Inheritance of AAT in *Picea abies* – Some Old and New Facts

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Summary

Based on extensive materials of seeds collected from single trees we verify the mode of inheritance of aspartate aminotransferase (AAT) in Norway spruce. Segregation analyses indicated that this enzyme system is coded by three different loci. Close linkage (r=0.058) exists between *Aat-2* and *Pgi-2* loci, and not *Aat-1* and *Pgi-2* as reported in other studies. This inconsistency of which AAT locus is linked to *Pgi-2*, may result from various notations of the same locus by different authors.

Key words: Picea abies, allozymes, AAT, inheritance, linkage.

Introduction

The mode of inheritance of more than 20 enzymes has been studied in Norway spruce (BERGMANN, 1974; LUNDKVIST, 1979; POULSEN et al., 1983; ALTUKHOV et al., 1986; MUONA et al., 1987; LAGERCRANTZ et al., 1988; MORGANTE et al., 1989; GONCHARENKO et al., 1995). The enzyme system of aspartate aminotransferase (AAT; EC 2.6.1.1) often referred to as glutamic-oxaloacetic transaminase (GOT) was among the first analyzed (LUNDKVIST, 1979). Usually four zones are observed on gels stained for AAT activity (*Fig. 1*). However, there are different, often confusing opinions about the genetic control of these zones (LUNDKVIST, 1979; POULSEN et al., 1983; ALTUKHOV et al., 1986; MUONA et al., 1987; LAGERCRANTZ et al., 1988; GONCHARENKO et al., 1995). The reason for this is that the first two zones (1 and 2) reveals low polymorphism, while the third and fourth zones has co-migrating bands. During isozyme studies on genetic variation of Norway spruce in Poland, we have analyzed seeds collected from about 1,000 individual tress. With such extensive material the mode of inheritance of AAT in Norway spruce could be investigated in detail.

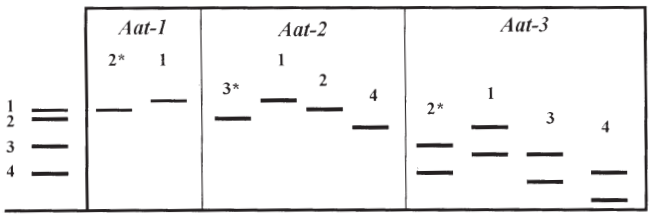

Figure 1. – Electrophoretic AAT phenotypes for macrogametophytes of *Picea abies*. The most frequent alleles are marked with asterisks.

Materials and Methods

Seeds samples were collected in 1998-2000 from 29 Polish populations of Norway spruce. The seeds were used for studies of genetic variation of Norway spruce in Poland (LEWANDOWSKI and BURCZYK, 2002). The allozyme genotypes of 13 enzyme systems encoding totally 27 loci were determined for 960 individual trees. Among those, there were several trees indicating polymorphism of AAT within staining zones 1 and 2, which were used for a profound analysis of AAT inheritance.

Macrogametophyte tissues were homogenized in 30 µl of Tris-HCl buffer pH 7.2 containing 15 % of 2-mercaptoethanol. Electrophoretic separation was carried out in 12 % starch gel by applying a buffer system according to RIDGEWAY et al. (1970) and gel slices were stained for AAT activity following CHELIAK and PITEL (1984). Segregation patterns and linkage relationships were evaluated using the chi-squared goodness-of-fit test (MATHER, 1951). Data for the same locus pair from individual trees used for linkage studies were pooled together, if they were homogeneous. Recombination fractions (R) were calculated by the binominal estimator: $R = r/n$, where r is the number of recombinant types observed and n is the total number of observations. The standard error of this estimate is given by $[R(1-R)/n]^{1/2}$ (RUDIN and EKBERG, 1978).

Results and Discussion

Analyses of the macrogametophyte of 960 Norway spruce trees indicated that haplotypes of AAT are represented by bands located in four zones (1,2,3 and 4) (*Fig. 1)*. Based on detailed segregation analyses we postulate that the zone 1 and zone 2 are coded by two different loci. In contrast, the bands observed within zone 3 and zone 4 are the co-migrating bands coded by a single locus. The three loci were numbered according to electrophoretic mobility of their allozymes. The most anodally migrating locus was named *Aat-1*, the next *Aat-2*, and the last one *Aat-3*. For the two first loci which were overlapping, the number was determined by relative positions of the most frequent allozymes. In our material we observed two and four alleles for *Aat-1* and *Aat-2*, respectively. The observed heterozygosities for the entire sample from Poland were low, 0.02 for *Aat-1* and 0.06 for *Aat-2*. This parameter was much higher for the *Aat-3* locus (0.52), which was represented by four alleles. No significant deviation from the expected Mendelian segregation ratio (1:1) nor heterogeneity among trees were observed at any of these loci (*Table 1*). Among all studied trees we found only one which was heterozygous at all three loci. Analyses of co-segregation patterns of allozymes indicated no linkage among the AAT loci (*Table 2*).

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

Locus	Allelic combination	Observed segregation	Deviation Chi^2 test (1 df)	Heterogeneity Chi^2 test (df)
Aat-1	1/2	55:48	0.48	1.44 (2)
Aat-2	1/3	16:13	0.31	-----
	2/3	11:16	0.93	-----
	3/4	15:15	0.00	-----
Aat-3	1/2	61:63	0.03	8.27 (10)
	1/3	9:12	0.43	-----
	1/4	17:10	1.81	-----
	2/3	24:18	1.24	0.38 (2)

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Table 2. – Tests for linkage among allozyme loci, with recombination frequency (R), its standard deviation (SD) and proportion of the studied trees with significant linkage.

Locus pair	Total seeds	Linkage Chi^2 test	Recombination frequency R (SD)	N linked/ / N studied
Aat-1 / *Aat-2*	18	0.89 ns	-- --	0 / 1
Aat-1 / *Aat-3*	18	0.00 ns	-- --	0 / 1
Aat-2 / *Aat-3*	18	2.00 ns	-- --	0 / 1
Aat-1 / *Lap-2*	57	29.49 ***	0.140 (0.046)	1 / 1
Aat-1 / *Pgi-2*	45	0.02 ns	-- --	0 / 3
Aat-2 / *Pgi-2*	86	67.16 ***	0.058 (0.025)	3 / 3

NOTE: *** – parameter significant at $p < 0.001$,
ns – parameter not significant.

Our interpretation of the AAT inheritance is comparable with earlier findings of POULSEN et al. (1983), ALTUKHOV et al., 1986, and LAGERCRANTZ et al. (1988). However, the later authors numbered the loci in opposite direction, so that the least anodally migrating locus was designated as *1*, and so on. LUNDKVIST (1979) similarly observed four zones, although his interpretation was different. He suggested that the two most anodally migrating zones (1 and 2) are coded by a single locus and not two separate loci, while the co-migrating bands of zones 3 and 4 reflect the expression of two different, closely linked loci. However, we support the conclusion of LAGERCRANTZ et al. (1988) that the zones 3 and 4 are coded by a single locus (named *Aat-3* in our study and *Aat-1* in the study of LAGERCRANTZ et al., 1988). The interpretation of that locus on zymograms is complicated by apparent posttranslational modifications, resulting in duplicate forms of the primary isozyme at this locus. As pointed out by O'MALLEY et al. (1979), it is more likely that the double-banded phenotypes are due to posttranslational modifications of the molecule than that they are coded by two tightly linked loci.

There were several authors reporting only three zones of the enzyme activity (MUONA et al., 1987; GONCHARENKO et al., 1995). We noticed that the most anodally migrating locus, designated in this study as *Aat-1*, stains faintly, thus it is possible that it was not scored by those authors. The different interpretation of AAT loci is reflected also in the description of linkage patterns between AAT and PGI (phosphoglucose isomerase, EC 5.3.9.1) loci. In our material, we found a close linkage between *Aat-2* and *Pgi-2*, with the recombination frequency of 0.058 (*Table 2*). This value is very similar to the recombination frequencies estimated between *Aat-1* and *Pgi-2* by MUONA et al. (1987; R = 0.062) and GONCHARENKO et al. (1994; R = 0.073). Slightly higher recombination was observed by GEBUREK and VON WUEHLISCH (1989; R = 0.15). Therefore we conclude that our locus *Aat-2* corresponds to locus *Aat-1* described by MUONA et al. (1987), GEBUREK and VON WUEHLISCH (1989) and GONCHARENKO et al. (1995).

Linkage between *Pgi-2* and one of the AAT loci in the *Pinaceae* family is well documented in the literature (GURIES et al., 1978; ADAMS and JOLY, 1980; NEALE and ADAMS, 1981; EL-KASSABY et al., 1982; KING and DANCIK, 1983; CHELIAK and PITEL, 1985; GONCHARENKO et al., 1994; LEWANDOWSKI, 1999). However, for genera *Pinus* and *Picea,* this linkage is usually reported between *Aat-1* and *Pgi-2* loci, and not *Aat-2* and *Pgi-2* as in our study. This inconsistency about which AAT locus is linked to *Pgi-2*, may result from various notations of the same locus, as noted earlier.

Similar difficulties in the interpretation of AAT inheritance may have concerned also other spruce species (KING and DANCIK, 1983; BOYLE and MORGENSTERN, 1985; BARRETT et al., 1987; RINGIUS and INNES, 1990), where authors describe genetic linkage between *Aat-1* and *Pgi-2*. CHELIAK and PITEL (1985) suggested that evolution from the last common ancestor between the *Pinus-Picea* group and other coniferous genera, like *Larix*, *Pseudotsuga* and *Abies*, caused the AAT system to progress to the point where accumulated mutations can now be observed as electrophoretic mobility differences. They suspected that what is called *Aat-1* in the *Pinus-Picea* group is functionally homologous to *Aat-2* in the other genera, and similarly *Aat-1* in *Pseudotsuga*, *Larix* and *Abies* is functionally homologous to *Aat-2* in the *Pinus-Picea* group. In our opinion this conclusion cannot be valid for Norway spruce, and maybe also for other spruce species. Our observations clearly indicate that in Norway spruce the linkage exists between *Aat-2* and *Pgi-2* loci, similarly as for genera *Abies*, *Pseudotsuga* and *Larix*, while alleles of *Aat-1* and *Pgi-2* segregated independently (*Table 2*).

The accuracy of our interpretation is supported by the observations of linkage between loci *Aat-1* and *Lap-2* (leucine aminopeptidase EC 3.4.11.1). This was possible, since we found in our material a single tree heterozygous for both loci. The frequency of recombination was estimated to be R = 0.14. (*Tab. 2*). SZMIDT and MUONA (1989) reported for Scots pine a recombination of similar magnitude, however it was noted for the pair of loci *Aat-2* and *Lap-2*. This confirms that locus *Aat-1* reported in this study for Norway spruce is functionally homologous to locus *Aat-2* in genus *Pinus*.

If the linkage pair of loci *Aat-1* and *Pgi-2*, frequently reported for other spruces could have resulted from a misinterpretations of zymograms, then the problem arises, whether the linkage pair reported in this study (*Aat-2* and *Pgi-2*) actually exists also among other spruce species. Answering to this question requires additional investigations, however finding individuals heterozygous for both loci might be difficult.

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Effects of Inbreeding on Coastal Douglas-fir: Nursery Performance

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Abstract

In advanced generation seed orchards, low levels of inbreeding may be inevitable as relatedness among individuals in breeding populations increases with each generation. Unlike selfing, low level inbreeding can produce relatively large number of viable seeds. Following previous study on the effects of inbreeding on coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) filled seed production, the present study investigated inbreeding on nursery performance over various cross-types, including outcrosses (inbreeding coefficient F=0), crosses between half-sibs (F=0.125), between full-sibs (F=0.25), between parents and offspring (F=0.25), and selfing (F=0.5). Significant differences were found among cross-types for germination, seedling mortality, seedling diameter and height, and nursery cull rate. Inbreeding also increased among-family genetic variability. Cumulative losses of seedlings at the nursery stage were 18, 33, 31, 36 and 43%, respectively for the above types of crosses. This result indicates that seeds with low levels of inbreeding may produce relatively large numbers of seedlings that meet nursery culling standards and could be used for reforestation, resulting in negative impacts on the genetic gain realized in field plantations.

Key words: inbreeding depression, nursery performance, cull rate, genetic variability

Introduction

Inbreeding depression is a common phenomenon in forest tree species, particularly in conifers (reviewed by WILLIAMS and SAVOLAINEN, 1996). Strong inbreeding depression has been observed at different life history stages in many forest trees, including seed development (GRIFFIN and LINDGREN, 1985; SORENSEN and CRESS, 1994), growth performance in nurseries (SNIEZKO and ZOBEL, 1988; SORENSEN, 1997), and at the early stage of field trials (DUREL et al., 1996; LUNDKVIST et al., 1987; ORR-EWING, 1976; SORENSEN and MILES, 1982).

In first generation seed orchards, self-pollination is typically the only form of inbreeding. It can reduce both seed yields and genetic gain. As self-fertility is low in many species, including Douglas-fir (SORENSEN, 1973; SORENSON, 1971), the selfing rate in viable seed produced in first generation seed orchards is low (BURCZYK, 1998; PRAT and CAQUELARD, 1995), even lower than that of seed lots collected from stands grown under natural conditions (PRAT and BURCZYK, 1998). Thus, the negative impact of inbreeding on genetic gain in offspring from first generation seed orchards is expected to be small.

Inbreeding may become more serious as advanced breeding programs generate a large number of trees sharing one or more common ancestors. The best individuals are often from the same families, and selection will result in some degree of relatedness. Thus, in advanced generation seed orchards, in addition to selfing, some low levels of related matings will likely occur. Low levels of inbreeding, such as mating between half-sibs or full-sibs, have much greater capabilities of producing viable seed than selfing (SORENSEN and CRESS, 1994; WOODS and HEAMAN, 1989). Thus, if the seeds with low levels of inbreeding can produce acceptable seedlings for reforestation, and if these seedlings grow slower than outcross trees as found in some other conifers (DUREL et al., 1996), then low levels of inbreeding may have greater impact on the growth and yield of field plantations than selfing.

In order to investigate the effects of various levels of inbreeding on seed production and growth performance, and the rela-

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