Inheritance of Isozyme Variants in *Austrocedrus chilensis* (D. DON) FLORIN et BOUTELJE

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1. Summary

Austrocedrus chilensis (D. DON) FLORIN et BOUTELJE, "Ciprés de la Cordillera" is a dioecious conifer (Cupressaceae) that occupies a very wide precipitation range in the southern Andes Cordillera of South America. The drastic reduction of its natural distribution area in the last two centuries and its actual importance as a forest tree, make it essential to know the present range of its genetic variation in order to conserve and use it properly. To solve this through an allozymic approach the first step is to determine gene markers.

Seeds of 368 trees from 15 populations mainly from the center of the natural distribution area, were collected. Megagametophytes (haploid tissue) from each tree were subjected to horizontal starch gel electrophoresis. Twenty enzyme systems were analyzed. Data from six polymorphic systems were tested for MENDELian segregation ratios resulting in 10 controlling loci codifying for 23 alleles. Thirty five percent of them are rare alleles with a frequency less than 0.01, determining minor polymorphism as the predominant genetic profile of this species for the analyzed loci.

Phosphoglucomutase (PGM), leucine-amino peptidase (LAP) and glutamate dehydrogenase (GDH) reveal invariant electrophoretic patterns. In glutamate-oxalacetate transaminase (GOT) three zones were found; one invariant and the other two controlled by one locus each with three and two alleles respectively. Superoxide dismutase (SOD) showed a multiplebanded phenotype where one gene-locus controlling two alleles is postulated. Two zones revealed 6-phosphogluconate dehydrogenase (6-PGDH), one of them controlled by one locus with three alleles. Five zones were found in malate dehydrogenase (MDH). Three of them showed variation with two, three and two alleles respectively. Isocitrate dehydrogenase (IDH) showed two zones, and both of them with two allelic variants. It was not possible to confirm statistically the variation observed in shikimate dehydrogenase (SDH). The importance of a planned sampling when determining gene markers is discussed.

Key words: Austrocedrus chilensis, allozyme markers, inheritance, Cupressaceae, genetic variation, sampling.

FDC: 165.3; 174.7 *Austrocedrus chilensis.*

2. Introduction

Austrocedrus chilensis, "Ciprés de la Cordillera", is a dioecious conifer (Cupressaceae) endemic to Argentinean and Chilean Subantartic Forest (BRION et al., 1993). In the eastern side of the Andean-Patagonian Cordillera, this species is naturally distributed from 43° 35' to 36° 30' S lat. (DEZZOTTI and SANCHOLUZ, 1991). It occupies the warmer sites through a very wide precipitation range in East-West direction, from less than 400 mm to more than 2500 mm of average annual precipitation. This distribution covers one of the biggest precipitation gradients in the world for a forest tree species.

Due to the direct and indirect benefits, this native cypress is a very important forest tree species in the region. Its wood and its function as watershed protector and landscape component are essential for the economy the area. Estimations suggest that the present 140.000 ha originated from 2.000.000 ha that would have existed in the past (SCHMALTZ, 1992). Its irrational exploitation since the end of last century, by cattle overgrazing, forest fires (both intentional and non-intentional) and a specific disease ("mal del ciprés"; HAVRYLENKO et al., 1989), were the principal causes for the loss of its valuable genetic potential.

A genetic variation study of this species, specially related to the precipitation gradient, has been included in the program "Use and conservation of Patagonian forest genetic resources" (GALLO, 1993). Determination of allozymic gene markers was planned as the first step before undertaking the population genetic analysis.

Conifers are the main forest tree group whose genetic variation is studied through isozyme gene markers. The haploid condition of the megagametophyte tissue in its seeds allows direct observation of MENDELian segregation at heterozygous isozyme loci, without the need for controlled crosses. Few studies of variation in Cupressaceae species have been published (COPES, 1981; HARRY, 1983 and 1986; CONKLE, 1987; YEH, 1988; PERRY and KNOWLES, 1989; MILLAR and MARSHALL, 1991; XIE et al., 1991 and 1992; PAPAGEORGIOU et al., 1993, 1994); and only two deal with *A. chilensis* in particular (GALLO and GEBUREK, 1994; and FERREYRA et al., 1996).

GALLO and GEBUREK (1994) analyzed the inheritance of isozyme variation in megagametophytes from 24 trees and FERREYRA et al. (1996) omitted such analysis in cotyledon (diploid tissue) zimograms of the progeny from 16 trees.

The limited number of analyzed trees and populations in these previous studies, the lack of genetic analysis in one of them, and the risk of assuming as valid the same controlling loci in other species even of this same family (GALLO and PASTORINO, in preparation), made it necessary to determine more confidently the genetic control of allozyme variants of *A. chilensis* before considering them as genetic markers for use in population genetic studies. The preliminary results of the present research have been presented in two congresses (PASTORINO and GALLO, 1995a and b).

3. Materials and Methods

Cones of 368 trees from 15 populations (from six to 35 trees per population) covering the entire precipitation gradient (from 330 mm to 2650 mm average annual precipitation) in three transects in the central part of the natural distribution area in Argentina, were collected during the fall of 1994, 1995, 1996 and 1997. Between 10 km to more than 30 km separate contiguous populations along the same transect.

Seeds were removed from the cones by hand and dried in oven at 37 °C for 21 hours. They were stored at 4 °C during six

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months untill they were used in the analysis. A reserve was kept at –15 °C (enzyme activity was observed until three years later). Horizontal starch gel electrophoresis was performed immediately after each seed harvest. Homogenates and buffer systems were prepared following the same routine as GALLO and GEBUREK (1994). Gels were prepared from hydrolyzed starch, with 10% w/v starch and 2% w/v sucrose. A constant amperage of 120 mA for Tris-Citrate continuous system (1) (about 3.5 hr.) and 70 mA for Poulik discontinuous system (2) (about 5.5 hr.) was utilized with a bridge distance of 6.5 cm. Staining solutions were prepared according to CHELIAK and PITEL (1985).

Twenty enzyme systems were analyzed. With buffer system (1): 6-phosphogluconate dehydrogenase (6-PGDH, E.C.1.1.1.44); malate dehydrogenase (MDH, E.C.1.1.1.37); shikimate dehydrogenase (SKDH or SDH, E.C.1.1.1.25); isocitrate dehydrogenase (IDH, E.C.1.1.1.42); menadione reductase (MR, E.C.1.6.99.2); phosphoglucomutase (PGM, E.C.2.7.5.1); glucose-6-phosphate dehydrogenase (G6PD, E.C. 1.1.1.49); alcohol dehydrogenase (ADH, E.C.1.1.1.1) (also assayed with buffer system (2)); malic enzyme (ME, E.C.1.1.1.40) and aconitase (ACO, E.C.4.2.1.3) were analysed. With buffer system (2): glutamate-oxalacetate transaminase (also called aspartate aminotransferase, GOT or AAT, E.C.2.6.1.1); superoxide dismutase (SOD, E.C.1.15.1.1); leucine-amino peptidase (LAP, E.C.3.4.11.1); acid phosphatase (ACP, E.C.3.1.3.2); glutamate dehydrogenase (GDH, E.C.1.4.1.3); diaphorase (DIA, E.C.1.6.4.3); esterase (EST, E.C.3.1.1.1); phosphoglucose isomerase (PGI, E.C.5.3.1.9); nicotinamide adenine dinucleotide dehydrogenase (NADHDH, E.C.1.6.99.3) and nicotinamide adenine dinucleotide phosphate dehydrogenase (NADPHDH, E.C.1.6.99.1) were analysed. The lower slice of the gel (3 mm thick) was used to get the best resolution in GOT. Only LAP required a presoaking solution before staining. Thirteen percent EDTA w/w was added to PGM.

Because of the higher isozyme activity of the megagametophyte, the entire seed could be used for the routine works (GALLO and GEBUREK, 1994). Eight seeds per tree were subjected to electrophoresis in a first screening. Thus, the probability of identifying an heterozygous tree as such is 0.992. Additional samples (up to 166 seeds from a single tree) were assayed to verify the MENDELian segregation in individuals.

The isozyme nomenclature proposed by HARRY (1983) was followed (e.g. the most anodal zone for GOT is: GOT1, and the locus codifying for it (once proved) is: *Got1*; and the most common allele of this locus is: *Got1-100*, while the other alleles are expressed relative to it). Null allele (lack of stain activity) is expressed as *-n*, and the alternate is *-100* regardless of its frequency.

Inheritance of isozyme variants within each proposed zone was verified quantitatively through analysis of segregation ratios from putative heterozygotes. Chi-square test (α: 0.05) was performed to test MENDELian segregation (1:1 ratio) in single trees. Homogeneity between segregation ratios of all the heterozygous trees for a particular locus was tested by loglikelihood G-test (SOKAL and ROHLF, 1979) (G_h, α: 0.05). When homogeneity was not rejected, the 1:1 segregation ratio of the pooled data was tested (G_p, α: 0.05).

4. Results and Discussion

Observed phenotypes are sketched in *figure 1* (monomorphics) and *figure 2* (polymorphics). The enzymes PGI, NADHDH, NADPHDH, ADH, G-6-PD, ACO, SAP and ME, presented weak or no resolution in the initial electrophoresis analysis, and therefore were discarded. Cathodal migration was analyzed for some enzyme systems in few trees but no staining activity was observed (6-PGDH, MDH, GOT, IDH, PGM, PGI, LAP and ADH).

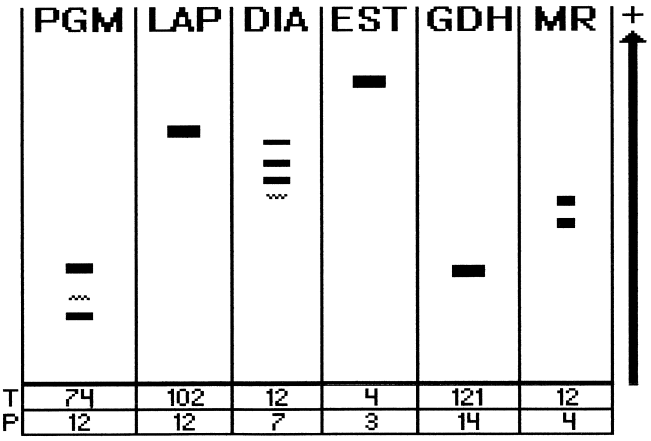

Figure 1. – Electrophoretic patterns of monomorphic isozyme systems found in *Austrocedrus chilensis* megagametophytes, and number of trees (T) and populations (P) analyzed in each.

4.1. Description of isozyme patterns and inheritance

4.1.1. Monomorphic enzyme patterns

Monomorphic patterns were observed in the initial electrophoresis analysis of six enzyme systems: PGM, LAP, DIA, EST, GDH and MR. The numbers of trees and populations analyzed are shown in *figure 1*. Because of lack of variation in the zymograms no genetic control assumptions are made for these systems. Sampling for DIA, EST and MR was too low (*Fig. 1*) to be discussed.

4.1.1.1. Phosphoglucomutase (PGM)

Two bands with a third faint one between them were found consistently in 74 trees from 12 populations. PERRY and KNOWLES (1989) proved the existence of one PGM locus in *Thuja occidentalis* L. megagametophytes coding for three alleles. PAPAGEORGIOU et al. (1993) found variation in two of three described zones in perisperm and embryo of *Cupressus sempervirens* L. seeds, coding for three and two alleles. XIE et al. (1991) observed two zones in megagametophytes of *Thuja orientalis* L. and proved the genetic control in one of them by a locus (with four single-banded alleles). In *Calocedrus decurrens* (TORR.) FLORIN megagametophytes, HARRY (1986) observed a single zone with four variants. Five variants of a single zone too were described by MILLAR and MARSHALL (1991) in the same tissue of *Chamaecyparis lawsoniana* (However, no genetic control performed in these studies).

4.1.1.2. Leucine-amino peptidase (LAP)

A single constant band was observed in 102 trees from 12 populations confirming what GALLO and GEBUREK (1994) had observed in their megagametophyte samples. COPES (1981), working with *Thuja plicata* DONN ex D. DON seedling homogenates, and XIE et al. (1991), working with *T. orientalis*, also found no polymorphism. In contrast, in cotyledon samples of *A. chilensis*, FERREYRA et al. (1996) described three electromorphs assumed as alleles belonging to a single locus, although they did not allow for inheritance analysis. Also without such analysis, CONKLE (1987) and MILLAR and MARSHALL (1991) reported one assumed locus with three alleles for endosperm homogenates from *Cupressus macrocarpa* HARTW. and from *Ch. lawsoniana*, respectively. In the other hand, HARRY (1986)

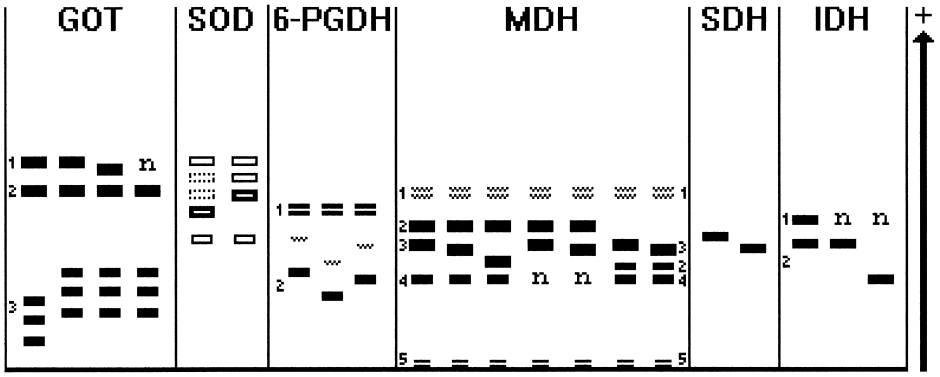

Figure 2. – Electrophoretic patterns of polymorphic isozyme systems found in *Austrocedrus chilensis* megagametophytes (n means null allele).

and PAPAGEORGIOU et al. (1994), proved the inheritance of the electromorphs of their zymograms: two variable loci coding for four and three alleles in *C. decurrens*, and one locus coding for six alleles in *Cupressus sempervirens* L., respectively.

4.1.1.3. Glutamate dehydrogenase (GDH)

A slow single band was observed for this enzyme system in 121 trees from 14 populations. HARRY (1986) and COPES (1981) also observed a single invariant band. Similarly PERRY and KNOWLES (1989) found a single band, but with an allelic variant (although in only one tree of 224 studied). PAPAGEORGIOU et al. (1993 and 1994) reported a single locus with four alleles for *C. sempervirens*, while XIE et al. (1991) also reported a single locus for *T. orientalis* but with two alleles. Similarly, a single locus with three alleles and with two alleles was assumed by MILLAR and MARSHALL (1991) and by CONKLE (1987) respectively.

4.1.2. Polymorphic enzyme patterns

Results of the analysis for tested segregation ratios are presented in *table 1.*

4.1.2.1. Glutamate-oxalacetate transaminase (GOT)

Three zones of activity are proposed for this enzyme system: two variable and one constant. The most anodal is codified by one locus with three alleles: *Got1-100, Got1-97* and *Got1-n* (*Fig. 2*). Both homozygotes of the first two alleles and the three possible heterozygotes were found. The null allele was inferred in two heterozygous trees from different populations. Since these heterozygotes were different (*100/null* and *97/null*) their segregation ratios were tested separately (*Tab. 1*). GALLO and GEBUREK (1994) verified the genetic control of alleles *Got1-100* and *Got1-97* but did not find the null allele.

The lowest triple-banded zone (*Fig. 2*) has already been described by GALLO and GEBUREK (1994) and is confirmed in

Table 1. – Genetic analysis of isozymes in *Austrocedrus chilensis.* Homogeneity of segregation ratios and MENDELian hypothesis proved through log-likelihood G-test in pooled data and through chi-square test in single tree data.

Locus	Putative Genotype	Nº Analyz. Trees	Nº Analyz. Populations	Nº Hetero-zygotes	Pool. Data Segr. Ratio	G_h	G_t	Individual Segr. Ratio	X^2
	100/97	336	15	74	432:384	80.5	83.3	45:49	0.17
Got1	*100/null*	336	15	1	--	--	--	56:52	0.15
	97/null	336	15	1	--	--	--	46:50	0.17
Got3	*100/50*	352	15	8	118:107	1.9	2.5	52:52	0
Sod	*100/115*	354	15	146	1014:965	157.1	158.3	62:74	1.06
6-Pgdh2	*100/78*	356	15	84	452:437	69.2	69.5	46:46	0
	100/92	356	15	2	89:84	1.9	2.1	54:60	0.32
Mdh2	*100/72*	353	15	7	158:164	4.8	4.9	84:82	0.02
Mdh3	*100/103*	353	15	61	411:226	53.3	108*	60:39	4.45*
	100/93	353	15	1	--	--	--	47:49	0.04
Mdh4	*100/null*	353	15	2	107:91	0.5	1.8	52:49	0.04
Sdh	*100/94*	174	9	50	232:229	77.1*	--	15:24	2.07
Idh1	*100/null*	128	14	1	--	--	--	61:51	0.89
Idh2	*100/65*	128	14	1	--	--	--	53:36	3.25

*) significant at $\alpha = 0.05$

the present work (*Tab. 1*). Although FERREYRA et al. (1996) interpreted the opposite, its genetic control has been elucidated by GALLO and GEBUREK (1994). Since *Got3-50* was observed in nine of 352 analyzed trees and only once as heterozygote, it is confirmed as a rare allele.

YEH (1988) and PERRY and KNOWLES (1989) observed two zones in megagametophytes of *T. plicata* and *T. occidentalis* respectively, but in both cases only one was proved to be controlled by a gene-locus. Both HARRY (1986), in *C. decurrens*, and XIE et al. (1991) in *T. orientalis*, found three variable zones codified by three different loci, and triple-banded the most cathodal as seen in the present work.

4.1.2.2. Superoxide dismutase (SOD)

This enzyme system revealed a negative multiple-banded phenotype where one codifying gene-locus with two alleles was determined. The most anodal band was clearly seen and two faintly stained bands appeared below it (*Fig. 2*). A slower darker fourth band belongs to a different zone whose genetic control was proved (*Tab. 1*). The slowest allele is the most common: *Sod-100*, and the fastest (*Sod-115*) appears to overlap the slowest of the upper faint bands, so it seems that a band is missing. Another alternative to explain this pattern would be to consider a null allele as shown in previous work (GALLO and GEBUREK, 1994; PASTORINO and GALLO, 1995a and b), but the greater intensity of the overlapping bands does not support this idea. Finally, a fifth invariant band was observed slightly below, which was useful to make easier the identification of the above described gene marker. Thus, two single-banded alleles were observed in heterozygous and both homozygous forms. The MENDELian segregation ratio was proved through both individuals and pooled data tests (*Tab. 1*).

Single invariant zone was observed in *T. occidentalis* (PERRY and KNOWLES, 1989) and in *T. orientalis* (XIE et al., 1991), whereas this system showed polymorphism in *C. decurrens* (HARRY, 1986) (three single-banded alleles) and *T. plicata* (YEH, 1988) (two alleles).

4.1.2.3. 6-Phosphogluconate dehydrogenase (6-PGDH)

One invariant and one variable zone as already described by GALLO and GEBUREK (1994) were confirmed (*Fig. 2*). This variable zone consists of a slow, dark band and another faint one located above the first at a fixed distance. The two marker alleles already determined in the work mentioned above were confirmed statistically in 84 heterozygous trees: *6-Pgdh2-100* and *6-Pgdh2-78*. Both homozygotes and the heterozygote were observed. A new allele with intermediate migration rate between the other two was found. This allele, *6-Pgdh2-92*, was observed in only two heterozygous (*100*/*92*) trees of the 356 analyzed, and verified statistically through both individuals and pooled data tests (*Tab. 1*). Two thin bands could be recognized in the invariant zone.

Since the embryo was rutinely homogenated together with the endosperm, two extra faint bands could sometimes be seen in the *6-Pgdh2* locus corresponding to the expected heterozygous genotype from diploid tissue in dimeric enzymes. Excised embryos were then run separately from their own endosperms and a phenotype with three bands (darker the one of the middle) verified this assumption. This additional proof confirmed the validation of the methodology of using the whole seed tissues, because of the higher enzyme activity of the endosperm (GALLO and GEBUREK, 1994).

PERRY and KNOWLES (1989) also found two zones in this enzyme: invariant the most anodal and with one locus codifying for three alleles the other one, as seen in the present work. HARRY (1986) and XIE et al. (1991) observed two loci too, but variable both of them. In *C. sempervirens*, PAPAGEORGIOU et al. (1993) found three staining zones but only one variable (with three alleles). Polymorphism was also reported by YEH (1988) too.

4.1.2.4. Malate dehydrogenase (MDH)

This is the most complex isozyme system found in *A. chilensis*. Five staining zones can be recognized on its multiple-banded zymograms (*Fig. 2*). The bands of the most anodal zone (MDH1) stained faintly and were invariant in all 353 analyzed trees. MDH2 showed variation with the slowest electromorph overlapping between MDH3 and MDH4. This hypothesis was proved statistically in the observed segregation ratio verifying the presence of two alleles: *Mdh2-100* and *Mdh2-72* (*Tab. 1*). This last allele was found exclusively in one of the 15 populations. In MDH3 three phenotypes were observed. Two of them (MDH3-100 and MDH3-103) were not easy to distinguish from each other, because of their relative proximity. In some very clear electrophoretic runs, they were obvious, but in general, the identification was unreliable. This difficulty hindered the statistical confirmation of the segregation because the expected segregation ratio tests (both pooled and single data) were refused (*Tab. 1*). A third phenotype of this zone was verified statistically (*Tab. 1*) as *Mdh3-93* allele. This allele is extremely rare since it was found in only one of 353 analyzed trees. The fourth zone was coded by a single locus with two alleles (*Tab. 1*). One of them verified as a null allele was observed in only two heterozygous trees. The most cathodal zone, MDH5, is an invariant double band very near of the starting point of the gel.

GALLO and GEBUREK (1994) described four zones and could prove a codifying gene-locus for their second varying zone, with two alleles, that they called *Mdh2-100* and *Mdh2-105*, and are assumed as the MDH3-100 and MDH3-103 phenotypes described in the present research. Neither our loci *Mdh2* or *Mdh4*, with two alleles each, nor the rare allele *Mdh3-93* were observed in the cited work.

HARRY (1983) proposed a very complex heredity system for this enzyme in *C. decurrens*. Three activity zones, one coded by a single locus with three alleles, other determined by two unlinked loci: structural and modifier, and the last one not reliably enough to be scored. Three zones were also reported by PERRY and KNOWLES (1989) in *T. occidentalis*: three alleles for the most anodal, invariant the intermediate zone, and with a null allele the slowest one. PAPAGEORGIOU et al. (1993 and 1994) observed in *C. sempervirens* three migrating zones too; the two slowest were variable, with three and two alleles respectively.

4.1.2.5. Shikimate dehydrogenase (SDH)

This enzyme system showed two electromorphs of an unique single-banded zone but they were so close and faint that they could not be clearly differentiated in all runs. One of the analyzed trees showed a segregation ratio close to the expected 1:1 (*Tab. 1*) but the pooled data could not be tested since the homogeneity of the observed segregations was rejected (*Tab. 1*).

GALLO and GEBUREK (1994) proposed one locus codifying for two alleles proving the MENDELian segregation hypothesis with individual and pooled data of seven heterozygous trees. This enzyme system revealed two variable zones in *C. decurrens*, coded by two loci with eight and five alleles respectively (HARRY, 1986), and three zones (XIE et al., 1991), but only the most anodal proved to be controlled by a locus, with three alleles.

4.1.2.6. Isocitrate dehydrogenase (IDH)

This enzyme system shows two variable zones. The most anodal varying in the presence or absence of one band: only one

from 128 analyzed trees were found to be heterozygote for IDH1 zone segregating in a 1:1 ratio of the proved genotype *Idh1-100/n* (*Tab. 1*). Lack of stain activity was assumed for embryo in this zone (twenty embryos of the heterozygous tree run separately revealed only the slowest band). The slowest zone is codified by a gene-locus with two alleles: *Idh2-100* and *Idh2-65* (*Tab. 1*). Only one individual of 128 analyzed revealed this last allele (heterozygote) and, as in *6-Pgdh2*, three faint bands could be seen as product of the embryo stain activity of this dimeric enzyme.

GALLO and GEBUREK (1994) did not find either *Idh1-100* or *Idh2-65* alleles, so they probably recognized only the slowest zone which was assumed as a single invariant band. In *C. decurrens* HARRY (1986) found also two zones: the most anodal presented an invariant single band, while the other was proved to be controlled by one locus with two alleles. Also two loci were verified by PERRY and KNOWLES (1989) in *T. occidentalis* and by XIE et al. (1991) in *T. orientalis*: the slowest with three alleles and the fastest with two (one of them null) for the first species, and both loci with three alleles for the last one.

4.2. The importance of planning the sampling

Due to the many misinterpretations that appear when assuming directly the zymograms of any species, even within the same taxonomic family, the determination of gene markers through inheritance analysis should be the first step before proceeding with population genetic studies. This means that the determination of gene markers should be a planned research activity and not just a secondary task of a variation study, as it seems to be in many cases. Only completely, precisely, and confidently determined gene markers can be used in later variation studies and comparative analysis among them.

One of the most important aspects of a good marker determination refers to the size and location of the sampling. When determining gene markers in conifers megagametophytes different levels of sampling are consciously or unconsciously considered. These include within individuals, populations and species sampling.

In the first case the minimum number of seeds per tree plays a role in relation to the probability of correctly identifying an heterozygote. This is given by the following formula: $1\text{-}[1/2^{n-1}]$, where n is the number of megagametophytes analyzed per mother tree. Some authors consider 5 or 6 seeds as a proper sampling size to detect the heterozygous individuals (e.g. in Cupressacea: HARRY, 1983; CONKLE, 1987; MILLAR and MARSHALL, 1991). In the present research eight seeds were run, this means that the probability of finding the two alleles of an heterozygote was 0.992. Even though, one heterozygous tree analyzed was first considered as homozygote because all eight megagametophytes showed the same phenotype. This means we were dealing with the rare case of occurrence with a 0.008 remnant probability. Because it was a rare allele expected to be present as heterozygote, extra runs were made, and correct identification was possible.

An interesting comparison of the results of our work in relation to sampling is with those of GALLO and GEBUREK (1994). This is because of the use of the same tissue and methodology in both of them but a very different number of trees collected from different populations. This means that the location and specially the size of the sample should be the main influencing factors for both the quantitative and qualitative differences in the compared results. Most of the 24 trees collected in the preliminary work of GALLO and GEBUREK (1994) were distributed mainly in the central part of the natural distribution area of the species but belonged to different populations than in the present work. All the alleles found in this previous work were also observed in our present work. On the contrary, the analysis of the larger sample of 368 trees collected systematically in the present work allowed the finding of seven rare alleles in heterozygous state. This made it possible to determine four new marker gene loci which resulted in a total of 11 more marker alleles than in the preliminary work.

If the genetic variation of this species had developed homogeneously then a sample of about 350 trees would have had a probability equal to 0.95 to detect rare alleles with a frequency of about 0.018 (HATTEMER et al., 1993). However, the distribution of the genetic variation is not homogeneous, specially in mountain regions. Therefore when determining allozyme gene markers the systematization of the sampling appears to be very important. In this strong differentiated species the collection along transects following the main environmental factor (precipitation gradient) provided a good screening method of the genetic variation.

A rare allele if considered the analyzed species level, but exclusive and rather common in one of the screened populations like *Mdh2-72* ($f > 0.20$) would have been probably found in the previous work if this population had also been chosen. In this case, the determining factor for detecting this locally rather frequent allele and marker gene locus was the location of the sampling and not the number of trees analyzed. On the other hand a higher number of trees per population are necessary to detect such rare alleles that appear in more than one of them like *Mdh4-n* or *6-Pgdh2-92*. As inferred from *table 1*, 35% of the determined marker alleles have to be considered as rare with a frequency less than 0.01. This means that monomorphism (fixation for a single allele) and minor polymorphism (one allele prevalent, at least one allele, no allele of intermediate frequency) (GREGORIUS and BERGMANN, 1995) were the predominant genetic profiles found at the population level in this species. The ecological genetic significance of this, deserves a special analysis and discussion to be included in a future analysis of genetic variation and differentiation of this species.

Before determining gene markers (before speculating about population genetics) it is essential to plan a good sampling scheme in the field. This means to have an *"a priori"* hypothesis of the distribution of the genetic variation of the species and to determine which populations and how many trees per population are going to be collected. The size and location of sampling are therefore very important in determining many and confident gene markers.

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Clonal Variation in Wood Quality and Growth in Young Sitka Spruce (*Picea sitchensis* (BONG.) CARR.): Estimation of Quantitative Genetic Parameters and Index Selection for Improved Pulpwood

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Summary

Repeatabilities and clonal correlations were estimated for wood properties (pilodyn and lignin content), stem straightness and growth characters (height, diameter and volume index) measured in young Sitka spruce clones, under fast growing conditions. The estimates of the quantitative genetic parameters were based on a random sample of 30 clones taken from a population of 253 clones at age 9 years from field planting. The magnitude of the clone-mean repeatability estimate indicated that the examined characters are able to respond well to selection based on clone means and subsequent vegetative propagation. However, for lignin content, the phenotypic variation was rather low (i.e. coefficients of variation of 3.7% and 2.9% on the individual ramet and clone mean bases, respectively), which may limit the magnitude of the genetic gain. The estimated clonal correlations between the wood properties and growth pointed out that clonal selection for reducing the population level in lignin content and/or increasing the whole-ring density is expected to result in an indirect genetic decline in growth.

Multiple trait selection indices were constructed to explore the possibilities of improving the yield and/or the quality of young Sitka spruce wood for pulp production. In the absence of quantitative economic data – and to ascertain the possible consequences of clone selection and clonal propagation for the proposed end use – contrasting multiple trait index selection options were compared on the basis of predicted genetic responses in wood properties, growth and stem straightness.

Key words: *Picea sitchensis*, lignin content, pilodyn, wood density, growth traits, stem straightness, repeatability, clonal correlation, index selection, pulp production.

FDC: 165.3; 165.441; 165.6; 181.64/.65; 232.13; 812.31; 813.11; 861.0; 174.7 *Picea sitchensis*; (489).

Introduction

In a tree breeding programme that aims at producing raw material with better qualities for a given wood processing industry, it is essential to include wood properties as potential selection criteria. The present work places particular emphasis on whole-ring density and lignin content in wood, and it is intended to provide information that can be especially relevant to the utilization of Sitka spruce (*Picea sitchensis* (BONG.) CARR.) wood for pulping purposes.

Sitka spruce wood has pale colour, good fiber characteristics and low resin and other extractive content, which make this

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