Genome **44**: 962–970 (2001). — LERCETEAU, E., PLOMION, C. and ANDERSON, B.: AFLP mapping and detection of quantitative trait loci (QTLs) for economically important traits in *Pinus sylvestris* : a preliminary study. Molecular Breeding **6**: 451–458 (2000). — LORBER, A. and KOWALSKI, B. R.: Appl. Spec. **44**, 1464 (1990). — MARQUES, C. M., VASQUEEZ-KOOL, J., CAROCHA, V. J. FERREIRA, J. G., O'MALLEY, D. M., LIU, B. H and SEDEROFF, R.: Genetic dissection of vegetative propagation traits in *Eucalyptus tereticornis* and *E. globulus.* Theor Appl Genet **99**: 936–946 (1999). — MARQUEZ-CEDILLO, L. A., HAYES, P. M., KLEINHOFS, A., LEGGE, W. G., ROSSNAGEL, B. G., SATO, K., ULLRICH, S. E. and WESENBERG, D. M.: QTL analysis of agronomic traits in barley based on the doubled haploid progeny of two elite North American varieties representing different germplasm groups. Theor Appl Genet **103**: 625–673 (2001). — MOREAU, L., CHARCOSSET, A., HOSPITAL, F. and GALLAIS, A.: Marker-assisted selection efficiency in population of finite size. Genetics **148**: 1353–1365 (1998). — O'MALLEY, D. M. and MCKEAND, S. E.: Marker-assited selection fro breeding values in forest trees. For Genet **1**: 207–218 (1994). — PAGLIA, G. P., OLIVIERI, A. M. and MORGANTE, M.: Towards second-generation STS (sequence-tagged sites) linkage maps in conifers: a genetic map of Norway spruce (*Picea abies* K.). Mol Gen Genet **258**: 466–478 (1998). — PANSHIN, A. J. and DE ZEEUW, C. E.: Textbook of wood technology: structure, identification, properties, and uses of the commercial woods of the United Sates and Canada, 4th edn. McGraw-Hill, New York (1980). — PLOMION, C., BAHRMAN, N., DUREL, C. E. and O'MALLEY, D. M.: Genomic mapping in *Pinus pinaster* (maritime pine) using RAPD and protein markers. Heredity **74**: 661–668 (1995). — PLOMION, C., DUREL, C. E. and O'MALLEY, D. M.: Genetic dissection of height in maritime pine seedlings raised under accelerated growth conditions. Theor Appl Genet **93**: 849–858 (1996). — REMINGTON, D. L., WHETTEN, R. W., LIU, B. H. and O'MALLEY, D. M.: Construction of an AFLP genetic map with nearly complete genome coverage in *Pinus taeda.* Theor Appl Genet **98**: 1279–1292 (1999). — RITTER, E., ARAGONES, A., MARKUSSEN, T., ACHERÉ, V., ESPINEL, S., FLADUNG, M., WROBEL, S., FAIVRE-RAMPANT, P., JEANDROZ, S. and FAVRE, J. M.: Towards Construction of An Ultra High Density Linkage Map for *Pinus pinaster.* Annals of Forest Science **59**: 637–643 (2002). — SCHNEIDER, K., SCHÄFER-PREGL, R., BORCHARDT, D. C. and SALAMINI, F.: Mapping QTLS for sucrose content, yield and quality in sugar beet population fingerprinted by EST-related markers. Theor Appl Genet **104**: 1107–1113 (2002). — SEWELL, M. M., BASSONI, D. L., MEGRAW, R. A., WHEELER, N. C. and NEALE. D. B.: Identification of QTLs influencing wood property traits in loblolly pine (*Pinus taeda* L.). I. Physical wood properties. Theor Appl Genet **101**: 1273–1281 (2000). — SEWELL, M. M., DAVIS, M. F., TUSKAN, G. A., WHEELER, N. C., ELAM, C. C., BASSONI, D. L. and NEALE, D. B.: Identification of QTLs influencing wood property traits in loblolly pine (*Pinus taeda* L.). Chemical wood properties. Theor Appl Genet **104**: 214–222 (2002). — UGLETTO, L., LERIQUE, D. and ROBERT P.: Une nouvelle méthode de détermination rapide des matières solubles. Application aux pulpes de bois pour pâte à papier. Informations Chimie n° 237, 3 p. (1997). — VAN OOIJEN, J. W.: Accuracy of mapping quantitative traits loci in autogamous species. Theor Appl Genet **84**: 803–811 (1992). — VERHAEGEN, D. and PLOMION, C.: Genetic mapping in *Eucalyptus urophylla* and *E. grandis* using RAPD markers. Genome **39**: 1051–1061 (1996).

Mode of Inheritance of Aspartate Aminotransferase in Silver-Fir (*Abies alba* Mill.)

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Abstract

In European silver fir (*Abies alba* Mill.) the enzyme system of aspartate aminotransferase (AAT) was supposed to be encoded by three gene loci. After applying improved extraction and electrophoretic separation procedures we succeeded in resolving four different AAT activity zones in zymograms which were found to be encoded by four loci. Whereas the two most anodal isozymes (AAT-A and AAT-B) and the most cathodal isozyme (now AAT-D) were already well-known, an intermediate AAT activity zone (now AAT-C) became for the first time visible following electrophoretic separation of seed tissue (megagametophyte) extracts. Possible associations of these four isozymes with different subcellular compartments were discussed.

Key words: Abies alba, AAT inheritance, aspartate aminotransferases, allozymes, megagametophyte.

Introduction

Aspartate aminotransferase (AAT or GOT, EC. 2.6.1.1) is an important enzyme, of the primary metabolism, which plays a key role in both nitrogen and carbon metabolism in many organisms (IRELAND and JOY, 1985). This enzyme system catalyzes the reversible reaction between an amino acid and a keto acid leading to the exchange between the α-amino group and the keto group:

$$\text{Aspartate} + \alpha\text{-ketoglutarate} \rightleftarrows \text{oxaloacetate} + \text{glutamate}$$

Aminotransferases are specific for acceptor and donor of an α-amino group, but aspartate aminotransferase besides L-aspartate and L-glutamate reacts also with L-tyrosinate, L-cysteine sulfonate, homocysteinate and also some aspartate analogous (KEESEY, 1987). The prosthetic group of aminotransferases is pyridoxal phosphate.

In conifers the AAT system is generally found to be encoded by three gene loci the isozymes of which are presumably confined to different subcellular compartments (CONKLE, 1981). Similarly, three AAT (or GOT) loci could be identified in several fir species (e.g. A. balsamea – NEALE and ADAMS, 1981; A. lasiocarpa – SHEA, 1988; A. pinsapo – PASCUAL et al., 1993), which was in accordance with the results on silver fir (A. alba) where three polymorphic loci (AAT-A – AAT-C) were identified in all seed and bud tissues (MEJNARTOWICZ, 1979, 1996; HUSSENDÖRFER et al., 1995; LEWANDOWSKI et al., 2001).

Based on improved extraction with non-ionic detergents and electrophoretic separation procedures it was possible to detect a fourth AAT activity zone in zymograms of seed tissue extracts. The investigation of the novel AAT patterns and their genetic control is presented in the following report.

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Material and Methods

For this study megagametophytes of seed samples collected from 260 open pollinated silver fir trees of 11 natural populations in the Polish Sudeten and Carpathian Mts. were analysed. For details of provenances see MEJNARTOWICZ (2000). The AAT isozymes were resolved by means of discontinuous horizontal starch gel electrophoresis, using. 0.2 M boric acid-lithium hydroxide, pH 8.1, as tray buffer and Tris/citric acid +10% electrode buffer and 2% sucrose, pH 8.3, as gel buffer. The starch-gel slabs (11,5%) had a distance of 12 cm and the separation was performed at constant power of 50 mA for about 5 hours.

The extraction buffer was a 0.1M Tris/HCl solution (pH 7.2) containing 150 µl mercaptoethanol / 100 ml buffer and in order to solve the membrane-bounded aminotransferases 200 µl of detergent Triton X-100 / 100 ml buffer was added. Seven megagametophytes from each individual tree were homogenized with this extraction buffer. The staining solution consisted of 100 ml 0.1 M dibasic sodium phosphate, pH 7.4, containing 100 mg Fast blue BB salt, 6.6 mg pyridoxal-5'-phosphate, 130 mg α-ketoglutaric acid, 230 mg L-aspartic acid and 6 mg Bovine albumin fraction V. The above methods are similar to those proposed by WENDEL and WEEDEN (1989).

Results and discussion

In almost all studies on isozyme inheritance and variation in silver fir, three distinct AAT (or GOT) zones have been identified so far, which were found to be controlled by three separate gene loci (MEJNARTOWICZ, 1979; FADY and CONKLE, 1993; HUSSENDÖRFER et al., 1995). Two zones (AAT-A, AAT-B or AAT-1, AAT-2) are migrating towards the anodal front in zymograms, whereas the third zone (AAT-C or AAT-3) is located near the origin of zymograms and is composed of two co-migrating bands (double-banded variants).

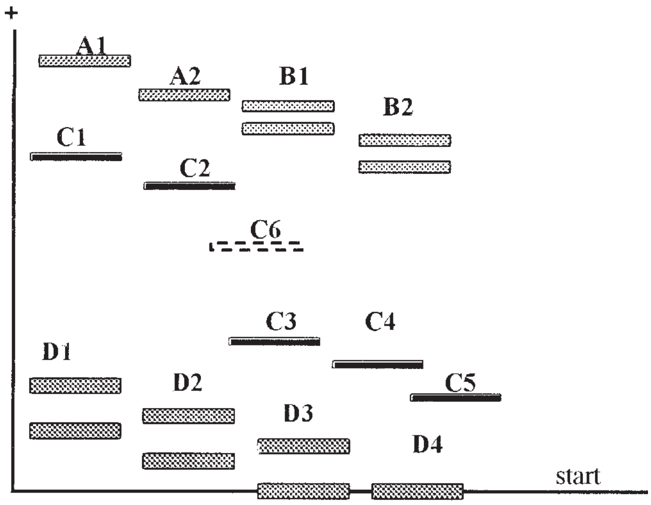

Figure 1. – Schematic illustration of the banding patterns found for AAT (GOT) in *Abies alba* megagametophyte. The diagram shows the position of all the bands observed and the genetic interpretation of the alleles of respective loci. Doted box C6 denoted recessive (null) allele.

After using the improved extraction and separation procedures, we have found in total four AAT activity zones in megagametophytes tissues of trees from several Polish mountain populations. Whereas the two more anodal zones correspond with the well-known AAT-A and AAT-B and possess two allelic variants each (A1, A2 and B1, B2), a new activity zone appeared in the middle part of zymograms and revealed a considerable amount of variation that was not affected by the band variation of the other AAT zones (*Fig. 1* and *Fig. 2*). This zone, now designated AAT-C, is found to be controlled by a separate gene locus, since two variants occurred alternatively and showed no significant deviation from a 1:1-ratio of segregation among the haploid megagametophytes of single putatively heterozygous trees e.g. Lądek No. 90, No. 82: and Międzygórze No. 62 (*Table 1*). In total five allozymes could be detected for AAT-C of which C1 is overlapping with allozymes of AAT-B and C5 with those of AAT-D (*Fig. 1*). Furthermore, one variant of AAT-C appeared to be a so-called null allele, and designated as: AAT-C6, since no activity could be detected in zymograms of haploid megagametophytes (*Fig. 2* and *Fig. 3*). The fourth AAT zone (former AAT-C) is now called AAT-D and showed the well-known double-banded variants near the cathodal end of zymograms (*Fig. 2* and *Fig. 3*).

Table 1. – Observed segregation of allozymes of zone AAT-C among seed megagametophytes from heterozygous trees of silver fir.

Tree No.	Allozyme variants	Observed segregation	N	χ^2-test (1 df)
Lądek 82	AAT-C1:C3	19 : 26	45	1.09 ns
Lądek 90	AAT-C3:C6	22 : 16	38	0.95 ns
Międzygórze 62	AAT-C2:C3	14 : 21	35	1.41 ns

Plant aminotransferases were found to be associated with four different subcellular compartments, such as plastids (pAAT), mitochondria (mAAT), glyoxisomes (gAAT) and cytosol (cAAT) (LIU and HUANG, 1977). Therefore, it is assumed that the four AAT loci now identified in silver fir encode the enzymes for these compartments. This result is in agreement with other studies on tree species where four AAT loci could be established, as for instance, in *Populus deltoides* and *P. nigra* (RAJORA, 1985), in *Ficus* (ELISIARIO et al., 1998) and in *Pinus roxburghii* (SHARMA and V. WUEHLISCH, 1998).

It is generally accepted that nuclear genes code for the isozymes functioning in different subcellular compartments (for review, see WADSWORTH, 1997), hence the results of the inheritance studies of AAT in silver fir are in agreement with this finding. However, it cannot be established which of the four loci is coding for which compartment isozyme in silver fir, since no cell fractionation experiments were performed. In many plants organelle-specific isozymes are often migrating faster than cytosolic forms and are displaying only relatively little variation, so it is imaginable that AAT-A and AAT-B are mitochondrial and/or plastid isozymes and the more variable AAT-D a cytosolic form. The isozyme AAT-C which could not be

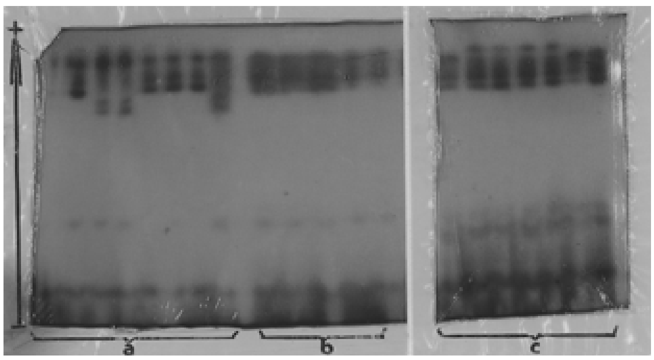

Figure 2. – Gel photograph showing *AAT* banding patterns of the haploid *Abies alba* Mill. megagametophyte from 3 trees. Genotypes *AAT* of tree *a*: A2A2B1B2C4C6D3D3, genotype of tree *b*: A2A2B1B1C4C4D3D3, and tree c: A1A2B1B1C4C4D1D1.

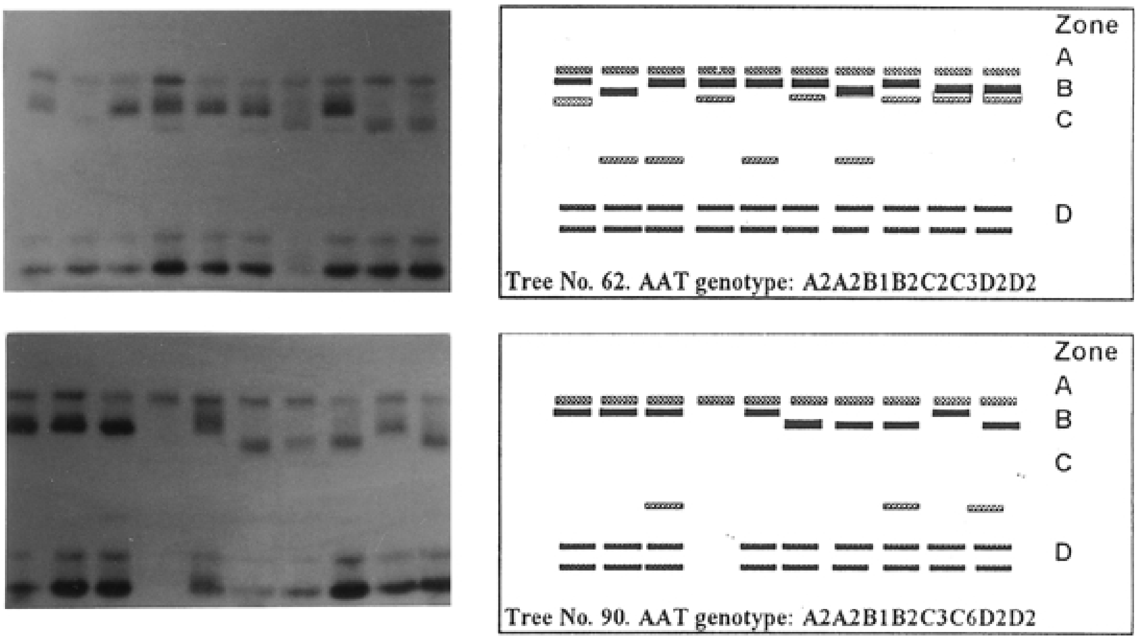

Figure 3. – Examples of AAT variation in locus AAT-C from 10 megagametophytes of Międzygórze tree No.62 and from Lądek population tree No. 90. Photos and schematic illustrations.

detected in earlier studies may be associated with the glyoxisomes (or peroxisomes) and becomes visible only after liberation from this organelle with the aid of a particular extraction procedure having non-ionic detergent as Triton X-100, solubilizing of membrane proteins and stabilizing the enzymes.

Conclusion

Based on the results of AAT electrophoresis of seed megagametophytes from silver fir, it is evident that the isozymes of this system are encoded by four loci. They are probably associated with different subcellular compartments, such as mitochondria, plastids, cytosol and glyoxisomes. At present state of knowledge it is not possible to establish which locus is coding for which subcellular isozyme. To solve this problem additional studies on cell fractionation of seed tissues from silver fir are needed.

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References

CONKLE, M. T.: Isozyme variation and linkage in six conifer species. *In:* Isozymes of North American Forest Trees and Forest Insects. General Techn. Report PSW-48, Berkeley, p.11–17 (1981). — ELISIARIO, P. J., NETO, M. C, CABRITA, L. F., LEITAO, J. M., AKSOY, U., FERGUSON, L. and HEPAKSOY, S.: Isozyme and RAPDs characterization of a collection of fig (*Ficus carica* L.) traditional varieties. Acta-Horticulturae: **480**: 149–154 (1998). — FADY, B. and CONKLE, M. T.: Allozyme variation and possible phylogenetic implications in *Abies cephalonica* Loudon and some related eastern Mediterranean firs. Silvae Genetica **42**, 351–359 (1993). — HUSSENDÖRFER, E., KONNERT, M. and BERGMANN, F.: Inheritance and linkage of isozyme variants of silver fir (*Abies alba* Mill.). Forest Genetics **2**, 29–40 (1995). — IRELAND, R. J. and JOY, K. W.: Plant transaminases. *In:* Transaminases, CHRISTEN, P. and METZLER, D. E. (eds.), John Wiley & Son, New York, pp. 376–384 (1985). — LEWANDOWSKI, A., FILIPIAK, I. and BURCZYK, J.: Genetic variation of *Abies alba* Mill. in Polish part of Sudety Mts. Acta Soc. Bot. Pol. **70**, 215–219 (2001). — KEESEY, J.: Glutamate-Oxalacetate-Transaminase (GOT). Biochemical Information. Boehringer Mannheim Biochemicals. Indianapolis: **33** (1987). — LIU, K. D. F. and HUANG, A. H. C.: Subcellular localisation and developmental changes of aspartate-a-ketoglutarate transaminase isozymes in the cotyledons of cucumber seedlings. Plant Physiol. **59**, 777–782 (1977). — MEJNARTOWICZ, L.: Polymorphism at the LAP and GOT loci in *Abies alba* Mill. populations. Bulletin de l'Académie Polonaise des Sciences. Série des sciences biologiques. Cl.V. Vol. 27(12): 1063–1070 (1979). — MEJNARTOWICZ, L.: Cisovka – the relic population of *Abies alba* and its relationship to man-made silver-fir stands in Białowieża Primeval Forest. Acta Soc. Bot. Pol. **65**(3-4): 319–328 (1996). — MEJNARTOWICZ, L.: Polish Sudeten and Carpathian Mountains Silver-fir (*Abies alba*) population genetic investigation. IUFRO WP: 1.05–16. *Ecology and Silviculture of European Silver Fir.* Proc. of the 9th International European Silver Fir Symposium. May 21–26, 2000 Skopje. Rep. of Macedonia: 49–54 (2000). — NEALE, D. B. and ADAMS, W. T.: Inheritance of isozyme variants in seed tissues of balsam fir (*Abies balsamea*). Canadian J. Botany **59**, 1285–1291 (1981). — PASCUAL, L., GARCIA, F. J. and PERFECTTI, L.: Inheritance of isozyme variations in seed tissues of *Abies pinsapo* Boiss. Silvae Genetica **42**, 335–340 (1993). — RAJORA, O. P.: Studies into genetics and species relationships of *Populus deltoides* Marsh., and *P. nigra* L. and *P. maxiimoviczii* Henry based on isozymes, pollen competition and leaf morphology. Ph.D. Thesis , University of Toronto (1985). — SHARMA, K. and V. WUEHLISCH, G.: Genetic interpretation of malate dehydrogenase (MDH) isozyme gene loci using a new staining approach and genetic control of ten other isozymes in *Pinus roxburghii* Sarg. Silvae Genetica **47**(5–6): 321–332 (1998). — SHEA, K. L.: Segregation of allozyme loci in megagametophytes of Engelmann spruce and subalpine fir. Genome **30**, 103–107 (1988). — WENDEL, J. F. and WEEDEN, N. F.: Visualization and interpretation of plant isozymes. *In:* Isozymes in Plant Biology. SOLTIS, D. E. and SOLTIS P. S. (Eds.). Chapman & Hall, London, pp. 5–45 (1989). — WADSWORTH, G. J.: The plant aspartate aminotransferase gene family. Physiologia Plantarum **100**: 988–1006 (1997).