorphic locus, and the evenness of allele frequencies within populations (Berg and Hamrick, 1997; Lee et al., 1997). Namely, the loci having more alleles and even allele frequency distributions (major polymorphism, refer to Lewontin, 1985) have higher H_e (and genetic diversity v) values than those loci which have less alleles and uneven allele frequency distributions. In the case of *Got-2* in this study, the S group has more even allele frequency distributions than the T group. This might make a contribution to higher H_e value for the S group at the gene locus *Got-2*. On the other hand, we could not rule out the possibility of sampling error because the sample sizes for each group might be too small to observe all genotypes. The estimates of genetic diversities (v) for each locus ranged from 1.000 to 2.388 within the S group, and from 1.069 to 2.990 within the T group.

The values for H_o averaged over all loci were 0.212 for the S group and 0.293 for the T group. The difference between the values of the two group is 0.081, which means an excess in favour of the T group of 38%. This tendency is also shown in the values for the H_e and the v. However, the proportions of excess in favour of the T group for the H_e and the v were less than that for the H_o (6.3% for H_e and 3.9% for v, respectively). As seen in the heterogeneity tests for allele and genotype frequency distributions, the number of alleles at each locus as well as the allele frequency distributions are similar in both groups. This is why the degree of differences between the S and the T groups in the H_e and the v is relatively small compared with that in the H_o. These results were also shown in other report (Müller-Starck, 1989). The gene pool diversities were 1.533 for the S group and 1.613 for the T group, respectively.

The hypothetical gametic multilocus diversity (v_{gam}) of the T group (43.716) was clearly larger than that (33.607) of the S group. In addition the value of LP was higher in the T group (7.19 for the T group vs. 4.75 for the S group). These reductions in the levels of v_{gam} and LP in the S group suggest a reduction in long-term evolutionary potential of the S group (Bergmann et al., 1990).

DISCUSSION

The genetic structures of forest trees have evolved through adaptation to various biotic and abiotic environmental factors and maintained a

considerable amount of genetic diversity (Hamrick and Godt, 1989). However, severe environmental changes can lead to abrupt changes of genetic structures of forest trees. The ubiquitous appearance of air pollution in developed countries including Korea should be regarded as a drastic change in the abiotic environment of forest tree species and consequently several tree species particularly sensitive to pollution effects may be undergoing a rapid decline. As a consequence, it is very important and urgent to study genetic structures of forest trees under the impacts of environmental stress and to prepare the proper strategy for the preservation of forest trees.

In order to determine the genetic structures under the impacts of air pollution, it is reasonable to choose enzyme systems which are involved in biochemical resistance mechanisms or pollutant-sensitive metabolic pathways. For this purpose, we selected 6 enzyme systems which have been known to be closely associated with such metabolic pathways (Bergmann and Scholz, 1989 and references therein). In contrast with our expectations, however, we could not find statistically significant differences in allele and genotype frequencies between the two groups at most loci, as already shown in the heterogeneity test (Table 2). This may be due to the fact that the number of sampled trees for each group is not enough to detect the differences in allele and genotype frequencies, because allele richness is strongly dependent on sample size (Berg and Hamrick, 1997). Additionally the *G*-test used for heterogeneity test in this study is likely to be unreliable in cases where expected frequencies of some classes are low - generally less than 5 (Sokal and Rohlf, 1981). At the same time, we cannot rule out the possibility that sampled trees for each group do not appropriately represent each group since the selective effects of the present environmental stress conditions may not be strong enough to affect tree growth and consequently not sufficient to detect the difference of the genetic structures between the sensitive and the tolerant trees. On the other hand, we could find genetic difference at the gene locus *Got-2* comparing the two groups differing in the field resistance. The enzyme GOT is essential for the aminoacid metabolism. The activity of this enzyme is known to be altered by SO_2 (probably one of the main stress factors in this study) and other toxic gases (Rabe and Kreeb, 1980). Accordingly we could deduce that GOT enzyme in *P. sargentii* also plays an important role in the variation of tolerance mechanism as already shown in other plants (Bergmann and Scholz, 1987; Geburek et al., 1987).

If the more sensitive trees to pollutant stress would be eliminated - naturally or artificially - from the populations prior to reproduction, certain alleles at several gene loci would decrease in frequency and may be lost due to random events, although at other loci the present genic diversity would be preserved. In this study we cannot estimate what extent the parental stand genetic diversity is expressed in the replacement stand, because the sampled stand has not yet regenerated. As a consequence, we are not able to suggest what the different genotype frequencies in each group may mean for the regenerating forest and we do not know whether the affected alleles have selective values. For providing evidences on this issue, we will have to study the genetic structure of the regenrated stand and then compare it with that of parents in the future.

In general, there are expectations that more heterozygous populatiopns will exhibit higher resistance to various stress factors. In other words, maintenace of genetic diversity in forest tree populations that are undergoing fundamental population changes, whether natural or human-induced, is seem to be the key to adaptability and continued evolution (Müller-Starck, 1985; Ledig, 1988; Namkoong, 1991; Oleksyn et al., 1994). There is mounting evidence that tree populations that have sustained genetic losses are more susceptable to productivity decline and loss of environmental fitness in the event of major environmental changes (Müller-Starck, 1985; Bergmann and Scholz, 1987; Bergmann et al., 1990; Oleksyn et al., 1994). This tendency was also shown in this study. All the genetic parameters studied in this study - genetic multiplicity, genetic diversity, and heterozygosity - showed that a higher level of genetic diversity is maintained in the T group. Especially,

the surplus of the hypothetical gametic multilocus diversities (v_{gam}) in the T group compared to that of the S group (on the average 30%) stresses the importance of genetic variation for the maintenance of viability. This measure reflects the ability of a population to create genetic variation in the next generation and is thus an important parameter in the determination abilities (refer to Gregorius et al., 1986 and Bergmann et al., 1990). Neverthless, it should be mentioned that associations between the level of heterozygosity and quantitative traits (especially growth charaters) as well as between the level of heterozygosity and the degree of tolerance to air pollutants are not always observable in forest trees (El-Kassaby, 1982; Scholz and Bergmann, 1984).

In the near future, we need to study how single pollutants such as SO_2, HF, O_3 etc. affect the genetic structures of forest tree species and estimate what extent of the parental stand genetic diversity is expressed in the regenerated stand. In these ways, we can deduce how the evolutionary forces such as selection operate under the impacts of air pollution. In addition we need to study genetic responses of *Prunus sargentii* in other industrial regions and/or other tree species to severe environmental stress. Comparing those data with the present results, we can provide more useful evidences on how to maintain forest tree populations under environmental stress. Finally, we have to make an effort to find isozyme and/or DNA markers which are closely linked to morph-physiological traits affected by environmental stress for further study on these issues.

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