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Linkage mapping and QTL analysis for growth of young *Pseudotsuga menziesii* plants

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Abstract

A controlled cross between two Douglas-fir trees, from Washington State, have been analysed for genetic mapping and for QTL detection. Their progeny have been measured for stem height, bud flushing, and polycyclism on the second and third year after sawing. A genetic map was drawn from segregations of AFLP markers recorded in 157 seedlings. These markers were obtained with 25 primer pairs tested in other Pinaceae species. Genetic map of the female parent consisted of 123 markers distributed in 24 linkage groups. QTLs have been detected by interval mapping for traits showing a normal distribution (stem height, stem elongation), and by a Kruskal and Wallis test for trait that did not show a normal distribution (bud flushing, polycyclism). QTLs have been detected for all investigated traits. QTLs for growth and flushing traits did not overlapped on the female genetic map. QTLs for vegetative flushing stage of terminal bud recorded in 2001 and for day of bud elongation recorded in 2002 did not overlapped. Further evaluations of quantitative traits are required to test time stability of QTLs

Introduction

Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, grows naturally in the western coast of North America and has been introduced in Europe in the XIXth century. Its growth and its adaptation (no important disease recorded up to now) led foresters to plant it in large areas. Native provenances have been tested and recommended for plantations (Christophe and Birot, 1983; Kleinschmit and Bastien, 1992). Douglas-fir has became the most important introduced forest tree species in Europe (more than 600,000 ha in Western Europe). Seeds used for plantation are now mostly produced in seed orchards. Clones are selected to provide a genetic gain for traits such as growth, adaptation, phenology, wood quality, stem straightness, etc.

Breeding programs require various information on genetic components of traits. Recent applications of molecular markers, which have firstly been developed for genetic variation analyses, deal with genetic improvement: construction of genetic maps (Grattapaglia and Sederoff, 1994) and localisation of loci controlling quantitative traits (QTL) open the perspectives of marker-assisted selection and provide the breeder with new information like the number of loci influencing the trait, and their position within the genome.

Several traits have been investigated for selection: height growth, late bud-flushing which can prevent damages produced by late spring frost, second flushing is unfavourably correlated with forking defects and induces defection of wood quality, it has a genetic component (Adams and Bastien, 1994), and is selected against. Wood quality is not really included in selected parameters, due to its late expression. Marker-assisted selection will favour selection for wood quality when QTL detection will be performed in large samples of mature trees. Wood quality has been analysed in order to provide selection criteria (Mandy et al., 1999).

In Douglas-fir, genetic maps have been obtained from segregations among megagametophytes (Krutovskii et al., 1998) or in a three generation pedigree (Jermstad et al., 1998). Markers used were mostly RAPD, which remain very difficult to transfer to an other pedigree. The genetic maps available

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are suitable for QTL detection but they consist of 16 or 17 linkage groups instead of the 13 expected ones. QTL have then been detected in Douglas-fir for vegetative bud-flush timing and cold-hardiness by Jermstad et al. (2001a, 2001b).

The present study deals with genetic mapping of Douglas-fir, using AFLP markers, and QTL detection of traits expressed in very young plants, like bud-flushing timing and stem height.

Materials and methods

Plant material

Two trees from coastal variety of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, var. *menziesii* have been crossed. The female parent, F3753, originated from seeds collected in the provenance Enumclaw (Washington State) while the male parent, M1764, derived from seeds collected in provenance Granite Falls (Washington State). F1 generation seeds have been sown in greenhouse in spring 2000, 200 plants have been transferred in nursery. Plants have then been transplanted in a test under homogeneous conditions at Peyrat-le-Château (Haute-Vienne, France).

Genetic mapping

DNA was extracted from needles of 157 plants. DNA was then hydrolysed with restriction enzyme and amplified with 25 primer pairs to produce AFLP markers according to Vos et al. (1995). These primer pairs were selected for amplification in several pine species in order to allow genetic map comparison.

Markers were mapped using the pseudo-testcross strategy developed by Grattapaglia and Sederoff (1994). Two maps were obtained, consisting of heterozygous markers in the female parent on one hand and in the male parent on the other hand. Distance between markers was assessed by the Kosambi (1944) function. Markers heterozygous in both parents were mapped in each map and showed homologies between both maps. A consensus map could be then drawn. Markers were distributed among linkage groups with a LOD score of 3.0 when segregating 1:1 (using Mapmaker) and at a LOD score of 5.0 when segregating 1:3 (using Joinmap).

Quantitative traits

Plants have been measured in 2001 and 2002. Vegetative flushing time is a stable trait that can be assessed early. In 2001, vegetative flushing stage of terminal bud (*F*) was assessed to a 6 level scale (small dormant bud, recorded 1, to elongated bud, recorded 6) at one observation in May. Stem height of plants (*H1*) was measured in autumn, after growth period end. In 2002, plants were observed each 2-3 days to determine the individual day of bud elongation (*D*). Total stem height (*H2*) was assessed at the end of 2002. In 2002, second flushing was noticed in several plants, presence of polycyclism (*P*) was recorded. Elongation of first (*f1*) and eventually second flush (*f2*) growth were measured in all plants. Total stem elongation (*TE*) in 2002 was then recorded. Quantitative traits were recorded in 115 plants.

QTL detection

When the trait did not show a normal distribution, QTLs were then detected using a non-parametric method using Kruskal and Wallis test (significant for $\alpha<0.005$). QTLs were located for traits showing a normal distribution by interval mapping according to Lander and Botstein (1989).

Results and discussion

Polymorphisms

One primer pair combination did not produce markers (Table 1). The 24 remaining primer pairs produced 475 polymorphic markers, 310 out of these showed a band in the female parent and 286 in the male parent. Markers showing a band in one parent and segregating 1:1 and showing a band in both parents and segregating 1:3 (absence:presence) were retained for mapping. Markers with significant deviation ($\alpha<0.01$) were discarded. The female parent was heterozygous for 126 markers segregating 1:1 and the male parent for 100 markers. In addition, 94 markers were heterozygous in both parents and segregated 1:3.

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Table 1. Polymorphims recorded with the AFLP primer pairs.

EcoRI / Mse I primer pair	Code	Number of polymorphic bands
E-ACA/ M-CCAC	a/AC	46
E-ACA/ M-CCAG	a/AG	13
E-ACA/ M-CCCA	a/CA	19
E-ACA/ M-CCCG	a/CG	22
E-ACA/ M-CCGC	a/GC	37
E-ACA/ M-CCGG	a/GG	13
E-ACA/ M-CCTG	a/TG	22
E-ACC/ M-CCAG	c/AG	0
E-ACC/ M-CCGT	c/GT	14
E-ACC/ M-CCTG	c/TG	17
E-ACG/ M-CCAA	g/AA	14
E-ACG/ M-CCAC	g/AC	40
E-ACG/ M-CCAG	g/AG	21
E-ACG/ M-CCAT	g/AT	26
E-ACG/ M-CCCA	g/CA	17
E-ACG/ M-CCGC	g/GC	19
E-ACG/ M-CCGT	g/GT	9
E-ACG/ M-CCTA	g/TA	18
E-ACG/ M-CCTC	g/TC	21
E-ACG/ M-CCTG	g/TG	15
E-ACGC/ M-CAC	gc/C	20
E-ACGC/ M-CCAC	gc/AC	17
E-ACGC/ M-CCGC	gc/GC	13
E-AAACA/ M-AGCG	ca/CG	12
E-AAACC/ M-AGCG	cc/CG	10

Genetic map

The genetic map of the female parent consisted of 120 markers (31 segregating 3:1 and 89 segregating 1:1) distributed in 24 linkage groups of at least two markers (Table 2). The linkage groups stretched on a total length of 1288 cM, 13 linkage groups were longer than 50 cM (up to 126.6 cM), 11 consisted of 5 markers or more (up to 11 markers). Eleven linkage groups contained markers segregating 1:1 and 3:1. The largest group and two small linkage groups (of two markers) consisted of markers segregating 1:3. In the largest group of 11 markers, alleles leading to amplification were bare on the same chromosome of the pair.

The genetic map of the male parent consisted of 54 markers (16 segregating 3:1 and 38 segregating 1:1) distributed in 15 linkage groups of at least two markers (Table 2). Their total length was of 509 cM. Only 2 linkage groups were longer than 50 cM (101.6 cM and 62.5 cM), and 4 consisted of 3 markers or more (up to 21 markers). Only three groups of markers segregating 3:1 were shared by both genetic maps. No other homology among linkage groups could be suspected since other markers heterozygous in both parent remained unlinked in at least one of them.

The female parent map appeared similar of those obtained by Jermstad et al. (1998) with 141 markers distributed in 17 linkage groups stretching 1062 cM. Krutovskii et al. (1998) produced a longer map, but the number of linked markers was higher (201).

Variation of quantitative traits.

The vegetative flushing stage (*F*) recorded in 2001 and the vegetative flushing date recorded in 2002 did not show a normal distribution (Figure 1). Most plants showed an early development. The stem height measured in 2001 (*H1*) and in 2002 (*H2*) presented a normal distribution, their variation coefficient were respectively of 0.21 and 0.31. A second flushing in some plants and a third flushing in

one plant has been observed in 2002. Most plants had a monocyclic growth. Elongation of first (*f1*) and second flush (*f2*) and total stem elongation (*TE*) showed a distribution close to a normal one.

Table 2. Characteristics of genetic maps.

Attribute	Female parent F3753	Male parent M1764
Number of markers segregating 1:1[a]	89	38
Number of markers segregating 3:1[a]	31	16
Number of linkage groups	24	15
Linkage groups with at least 5 markers	11	2
Linkage groups of more than 50 cM	13	2
Average distance between consecutive markers	13 cM	13 cM
Total length of linkage groups	1288 cM	509 cM

a: markers included in linkage groups of at least two markers

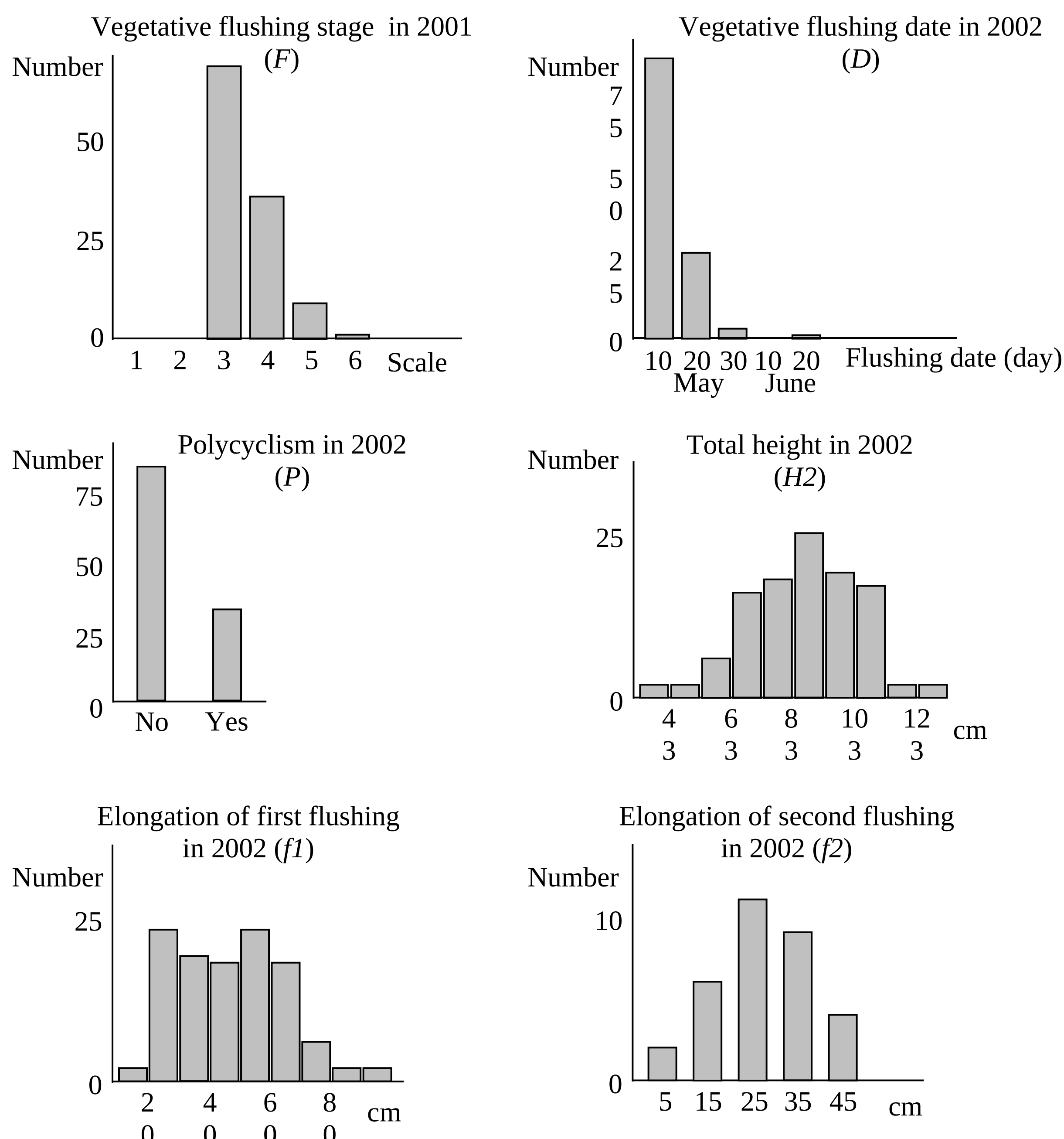

Figure 1. Distribution of the phenotypical values for several traits under investigation.

QTLs

Two markers located on two female parent linkage groups (F4 and F5), and 4 unlinked markers (3 for the female parent and 1 for the male parent) were linked to QTLs controlling flushing stage of terminal bud (*F*) measured in spring 2001 (Table 3). Three different QTLs were detected for vegetative flushing time (*D*) recorded in 2002, one on female linkage group F18 and two in male linkage groups M1 and M2 (Figure 2). Jermstad et al. (2001a) observed 9 QTLs for terminal bud flushing time in Douglas-fir. This trait with a high heritability appeared also in the present study under the control of various QTLs.

Height at the end of 2001 (*H1*) was controlled by QTLs located on female linkage group F7 (LOD score of 2.27) and male linkage group M1 (LOD score of 2.33), they were confirmed by Kruskal and Wallis test, that revealed in addition two unlinked markers of the male parent (one highly significant: α<0.0005). QTLs for height at the end of 2002 (*H2*) were detected on female linkage group F2 and male linkage group M1 (LOD score of 2.58) which are homologous, but not exactly in the same position than for height at the end of 2001. An additional marker located on female linkage group F6 was detected by Kruskal and Wallis test.

Table 3. Detection of QTLs by interval mapping or Kruskal and Wallis test.

Trait	Marker close to QTL	Linkage[a] group	Kruskal and Wallis test (χ^2)[b]		LOD score	Variance explained (%)
F	g/AC-55	F4	8.078	****		
	a/CG-18	F5	8.402	****		
	g/TA-4	F unlinked	8.554	****		
	g/TG-7	F unlinked	12.109	*****		
	gc/GC-7	F unlinked	9.701	****		
	a/GG-12	M unlinked	8.063	****		
D	gc/C-3	F18	11.745	*****		
	gc/C-12	M1	8.826	****		
	a/CA-11	M2	8.016	****		
H1	g/AC-1	F7	7.928	****	2.27	5.2
	gc/C1-2	M1	10.393	****	2.33	9.9
	c/TG-17	M unlinked	12.153	******		
	gc/AC-25	M unlinked	8.636	****		
H2	g/AC-72	F2	NS		2.58	42.9
	gc/C-7	F6	9.217	****		
	g/AA-28	M1	NS		2.58	43.2
f1	g/TC-14	F1	7.965	****		
	g/AC-68	F1	8.022	****	2.31	12.4
	g/AA-3	F1	11.780	*****		
	g/AT-8	F7	NS		2.24	33.8
	g/TA-3	F14	11.863	*****	2.69	10.4
	ca/CG-7	F17	NS		2.47	52.3
f2	g/TA-1	F5	NS		3.23	79.9
	g/AT-8	F7	10.519	****	3.64	60.5
TE	g/AT-12	F7	NS		2.81	41.5
	a/CG-12	F14	8.539	****	2.38	9.2
	a/GC-9	M1	10.527	****		
	a/GC-2	M1	9.468	****	3.03	14.1
P	a/GC-2	M1	10.755	****		

a, F: female; M: male;

b, ****,*****,******: respective probability of 0.995, 0.999, 0.9995, NS not significant.

Figure 2. Loaction of QTLs detected for investigated traits on the genetic map of the female parent. ▬ : segregation 1:1; ‐ ‐ ‐: segregation 3:1

First flushing (*f1*) QTLs (LOD score of 2.24-2.69) were located on four female linkage groups (F1, F7, F14, F17), two of them were confirmed by Kruskal and Wallis test (three close markers on linkage group F3). Second flushing (*f2*) QTLs were suspected on female linkage groups F5 and F7, as only a part of plants showed second flushing, the sample size was very reduced. The stem elongation in 2002 (*TE*) was controlled by QTLs located on female linkage groups F7 (LOD score of 2.81) and F14 (LOD score of 2.38) and male linkage group M1 (LOD score of 3.03). A single marker was found to be linked with polycyclism (*P*), located on male linkage group M1 by Kruskal and Wallis test ($\alpha<0.005$).

Conclusions

The parents of the progeny were genetically close since they were from provenances of Washington State and belonged to the coastal variety. This led to a high proportion of markers shared by the parents and segregating 3:1. Unfortunately these markers did not allow to align many linkage groups of the male and of the female genetic maps. QTLs were detected for traits recorded on young seedlings. The year to year stability of the QTLs was not established. Further analysed are required to test it. Even the vegetative phenology, analysed two consecutive years, by different approaches, did not led to the same localization of QTLs. The number and the distribution of QTLs for vegetative phenology would show that this trait with usually a high heritability could be controlled by numerous genes. The estimation of the genetic components in the observed phenotypic variation could be obtained with a cloned progeny like those analysed by Jermstad et al. (2003). Stable QTLs, independent to the year and to the environment, will be considered for further marker-assisted selection.

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References

Adams W.T., Bastien J.C. 1994. Genetics of second flushing in a French plantation of coastal Douglas-fir. Silvae Genet., 43: 345-352.

Christophe C., Birot Y. 1983. Genetic structures and expected genetic gains from multitrait selection in wild populations of Douglas fir and Sitka spruce. II. Practical application of index selection on several populations. Silvae Genet., 32: 173-181.

Grattapaglia D., Sederoff R. 1994. Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. Genetics, 137: 1121-1137.

Jermstad K., Bassoni D., Jech K., Ritchie G., Wheeler N., Neale D. 2003. Quantitative trait loci controlling adaptive traits in coastal Douglas-fir. III. QTL by environment interactions and verification. Plant and Animal Genome XI, San Diego, Poster 563.

Jermstad K.D., Bassoni D.L., Wheeler N.C., Neale D.B. 1998. A sex-averaged genetic linkage map in coastal Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco var *'menziesii'*) based on RFLP and RAPD markers. Theor. Appl. Genet., 97: 762-770.

Jermstad K.D., Bassoni D.L., Jech K.S., Wheeler N.C., Neale D.B. 2001a. Mapping of quantitative trait loci controlling adaptive traits in coastal Douglas-fir. I. Timing of vegetative bud flush. Theor. Appl. Genet., 102: 1142-1151.

Jermstad K.D., Bassoni D.L., Wheeler N.C., Anekonda T.S., Aitken S.N., Adams W.T., Neale D.B. 2001b. Mapping of quantitative trait loci controlling adaptive traits in coastal Douglas-fir. II. Spring and fall cold-hardiness. Theor. Appl. Genet., 102: 1152-1158.

Kleinschmit J., Bastien J.C. 1992. IUFRO's role in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) tree improvement. Silvae Genet., 41: 161-173.

Kosambi D.D. 1944. The estimation of map distances from recombination values. Ann. Eugenics, 12: 172-175.

Krutovskii K.V., Vollmer S.S., Sorensen F.C., Adams W.T., Knapp S.J., Strauss S.H. 1998. RAPD genome maps of Douglas-fir. J. Hered., 89: 197-205.

Lander E.S., Botstein D. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics, 121: 185-199.

Mamdy C., Rozenberg P., Franc A., Launay J., Schermann N., Bastien J.C. 1999. Genetic control of stiffness of standing Douglas fir; from the standing stem to the standardised wood sample, relationships between modulus of elasticity and wood density parameters. Part I. Ann. For. Sci., 56: 133-143.

Vos P., Hogers R., Bleeker M., Reijans M., Van De Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M., Zabeau M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res., 23: 4407-4414.