Realized Genetic Gains Observed in Progeny Tolerance of Selected Red Pine (*Pinus densiflora*) and Black Pine (*P. thunbergii*) to Pine Wilt Disease

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Abstract

Realized genetic gains in progeny tolerance of red pine (*Pinus densiflora*) and black pine (*P. thunbergii*) that were selected in a breeding project of resistance to pine wilt disease was evaluated by analyzing ten years' results of artificial inoculation tests with pine wood nematode at a nursery of Kyushu Regional Breeding Office. According to the result of analysis of variance across the ten years' tests, family variation was highly significant with 0.836 of family-mean heritability. The realized gains in the progeny tolerance were calculated as 0.18 increase in the nursery survival rate for red pine and 0.35 for black pine as compared to the survival of non-selected populations. Those increases brought about the average levels of progeny tolerance up to 0.65 for red pine and 0.51 for black pine, both of which are greater than that of loblolly pine (*P. taeda*) population: 0.47.

Key words: Breeding for resistance, Pinus densiflora, P. thunbergii, artificial inoculation, pine wood nematode, progeny tolerance

Introduction

During the last four decades, pine forests have been heavily damaged by pine wilt disease that firstly occurred in southwestern part of Japan and gradually spreading north to cover the whole natural range of the two major pine species: Japanese red pine and black pine (NAKAMURA and NOVOTNY, 1999). Those pines were once a major plantation species that are adaptable to infertile soils or coastal region where no other species can be used (SATO, 1971). For this reason a breeding project for resistance to pine wilt disease was started in 1978 in cooperation with three regional offices of Forest Tree Breeding Center and fourteen prefecture organizations in southeastern Japan (FUJIMOTO and OHBA, 1981).

The breeding project has identified 92 clones of red pine and 16 clones of black pine whose tolerance is greater than that of loblolly pines that would survive under the current epidemic situation in Japan (FUJIMOTO et al., 1989). Those clones were used to establish clonal orchards of both pines: 34 orchards at 21 prefectures (TODA et al., 1993). The supply of seedlings has now started for operational plantings due to an urgent demand for establishing plantations with those tolerant pines, however, average levels of tolerance of these pines have not fully investigated yet.

This paper will examine progeny tolerance to the disease by analyzing ten years' results of artificial inoculation tests with pine wood nematode at a nursery of Kyushu Regional Breeding Office. Then the realized gains as well as the family variations were investigated in relation with the future deployment of the project.

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Materials and Methods

Tree breeding project of pines tolerant to pine wilt disease

This project was started in 1978 by selecting apparently healthy trees from heavily infested stands in southeastern part of Japan. Candidate tree selection was practiced during the first three years resulting in 11,466 trees of red pine and 14,620 trees of black pine being selected (TODA et al., 1989). The target stands and areas for selection were determined before the start of project to select as many tolerant pines as possible. The criteria for stand selection were stand mortality (more than 70%), stand age (more than 30 years old), and elevation (lower than 400 m where the pine wilt disease was most serious).

Grafted ramets of candidates were used for clonal testing. Artificial inoculation with pine wood nematode was done twice. In the first screening, each of ten ramets per candidate were artificially inoculated in the green houses with 0.1cc of suspension containing 10,000 of pine wood nematode. The second screening was made using only 7.4% of the red pine candidates and 1.5% of the black pine candidates that showed better survival at the first screening. Each of twenty ramets of those candidates were inoculated in the same manner as in the first screening using five loblolly pine families as a check. 92 clones of red pine and 16 clones of black pine were determined in 1984 as tolerant to pine wilt disease. Thus the final selection rates throughout the two stages of screenings were resulted in 0.0080 for red pine and 0.0011 for black pine. The tolerance of those clones is regarded as the same or better than that of loblolly pine.

Progeny tolerance to pine wilt disease

Progeny tolerance of the selected pines was evaluated by analyzing data on survival after artificial inoculation with pine wood nematode. The data used for this analysis are records on survival ratio of open pollinated families tested at the nursery of Kyushu regional breeding office from 1984 to 1997 (*Table 1*). Groups of families tested each year were different, but the same set of Loblolly pine families was inoculated in most of the years as checks. Inoculation test to the open pollinated progenies of selected pines was started in 1990 for red pine and in 1992 for black pine. Most of the seed used for the progeny tests were from the clone bank at Kyushu regional breeding office. The non-selected populations were open pollinated families of plus trees that were selected for their growth and stem form in the early of 1950's before the outbreak of pine wilt disease.

Progeny testing for tolerance to pine wood nematode was repeated in every year using essentially the same procedure as mentioned below. Each family was planted in a plot of ten to sixty seedlings in a randomized complete block design with three replications. Artificial inoculation was made on two years old seedlings in the middle of July using a 0.05 ml suspension with 5,000 pine wood nematodes. Survival ratio of each plot

Table 1. – Number of open pollinated families tested and average survival rates after the inoculation tests since 1984 until 1997.

Year	Total		Red pine				Black pine				Loblolly pine	
			Selected		Non-select		Selected		Non-select			
1984	31	0.59			17	0.71			9	0.39	5	0.44
1985	52	0.21			15	0.36			37	0.15		
1990	58	0.60	42	0.68					11	0.15	5	0.88
1991	80	0.60	50	0.66	25	0.52					5	0.37
1992	101	0.55	86	0.57			8	0.48	2	0.06	5	0.47
1993	112	0.76	89	0.80	2	0.54	10	0.69	6	0.20	5	0.77
1994	96	0.58	79	0.60	5	0.34	6	0.62	1	0.55	5	0.41
1995	38	0.50	30	0.55			3	0.33			5	0.30
1996	94	0.37	77	0.40			14	0.28			3	0.16
1997	99	0.68	87	0.71			12	0.47				
Average		0.58		0.62		0.55		0.47		0.22		0.46

Note) Number of families tested and their average survivals were given in each column.

was calculated by counting living trees three months after the inoculation.

Analysis of variance was made using data on survival ratio of each plot after the arcsine square root transformation. Since the families tested were different each year, analysis of variance was made using least square method with the following linear model (HARVEY, 1979).

$$y_{ijk} = \mu + Y_i + R/Y_{ij} + F_k + Y^*F_{ik} + e_{ijk} \qquad (1).$$

y_{ijk} is a arcsine transformed survival ratio of the k^{th} family plot at j^{th} replication in the i^{th} year. μ, Y_i, R/Y_{ij}, F_k , Y^*F_{ik} and e_{ijk} are population mean, year-effect, replication-effect, family-effect, year and family interaction, and experimental error respectively.

Tolerance for each family was the least square estimate of survival ratio calculated from the analysis of variance. To check the accuracy of the estimates, family-mean heritability on the nursery survival was calculated by the following formula (ZOBEL and TALBERT, 1984).

$$h_f^2 = \sigma_f^2 / [\sigma_f^2 + \sigma_{yf}^2 / y + \sigma_e^2 / (y \cdot r)] \qquad (2).$$

where σ_f^2, σ_{yf}^2, σ_e^2 are the variance components for family, year x family interaction, and experimental error, respectively and y and r are the number of years tested and number of replications.

Results and Discussion

Average survival rates after the inoculation for each of the ten years' tests were given in *Table 1*. The average rates varied greatly among the years: ranging from 0.21 to 0.76 with an average of 0.58. This is due to the fact that an annual fluctuation of climatic condition was the primary factor to affect the survival, because the inoculation tests were conducted in an open nursery condition. The difference in the survival rate was also evident among the species as well as the population within the species where the survivals of selected population were consistently higher than those of the non-selected population.

Analysis of variance across the ten years' results was given in *Table 2*. Although year x family interaction was statistically significant, family variation for survival rate was highly significant, and the family-mean heritability was 0.836. This is because the interaction variance (σ_{yf}^2 = 0.0094) was less than one half of the family variance (σ_f^2 = 0.0236) and the effect was further reduced by several years' repetition of the tests as shown in the denominator of formula (2). This is especially true to the selected populations where average numbers of the tests per family were 5.9 for red pine and 3.6 for black pine. Thus the estimates of survival ratio for the families of selected pines might be sufficiently accurate to evaluate the level of tolerance. On the other hand, each estimate of the families in non-selected populations would be less reliable, because most of the families were tested only once; average number of the tests per family was 1.3 for both red pine and back pine. However, the average level of tolerance for the non-selected populations might be accurate enough to evaluate the result selection, because these averages were derived from the estimates of more than fifty families in both species (*Table 3*).

Table 2. – Analysis of variance table on survival ratio across the ten years results of artificial inoculation with pine wood nematode.

Source of variation	d.f.	Mean square	Expected components
Year	9	4.3219	-
Replication / year	16	0.1344	-
Family	210	0.2561 **	$\sigma_e^2 + 2.37\,\sigma_{yf}^2 + 9.09\,\sigma_f^2$
Year × family	539	0.0443 **	$\sigma_e^2 + 2.62\,\sigma_{yf}^2$
Error	1159	0.0195	σ_e^2

Note) Arcsine square root transformation was made before the calculation. **: significant at the 1% level.

Around 0.18 of increase in nursery survival rate was achieved by the selection as compared to the none-selected populations for red pine (*Table 3*). The difference in the level of tolerance was clearly observed in frequency distributions for the two populations: selected and none-selected, both of which were drawn using the estimates of red pine families (*Figure 1*). However, the two distributions seemed to be overlapping due to the wide variation of non-selected population, which is probably inflated by the interaction effect. This suggests that the survival of selected population currently used in clonal seed orchards can be further improved by culling clones that show poor progeny survival. It also indicates a possibility to select the most tolerant genotypes from the non-selected population to add into the selected population for resistance.

Table 3. – A summary of the realized gains in progeny tolerance of nursery survivals for red pine and black pine after inoculation test with pine wood nematode

Species	Selected population		Non-selected population		Realized gain
Red pine	0.650	(91)	0.473	(50)	0.177
Black pine	0.514	(14)	0.166	(51)	0.348
Loblolly pine			0.474	(5)	

Note) Numbers in the parenthesis were those of the families to calculate each population mean.

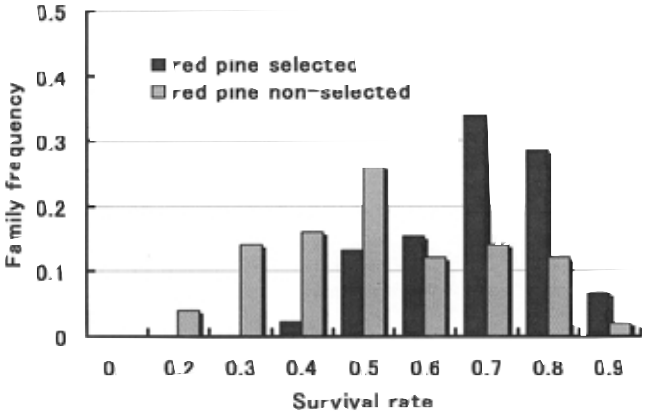

Figure 1. – Nursery survival rate of red pine families after the artificial inoculation with pine wood nematode.

With black pine, the increase in nursery survival was 0.35 of rate as compared to the non-selected population (*Table* 3). This amount of progress is much greater than that of red pine, although the mean survival of the selected black pine population was still lower than that of red pine. This is because a species level of tolerance for black pine was much lower than that of red pine as indicated by an inverse J shaped frequency

distribution (*Figure 2*). Hence the very stringent selection had to be applied to black pine to achieve the predetermined level of tolerance: an equal tolerance with the loblolly pine. The problem with the result of this selection is the size of the selected population; only sixteen clones which is too small to conduct a recurrent selection program to improve tolerance over generations.

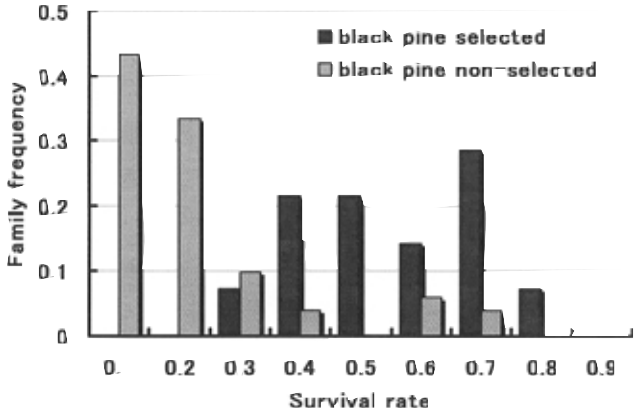

Figure 2. – Nursery survival rate of black pine families after the artificial inoculation with pine wood nematode.

In conclusion, progenies of red pine and black pine that were once screened using grafts inoculated with pine wood nematode proved to be as tolerant as loblolly pine that was the predetermined level of the tolerance for this project. In the case of red pine whose tolerance was relatively high, the level of tolerance for the selected population was well exceeded the target rate with a half of the realized gain of black pine by retaining potentials for further improvement. On the other hand, for black pine, a very stringent selection had to be applied to achieve target tolerance because of its inherent susceptibility to the disease. This stringent selection had brought about an apparent increase in the tolerance, while it also resulted in an excessive reduction in the population size for breeding.

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Buchbesprechung

Current Trends in the Embryology of Angiosperms. Edited by S. S. BHOJWANI and W. Y. SOH. 2001. Kluwer Academic Publishers, Dordrecht, Niederlande. ISBN 0-7923-6888-6. 533 pages.

This comprehensive book covers all processes involved in embryo formation of angiosperms. Besides reviewing classical data in this field, a considerable amount of new information is offered. This includes advances and perspectives that are due to a tremendous progress in modern technologies such as electronic and confocal laser microscopy, immunohistochemistry, gene expression analysis including microarray-technologies as well as transformation technologies. As the editors state 'this has changed the face of the Embryology of Angiosperms from a descriptive to an experimental and applied science'.

The book includes 21 chapters reviewing significant results in all relevant *in vivo* and *in vitro* aspects of embryo formation. The chapters are in line with a developmental/ontogenetic chronology and start with processes of gametogenesis followed by fertilisation processes. Basic and up-to-date information is gathered on subjects like sexual incompatibility, parthenocarpy and apomixis. Chapters with high relevance for bio- and genetechnology are dealing with advances in synthetic seed technology, somatic embryogenesis and the regeneration of haploid plants from male and female gametophytes.

The important topics are illustrated, topics others than headlines can be easily found in the subject and plant index at the end of the book. The book addresses a broad readership in botany, researchers as well as teachers and students. It is highly recommendable to the tree science community, in particular, as there is a lot of valuable information for further progress in basic but also e.g., in applied hybrid and breeding research.

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