Inheritance and Linkage of Allozymes in *Pinus armandii* FRANCH.

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Summary

Ten enzyme systems (ADH, FDH, FLE, LAP, MDH, MEN, 6PGD, PGI, PGM, SOD) coding for 20 loci were investigated in *Pinus armandii.* MENDELian inheritance was confirmed for allozymes at 12 loci by testing the fit of band-pattern segregation in macrogametophytes from heterozygous trees to the expected 1:1 ratio. Linkage relationships were examined for 41 pairs of allozyme loci. Four pairs of loci appear to be linked: Pgi2-Sod2 with a recombination frequency (R) = 0.11; Adh2-Men2, with R = 0.13; Men2-Sod2, with R = 0.18, and Adh2-Sod2, with R = 0.29.

Key words: Pinus armandii, allozymes, inheritance, linkage.

Introdoction

Pinus armandii FRANCH. is a widely distributed haploxylon pine of western and southwestern China (CRITCHFIELD and LITTLE, 1966), where it is one of the important reforestation tree species in sub-alpine regions. Its natural range spreads over a large area from the south subtropical region to the northern grasslands. (MA CHANG-GENG, 1989).

In the last decade allozymes have become very popular as genetic markers in forest genetic studies. They have been used to map the genome, quantify genetic variation and differentiation, analyze the mating system, study introgression and phylogenetic relationships between species. Knowledge of inheritance and confirmation of genetic control of allozymes with simple MENDELian inheritance is the first step facilitating their use as gene markers in population genetics.

This report describes inheritance and linkage of allozymes in *Pinus armandii.*

Material and Methods

Seeds for electrophoresis were collected from 15 trees growing in the Kórnik Arboretum, Poland. Seven macrogametophytes from each of the studied trees were first sampled to define genotypes. Afterwards, additional 15 to 31 macrogametophytes per tree were analysed from highly heterozygous trees that were selected to study linkage and to verify the segregation ratio.

Macrogametophyte tissues and embryos were isolated separately from the seeds and homogenized in TRIS-HCl buffer pH 7.2 with the addition a 0.15% 2-mercaptoethanol. Homogenates were subjected to horizontal 12% starch gel electrophoresis applying buffer system I (RIDGEWAY et al., 1970) and buffer system II (SICILIANO and SHAW, 1976). Gel slices were stained for activity of 10 different enzymes using recipes described by CHELIAK and PITEL (1984). The following 10 enzymes were investigated in this study (their abbreviations, enzyme commission codes and buffer system upon which they were run are in parenthesis): alcohol dehydrogenase (ADH, EC 1.1.1.1, II), fluorescent esterase (FLE, EC 3.1.1.1, I), formate dehydrogenase (FDH, EC 1.2.1.2, I), leucine aminopeptidase (LAP, EC 3.4.11.1, I), malate dehydrogenase (MDH, EC 1.1.1.37, II), menadione reductase (MEN, EC 1.6.99.2, II), 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44, II), phosphoglucose isomerase (PGI, EC 5.3.1.9, I), phosphoglucomutase (PGM, EC 2.7.5.1, I), superoxide dismutase (SOD, EC 1.15.1.1, II).

Inheritance of allozyme polymorphism in haploid tissue from heterozygous trees was tested for confirmation with the expected 1:1 ratio, using the chi-square test. The chi-square test was also used for the estimation of heterogeneity of results pooled

Table 1 – Observed allozyme segregation in macrogametophytes of heterozygous trees and chi-square tests for goodness of fit to 1:1 ratio and heterogeneity among employed trees.

Locus	Allelic combination	Observed segregation	Deviation chi-square test (1 df)	Heterogeneity chi-square test (df)
Adh 2	1/2	18 : 20	0.10	--
Fdh	1/2	28 : 32	0.27	1.48 (1)
Fle	1/2	23 : 34	2.12	0.23 (1)
Lap2	1/3	24 : 20	0.36	3.28 (1)
	1/2	13 : 9	0.73	--
	2/3	22 : 16	0.95	--
Mdh2	1/2	14 : 8	1.64	--
Men2	1/2	14 : 24	2.63	--
6Pgd1	1/2	41 : 35	0.47	0.06 (1)
6Pgd2	1/3	38 : 43	0.31	1.55 (2)
	2/3	10 : 12	0.18	--
Pgi2	1/2	22 : 16	0.95	--
Pgm1	1/2	21 : 19	0.10	2.08 (1)
Pgm2	1/2	48 : 34	2.39	0.40 (2)
Sod2	1/2	67 : 59	0.51	1.01 (3)

non of the chi-square tests was statistically significant.

over all trees (MATHER, 1963, p. 13 to 25 and 69 to 90). The statistical evaluation of linkage relationships was done using chi-square tests as described by MATHER (1963, p. 32 to 55). The frequency of recombination (R) between loci was calculated by the formula: R= r/n, where r is the number of recombinants and n is the total number of macrogametophytes analyzed. The standard error of this estimate is given by: $[R(1-R)/n]^{1/2}$ (RUDIN and EKBERG, 1978).

Results and Discussion

Inheritance of isozyme patterns

Twenty loci were identified from the 10 enzyme systems analyzed (*Fig. 1*). MENDELian inheritance was tested for the twelve polymorphic allozyme loci. In summary, no significat deviation from the expected 1:1 segregation ratio nor chi-square heterogeneity were observed at any of the loci studied (*Table 1*), indicating that these allozymes exhitited distinct, co-dominant expression and simple MENDELian segregation in their mode of inheritance. I have not found any previous information about the mode of inheritance of allozyme loci in *Pinus armandii.*

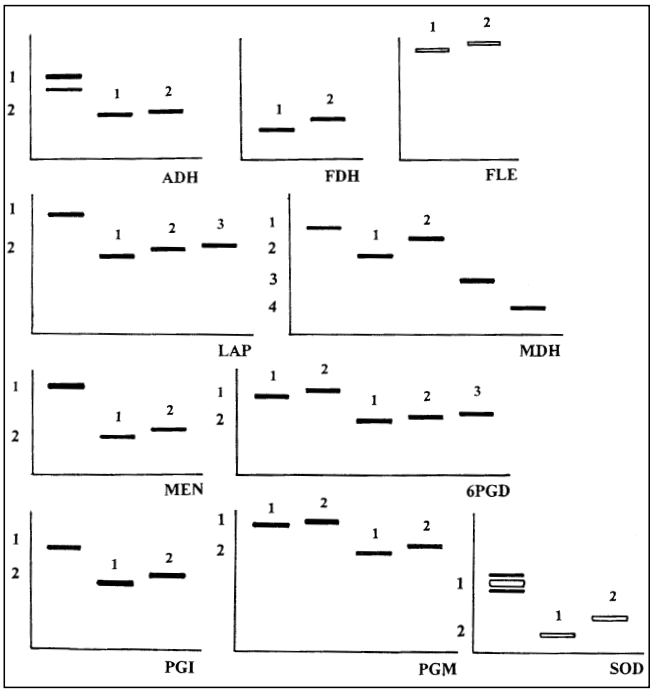

Figure 1. – Enzyme phenotypes found in *Pinus armandii.*

Description of the observed isozyme patterns is presented below.

Alcohol dehydrogenase (ADH)

There were two zones of activity on gels stained for ADH. The most anodal zone, designated as Adh1 was invariant and double-banded. The lower zone (Adh2) had two single-banded variants. Genetic control of ADH by two independent loci has been reported also for other pines (RUDIN and EKBERG, 1978; O'MALLEY et al., 1979; STRAUSS and CONKLE, 1986; WANG et al., 1991; GONCHARENKO et al., 1994).

Fluorescent esterase (FLE)

One zone of activity, with two variants, was evident on gels stained for this enzyme. In other pines also one zone of FLE activity have usually been scored (STRAUSS and CONKLE, 1986; WANG et al., 1991; GONCHARENKO et al., 1992, 1994).

Formate dehydrogenase (FDH)

Also one zone of activity, with two variants, was evident on gels stained for FDH. A single zone of activity for this enzyme was reported in *Pinus sylvestris* (MEJNARTOWICZ and BERGMANN, 1985), *Larix decidua* (LEWANDOWSKI and MEJNARTOWICZ, 1990) and *Pseudotsuga menziesii* (LEWANDOWSKI and MEJNARTOWICZ, 1992).

Leucine aminopeptidase (LAP)

Two zones of activity were detected on LAP gels. The faster region (Lap1) was monomorphic. Lap2 contained three well distinguished variants. Genetic control of LAP by 2 independent loci has been reported for several other pines (RUDIN, 1977; O'MALLEY et al., 1979; ADAMS and JOLY, 1980; STRAUSS and CONKLE, 1986; GONCHARENKO et al., 1992, 1994).

Malate dehydrogenase (MDH)

Four zones of activity were evident on gels stained for MDH. The most anonal zone (Mdh1) stained less intensely, and similary to Mdh3 and Mdh4 were invariant in the investigated material. Two variants exist in Mdh2. Contrary to most other conifer tree species, heterodimeric bands between Mdh2 and Mdh3 have not been observed. Similary, heterodimeric band does not exist in pines of subsection *Cembrae* (GONCHARENKO et al., 1992).

Menadione reductase (MEN)

Two zones of activity were detected on MEN gels. The faster region Men1 was monomorphic. Men2 contains two variants. Similary to other coniferous tree species (LEWANDOWSKI and MEJNARTOWICZ, 1990), identical zymograms were observed when 2.6-dichlorophenolindophenol was used as substrate for diaphorase. It seems, that both substrates gave a picture of the same enzyme or enzymes with low substrate specifity. For the two zones of this enzymatic activity observed in *Pinus armandii* I left arbitrarily the name menadione reductase. Two or three zones of menadione reductase or diaphorase appear to exist in the majority of *Pinus* species (MILLAR, 1985; STRAUSS and CONKLE, 1986; GONCHARENKO et al., 1994; SHARMA and VON WUEHLISCH, 1998).

Phosphoglucose isomerase (PGI)

Two zones of activity were evident from the gels stained for PGI. The faster migrating zone was invariant. Pgi2 exhibited two variants. One or two zones of Pgi activity have usually been reported in pines (GURIES and LEDIG, 1978; O'MALLEY et al., 1979; ADAMS and JOLY, 1980; MILLAR, 1985; STRAUSS and CONKLE, 1986; WANG et al., 1991; GONCHARENKO et al., 1994; SHARMA and VON WUEHLISCH, 1998).

Phosphoglucomutase (PGM)

PGM had two polymorphic zones of activity. Two allozymes were observed for each zone. The anodal zone stained intensely whereas the cathodal one was faint. Two PGM loci appear to exist in the majority of *Pinus* species (GURIES and LEDIG, 1978; ADAMS and JOLY, 1980; STRAUSS and CONKLE, 1986; WANG et al., 1991; GONCHARENKO et al., 1992, 1994; SHARMA and VON WUEHLISCH, 1998).

6-phosphogluconate dehydrogenase (6PGD)

Gels staining for 6PGD had two intensely stained zones. The most catodal zone (designated as 6Pgd2) had three variants, whereas the most anodal zone (designated as 6Pgd1) only two. Additionaly, in the middle region three less intensely staining bands exist. It is possible that this region is under control of two additional loci, similarly to some other species of sect.

Table 2. – Two locus combinations and number of trees employed for the linkage analysis (upper half) and results of statistical testing (lower half).

Locus	Adh2	Fdh	Fle	Lap2	Mdh1	Men2	6Pgd1	6Pgd2	Pgi2	Pgm1	Pgm2	Sod2
Adh 2		–	–	1	–	1	–	1	–	–	–	1
Fdh	–		2	–	1	–	1	1	–	–	–	1
Fle	–	NS		–	1	–	1	1	–	–	–	1
Lap2	NS	–	–		1	1	1	2	1	2	2	3
Mdh1	–	NS	NS	NS		–	1	2	–	1	–	1
Men2	***	–	–	NS	–		–	1	–	–	1	1
6Pgd1	–	NS	NS	NS	NS	–		1	1	1	1	2
6Pgd2	NS	NS	NS	NS	NS	NS	NS		–	1	1	2
Pgi2	–	–	–	NS	–	–	NS	–		–	1	1
Pgm1	–	–	–	NS	NS	–	NS	NS	–		1	1
Pgm2	–	–	–	NS	–	NS	NS	NS	NS	NS		2
Sod2	**	NS	NS	NS	NS	***	NS	NS	***	NS	NS	

– two-locus combinations not tested

NS not significant

** significant linkage at p <0.01, *** significant linkage at p<0.001

Table 3. – Significantly linked pairs of allozyme loci in *Pinus armandii* with recombination frequency (R), its standard deviation (SD), chi-square test for linkage and proportion of trees studied with significant linkage.

Pair of allozyme loci	Total seeds	Recombination frequency R (SD)	Chi-square-test	N linked/N studied
Adh2/Men2	38	0.13 (0.05)	20.63 ***	1/1
Adh2/Sod2	38	0.29 (0.07)	6.74 **	1/1
Pgi2/Sod2	38	0.11 (0.05)	23.68 ***	1/1
Men2/Sod2	38	0.18 (0.06)	15.16 ***	1/1

– Significant levels: ** –p <0.01, ***p <0.001

Strobus (BERGMANN and GILLET, 1997). However, lack of variation in my material does not allow me to verify this presumption. Two or sometimes three loci of 6PGD were described in other pines (GURIES and LEDIG, 1978; O'MALLEY et al., 1979; MILLAR, 1985; SZMIDT and YAZDANI, 1984; STRAUSS and CONKLE, 1986; GONCHARENKO et al., 1994; SHARMA and VON WUEHLISCH, 1998).

Superoxide dismutase (SOD)

Two zones of activity were detected on SOD gels. In the faster region three bands exist. It is possible that this region is under control of two or more loci, but lack of variation in the investigated material does not allow to verity this presumption. In the most catodal zone (designated as Sod2) two variants were observed. Two zones of activity for this enzyme were reported for *Larix decidua* (LEWANDOWSKI and MEJNARTOWICZ, 1990) and *Taxus baccata* (LEWANDOWSKI et al., 1992),

When an allozyme locus was clearly expressed in the embryo and when heterozygous embryos were found, the quaternary structures of allozymes were infered. On gels stained for Lap2, Pgm1, and Pgm2, only one or two-banded patterns were found indicating that these enzymes are monomers, which is in accordance with the results reported for other conifer taxa (RUDIN, 1977; ADAMS and JOLY, 1980; MILLAR, 1985; WANG et al., 1991; LEWANDOWSKI et al., 1992). On the other hand, heterozygous embryos stained for Fdh, Fle, Mdh2, 6Pgd1, 6Pgd2, Pgi2 and Sod2 showed an additional third band of intermediate mobility, which suggest dimeric structure of these enzymes. Such suggestions have also been described for other conifer taxa (GURIES and LEDIG, 1978; ADAMS and JOLY, 1980; MILLAR, 1985; WANG et al., 1991; LEWANDOWSKI et al., 1992).

Linkage

Out of the 66 possible two-locus combinations which can be formed from 12 polymorphic loci, among the available trees, 41 pairs of allozyme loci were possible to compare. *Table 2* shows the combinations of allozyme loci tested and the number of trees studied. Linkage was found between four pairs of allozyme loci, with recombination frequencies from 0.11 (between Pgi2 and Sod2) to 0.29 (between Adh2 and Sod2) (*Table 3*). These four loci formed one linkage group. The order of the loci of the established linkage group is: Pgi2, Sod2, Men2 and Adh2. The order of 3 loci (Pgi2, Men2 and Adh2) and the approximate distances between them in *Pinus armandii* are like those reported in other *Pinus* species (CONKLE, 1981; STRAUSS and CONKLE, 1986; NIEBLING et al., 1987; GONCHARENKO et al. 1998). Presence in this linkage group of locus Sod2 is interesting. It is localizated in *Pinus armandii* between Pgi2 and Men2. Earlier one locus of SOD has been found in the same linkage block in two species of *Pinus* (CONKLE, 1981) and in *Taxus baccata* (LEWANDOWSKI et al., 1992). However, CONKLE (1981) localized the Sod locus to the right of ADH loci in lodgepole pine and to the left of Pgi2 in Jeffrey pine.

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A Breeding Strategy for the New Zealand Radiata Pine Breeding Cooperative

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Abstract

This paper documents the breeding strategy of the New Zealand Radiata Pine Breeding Co-operative (NZRPBC) following a revision in 1997 to 1999. This co-operative serves 15 members in New Zealand and south-eastern Australia, and provides improved genetic material used for planting throughout New Zealand and in parts of Australia. In the revised strategy, emphasis on recurrent selection for general combining ability (GCA), a 2-superline structure, main population and breeds are maintained. A non-regionalised breeding programme, and a final selection around age eight years are also maintained. A new Structural Timber breed is to be formed, along with a Clear Cuttings breed (with modified emphases compared to the long-standing Long Internode breed). The Growth and Form breed will be expanded, recombining new superior parent clones. The existing Dothistroma-resistant breed will also be progressed, while the existing Long Internode and High Wood Density breeds will be used as sources of selections. Good parents not selected for the breeds will be used in the main population. A Guadalupe breeding population is also to be established.

The combined populations are to have a census number near 550 and a target status number of 400. The role of the breeds is to get optimum genetic gain while delaying the build-up of inbreeding, and to be the main source of new selections for seed orchards. The main population will serve as a reservoir of genetic diversity, as a source of candidates for existing and future breeds, and as a form of 'genetic insurance'. Candidates within breeds will be crossed in disconnected factorials and tested as seedlings or as clones within families. Candidates within the main and Guadalupe population will be tested as seedlings.

The previous full review of the New Zealand breeding strategy for radiata pine was in 1986. The strategy was revised because of subsequent developments that impacted on breeding, including: new information and new tested parents; adopting a collaborative government-industry approach to breeding; advances in breeding strategy, forest genetics and propagation techniques; and changes in emphases and practices in the forestry sector. The 1986 strategy had several key results, namely an emphasis on recurrent selection for general combining ability, a 2-superline breeding population with relatedness kept within superlines, stratifying the breeding population to an unspecialised main population and specialised breeds (with some overlap), a single breeding programme for all New Zealand, and separate crossing for recombination and for estimation of GCA.