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Genetic Control of Heartwood Content in Larch

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(Received 10th October 2000)

Abstract

Genetic variability for heartwood/sapwood extent in larch was examined at the population, progeny and clonal levels. For the study, increment cores were taken in 2 provenance trials (one of European larch at 34 years and one of Japanese larch at 36 years), and in 3 hybrid larch trials at around 15 years old (2 full-sib progeny trials and 1 clonal trial).

Heartwood appears early in larch and seems to progress quickly: at 15 years, it represents already more than 60% (along the radius) for hybrid larch and more than 70% for more mature (34 to 36 year old) European and Japanese larch. Genetic variability of about the same magnitude as that for radial growth was observed for heartwood/sapwood dimensions both at the provenance level (for European larch but not for Japanese larch) and at the progeny or clonal level (for hybrid larch). Broad-sense heritability levels for heartwood traits were high (0.75 to 0.92 for heartwood length, 0.63 to 0.99 for heartwood proportion). Heartwood and, to a lesser extent sapwood content, were positively linked to diameter growth in the different genetic entries studied (genetic correlations: 0.87 to 0.96 for heartwood length); heartwood extent was mostly independent of or positively correlated with sapwood content.

The proper choice of the species (Japanese versus European larch), of the origin (Central European larch populations rather than alpine ones), or of hybrid larch progenies or clones can result in significant combined genetic gains for both heartwood content and growth.

Key words: heartwood, sapwood, *Larix,* provenance, progeny, clone, hybrid, heritability, genetic variability.

Introduction

Among wood of coniferous species, European larch (*Larix decidua* MILL.) is much appreciated for its good mechanical properties but even more for the high natural durability of its heartwood (COLLARDET and BESSET, 1988).

Natural durability of wood is linked to its anatomical features (determining, for example, water permeability) and to its (qualitative and quantitative) content of extractives. Heartwood and sapwood are in many cases clearly distinguished by the amount of extractives; in European larch, KEITH and CHAURET (1988) have shown an increase of both water-soluble and alcohol-benzene extractives from the pith to the heartwood-sapwood boundary, and then a marked decrease in the sapwood. In this respect, the sapwood of larch is not durable and thus of low value as lumber but, in the native wood resource from the Alps, its extent is usually negligible: about 10% of a breast height (BH) diameter of 40 cm (RINGARD, 1980; COLLARDET and BESSET, 1988).

Wood from lowland plantations might not present the same characteristics in particular for the proportion of heartwood. Besides their establishment in more favourable ecological conditions, these stands are planted either with an exotic species (Japanese larch, *Larix kaempferi* (Lamb.) Carr.) or with varieties (populations from Central Europe or interspecific hybrids) selected so far mostly for their adaptability to biotic and abiotic factors and for their fast growth (Pâques, 2000).

As pointed out by Hillis (1971), the extractive content of conifers is related to heartwood formation, which is in turn a function of both age and growth rate. Heartwood formation in larch is known to start very early, at around 5 years of age (Hirai, 1952). Natural or artificial environmental factors such as those affecting the crown size (*e.g.* competition, thinning, pruning) or directly enhancing growth rate (*e.g.* fertilization, irrigation) have been shown to influence sapwood/heartwood areas (Sellin, 1994; Takei, 1996; Mörling and Valinger, 1999; Margolis *et al.,* 1988; Polge, 1982).

Genetic factors also seem to control content of heartwood in conifers. Considerable genetic variability has been reported by several authors at the origin, progeny or clonal levels. Among conifers, examples are mostly for pines (e.g. for *Pinus radiata:* Nicholls and Brown, 1974; Cown *et al.,* 1992; Nyakuengama *et al.,* 2000; for *Pinus sylvestris*: Schultze-Dewitz and Götze, 1987; Fries and Ericsson, 1998; Ericsson and Fries, 1999; for *Pinus banksiana:* Magnussen and Keith, 1990), but also for Douglas-fir (*Pseudotsuga menziesii*) (e.g. Lausberg *et al.,* 1995), and for giant sequoia (*Sequoiadendron giganteum*) (Knigge, 1993). For larch too, genetic variation among provenances of European larch has been shown to be large but the number of studies is very limited (Schreiber, 1944; Leibundgut, 1983; Kulej and Niedzielska, 1990). Genetic variation has been also reported by Sachsse *et al.* (1978) among clones of Japanese larch.

The objective of this study is to investigate the variation in heartwood content at different genetic levels, to determine its heritability and its relationships with other growth traits. Consequences for breeding will be then discussed.

Material and Methods

Five experimental trials representing different *Larix* species and types of genetic entries were analysed for this study. They were one provenance trial of European larch, one provenance trial of Japanese larch; two full-sib progeny- and one clonal-trial of hybrid larch (*Larix x eurolepis*). A brief description of these trials is given in *table 1*. Their ages were respectively 34, 36, 15, 15 and 16 years old at the time of sampling. Average stand density was 300 stems/ha and 470 stems/ha respectively in the two provenance trials and 1100 stems/ha in the other trials, which had not yet been thinned at the time of sampling. A more detailed description of the material and experimental designs can be found in Pâques (1992, 1996a and b).

Radial increment cores were collected at breast height (1.30 m) from a sample of trees (more than 2400 trees in total, *Table 1*); trees of each genetic entry in each trial were chosen at random. Special care was taken to have cores passing through the pith. The sampling procedure is supported by the work of Hakkila and Winter (1973), who concluded that the average percentage of heartwood in a stem of larch can be estimated with great accuracy from a sample taken at breast height.

The total length of each core (from pith to bark) as well as the length of the sapwood (based on visual colour observations) were recorded. The length of the heartwood radius and the proportion of heartwood along the radius were computed. The number of sapwood rings was determined only in the Japanese larch provenance and in the hybrid larch clonal trials. Rings partially occupied by heartwood were excluded from the sapwood ring number.

Heartwood/sapwood traits were then related to growth parameters such as total height and radial growth recorded at time of core sampling.

Analysis of variance was performed on data for each trial according to the following model:

$$Y_{ijkl} = \mu + B_i + G_j + GxB_{ij} + e_{ijkl}$$

where

Y_{ijkl}: Individual observation,

μ: Overall mean,

B_i: Block effect (fixed),

G_j: Genotype effects (*i.e.* provenance, progeny or progeny + clone/progeny effects) (random),

GxB_{ij}: Interaction effect,

e_{ijkl}: Residual error

Data were adjusted for the fixed block effects and variance components of total variation were then estimated using OPEP software package (Baradat and Labbé, 1994).

For hybrid larch progeny and clonal trials, broad-sense heritabilities (H^2_G) were computed at the progeny mean level (1) or at the clone mean level (2) as :

$$H^2_G = \sigma^2_F / (\sigma^2_F + \sigma^2_w/n) \qquad (1)$$

$$H^2_G = \sigma^2_{Cl} / (\sigma^2_{Cl} + \sigma^2_w/n) \qquad (2)$$

where s^2_F, s^2_{Cl}, s^2_w = respectively estimated progeny, clone and residual variance components and n, expected mean square coefficient for progeny or clone estimate.

Table 1. – Description of test sites (EL = European larch, JL = Japanese larch, HL = hybrid larch).

Forest stand	Region	Elevation (m)	Species	Genetic entry	Nber of genetic entries studied	Nber of trees sampled	Average nber of trees sampled/genetic entry	Initial plot size	Nber of blocks	Sowing year	Age at evaluation (yrs)
F.D. Coat-An-Noz (22)	Bretagne	200	EL	provenances[1]	17	341	19	11 x 11	11	1958	34
F.D. Coat-An-Noz (22)	Bretagne	200	JL	provenances[1]	12	155	12	2 x 2	6	1957	36
Peyrat-Le-Château (87)	W. Massif Central range	460	HL	full-sib progenies	10 (8 HS[2])	375	28	2 x 5	8	1980	15
Beaumont-du-Lac (87)	W. Massif Central range	540	HL	full-sib progenies	54	1125	21	1	40	1983	15
F.D.Eclache (63)	Central Massif Central range	1000	HL	clones (5 FS[2] families x 10 clones)	50	455	9	2 (non-contiguous)	9	1982	16

[1] IUFRO trials

[2] FS = full-sib progeny, HS = half-sib progeny

For provenance trials, a parameter comparable to a broad-sense heritability was computed as in (1) following NANSON (1970). It represents the reproductible part of the total variance attributed to the mean population effects in panmictic conditions.

Standard errors of heritabilities were computed as suggested by ANDERSON and BANCROFT (1952).

Results and Discussion

1) Genetic variability and heritability

a) At the provenance level (European and Japanese larch)

At 34 years of age, the proportion of heartwood is very high and reaches 71% in European larch for trees of over 19 cm on average in diameter under bark; it is even greater in Japanese larch for which heartwood represents 80% (*Table 2*); the number of sapwood rings for the latter is around 9. As indicated by HAKKILA and WINTER (1973), Japanese larch appears to produce the highest proportion of heartwood among larch species.

Highly significant differences among provenances are observed for all traits in European larch but not in Japanese larch. For European larch, heartwood proportion ranges from 60% to 76% and tends to increase from the SW to the NE of the native range (*Figure 1*). South-western alpine larch origins (*e.g.* origin numbers 22, 23, 24, 26 in *Figure 1*) show a lower heartwood proportion than interior, northern and eastern alpine ones (*e.g.* 3, 7, 11), which themselves have less heartwood than Sudetan mountains (*e.g.* 39, 40, 106, 107) or Central Poland (104) populations. For Japanese larch, variability among provenances is nearly non-existent (*Figure 1*).

For both species, among provenance variability is similar or even higher for sapwood and heartwood length than for radius

Table 2. – Trait mean and phenotypic and between-provenances coefficients of variation, provenance component of total variation and its level of significance, and broad-sense heritability (according to NANSON, 1970) in the European (EL) and Japanese (JL) larch provenances trials.

Traits	Trials	Mean	Individual phenotypic coeff.var. (%)	Between-provenance coeff. var. (%)	Genetic component of total variation (%)	BS heritability (se)
Radius length (mm)	EL	96.8	27	20.5	41.1***	0.926 (0.024)
	JL	128.8	17	2.7	2.7ns	1)
Sapwood length (mm)	EL	27.4	29	30.1	55.0***	0.957 (0.014)
	JL	24.9	24	6.5	7.4*	0.499 (0.093)
Nber of sapwood rings	EL	–	–	–	–	–
	JL	9.2	22	5.8	7.1*	0.486 (0.095)
Heartwood length (mm)	EL	68.9	34	24.8	40.3***	0.924 (0.025)
	JL	103.8	21	3.1	2.4ns	1)
Heartwood proportion (%)	EL	71	8	11.8	58.3***	0.988 (0.005)
	JL	80	8	1.4	3.2ns	1)

*,**,*** : F-test for provenance effects significant respectively at a = 5%, 1% and 0.1%

1) not given because of absence of significant provenance effects

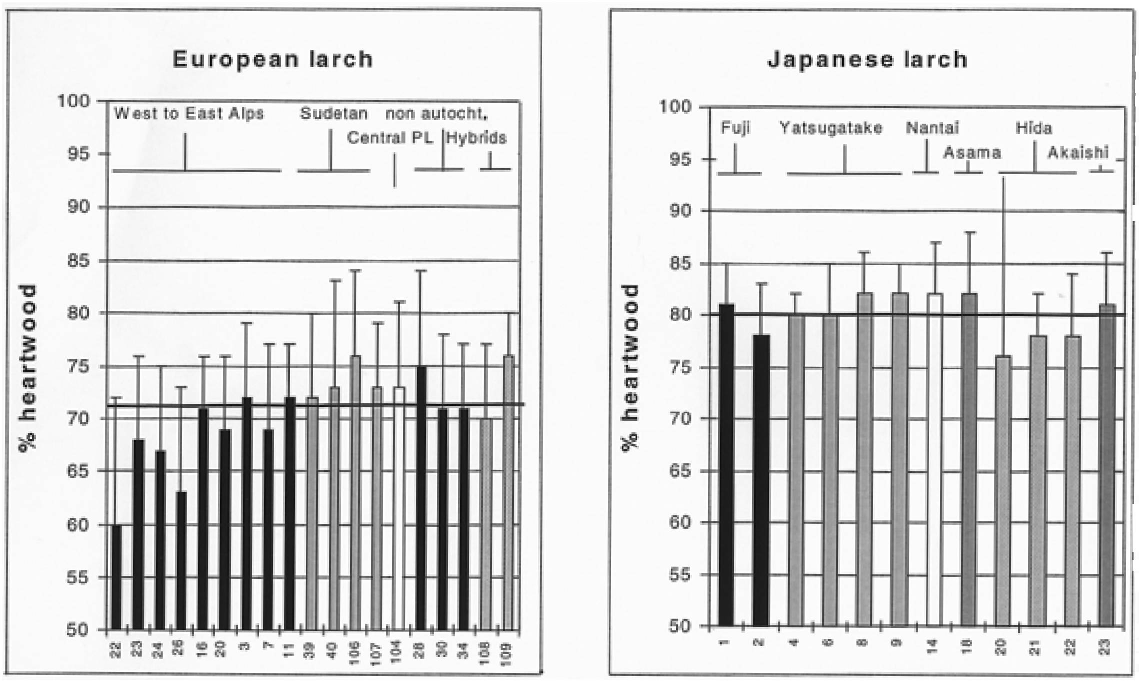

Figure 1. – Mean (and SE) heartwood proportion for European and Japanese larch provenances (Coat-An-Noz : IUFRO international provenance trials).
The solid line indicates the trial mean.

length and it is greater for sapwood length than for heartwood length. For heartwood proportion, coefficients of variation are much smaller. The genetic control parameters (broad-sense heritabilities following Nanson (1970)) exceed 0.92 for all traits in European larch (with the largest value of 0.99 for heartwood proportion) but they are small for Japanese larch due to the low variability among provenances and are estimated with a low precision.

Considerable variability among European larch provenances has also been observed by Kulej and Niedzielska (1990): at 22 years old, the proportion of heartwood among Polish origins tested varied from 53.1% to 78.4% with an average of 61.8%; the number of sapwood rings was on average 8 with a range of 7 to 9. In that study, there is no indication of superiority of either Central Poland or Sudetan Mountains races for these traits. Conversely, Schreiber (1944)'s results (cited by Sindelar, 1992) indicated higher values for heartwood volume proportions (over bark) at 25 years for Sudetan larch (47%) than for Polish larch (25% to 39%); Alpine origins having intermediate values. Similarly, Leibundgut (1983)'s results at ages from 24 years to 35 years indicate that Sudetan origins produce more heartwood (40% proportion by area) than Alpine origins (28%); he observed that origins from low elevations have more heartwood than those from high elevations and he concluded that origins growing faster (*i.e.* Sudetan larch versus Alpine larch) start heartwood formation earlier. Our results confirm this trend.

In contrast to European larch, Japanese larch has shown little variation among provenances for various traits including growth and stem form (Schober and Rau, 1991; Pâques, 1996b). The same conclusion is reached in this study for sapwood/heartwood content with the lowest coefficient of variation among provenances for heartwood proportion (CVpr = 1.4%). At about the same age, Takata and Hirakawa (1995) also found no significant differences in heartwood proportion among 24 provenances of Japanese larch across 3 IUFRO trials in Japan. Variability in that species is mostly at the individual tree level.

b) At the progeny and clonal levels (hybrid larch)

At around 15 years of age, the proportion of heartwood along the radius is already high (more than 60%) and is consistent across the 3 sites (*Table 3*). It ranges from 53% to 70% for hybrid larch progenies. It is of the same magnitude for clones (50% to 72%) and can be compared to the clonal range observed by Sachsse *et al.* (1978) at about 20 years for Japanese larch (35.4% to 54.9% of cross-sectional area or 60% to 74% of radius length).

Compared to that of European larch in the Bretagne trial, the hybrid larch growth is much faster: the mean annual ring width (6 mm) is more than twice that of European larch and the average BH diameter is nearly the same at 15 years to 16 years as that of European larch at 34 years of age. Nevertheless at similar diameter, the proportion of heartwood in hybrids is still lower, indicating the importance of age in heartwood formation (Hillis, 1987).

Phenotypic individual variability is high, especially for heartwood and sapwood length, and it is greater than for radius length itself. At the genetic level, variability for sapwood and heartwood length is also of similar size or greater than for growth itself but it is usually smaller for heartwood proportion, as indicated by genetic coefficients of variation (*Table 3*).

Highly significant differences exist among hybrid families for radius length and sapwood length. For heartwood length and heartwood proportion, differences are highly significant at the progeny level at Beaumont, but at neither the Peyrat nor the Eclache site. However at the last site, highly significant differences are found at the clonal level for these 2 traits and for the number of sapwood rings. Except on the Peyrat site, all traits seem to be under strong genetic control: broad-sense heritabilities are 0.78 to 0.86 for radius length, 0.67 to 0.82 for sapwood length, 0.75 to 0.83 for heartwood length and 0.63 to 0.78 for proportion of heartwood.

These results can not be compared to similar published results on larch because they are non-existent. Nevertheless, some other studies on conifers (mostly pines) have shown good genetic control for heartwood content in progeny trials. In Scots pine, Fries and Ericsson (1998) and Ericsson and Fries (1999) found narrow-sense heritabilities for heartwood diameter ranging from 0.30 to 0.54, compared to 0.14 to 0.03 for stem diameter. In radiata pine, Nicholls and Brown (1974) indicate narrow-sense heritability of 0.20 for heartwood area proportion while in a study by Nyakuengama *et al.* (2000), it reached 0.98 for heartwood area compared to 0.23 for basal area. In a jack pine study, Magnussen and Keith (1990) found a narrow-sense heritability of 0.23 for heartwood area percentage, which was much higher than for stem diameter. At the clonal mean level, Nicholls and Brown (1974) found heritabilities for heartwood area proportion in radiata pine ranging from 0.34 to 0.45.

2) Relationships among traits

As indicated in *table 4,* heartwood content is directly linked to radial growth. Correlations with heartwood length are posi-

Table 3. – Trait mean and phenotypic and between-progeny coefficients of variation, progeny component of total variation and its level of significance, and broad-sense heritability in the 2 hybrid larch progeny trials (BE. = Beaumont, PE. = Peyrat) and in the hybrid larch clonal trial (EC = Eclache).

Traits	Trials	Mean	Phenotypic coeff.var. (%)	Between progeny coeff. var. (%)	Genetic component of total variation (%)	BS heritability (se)
Radius length (mm)	BE	78.4	17	7.5	23.0***	0.86 (0.03)
	PE	90.8	19	4.8	7.4***	0.69 (0.13)
	EC	92.7	16	5.5-6.9[2]	11.3**-18.1***[2]	0.78 (0.05)
Sapwood length (mm)	BE	31.1	24	6.7	8.9***	0.67 (0.06)
	PE	32.1	19	8.6	13.7***	0.82 (0.08)
	EC	35.7	21	8.5-7.7[2]	16.4***-13.6***[2]	0.76 (0.05)
Nber of sapwood rings	EC	4.1	20	0.0-7.3[2]	0.0ns-62.0***[2]	0.58 (0.09)
Heartwood length (mm)	BE	47.3	24	9.7	18.7***	0.83 (0.03)
	PE	58.7	25	3.5	2.3ns	[1]
	EC	57.0	25	5.0-11.5[2]	4.0ns-21.6***[2]	0.75 (0.05)
Heartwood proportion (%)	BE	60	14	5.3	14.3***	0.63 (0.06)
	PE	64	15	1.8	2.5ns	[1]
	EC	61	13	2.7-6.0[2]	4.1ns-20.3***[2]	0.78 (0.02)

*,**,*** : F-test for progeny/clone effects significant respectively at a = 5%, 1% and 0.1 %

[1] Not given because of absence of significant progeny effects

[2] The first figure refers to the progeny level and the second to the clone level.

Table 4. – Coefficients of phenotypic (r_P) and genetic (r_G) correlation between radial growth and sapwood/heartwood traits.

		Sapwood length	Heartwood length	Heartwood proportion
European and Japanese larch provenance trials				
EL	r_P	0.527***	0.953***	0.451***
	r_G	0.467	0.928	0.405
JL	r_P	0.283***	0.963***	0.475***
	r_G	0.238	0.963	0.524
Hybrid larch progeny and clonal trials				
BE	r_P	0.515***	0.829***	0.200***
	r_G	0.733	0.953	0.522
PE	r_P	0.519***	0.841***	0.182***
	r_G	0.456	0.878	0.485
EC	r_P	0.362***	0.874***	0.424***
	r_G	0.438	0.872	0.375

*** = significantly different from 0 for a = 0.1 %

tive and high both at the phenotypic and at the genetic (provenance, progeny or clonal mean) levels; sapwood length is also positively linked to radial growth but to a lesser extent, and correlations with heartwood proportion are intermediate. Positive and mostly strong genetic correlations between diameter growth and heartwood/sapwood content have been found by many authors for pines (at the origin level: FRIES, 1999; at the progeny level: FRIES and ERICSSON, 1998; at the clonal level: NYAKUENGAMA *et al.* 2000). Therefore, it seems clear that fast growth is associated with heartwood content both in absolute and relative terms.

Relationships between heartwood and sapwood extent (not presented) are much weaker and range from –0.138 to 0.245 for phenotypic correlation coefficients and from –0.059 to 0.491 for genetic correlation coefficients. In all cases, they are either not significantly different from 0 or significantly different but then positive. FRIES (1999) and NYAKUENGAMA *et al.*'s (2000) results go mostly in the same direction; in the first study, there were no relationships between heartwood and sapwood lengths and in the second, there was a positive correlation. So, increased sapwood content does not seem to prevent heartwood formation.

3) *Consequences for breeding*

a) Expectation of genetic gains through selection

As mentioned above, larch wood is mostly used for lumber, including outdoor construction and the heartwood is the most valuable part of the timber. A breeding goal could thus be to increase through selection the heartwood content both in absolute and possibly in relative terms. In any case, selecting for fast growth as a prime criterion should not a *priori* reduce heartwood content.

Results presented above are mostly encouraging. They clearly show that it is possible to improve growth and at the same time to enhance heartwood length and proportion. Correlations are positive and heritabilities are high enough to ensure genetic gains in both traits at the provenance, progeny and clone levels.

Japanese larch has in this study a small advantage over European larch both in growth and heartwood content. The best origins of European larch (namely Sudetan Mountains and Central Poland larch) selected in most breeding programmes for their fast growth and broad adaptability (SCHOBER, 1985; PÂQUES, 2000) show also the highest heartwood content.

For hybrid larch, expected genetic gains from progeny and clonal selection are presented in *table 5* for heartwood traits together with growth traits. Gains for heartwood length are mostly superior to those for total height or girth; for heartwood proportion, they are slightly inferior but still of considerable interest: indeed if one considers a selection intensity of 1% (i = 2.665) which would be more realistic in this example, gains would reach 23.4% and 11.2% respectively for heartwood length and proportion for the hybrid larch progeny trial at Beaumont and 28.2% and 17.3% respectively for the hybrid larch clonal trial at Eclache. Gains for the Peyrat progeny trial (not presented) are much more limited due to the narrow genetic base of the material.

Table 5. – Expected genetic gains from selection on a progeny (BE) and a clonal (EC) mean basis (gains expressed in % of mean, for a selection intensity of 1 %).

	BE	EC
Direct selection		
Total height	5.2	4.0
BH girth	6.8	7.0
Individual stem volume	15.7	16.1
Heartwood length	8.8	10.6
Heartwood proportion	4.2	6.5
Indirect selection on total height		
for heartwood length	4.5	2.9
for heartwood proportion	2.6	2.6
Indirect selection on BH girth		
for heartwood length	8.3	9.3
for heartwood proportion	2.3	2.5

Direct selection for heartwood characteristics themselves will not be practical on a large scale as it would mean sampling all trees by taking increment cores. The positive correlations between heartwood traits and BH diameter suggest that indirect selection on BH girth would be efficient: indirect selection for heartwood length would give nearly the same gains as direct selection; for heartwood percentage, they would be smaller but still they would exceed 6% (for i = 2.665). Indirect selection on total height (less subject to competition effects than diameter) will also influence positively heartwood content.

b) Limitations of this study and further information needed for efficient breeding

These early results (at 15 to 16 and 34 to 36 years) are of course preliminary and are based for each of the 5 experiments on a unique site: as a consequence, genetic parameters such as heritabilities or expected gains are probably over-estimated. However, their high levels allow us to be optimistic about the selection possibilities for increasing heartwood sections in stems.

Further information is needed to ensure that breeding will be successful. Firstly, to be fully usable, these results will need to be supported by some evidence that the observed genotype differences in heartwood content are stable over sites and over time. Site effects have been shown in many studies to be important in heartwood content but genotype by site (GxE) interactions are poorly documented. Favourable indications of stability are provided by Malan and Verryn (1996): in a study with 31 clones of eucalypts, tested over 26 sites, they show that most (all except two) clones were insensitive to the environment changes or of average stability for heartwood content.

Time is also known to play an important role in heartwood extension (Hillis, 1987) but really the question for breeders is whether the advantage in heartwood content, observed for some genotypes at these early ages, will be maintained until the end of the rotation, fixed nowadays more by commercial diameter sizes than by tree maturity. No information seems to be available in the literature on this genotype x year stability. It can only be hypothesized that if heartwood content is really strongly linked to BH diameter, and if juvenile-mature correlations are strong for stem diameter, ranking for heartwood content would be stable over years. Such conditions have been found in the European larch provenance trial used in this study. Age-age correlations for diameter have been found to be moderate to high: 0.60 between 13 and 34 years but 0.94 between 26 and 34 years old (Pâques, 1996a) and as shown in this study, heartwood is strongly correlated with BH diameter. Nevertheless, the relationship might not be simple, as genotypes growing faster will meet earlier competition, which might reduce growth rate and modify tree architecture. In any case, the need for clear demonstration of genetic differences in the heartwood extension rate and of its stability over years remains.

A second condition on which the breeding goal of increasing heartwood content relies is that the speed of heartwood formation does not significantly alter qualitatively or quantitatively, the content of extractives which determine natural durability and wood coloration (important for aesthetic reasons). This major question is actually under study.

Conclusion

Forest tree breeders have concentrated their efforts to date mostly on breeding for adaptability and for growth. Wood properties have been in comparison little worked on. Genetic experiments have been in many cases too young for measuring wood properties and for many species, the selection criteria for wood quality are not yet always well defined. That is typically the case for larch and more especially for larch bred for lowland plantations. However, improvement of larch varieties for heartwood content does not seem ridiculous because it is traditionally the most appreciated part of the timber. This should only be done on condition that fast heartwood formation does not alter its anatomical and chemical properties to such a level that its aesthetics (colour) and natural durability would be depreciated. This possibility has to be checked carefully.

Meanwhile, this study shows that genetic variability at the population, at the progeny or at the individual level together with the estimated heritabilities are large enough to make selection efficient for heartwood content. Additionally, the favourable correlations between radial growth and heartwood length indicate that selection for both fast growth and rapid heartwood extension is possible for larch species and hybrids.

Acknowledgements

This study has been financially supported by the European Commission, project EU-FAIR CT98-3354. The author is grateful to the INRA-Orléans technical team and specially to F. Millier who has realized most of the measurements and to an anonymous reviewer for his helpful comments to improve this paper.

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Identification of Candidate Genes For Use in Molecular Breeding – A Case Study With the Norway Spruce Defensin-like Gene, *Spi 1*

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(Received 23th October 2000)

Abstract

In this study we have investigated whether the defensin-like gene *spi 1*, isolated from Norway spruce, contributes to quantitative disease resistance and is a suitable candidate for utilisation in Norway spruce breeding programmes. The following questions have been raised: (1) Can the putative defense gene, *spi 1*, improve the defense towards microbial pathogens in a model plant species, tobacco? (2) Is it possible to produce transgenic plants of Norway spruce that overexpress *spi 1* and are less susceptible to the pathogenic fungus *Heterobasidion annosum*? Compared to control plants, tobacco plants expressing *spi 1* under an enhanced CaMV 35S promoter permitted less growth of the bacterial pathogen *Erwinia carotovora*. Embryogenic cultures of Norway spruce were transformed with a similar construct. The general phenotype of regenerated transgenic plants was normal, although it was difficult to maintain certain sublines in culture owing to poor initial growth. Among the transformed plants those with the highest content of SPI 1 displayed reduced fungal growth in the sapwood after inoculation with *H. annosum*. In conclusion, the *spi 1* gene increases resistance in both homologous and heterologous systems.

Key words: Molecular breeding, Norway spruce, plant defensin, transformation.

Introduction

During the last decade biotechnology has been integrated into the breeding programmes of several agricultural and horticultural species. The use of DNA markers for the selection of valuable traits is today an established method (KELLY and MIKLAS, 1998; YOSHIMURA, et al., 1995), and genetic engineering can be applied to several herbaceous species (BIRCH, 1997). However, the use of biotechnology for the breeding of tree species has until now been very limited. This is especially true of the economically important coniferous forest tree species, despite the fact that the benefits for these will be considerable. The breeding cycle of most crop plants takes less than one year, while for tree species it spans several years, for example in Norway spruce one breeding cycle is about 25 years. Anything that can be done to speed up the breeding cycle or improve the genetic gain per cycle will be an important input.

Conifers are outbred and strongly heterozygous, and the traits bred for are quantitative, continuously varying in the population. At least some of the quantitative traits are regulated by major genes, which can be determined by the association of a locus (quantitative trait locus, QTL) with the trait in a segregating population in linkage disequilibrium. In some cases, 'candidate' major genes putatively regulating a quantitative trait have been identified that map close to a known QTL associated with the trait. An example in conifers is the *cad-n1* locus of loblolly pine, which alters the composition of the lignin and maps at or close to a QTL regulating growth (WU et al., 1999). For quantitative disease resistance, candidate gene analysis has been applied successfully to wheat (e.g. FARIS et al., 1999). As a complement to such mapping studies, the importance of specific genes can be tested in transgenic plants that over- or underexpress the gene of interest. This approach

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