Identification of QTLs Controlling Growth, Chemical and Physical Wood Property Traits in *Pinus pinaster* (Ait.)

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Abstract

Growth characteristics and wood quality represent important criteria in forestry. Dissection of these quantitative traits into single genetic components using molecular markers and linkage maps, provide useful information for breeders about the genetic determinism of polygenic traits. In order to apply marker assisted breeding in early selection procedures, QTL analyses for growth traits and physical and chemical wood properties were performed in a *Pinus pinaster* progeny. A high-density linkage map of *P. pinaster* was used for this purpose. No significant correlations were found between wood quality and growth traits. A total of 10 QTLs were detected for the growth traits height and diameter and 40 QTLs for seven wood parameters including α-cellulose content, lignin content, pulp yield, brightness and wood densities. QTLs were spread across the genome. The percentages of phenotypic variances explained by individual QTLs ranged from to 4.3% to 18.4% and the sum of trait-specific QTLs explained 20 to 53% of the total variance. The usefulness of marker assisted selection for wood quality traits is discussed.

Key words: Pinus pinaster, QTL analyses, growth properties, α-Cellulose, lignin content, CIE, wood density, pulp yield, marker assisted selection

Introduction

Pinus pinaster is native to the western Mediterranean basin and the atlantic coast of north Africa and Europe, from western France to Morocco. Within its natural range the species is split into numerous scattered provenances occurring in highly variable edaphic and climatic conditions. It has been intensively used for reforestation in south-western Europe (France, Spain, Portugal) where it currently stretches on over four million ha, and secondarily introductions have been made in South America (Chile, Argentina), Australia, New Zealand and South Africa.

The breeding programs concerning this species were initially started in the sixties by INRA-France. Provenance tests were set up using material collected from the whole natural distribution area, allowing a description of the genetic variation of characters affecting growth, cold hardiness, drought tolerance and disease sensitivity. Improved material was produced using classical breeding methods resulting in a twofold increase of productivity during the three last decades (from 4.5 to 9 m^3/ha/year).

Meanwhile, the priorities of national breeding programs have changed. Special emphasis has been put on wood quality, and the main issue expected in the next future, will be the production of improved clones specifically targeted for growth, environmental adaptability, wood properties and disease resistance traits, or for selected combinations of some of these characters, according to the needs of foresters and industry. In this context, particular attention is dedicated to wood properties. Industry increasingly requires that breeding programs closely adapt their programs to the needs of the production processes, and the availability of raw material with uniform physical and chemical characteristics is a crucial objective. Therefore, investigation of genetic control of wood quality, involving identification of genes that govern these complex traits, is of key importance. Detection of quantitative trait loci (QTLs) is a promising method to reach this aim, as several examples in different crop species show (Hittalmani et al., 2002, Schneider et al., 2002, Kato et al., 2000, Marquez et al., 2001).

An essential pre-requisite for the establishment of QTL's is the availability of detailed linkage maps. These allow to QTL positions to be determined, to identify the magnitudes of their effect(s), and to detect the mode of gene action (Lander and Botstein, 1989). Genetic maps have been established in recent years for several important forest species, including the main conifer species of economic importance, including *Larix* (*L. decidua* and *L. kaempferi*, Arcade et al., 2000), *Picea* (*P. abies*, Binelli et al., 1994, Paglia et al., 1998; *P. glauca*, Gosselin et al., 2002), and *Pinus* (*P. radiata*, Devey et al., 1996; *P. taeda*, Remington et al., 1999; *P. sylvestris*, Lerceteau et al., 2000; *P. pinaster*, Plomion et al., 1995, Costa et al., 2000, Chagné et al., 2002, Ritter et al., 2002). QTL analyses subsequently developed in these species allowed the detection of individual loci controlling the variation of tree growth, form and environmental adaptability, as well as physical and chemical wood properties. Groover et al. (1994) and Sewell et al. (2000, 2002) identified several QTLs influencing wood properties in Loblolly pine. In *Pinus sylvestris*, Hurme et al. (2000) located QTLs for bud set and frost hardiness, and Lerceteau et al. (2000) detected loci controlling wood volume. Some QTLs involved in the control of adaptive traits such as the timing of vegetative bud set, spring and fall cold hardiness have been mapped also in Douglas fir (Jermstad et al., 2001a, 2001b).

The present work describes QTL analysis carried out in *Pinus pinaster* based on a high-density map composed of AFLP-, SSR- and EST- markers as published by Ritter et al. (2002). The results concern growth characters and also, for the first time in this species, physical and chemical wood properties. The only QTLs which were published to date in this species, considered total height and germination date (Plomion et al., 1996). The novel QTL information provided will help in the development of marker assisted breeding programs.

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Material and Methods

Plant material

The *Pinus pinaster* hybrid family n° 161 was selected for linkage mapping and QTL analyses. This family was descended from an interracial hybridization between parental clone 0024 (Landes provenance, selected for growth) and clone C803 (Corsican provenance, selected for straightness) in order to combine these particular characteristics. A F1 full-sib progeny of 80 individuals (established in the field in 1990) was available for the analyses.

Estimation of growth characteristics and wood properties

Height and diameter

Height and diameter of the progeny was determined in autumn 1999 at an age of 9-years. Height was measured with a graduated pole for heights up to 8 m and with a vertex (Haglöf, Sweden) for heights beyond 8 m. Diameter data were calculated from circumference data measured at breast height with a ribbon rule (AFNOR, 1984).

Chemical composition

I. Extractives content

Extraction of wood sawdust (40–60 mesh fraction) was carried out using an automatic Soxtec apparatus (extraction unit 2050 by Foss Tecator, Denmark). Water-soluble compounds were extracted first for 6 hours, followed by a 3-hour extraction cycle with acetone. Total extractives contents were determined as weight ratios between both, water- and acetone-soluble compounds, and non-extracted oven dried wood (Ugletto et al., 1997).

II. Lignin and α-cellulose content prediction by near infrared spectroscopy

The measurements were performed with a BIORAD spectrometer which was configured to near-infrared or middle-infrared. The NIR spectra were measured in diffuse reflectance mode. NIR method requires samples to be milled (40–60 mesh fraction) and extracted before spectra acquisition. The principle of properties determination is based on the creation and the validation of a calibration model, which correlates spectroscopic results to laboratory measurements. All calibration models were developed using QUANT software. A large number of samples (510) was used for the calibration model construction. Three quarters of the total number of samples were used for this purpose, the remaining fourth was reserved validation purposes. This calibration was performed by a multivariate analysis with the PLS (Partial Least Squares) regression procedure on a specific region of the IR spectra. After the calibration model was obtained, two different validations procedures were performed. The first one, called cross-validation consisted of removing 10 random samples from those used for the calibration and re-validating the calibration with the remaining samples. The procedure was repeated several times, in order to check all points and to remove aberrant points from the calibration. The best correlation was then adopted. The second validation procedure consisted of using the calibration model adopted after the cross-validation test to predict the properties of the remaining fourth set of samples and to compare the predicted values with laboratory measurements (Lorber and Kowalski, 1990).

III. Kraft cooking and pulp colour

Kraft cooking runs were carried out in micro-digesters of 12 ml, using 1.5 g of oven dried wood. Micro-digesters were placed in a rotating device immersed in an oil bath that allows a large number of simultaneous cookings to be performed under the same conditions. Cooking conditions were: liquor alkaliny/sulphidity = 22/32, liquor/wood ratio = 4; H-factor = 1600, temperature profile : 20–170 °C in 90 min. followed by a plateau at 170 °C for 60 min. and a rapid cooling with cold water. Yield was determined as the ratio of the amount of pulp recovered over cooked wood. The amount of pulp was too small to determine the residual lignin content by the kappa number method. Therefore, only CIE color indexes (L*, a*, and b*) were determined by using a Minolta CM-3630 spectrophotometer. L* is a gray level scale (0 being black and 100 being white), a* is a green (negative values) – red (positive values) axis and b* is a blue (negative values) – yellow (positive values) axis (Hunter, 1975).

Wood density

In order to obtain microdensitometric profiles, 5 mm diameter longitudinal wood cores from the logs were sampled. Then 2 mm thick segments were cut from the cores by using twin saws. The samples were x-rayed and the radiographic films digitalized with a high resolution scanner. Density levels were associated to gray levels in the image by means of a calibration curve which had been previously established. Windendro® software was used to delimitate the annual growth ring and to calculate the microdensitometric profiles. The following parameters were analyzed: ring width, minimum density, maximum density, average density and the standard deviation. From these densitometry data, average wood density and biomass weight were calculated for the disks of the corresponding the cores (Chantre et al., 2000).

QTL analyses

The complete high density linkage map published by Ritter et al. (2002) was used to identify genomic regions controlling growth rate, chemical and physical wood properties of *P. pinaster*. The traits for which QTL analyses were performed are presented in *table 1A*. QTLs were mapped using least square interval mapping method developed for backcross progenies according to Knapp et al. (1990) and Knapp and Bridges (1990), and applied to all intervals composed of individual markers from both parents. SAS software (SAS Institute Inc. 1989) and, in particular, the procedure PROC NLIN was used for computational analysis.

The model is described in detail in the cited literature and consists of solving for each interval defined by two adjacent marker loci A and B and a putatuve QTL located within this interval a set of non linear equations. Model equations for the observed phenotypic value of each progeny genotype are based on the distribution of expected frequencies of the different genotypes at the QTL within each marker class. These expected frequencies depend on the recombination frequency r1 between marker locus A and the putative QTL. The unknown parameters to be estimated are (i) the expected mean values in terms of trait expression of the different genotypes at the QTL; and (ii) the recombination frequency r1 (the recombination frequency between loci A and B is known).

If valid estimates of the parameters are obtained, the significance of this "full model" can be tested based on the L-value of a likelihood ratio test (Knapp and Bridges, 1990; Galant, 1987). For this purpose, a separate analysis under the assumption that no QTL is present in the interval is performed, based on a simple regression analysis (= reduced model), in order to obtain the sum of squares of the corresponding error.

The test parameter L is obtained by:

$$L = \frac{(SSEr - SSEf)\, dff}{(dfr - dff)\, SSEf}$$

where *SSEf* and *SSEr* are the error sums of squares of the full and reduced models, respectively, and dff and dfr are the corresponding degrees of freedom. The *L* value is tested against the F-value with dfr and dff degrees of freedom. If the resulting probability is less than ∝ (Type I probability), the null hypothesis of no QTL can be rejected. The percentage of total variance explained by the sum of the individual QTLs was calculated by performing simple multiple regression analysis on the corresponding intervals (KEARSEY and HYNE, 1994). This procedure for QTL analyses has been applied also recently by HERRÁN et al. (2000), LEBRUN et al. (2001) and LARRAYA et al. (2002).

Results

Trait analyses

Means and coefficients of variation for the traits under study are presented in *table 1A*. The observed coefficients of variation were in general low for all characters and ranged between 2 and 12%, except for extractive contents (22.7%).

The correlation coefficient between alpha cellulose and lignin content showed a significant positive correlation. Both traits were highly negatively correlated with extractives content and to a lesser degree with brightness. CIE brightness showed a positive correlation with extractive content, and mean, minimal and maximal wood density were also significantly correlated. No significant phenotypic correlations between growth characteristics and wood quality traits were detected, except a slight negative correlation between diameter and brightness.

QTLs for Growth characteristics

Ten putative QTL were detected for the growth traits height and diameter at significant levels of P<0,05 (*Table 2*). Six were descended from parent 0024 while the others were from parent C803. Seven QTL controlled stem diameter and were distributed on 5 linkage groups (*figure 1*). The explained variation associated with single QTL ranged from 5 to 14.5%. In total, the seven QTL accounted for 48.5% of the phenotypic variance. Three putative QTLs were detected for height and were located on linkage groups 2, 6 and 10. They explained 22% of the total variation. The effects of QT allele differences relative to the corresponding trait means varied between 6.2 and 7.9%.

QTL for wood quality

In total, forty significant QTLs (α=5%), were detected for wood quality characters. Sixty percent of mapped QTLs originated from the male parent C803 (*Fig. 1*). For each trait, between two and eight QTLs were located on the *Pinus pinaster* map (*Tab. 2*). The highest number of QTLs were detected for mean wood density, followed by pulp yield (7 QTLs), whereas the lowest number of QTLs were observed for extractives content. The strongest QTL (Q3.4) was identified for pulp yield, accounted for 18.43% of the trait variance and showed the highest confidence level (probability value) of all QTLs identified in this study. The QTL (Q4.1) which explained the least of the total variance (4.3%) was identified for extractive content. The lengths of the intervals between the QTLs located, varied between 1.0 cM (Q6.5) and 17 cM (Q2.1). The proportion of phenotypic variance explained by the QTL ranged from 4.3 to 18.4%. The total variances explained by all QTLs corresponding to each trait are also given in table 2. Their highest values were detected for mean wood density (52,5%), followed by pulp yield (47.3%). The relative effects of the QT allele differences varied in general between 1.8 and 9.7% but were somewhat higher for QTLs related to extractive contents (>14%).

QTLs were distributed over all of the 12 linkage groups and all traits showed QTL locations on several different linkage groups. Some linkage groups such as LG3 and LG10 harboured QTLs for six and seven different traits, while on LG11, LG1, LG4, LG5 and LG9 only QTLs for one or two different characters were present. However, frequent closely linked (or probably identical) QTLs for different traits were detected. Six pairs of QTLs were identified even with the same linkage group interval (*Fig. 1*). For wood density, three paired QTLs within identical intervals were detected on LG1 (Q6.1, Q8.1), LG3 (Q6.3, Q7.2) and LG12 (Q6.8, Q7.3), respectively. The same was true for a QTL for α-cellulose (Q1.3) and a QTL for extractives content (Q4.1) on LG8. Further QTLs located within same interval were found for lignin content (Q2.6) and extractives content (Q4.2) on linkage group 10, and for pulp yield (Q3.1) and mean wood density (Q6.2) on LG3. Moreover, in several cases, closely linked QTLs were found in overlapping, adjacent

Table 1. – Characteristics of the traits used for QTL analyses in the *P. pinaster* progeny 0024 x C803.

A) Mean values, ranges and coefficients of variation

Code[a]	Property	Scale	Mean	Min	Max	CV
Q1	Alpha cellulose content	%	44.34	41.12	46.98	2.84
Q2	Lignin content	%	26.60	23.75	29.20	3.97
Q3	Pulp yield	%	37.58	33.25	40.38	3.80
Q4	Extractives content	%	9.96	3.98	17.94	22.7
Q5	CIE Brightness	-	71.71	67.61	75.07	2.1
Q6	Mean wood density	kg/dm^3	0.408	0.341	0.500	7.5
Q7	Minimum wood density	kg/dm^3	0.264	0.224	0.301	7.3
Q8	Maximum wood density	kg/dm^3	0.803	0.684	0.956	7.3
Q9	Diameter	cm	47.13	29	61	11.8
Q10	Growth height	dm	77.26	58	105	11.4

a: Code used for detected QTLs in *Table 2* and *Figure1.*

B) PEARSON's correlation coefficients and significance levels for relationships between characters used for QTL analyses in the *P. pinaster* progeny 0024 x C803.

	AC[a]	LC[b]	PY[c]	EC[d]	CIE-B[e]	MEWD[f]	MIWD[g]	MAWD[h]	DIA[i]	HG[j]
AC	-	0.36**	ns	-0.77**	-0.32*	ns	ns	ns	ns	ns
LC		-	ns	-0.81**	-0.31*	ns	ns	ns	ns	ns
PY				ns	Ns	ns	ns	ns	ns	ns
EC				-	0.40**	ns	ns	ns	ns	ns
CIE-B					-	ns	ns	ns	-0.26*	ns
MEWD						-	0.72**	0.39**	ns	ns
MIWD							-	0.36**	ns	ns
MAWD								-	ns	ns
DIA									-	ns
HG										-

a: Alpha cellulose content; b: Lignin content; c: Pulp Yield; d: Extractives content; e: CIE-Brightness; f: Mean wood density; g: Minimum wood density; h: Maximum wood density; i: Diameter; j: Height growth. Significant at * P = 0.05, ** P=0.001, ns = not significant.

or closely linked intervals. This was the case for two QTLs for lignin content which were located beside two QTLs for stem diameter on LG 7 and LG 10 (Q2.3 and Q9.4; Q2.5 and Q9.7, respectively). Co-locations of a QTL for height and minimum wood density on linkage group 2 (Q10.1, Q7.1) and for pulp yield and maximum wood density (Q3.2, Q8.3) on LG5 were observed. Also two QTLs for mean wood density (Q6.6, Q6.4) on LG 10 were closely linked to QTLs for lignin (Q2.6.) and extractives contents (Q4.2).

In general all interval flanking QTL markers were AFLP markers. However, for two QTLs controlling minimum and maximum wood density (Q7.1 on LG2 and Q8.3 on LG5) two codominant SSR markers are directly available (RTPEST11 and PR092a, respectively).

Table 2. – List of QTLs detected in the *P. pinaster* progeny 0024 x C803.

Code	Effect	RE[a]	LG	Interval	R[b]	R1[c]	PRL[d]	R2[e]	R2 total
Alpha-cellulose content									
Q1.1	0.82	1.85	2	242/6-22/2	16.7	16.7	3.54	5.98	
Q1.2	0.84	1.89	7	52/4-232/1	9.5	9.5	2.26	9.42	
Q1.3	0.92	2.07	8	77/12-73/3	8.4	0.0	1.44	12.24	
Q1.4	0.84	1.89	10	228/3-172/6	1.7	0.1	3.17	10.56	
Q1.5	0.81	1.83	12	105/6-184/4	1.5	0.0	3.28	9.76	39.31
Lignin content									
Q2.1	0.69	2.59	3	68/1-150/1	17.0	17.0	4.23	5.98	
Q2.2	0.62	2.33	6	255/2-107/9	10.9	10.9	4.80	7.95	
Q2.3	0.75	2.82	7	241/11-54/4	13.7	1.4	2.12	10.09	
Q2.4	0.64	2.41	9	257/7-254/5	3.7	0.0	4.40	7.07	
Q2.5	0.61	2.29	10	152/11-58/9	1.3	1.3	4.79	7.96	
Q2.6	0.81	3.05	10	165/1-152/17	2.8	2.8	0.84	13.17	38.72
Pulp Yield									
Q3.1	0.98	2.61	3	191/1-157/3	8.7	4.6	2.99	10.67	
Q3.2	0.90	2.39	5	149/1-58/1	1.9	0.7	3.22	9.65	
Q3.3	0.88	2.34	6	166/1-60/6	6.3	4.2	3.15	10.98	
Q3.4	1.23	3.27	6	181/4-32/4	1.4	1.4	0.17	18.43	
Q3.5	1.23	3.27	6	13/4-76/4	11.6	11.6	0.47	14.38	
Q3.6	1.13	3.01	8	69/1-147/17	9.7	0.0	0.28	9.79	
Q3.7	1.01	2.69	11	158/2-180/6	15.4	15.4	1.11	10.28	47.25
Extractives content									
Q4.1	1.36	13.65	8	77/12-73/3	8.4	0.0	4.27	4.26	
Q4.2	1.69	16.97	10	165/1-152/17	2.8	0.6	1.38	13.36	20.32
CIE-Brightness									
Q5.1	1.17	1.63	3	78/1-8/5	7.1	5.5	2.09	13.02	
Q5.2	0.97	1.35	6	157/1-239/5	1.8	0.0	2.20	7.76	
Q5.3	1.16	1.62	7	58/7-148/10	3.2	2.8	0.35	13.74	32.09
Mean wood density									
Q6.1	0.021	5.15	1	147/5-63/8	5.6	5.6	1.95	12.33	
Q6.2	0.021	5.15	3	19/1-157/3	8.7	7.9	1.83	9.34	
Q6.3	0.024	5.88	3	29/3-52/2	3.9	3.9	0.76	6.17	
Q6.4	0.021	5.15	10	220/1-156/6	6.7	5.0	1.53	11.52	
Q6.5	0.019	4.66	10	143/4-169/6	1.0	0.0	2.96	9.21	
Q6.6	0.021	5.15	10	144/1-165/1	4.4	2.6	3.74	10.96	
Q6.7	0.024	5.88	12	57/8-147/19	10.1	1.5	1.05	11.76	
Q6.8	0.022	5.39	12	239/4-240/8	1.1	1.4	1.04	12.95	52.54
Minimum wood density									
Q7.1	0.011	4.17	2	RTPEST11-54/7	0.8	0.0	4.98	7.67	
Q7.2	0.013	4.92	3	29/3-52/2	3.9	3.9	2.23	5.09	
Q7.3	0.011	4.17	12	239/4-240/8	1.1	1.1	5.34	8.58	18.49
Maximum wood density									
Q8.1	0.038	4.73	1	147/5-63/8	5.6	0.0	2.61	9.77	
Q8.2	0.037	4.61	4	255/7-232/2	9.9	0.0	4.80	7.50	
Q8.3	0.048	5.98	5	31/4-Pr092a	3.9	3.2	0.41	14.39	
Q84	0.038	4.73	5	253/5-53/4	2.4	1.5	3.19	10.68	
Q8.5	0.046	5.73	7	40/3-198/4	1.3	1.3	0.77	14.51	
Q8.6	0.038	4.73	10	252/1-30/1	7.1	0.0	2.39	10.25	42.29
Diameter (BHD)[f]									
Q9.1	3.41	7.24	3	229/3-171/7	2.5	0.0	2.96	8.54	
Q9.2	3.35	7.11	4	143/10-154/3	5.1	0.0	2.80	7.53	
Q9.3	3.98	8.44	4	145/3-60/3	2.0	0.0	0.62	9.27	
Q9.4	3.13	6.64	7	54/4-170/5	9.0	0.0	4.87	5.04	
Q9.5	4.59	9.74	9	147/12-30/6	6.6	4.8	0.39	14.51	
Q9.6	3.18	6.75	9	90/2-65/1	7.6	0.8	4.21	7.61	
Q9.7	3.71	7.87	10	58/9-235/3	1.2	1.2	0.97	10.70	48.48
Height growth									
Q10.1	5.63	7.29	2	23/7-40/9	10.5	2.4	2.97	9.78	
Q10.2	4.81	6.23	6	240/2-8/9	6.3	0.0	5.65	4.93	
Q10.3	4.25	5.50	10	65/4-147/10	5.6	0.0	5.92	5.80	21.84

[a] RE = relative effect of QT allele differences in percentage of the trait mean. [b] interval length in the linkage map (*Fig. 1*). [c] position with respect to upper flanking marker in the map (*Fig. 1*). [d] probability for null hypothesis of no QTL (%). [e] proportion of total variance explained by the QTL. f BHD = breast height diameter. R2 total indicate the percentage of total variance explained by the sum of the individual QTLs detected for a trait. QTL code in bold italics indicate QTLs descending from the seed parent 0024.

Discussion

Most QTL studies have been carried out for growth property traits in forest tree species (PLOMION et al., 1996, BRADSHAW and STETTLER, 1995; GRATTAPAGLIA et al., 1995). Only few QTL analyses are available for chemical and physical wood properties (GROOVER et al, 1994; SEWELL et al. 2000, 2002; ARCADE et al., 2002), and to our knowledge, this is the first report of QTL mapping for *Pinus pinaster* which also considers pulp yield.

We detected two to eight QTLs for growth traits, pulp yield and wood quality traits spread over all the 12 linkage groups, which correspond to the 12 chromosomes of the *P. pinaster* genome (RITTER et al., 2002). Individual QTLs accounted for 4 to 18% of the phenotypic variance and for most traits, the total phenotypic variation explained by all trait-specific QTLs was higher than 30%, raising up to 53% for mean wood density. For some traits such as extractives content, CIE brightness, or height only two or three QTL were detected, suggesting that only a few genes might control the expression of these characters. However, results of QTL analyses are generally influenced by factors such as degree of homozygousity or effects of QT allele differences at a QTL or by progeny size and genotype-environment interactions (VAN OOIJEN, 1992).

Ten putative QTL were found to be significantly associated with stem growth variables and spread across 6 of the 12 linkage groups. The variance explained by single QTLs ranged from 7.5% to 14.5%. These results are comparable with those previously published for other pine species such as *Pinus sylvestris* (LECERTEAU et al., 2000) and *Pinus taeda* (SEWELL et al., 2000).

Forty QTL spread over all linkage groups were found for seven physical and chemical wood properties. Trait specific QTLs were always located on several, different linkage groups. As with loblolly pine, dispersed genomic regions harboring QTL influencing wood specific gravity and chemical wood property, respectively, were detected by GROOVER et al. (1994) and SEWELL et al. (2002).

In our study extractive contents was negatively correlated with alpha cellulose and lignin content, and these latter two characters showed a positive correlation. These findings are reflected in the co-location of QTLs for these traits on linkage groups 8 and 10. Considering that wood is composed of approximately 95% lignin and holocellulose (α-cellulose and hemicellulose) the detected positive correlation of lignin and α-cellulose contents was unexpected. One might expect these characters to be inverse related (PANSHIN and de ZEEUW, 1980) as it was the case in different studies (SEWELL et al., 2002, HU et al., 1999).

Precise knowledge of QTL locations for traits of interest, the magnitude of the effects of the allelic differences at these QTLs and their biological significance, is necessarily required for an efficient application of marker assisted selection (MAS) in tree breeding (O'MALLEY and MCKEAND, 1994; HOSPITAL et al., 1997; MOREAU et al., 1998). Wood quality represents one of the most important criteria in forestry for which MAS may lead to genetic improvement, since this trait shows high heritability and requires several years to reach full expression. Applying MAS on a combined selection on diameter and wood density for

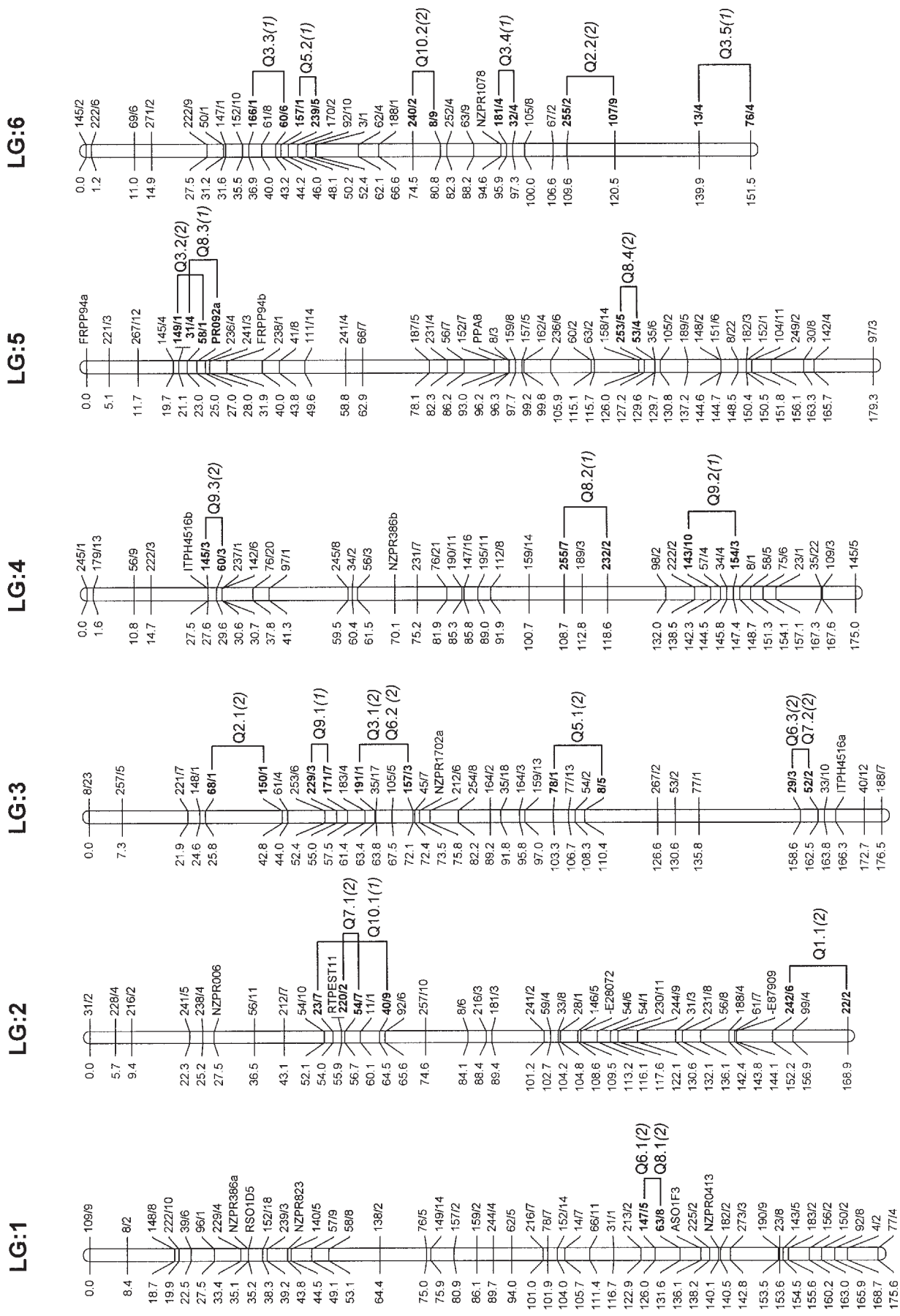
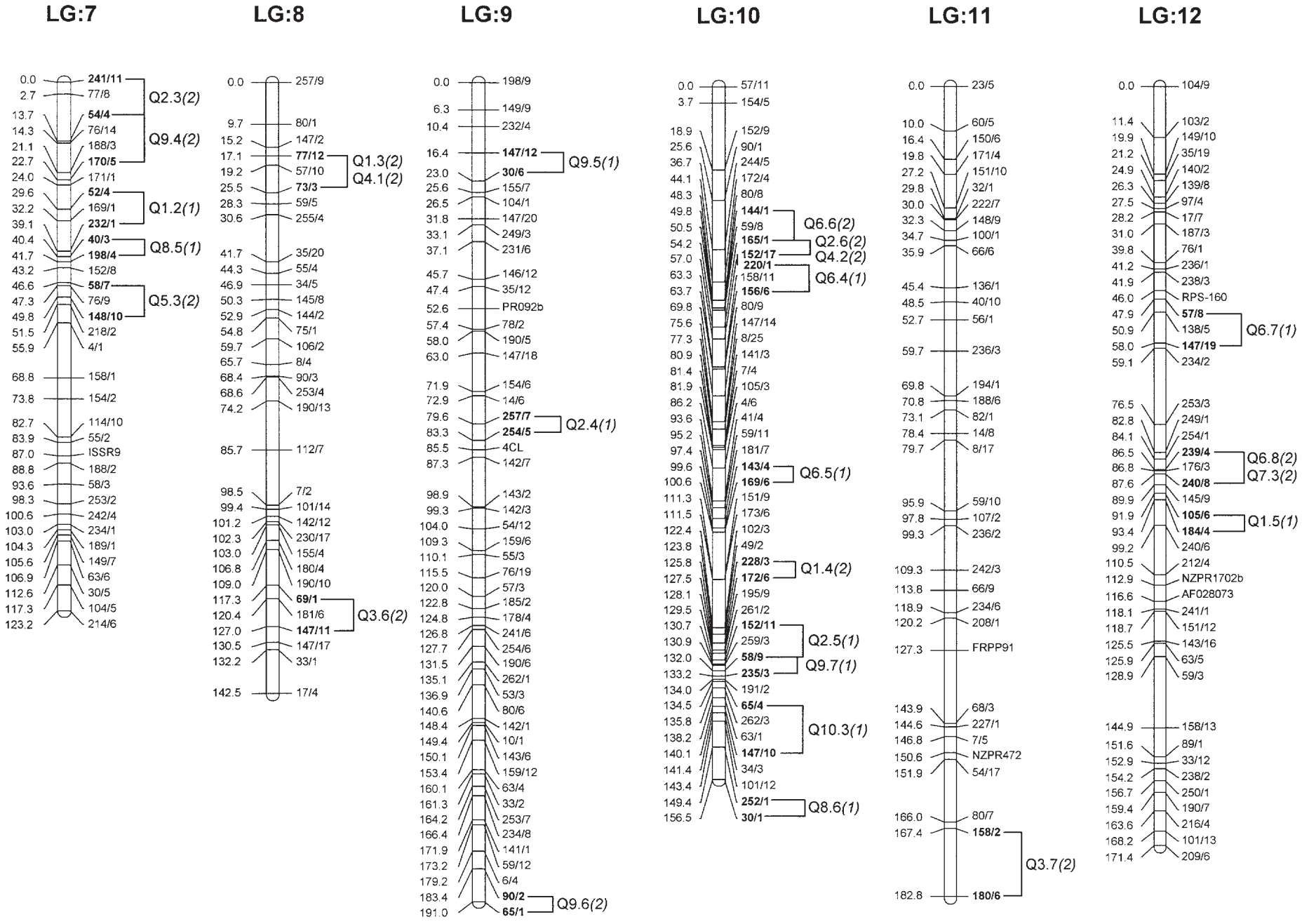


Figure 1. – Integrated linkage map of *Pinus pinaster* derived from population 0024 x C803 and QTL locations for growth characters and wood quality. QTL names refer to those described in detail in *Table 2*. Values in brackets behind the QTL name indicate the allocation of the QTL: 1: seed parent 0024 ; 2: pollen parent C803.


LG:7
0.0 241/11
2.7 77/8
13.7 54/4
14.3 76/14
21.1 188/3
22.7 170/5
24.0 171/1
29.6 52/4
32.2 169/1
39.1 232/1
40.4 40/3
41.7 198/4
43.2 152/8
46.6 58/7
47.3 76/9
49.8 148/10
51.5 218/2
55.9 4/1
68.8 158/1
73.8 154/2
82.7 114/10
83.9 55/2
87.0 ISSR9
88.8 188/2
93.6 58/3
98.3 253/2
100.6 242/4
103.0 234/1
104.3 189/1
105.6 149/7
106.9 63/6
112.6 30/5
117.3 104/5
123.2 214/6
Q2.3(2)
Q9.4(2)
Q1.2(1)
Q8.5(1)
Q5.3(2)
LG:8
0.0 257/9
9.7 80/1
15.2 147/2
17.1 77/12
19.2 57/10
25.5 73/3
28.3 59/5
30.6 255/4
41.7 35/20
44.3 55/4
46.9 34/5
50.3 145/8
52.9 144/2
54.8 75/1
59.7 106/2
65.7 8/4
68.4 90/3
68.6 253/4
74.2 190/13
85.7 112/7
98.5 7/2
99.4 101/14
101.2 142/12
102.3 230/17
103.0 155/4
106.8 180/4
109.0 190/10
117.3 69/1
120.4 181/6
127.0 147/11
130.5 147/17
132.2 33/1
142.5 17/4
Q1.3(2)
Q4.1(2)
Q3.6(2)
LG:9
0.0 198/9
6.3 149/9
10.4 232/4
16.4 147/12
23.0 30/6
25.6 155/7
26.5 104/1
31.8 147/20
33.1 249/3
37.1 231/6
45.7 146/12
47.4 35/12
52.6 PR092b
57.4 78/2
58.0 190/5
63.0 147/18
71.9 154/6
72.9 14/6
79.6 257/7
83.3 254/5
85.5 4CL
87.3 142/7
98.9 143/2
99.3 142/3
104.0 54/12
109.3 159/6
110.1 55/3
115.5 76/19
120.0 57/3
122.8 185/2
124.8 178/4
126.8 241/6
127.7 254/6
131.5 190/6
135.1 262/1
136.9 53/3
140.6 80/6
148.4 142/1
149.4 10/1
150.1 143/6
153.4 159/12
160.1 63/4
161.3 33/2
164.2 253/7
166.4 234/8
171.9 141/1
173.2 59/12
179.2 6/4
183.4 90/2
191.0 65/1
Q9.5(1)
Q2.4(1)
Q9.6(2)
LG:10
0.0 57/11
3.7 154/5
18.9 152/9
25.6 90/1
36.7 244/5
44.1 172/4
48.3 80/8
49.8 144/1
50.5 59/8
54.2 165/1
57.0 152/17
63.3 220/1
63.7 158/11
69.8 156/6
75.6 80/9
77.3 147/14
80.9 8/25
81.4 141/3
81.9 7/4
86.2 105/3
93.6 4/6
95.2 41/4
97.4 59/11
99.6 181/7
100.6 143/4
111.3 169/6
111.5 151/9
122.4 173/6
123.8 102/3
125.8 49/2
127.5 228/3
128.1 172/6
129.5 195/9
130.7 261/2
130.9 152/11
132.0 259/3
133.2 58/9
134.0 235/3
134.5 191/2
135.8 65/4
138.2 262/3
140.1 63/1
141.4 147/10
143.4 34/3
149.4 101/12
156.5 252/1
30/1
Q6.6(2)
Q2.6(2)
Q4.2(2)
Q6.4(1)
Q6.5(1)
Q1.4(2)
Q2.5(1)
Q9.7(1)
Q10.3(1)
Q8.6(1)
LG:11
0.0 23/5
10.0 60/5
16.4 150/6
19.8 171/4
27.2 151/10
29.8 32/1
30.0 222/7
32.3 148/9
34.7 100/1
35.9 66/6
45.4 136/1
48.5 40/10
52.7 56/1
59.7 236/3
69.8 194/1
70.8 188/6
73.1 82/1
78.4 14/8
79.7 8/17
95.9 59/10
97.8 107/2
99.3 236/2
109.3 242/3
113.8 66/9
118.9 234/6
120.2 208/1
127.3 FRPP91
143.9 68/3
144.6 227/1
146.8 7/5
150.6 NZPR472
151.9 54/17
166.0 80/7
167.4 158/2
182.8 180/6
Q3.7(2)
LG:12
0.0 104/9
11.4 103/2
19.9 149/10
21.2 35/19
24.9 140/2
26.3 139/8
27.5 97/4
28.2 17/7
31.0 187/3
39.8 76/1
41.2 236/1
41.9 238/3
46.0 RPS-160
47.9 57/8
50.9 138/5
58.0 147/19
59.1 234/2
76.5 253/3
82.8 249/1
84.1 254/1
86.5 239/4
86.8 176/3
87.6 240/8
89.9 145/9
91.9 105/6
93.4 184/4
99.2 240/6
110.5 212/4
112.9 NZPR1702b
116.6 AF028073
118.1 241/1
118.7 151/12
125.5 143/16
125.9 63/5
128.9 59/3
144.9 158/13
151.6 89/1
152.9 33/12
154.2 238/2
156.7 250/1
159.4 190/7
163.6 216/4
168.2 101/13
171.4 209/6
Q6.7(1)
Q6.8(2)
Q7.3(2)
Q1.5(1)

instance, would allow a significant increase in the selection intensity within families at the seedling stage. This would improve efficiency of selection save considerably time during field trials for clonal testing.

Application of selective QTL markers for pulp yield and α-cellulose, lignin and extractives contents can be economically relevant for the paper industries. The amount of α-cellulose in paper or board is an indicator of its stability and longevity, whereas the presence of non-cellulosic components contributes to the degradation of these materials. The pulp yield as well as the proportion of lignin is highly important for mill profitability. Pulp cooking is not possible until all lignin has been dissolved, which causes detrimental effects on both, yield and pulp strength. High amounts of wood extractives cause operational and quality problems in pulp and paper production due to the formation of spots, specks and other product defects. Pitches are commonly applied to wood extractives and other deposits in softwood pulping and papermaking processes. However, they disrupt the runnability of the paper-making machinery (ALLEN, 1980).

The SSR markers RTPEST11 and Pr092a for QTLs related to wood density (Q7.1, Q8.3) have been mapped in *P. taeda* (DEVEY et al, 1999) and *P. radiata* (C. ECHT, pers. comm.). Codominat SSR markers are transferable within and to a certain degree also between species. They could be used directly for introgression studies of wood density related QTLs in *Pinus pinaster* and other forest species. Other AFLP markers for important QTLs detected in this study will be isolated and converted into allele specific markers (CAPs). They will be useful to perform comparative QTL analyses in different forest species. There is evidence that trait specific QTLs are located on homologous chromosomal regions in different coniferous forest species (VERHAEGEN and PLOMION, 1996, GRATTAPAGLIA et al, 1996). In these studies, QTLs for wood density and vigor were detected in homologous linkages groups for Eucalyptus.

Also the ongoing efforts to align progressively individual linkage maps using subsets of common markers will help to clarify the relationships of such QTLs in different forest species. Determination of genomic regions associated with trait-specific QTLs across different species would improve considerably the efficiency and stimulate the application of marker assisted selection in forest breeding programs (MARQUES et al., 1999).

Despite of the relatively small size of the mapping population, a number of QTLs were detected for all traits under study, since a considerable amount of variation is present in the data as reflected in the corresponding coefficients of variation. However, the small progeny size decreases the sensitivity of detecting QTLs and only main QTLs can be detected, while smaller ones might remain unrevealed. This effect is also visible in the reduced number of significant, adjacent intervals pointing to a QTL in the neighbourhood (results not shown). On the other hand, smaller QTLs might be less stable across different environments and different germplasm and, therefore, their use is also limited.

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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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