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Allozyme Variation of *Pinus rigida* Mill. in an F_1 -Hybrid Seed Orchard and Estimation of the Proportion of F_1 -Hybrid Seeds by Allozyme Analysis[1]

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雜種 採種園에서 리기다소나무의 Allozyme 變異와 Allozyme 分析에 依한 雜種種子 發生率의 推定[1]

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ABSTRACT

Allozyme study for open pollinated seeds of forty nine pitch pine families in an F_1-hybrid seed orchard demonstrated that allozyme variants in aspartate aminotransferase(AAT), glutamate dehydrogenase(GDH) and leucine aminopeptidase(LAP) systems are encoded by at least eight loci; five for AAT, one for GDH and two for LAP. Allozyme variations showed polymorphisms at seven of the eight loci, except GDH. Average number of alleles examined over six loci were 2.33 and 2.67 for maternal and progeny groups, respectively. Average heterozygosity and genetic diversity computed over six loci were, respectively, 0.235 and 5.409 for maternal tree group, 0.238 and 5.569 for progeny group. The proportion of F_1-hybrid seeds estimated by allozyme analysis was 0.77%. The estimated proportion of F_1 hybrid seeds by allozyme study is in good agreement with the value 0.73% estimated by morphological study for the proportion of pitch x loblolly F_1 hybrid seedlings at a nursery. Indications for Wahlund effect, high levels of self-fertilization and for non-random matings in the F_1 hybrid seed orchard call for cautions in estimating allele frequency changes and mating probabilities for the parental and progeny groups.

Key words: Allozyme variation; hybrid seed orchard; mating probability; Pinus rigida *Mill.*

要 約

雜種採種園上의 리기다소나무 49家系로부터 種子를 채취하여 種子의 胚乳 및 胚에 대한 Aspartate aminotransferase (AAT), Glutamate dehydrogenase (GDH) 및 Leucine aminopeptidase (LAP) 등의 Allozyme 變異를 조사하여 다음과 같은 결과를 얻었다. 이들 세 가지 Allozyme system에서 AAT에 5개, GDH에 1개 및 LAP에 2개, 모두 8개의 遺傳子座(Locus)가 발견되었으며 GDH를 제외한 모든 유전자좌에서 Allozyme Polymorpshism을 발견하였다. 각 유전자좌에 있어서 평균 對立遺傳子 數는 種子母樹集團에서 2.33개, 次代集團에서 2.67개였다. 平均異型接合性은 종자모수집단이 0.235, 次代集團이 0.238이었

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고 유전자의 遺傳的 多樣性은 종자모수집단이 5.409, 次代集團이 5.569로서 같은 수종의 다른 집단 또는 다른 집엽수 수종에 비하여 비교적 낮은 값을 나타냈다. Allozyme 분석에 의하여 잡종채종원의 리기다소나무에 있어서 一代雜種 種子 發生率을 추정해 본 결과 일대잡종 종자의 발생빈도는 0.77%로서 묘포에서 조사한 일대잡종묘의 발생율 0.73%와 거의 일치하였다. 잡종채종원상의 종자모수 리기다소나무 및 이대 차대들 Allozyme 변이에 있어서 Wahlund 效果, 비교적 높은 수준의 自家受精 및 Non-random mating 등의 가능성이 발견되어 이들에 대한 Allozyme 변이 연구에 깊은 주의가 필요하다.

INTRODUCTION

Outline of the F_1-hybrid seed orchard and flowering characteristics of *Pinus rigida* Mill. (Maternal parent) and *Pinus taeda* L.(Paternal parent) in the F_1-hybrid seed orchard has been reported elsewhere by the present author[4]. In the beginning of nineteen seventies the author investigated the proportion of F_1-hybrid seedlings that originated from maternal pitch pine(*Pinus rigida* Mill.) trees of the F_1-hybrid seed orchard.

Morphological characteristics of the F_1-hybrid *Pinus rigida* x *Pinus taeda* seedlings were distinctive from those of pitch pine at 2-0 nursery seedling stage. Therefore, in most cases, there was no difficulty in distinguishing F_1-hybrid seedlings from those of pitch pines except in some maternal pitch pines that produced very vigorous pitch pine seedlings. The early observations at nurseries showed that most of the pitch pine trees in the F_1-hybrid seed orchard produced not a single F_1-hybrid seedling but a few early flowering trees that produced very few F_1-hybrid seedlings among several hundreds of the offsprings. It also appeared that the failure of F_1-hybrid seed production in this F_1-hybrid seed orchard was due mainly to difference in flowering time between the two parental tree species.

For a future breeding program it is interesting to know the genetic variation of parental tree species, of the offsprings and mating systems in seed orchards. For this purpose many researchers have conducted isozyme studies on conifer species in seed orchards[1,3,13,14,15,19,20,24] and in natural populations[6,9,10,11,12,17,23,26,27,29].

The aim of this study was to investigate genetic variation of *Pinus rigida* Mill. in an F_1-hybrid seed orchard and to find out a possible method for estimating the proportion of F_1-hybrid seeds produced from the F_1-hybrid seed orchard.

MATERIALS AND METHODS

Open-pollinated seeds of forty nine pitch pine (*Pinus rigida* Mill.) trees from the F_1-hybrid seed orchard were collected in the fall of 1981. The seeds were screened and stored in a refrigerator until use for the analysis. Seeds were germinated at room temperatures(22°C–26°C) on moist sands in petri dishes. Each macrogametophytes and germinating embryos of eight seeds per tree were separated and homogenized in 0.09 Mol. trisaminometane, 0.27 Mol. borate, 0.004 Mol. EDTA buffer (pH 7.4).

Allozymes for aspartate aminotransferase(AAT), glutamate dehydrogenase(GDH) and leucine aminopeptidase(LAP) were separated by running the crude extracts of macrogametophytes and the corresponding embryos of the same seeds, side by side, in a horizontal starch gel zone electrophoresis system. The probability of erroneous judgement of a heterozygote as a homozygote is $(1/2)^7$ in the allozyme analysis with eight macrogametophytes per tree.

Buffer systems and gel preparations are as follows;

(1) Electrode buffers

for cathode; H_3BO_3 0.2 M., LiOH 0.04 M., pH 8.1

for anode; H_3BO_3 0.2 M., LiOH 0.05 M., pH 8.3

(2) Gel buffer

Trisaminometane 0.05 M., Citrate 0.008 M.

(3) Gel preparation

Gel buffer	500 ml
Electrode buffer(cathodal)	16 ml
H_2O	55 ml

Starch(hydrolized) . . . . . 64 g
Saccharose . . . . . 1 g

Electrophoresis was run at constant current of 65 mA for fourteen hours for four gels of 12 cm long(from cathodal to anodal bridge connections), 22 cm wide and 8 mm thick. GDH, LAP allozymes were stained according to the methods described by Shaw and Prasad(22) and of AAT by Siciliano and Shaw(25).

RESULTS AND DISCUSSION

Number of loci and alleles

There are at least five zones of enzyme activity stained for AAT in *Pinus rigida* Mill.(Fig. 1). Alleles at loci A,B,C and allele D_1 at locus D migrate anodally while allele D_2 at locus D and alleles at locus E migrate cathodally in the buffer systems used in this present study. There are three alleles A_1, A_2 and A_3 at locus A, four at locus B(only three alleles, B_1, B_3, B_4 were found at present study) and two at each locus C, D and E. The alleles at loci C, D and E appeared to be tightly linked thus allele C_1 always associated with D_1 and E_1, and C_2 with D_2 and E_2 respectively. Allele B_2 for AAT found in another pitch pine population (under study now) was not found for pitch pine trees in this F_1-hybrid seed orchard. Alleles at loci D and E were not included for statistical analysis because the alleles at these loci often stained faintly in embryos.

The result obtained in this present study for AAT appeared similar to the one that reported by Guries and Ledig[8], except for locus B. on the same tree species. Guries and Ledig reported three and two alleles at AAT(GOT) 1 (This locus appeared correspond to be locus A at present study) and alleles at AAT(GOT) 2 (This locus appeared correspond to be locus C at present study), respectively, in pitch pine. The above authors also reported a third zone of AAT(GOT) activity which migrates cathodal to the origin and which displays a segregation pattern that coincides precisely with that of AAT(GOT) 2(AAT C at present study). This locus seems to correspond to the locus D that was found at present study. On the other hand, Hwang[5] reported four and five alleles respectively, for A and B loci in AAT(GOT) on his allozyme study for pitch pine originated from twenty four provenances.

In many cases, alleles at AAT B locus in macrogametophytes are silent at early stages of seed germination but sometimes the allozyme bands at the same locus appear at later stages of germination. The alleles at AAT B locus are visible at any stage of seed germination in embryos. Adams and Joly[1] also reported that a gene may not necessarily be detectable in both seed tissues, the macrogametophyte and the embryo. The zones of allele distribution at AAT A and B loci overlap each other, thus the reading of some allozyme patterns at these two loci is rather difficult if we do not compare allozyme banding patterns of macrogametophytes (maternal origin in conifer species) and the corresponding embryos(maternal and paternal origins).

Banding pattern of AAT in pitch pine indicated that the enzyme structure of AAT is dimeric as shown in other tree species[1,3,18]. In macrogametophytes, homozygotes comprise of a single band and heterozygotes comprise either of two different kinds of single band which segregate one to one ratio at a locus. In embryos, homozygotes comprise of a single band just same as in macrogametophytes while heterozygotes comprise of three bands, two of which are parental and one intermediate hybrid enzyme.

GDH in pitch pine appears to be monomorphic with only one allele A_2 (allelic name A_2 is given instead of A_1 for pitch pine though it has only one allele because paternal loblolly pine and the pitch x loblolly hybrid pine (under study now) have common allele A_2 and additional allele A_1 which migrates a little faster than A_2 to the anode) (Fig. 2). Adams and Joly[1] reported that GDH is controlled by one locus with five alleles in loblolly pine(*Pinus taeda* L.). However, Mitton and others[12] found that a homozygous genotype has a single, well resolved band and a heterozygote, a diffuse broad band.

Gels stained for LAP showed two polymorphic zones(loci A and B, Fig. 3) of enzyme activity as found in other pine species[1,3,15,17,19]. Three alleles were found at locus A, one of which was silent. But three functional alleles were found at locus B. The result obtained in this present work for LAP is very similar to those of the same species reported by Guries and Ledig[8] and by Hwang[5]. Hwang found four and three alleles for LAP A and B respective loci in pitch pine. The same kinds and number of alleles for LAP reported by Hwang in pitch pine were found in loblolly pine by Adams and Joly[1] and by the present author(unpublished).

Guries and Ledig[8] discussed the visualization of a silent allele for LAP in a certain buffer system, the allele of which was silent in buffer system described by Scandalios[21]. In this present work the allele which believed to be silent stained very faintly at two places; one above allele A_1 and the other near allele B_1. Functional allele in LAP usually show a distinctively stained band.

Average number of alleles examined over six loci

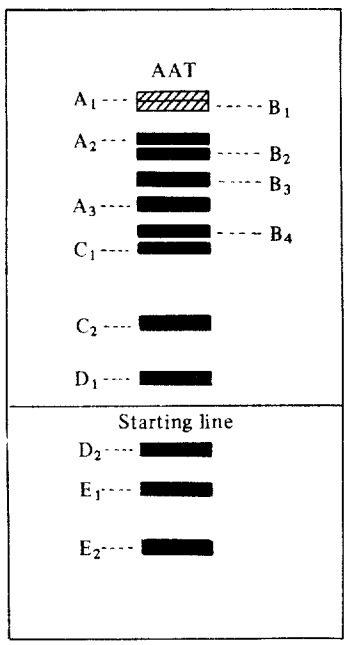

Fig. 1. Allozyme pattern of aspartate aminotransferase in macrogametophytes (Total zymogram).

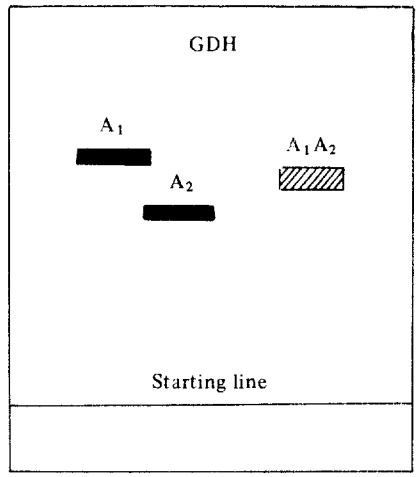

Fig. 2. Total zymogram of glutamate dehydrogenase.

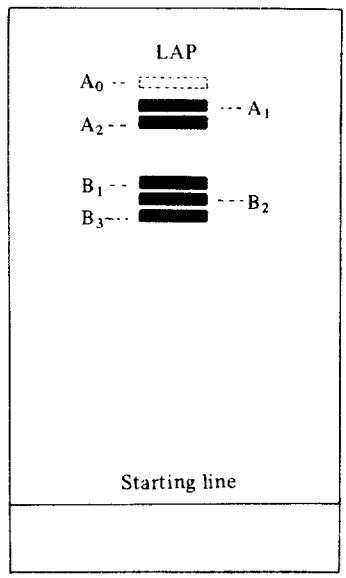

Fig. 3. Total zymogram of leucine aminopeptidase. A_0 ; a silent allele.

were 2.33 for maternal tree group and 2.67 for the progeny.

Allele and genotype frequencies

All the allozyme patterns except GDH showed a polymorphism. The most common alleles are A_2 and C_2 alleles for AAT, and A_1 allele for LAP. Both B_1 and B_3 alleles for AAT, and B_1 and B_2

alleles for LAP are almost equally common in parental trees and in the progenies(Table 1). Because, in many cases, the alleles at locus B for AAT in macrogametophytes were silent, theoretical allele frequencies at locus B for AAT in maternal group was estimated from the allele frequencies of the progeny group. The calculation was made under the assumption that the genotypic frequency of maternal group was under Hardy-Weinberg equilibrium and there was no change in gene frequencies in progeny group from those of maternal ones.

Total phenotypic gamete segregations followed the Mendelian mode of inheritance. However paternal allele frequencies at LAP locus A deviated significantly from those of maternal(macrogametophytes) ones(x^2 = 32.36, df = 2, $p < 0.01$). The same tendency was found for allele frequencies at LAP locus A between maternal and progeny groups (x^2 = 7.40, df = 2, $p < 0.05$), and between the total progeny group and paternal one (x^2 = 15.50, df = 2, $p < 0.01$). Deviation of paternal allele frequencies from those of maternal ones at LAP locus

Table 1. Allele frequencies for AAT, GDH and LAP in maternal (macrogametophytes), paternal (pollen) and the progeny group

Source	(Allele frequency) AAT							
	A_1	A_2	A_3	B_1	B_3	B_4	C_1	C_2
Maternal	0.02	0.96	0.02	0.49	0.51	–	0.04	0.96
Paternal	0.02	0.97	0.01	–	–	0.00	0.07	0.93
Progeny	0.02	0.96	0.02	0.49	0.50	0.01	0.06	0.94

Source	GDH		LAP					
	A_1	A_2	A_0	A_1	A_2	B_1	B_2	B_3
Maternal	–	1.00	0.09	0.87	0.04	0.46	0.52	0.02
Paternal	0.01	0.99	0.01	0.90	0.09	0.43	0.55	0.02
Progeny	0.00	1.00	0.06	0.88	0.06	0.45	0.53	0.02

may be caused partly by the silent allele A_0 which is usually masked by the other functional alleles under heterozygous conditions. This means that many of the silent A_0 alleles under heterozygous conditions might have not been detected as an A_0 allele and thus it give a lower allele frequency for A_0 than that of the actual one in embryos (alleles of paternal origins can be detected only in embryos). Also there is a possibility of gametic and/or of zygotic selection against pollen with A_0 allele or A_0A_0 genotype in embryos.

Deviation of paternal allele frequencies from those of maternal ones at a certain locus apparently indicated that mating was not at random and/or gametes production in the F_1-hybrid seed orchard was not in proportion to allele frequencies of the maternal tree group. Muller-Stark[14] and Ziehe[30] discussed non-random mating as a sexually asymmetric fertility selection. The F_1-hybrid seed orchard was established by species row to row planting of the two parental tree species along the line of contour. Loblolly pines have outgrown from sapling stage and suppressed pitch pines(Fig. 4). Intervened tree rows of loblolly pines apparently functioned as effective barriers against movement of pitch pine pollen during flowering seasons at reproductive stage. Furthermore, wind usually blows downwards at night and upwards in day time the direction of which meets at right angles to the barrier lines of loblolly pine trees during flowering seasons. Matings among pitch pines under these conditions would not have been random and thus would have caused a change in allele frequencies at polymorphic loci.

Self-fertilization within certain individuals of maternal tree group and cross-fertilization between pitch and loblolly pine trees may also change allele frequencies of progeny group from those of ma-

ternal ones. These were indications for high levels of self-fertilization in maternal trees with rare alleles. The proportion of discernible outcrossed progeny was only 21%. In other words, 79% of the progenies are originated partly from self-fertilization and partly from cross-fertilization by pollen of other trees that carry alleles like those of the maternal parent.

New recombinant genotypes appeared among progenies, however, there was no significant change in genotype frequencies for progeny group from those of parental group. The genotype frequencies in the parent(maternal) and progeny groups are presented in Table 2.

Table 2. Genotype frequencies in the parent (M) and progeny (F) groups.

Enzyme	AAT											
Genotype	A_1A_2	A_2A_2	A_2A_3	A_3A_3	B_1B_1	B_1B_3	B_3B_3	B_3B_4	B_4B_4	C_1C_1	C_1C_2	C_2C_2
M	0.04	0.92	0.04	–	0.24	0.49	0.27	–	–	–	0.10	0.90
F	0.04	0.93	0.03	0.00	0.28	0.40	0.30	0.01	0.01	0.01	0.11	0.88

Enzyme	GDH		LAP										
Genotype	A_1A_2	A_2A_2	A_0A_0	A_0A_1	A_0A_2	A_1A_1	A_1A_2	A_2A_2	B_1B_1	B_1B_2	B_1B_3	B_2B_2	B_2B_3
M	–	1.00	–	0.18	–	0.76	0.04	0.02	0.33	0.26	–	0.37	0.04
F	0.01	0.99	0.01	0.09	0.00	0.80	0.08	0.02	0.37	0.14	0.01	0.45	0.03

Alleles both in the trees of parental and progeny groups are under Hardy-Weinberg equilibrium except for the alleles at LAP loci of progeny group. The alleles at LAP A($x^2 = 11.52$, df = 5, $p < 0.05$) and B($x^2 = 109.02$, df = 4, $p < 0.01$) loci in the trees of progeny group deviate significantly from Hardy-Weinberg equilibrium. Generally, there were more homozygotes than expected for LAP A and B loci in the progenies. This result indicates that the maternal tree group may not be originated from the same parental population. If this is true, Wahlund effect[28] might have led to an increase of homozygotes at certain loci in the progeny group. High levels of inbreeding within the maternal group may also lead to an increase of homozygotes.

Average heterozygosity[16] and genetic diversity[7]

Table 3. Average heterozygosity(H) and genetic diversity(Vp) of the maternal (M) and progeny(F) groups computed over six loci.

Group	N	H	Vp
M	49	0.235	5.409
F	784	0.238	5.569

Estimation for the proportion of F_1-hybrid seeds produced in the F_1-hybrid seed orchard.

for the pitch pine groups (Table 3) appeared to be rather low compared to other tree groups of the same tree species[5] or to other coniferous tree species[2,3,16]. Hwang[5] reported values of 0.30 (0.21–0.37) and 7.89 (4.06–14.41), respectively for average heterozygosity and genetic diversity on the same species of pitch pines that consisting of 24 provenances from the northern parts of U.S.A.

Both pitch and loblolly pines have many common alleles that exactly coincide with each other over those six allozyme loci(unpublished). Some alleles at loci GDH and LAP appeared to be useful for estimating the frequency of F_1-hybrid seeds produced in the F_1-hybrid seed orchard. In loblolly pines both A_1 and A_2 alleles at LAP locus are present in different frequencies. Frequency of allele A_2 is much higher than A_1 in loblolly pines while allele A_2 is rare at LAP locus A in pitch pines (Table 1).

The proportion of F_1-hybrid seeds originated from species hybridization was calculated by subtracting the proportion of individuals that are likely to be produced by within maternal(pitch pine) species matings from the total number of A_1A_2 or A_2A_2 phenotypes for LAP. The proportion of A_1A_2 or A_2A_2 phenotypes produced by

within maternal species matings will be the same as the proportion of allele A_2 in the maternal gametes pool. Progenies originated from maternal trees that have allele A_2 were excluded from the above calculations because there were indications of high levels of selfing in those trees. In case of GDH allozyme locus all of A_1A_2 phenotype are originated from species hybridization because no A_1 allele was found in the maternal gametes pool. Allele A_1 for GDH was found in loblolly pines (see part for "Number of loci and alleles" on page 6).

The proportion of F_1-hybrid seeds calculated in this way was 0.77% of the total. The approximate estimation for F_1-hybrid seeds proportion by allozyme study is in good agreement with the value 0.73% estimated by morphological study for F_1-hybrid seedlings at a nursery in 1974(Table 4).

Table 4. Proportion of pitch x loblolly F_1-hybrid pine seedlings determined by morphological characteristics from 33 open pollinated families (2-0 seedling stage at a nursery bed).

Group	No. of families	Total no. of seedlings exam.	Mean & range	No. of F_1-hybrid seedlings	% of F_1-hybrids
I	15	3,372	224.80 43-405	51	1.51 0.71-4.65%
II	18	3,583	199.06 40-350	0	0.00 %
Sum	33	6,955		51	M = 0.73%

Group I: F_1-hybrid producing family. Group II: non-producing family.

Fig. 4. Growth of parental trees in an F_1-hybrid seed orchard. Left and right rows; *Pinus taeda* L. central row ; *Pinus rigida* Mill.

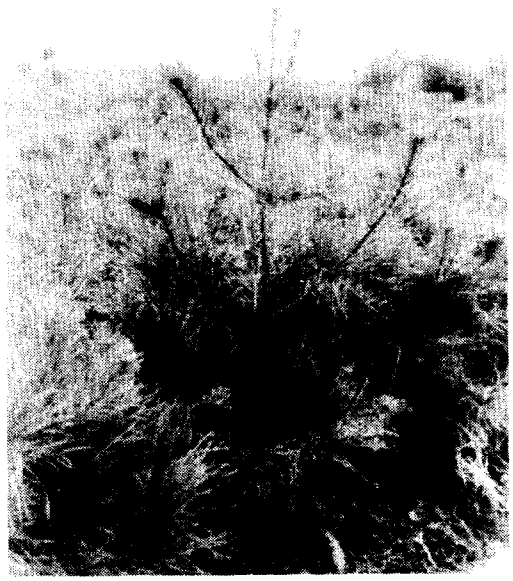

Fig. 5. Occasionally occuring F_1-hybrid seedling in a nursery bed. Seedlings are originated from maternal pitch pines in an F_1-hybrid seed orchard for pitch x loblolly hybrid seed production.

Seedlings (from 1971 seed year) for open-pollinated 33 pitch pine families were raised by 10cm x 10cm spot seeding at a nursery in Suweon during 1972–1973 years. In March 1974 the 2–0 seedlings of each pitch pine family were examined for their morphological characteristics to determine the proportion of F_1-hybrids. In most cases, pitch x loblolly F_1-hybrids could readily be recognized by their morphological characteristics (particularly by the seedling height and size of needle leaves. Fig. 5) from those of pitch pine seedlings of within species matings at 2-0 seedling stage.

Although an approximate estimation for the proportion of F_1-hybrid seeds produced in an F_1-hybrid seed orchard was made by allozyme analysis, a further study is required for a more precise estimation of the value. Indications for Wahlund effect, high levels of self-fertilization and sexually asymmetric fertility selection in the F_1-hybrid seed orchard call for cautions in estimating allelic frequency changes and mating probabilities for the parental and progeny tree groups.

LITERATURE CITED

1. Adams, W. T. and R. J. Joly. 1980. Genetics of allozyme variants in loblolly pine. The Jour. of Heredity 71: 33-40.
2. Conkle, M. T. 1979. Amount and distribution of isozyme variation of in various conifer species. Proc. 17th Meet. Canadian Tree Improv. Assoc. Part 2. Gander, Newfoundland, Canadian For. Serv., Ottawa. 109-117.
3. Chung, M. S. 1981. Biochemical method for determining population structure in *Pinus sylvestris* L. Acta Forestalia Fennica 173: 28 pp.
4. Chung, M. S. 1983. Flowering of *Pinus rigida* Mill. and *Pinus taeda* L. in an F_1-hybrid seed orchard. Jour. Korean For. Soc. 59: 31-36.
5. Hwang, J. W. 1983. Provenance variation in growth, morphology, wood properties and allozymes of *Pinus rigida*. Doctoral thesis, Seoul National University, College of Agriculture(Korean with English summary). 72 pp.
6. Krzakowa, M. and J. Szweykowski. 1979. Variation of 6-PGD in the populations of Polish Scots pine (*Pinus sylvestris*). Proc. Conf. Bioch. Genet. For. Trees Umea, Sweden. 1978. 86-98.
7. Gregorius, Hans-Rolf. 1978. The concept of genetic diversity and its formal relationship to heterozygosity and genetic distance. Mathematical Biosciences 41: 253-271.
8. Guries, R. P. and F. T. Ledig. 1978. Inheritance of some polymorphic isozymes in pitch pine (*Pinus rigida* Mill.). Heredity 40(1): 27-32.
9. Lundkvist, K. 1979. Allozyme frequency distribution in four Swedish populations of Norway spruce(*Picea abies* K.) 1. Estimation of genetic variation within and among populations, genetic linkage and a mating system parameter. Hereditas 90: 127-143.
10. Lundkvist, K. and D. Rudin. 1977. Genetic variation in eleven populations of *Picea abies* as determined by isozyme analysis. Hereditas 85: 67-74.
11. Mitton, J. B., Y. B., Linhart J. L. Hamrick and J. S. Beckman. 1977. Observations on the genetic structure and mating system of Ponderosa pine in the Colorado front range. Theor. Appl. Genet. 51: 5-13.
12. Mitton, J. B., Y. B. Linhart, K. B. Sturgeon and J. L. Hamrick. 1979. Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. The Jour. Heredity 70: 86-89.
13. Muller-Starck, G. 1979. Estimates of self- and cross-fertilization in a Scots pine seed orchard. Proc. Conf. Bioch. Genet. For. Trees. Umea, Sweden. 1978. 170-181.
14. Muller-Starck, G. 1982. Sexually asymmetric fertility selection and partial self-fertilization 2. Clonal gametic contributions to the offspring of a Scots pine seed orchard. Silva Fennica 16(2): 99-106.
15. Muller-Starck, G. 1982. Reproductive systems in conifer seed orchard 1. Mating probabilities in a seed orchard of *Pinus sylvestris* L. Silvae

Genetica 31 (5-6): 188-197.

16. Nei, M. 1975. Molecular population genetics and evolution. North-Holland Pub. Co. Amsterdam. 288 pp (p. 128-132).
17. Nikolic, D. and N. Tucic 1983. Isozyme variation within and among populations of European black pine (*Pinus nigra* Arnold). Silvae Genetica 32 (3-4): 80-89.
18. Rudin, D. 1977. The isozyme technique - a shortcut to the genes of our forest trees? Illustrations using *Pinus sylvestris* L. Doctoral thesis, Univ. Umea, Sweden. 53 pp.
19. Rudin, D. 1977. Leucine-aminopeptidase(LAP) from needles and macrogametophytes of *Pinus sylvestris* L. Inheritance of allozymes. Hereditas 85: 219-226.
20. Rudin, D. and Lindgren, D. 1977. Isozyme studies in seed orchards. Studia Forestalia Suecica 139: 23 pp.
21. Scandalios, J. G. 1969. Genetic control of multiple molecular forms of enzymes in plants; a review. Biochem. Genet. 3: 37-79.
22. Shaw, C. R. and R. Prasad 1970. Starch gel electrophoresis of enzymes – A compilation of recipes. Bioch. Genet. 4: 279-310.
23. Shaw, D. V. and W. Allard 1982. Isozyme heterozygosity in adult and open-pollinated embryo samples of Douglas-fir. Silva Fennica 16(2): 115-121.
24. Shen, H. H., D. Rudin and D. Lindgren. 1981. Study of the pollination pattern in a Scots pine seed orchard by means of isozyme analysis. Silvae Genetica 30(1): 7-15.
25. Siciliano, M. J., C. R. Shaw. 1976. Separation and visualization of enzymes on gels. In Chromatographic and Electrophoretic Techniques 2. Zone Electrophoresis (ed. Smith, I.). William Heinemann Medical Books Ltd. London. 185-209.
26. Tigerstedt, P. M. A. 1973. Studies on isozyme variation in marginal and central populations of *Picea abies*. Hereditas 75: 47-60.
27. Tigerstedt, P. M. A., D. Rudin, T. Niemela and J. Tammisola. 1982. Competition and neighbouring effect in a naturally regenerating population of Scots pine. Silva Fennica 16(2): 122-129.
28. Wahlund, S. 1928. Zuzammensetzung von Populationen und Korrelationserscheinungen vom Standpunkt der Vererbungslehre aus betrachtet. Hereditas 11: 65-106.
29. Woods, J. H., G. M. Blake and F. W. Allendorf. 1983. Amount and distribution of isozyme variation in ponderosa pine from estern Montana. Silvae Genetica 32(5-6): 151-157.
30. Ziehe, M. 1982. Sexually asymmetric fertility selection and partial self-fertilization 1. Population genetic impacts on the zygotic genetic structure. Silva Fennica 16(2): 94-98.