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Variation and Inheritance of Manoyl Oxid Acid in Pinus sylvestris (L.)

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Summary

The occurrence of manoyl oxid acid in clones and controlled crosses of *Pinus sylvestris* was investigated. The occurrence of manoyl oxid acid is under genetic control but there is not a simple Mendelian monogenic mechanism. It is suggested that manool is a precusor for manoyl oxid acid.

Key words: Pinaceae, Scots pine, genetics, biosynthesis.

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Introduction

In Scots pine needles pinifolic acid and its monomethyl ester are the dominating diterpenoids together with minor amounts of abietic, pimaric and 4-epiimbricatolic acids (ENZELL and THEANDER, 1962; TOBOLSKI and ZINKEL, 1982; ANDERSSON et al,. 1990). In some individual pine trees the diterpene alcohol isoabienol is the dominating compound while it is totally lacking in others (GREF, 1981). Manoyl oxid acid, the major resin acid of *Pinus resinosa* needles, has been found to be the principal resin acid of *Pinus nigra* and of *Pinus sylvestris* in central Europe (TOBOLSKI and ZINKEL, 1982; ZINKEL et al., 1985) but very seldom in Scots pine native to Scandinavia (ANDERSSON et al., 1990).

In genetical research of conifers, needle monoterpenes have often been used as markers (e.g. HILTUNEN, 1975; HILTUNEN et al., 1975; VON RUDLOFF, 1984; WHITE, 1983, 1984). Because of their physiological stability and ease of analysis they are well suited for this purpose. Although the diterpenes possess structural variety and occur in high concentrations, sometimes up to 2% to 3% of needle dry weight they have very seldom been used as genetical markers. This can partly be attributed to lack of suitable analytical techniques, but also to the fact that the diterpenes are to some extent affected by such environmental factors as light and nutrients (GREF and TENOW, 1987; BJÖRKMAN et al., 1991).

In our laboratories isoabienol, pinifolic acid and monomethyl pinifolate have been extensively studied in the search for genetical markers in resistance breeding. These studies have shown that neither isoabienol (GREF et al., 1985) nor pinifolic acid/monomethyl pinifolate (GREF and LINDGREN, 1984) are powerful as markers although the number of trees with or without isoabienol often segregated in agreement with a monohybrid pattern. Moreover, the occurrence of isoabienol also showed a distinct geographical variation pattern.

This study was done in order to determine seasonal variations and inheritance patterns of manoyloxid acid in the needles of Scots pine native to Scandinavia.

Materials and Methods

Plant Materials

Material 1

The Scots pine trees used in this investigation consisted of 15-year-old grafts growing in a nursery at Hörnefors (63° 28' N, 19° 54' E), 30 km SW of Umeå. In this nursery there are 103 clones, each represented by at least 8 grafted trees. Previous investigations have shown that only 2 clones, AC 5045 and BD 5056 contain manoyl oxid acid. There are no traces of this compound in any other clone. These 2 clones were analyzed for within-tree and maturational variations of manoyl oxid acid in May, July and October 1989 and in February 1992.

Material 2

In the spring of 1989 a number of controlled crosses between the clones with monoyl oxid acid, AC 5045 and BD 5056 and 2 clones without this acid, AC 3008 and BD 5027 were performed. The trees were crossed in all possible combinations.

Mature cones from these crosses were collected in September 1991. Seeds were extracted from the cones. In February 1992 the seeds were sown in cultivation boxes filled with peat in the greenhouse in Umeå. In May 1992 the seedlings were moved outdoors. In October and December 1992 needles from 10 of these seedlings were sampled for maturational variation and inheritance studies.

Extraction and Analysis

For the chemical analysis 200 mg of fresh needles were cut to small pieces, put into a screw cup test tube and extracted with 2ml of petroleum ether-diethyl ether (1:1) in an ultrasonic bath for 2 hours. The extractive solution was transferred to a new test tube and the solvent was evaporated under a stream of nitrogen. The extractives were redissolved and methylated with diazomethane prior to the gas chromatographic (GC) or mass spectroscopic (MS) analysis. The terpenoids were quantified by adding heptadecanoic acid to the extracts.

GC was performed with a gas chromatograph equipped with a flame ionisation detector and a fused silica DB-1 capillary column (15 m x 0.25 mm). The chromatograph was run isothermally at 210° C and hydrogen was used as a carrier gas. The individual compounds were calculated on a relative percentage basis of eluted diterpenes. The mass spectra were performed on a double focusing Jeol JMS-SX102 mass spectrometer connected to a gas chromatograph equipped with a fused silica SE-30 column (25m x 0.25mm) or on a Finnigan 4000 with a GC equipped with a BP-25 (26m x 0.25mm). Ions were generated with 70 eV and mass spectra were obtained for a mass range of 50 to 800 a.m.u.. All data were processed by Jeol MS-MP 7000 D or Incos data systems.

Results and Discussion

All grafts of BD 5056 and AC 5045 have shown presence of manoyl oxid acid but no other graft. Thus it seems evident that the absence or presence of this compound is under genetic control.

In fully outgrown mature needles of the clones BD 5056 and AC 5045 manoyl oxid acid was the major resin acid constituting 50% to 60% of the detected resin acids (1% to 1.2% needle dry weight) while pinifolic acid was found to be a minor compound. No diterpene alcohol was found in mature needles of these clones. In the non manoyl oxid acid clones pinifolic acid was the principal compound and there were no traces of diterpene alcohols in these clones at any time. Within-clonal variation in quantitative resin acid compositions was insignificant.

In young emerging foliage of the clones AC 5045 and BD 5056 manoyl oxid acid was the principal compound but in much lower concentrations than in mature needles (data not shown). These findings are in agreement with published works (TOBOLSKI and ZINKEL, 1982). A diterpene

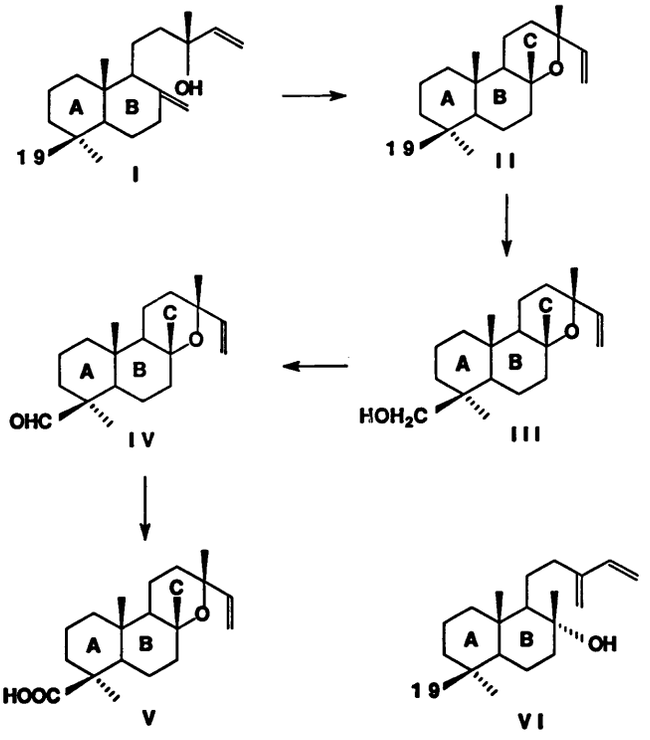

Figure 1. — Proposed biosynthetic pathway for manoyl oxid acid (V) from manool (I) via intermidiates (II, III and IV). Compound VI is isoabienol.

Table 1. — Segregation ratios for the crosses.

Cross Mother	Father	Progeny type	+	-	Probability[a] 1:1	1:3	Empty seeds
BD 5027	AC 3008	-x-	0	10	.001	.056	18
BD 5027	Wind poll.	-x-?	0	10	.001	.056	21
AC 5045	AC 3008	+x-	0	10	.001	.056	15
AC 5045	BD 5027	+x-	0	10	.001	.056	89
AC 5045	Wind poll.	+x-?	0	10	.001	.056	20
BD 5056	AC 3008	+x-	1	9	.011	.244	11
BD 5056	BD 5027	+x-	2	8	.055	.526	57
BD 5056	AC 5045	+x+	3	7	.172	.776	7
BD 5056	Wind poll.	+x-?	1	9	.011	.244	8

+ = with manoyl oxid acid; — = without.
a) probability that so few or fewer + cases would occur if true segregation were 1:1 or 1:3.

alcohol identified by MS most likely as manool (I) in *figure 1*, manoyl oxid (II) and 2 diterpenes of putative structures (III and IV) always occurred together with manoyl oxid acid (V) in emerging needles. During maturation of the young needles from May to October manoyl oxid acid concentration increased from 29% to 50% of total diterpenes detected at the expence of manool. No manool could be detected at the last sampling occasion in December. Identical trends were also observed for the young seedlings of manoyl oxid acid type. It is interesting to note that none of the clones or seedlings with manoyl oxid acid contained isoabienol (VI). To our knowledge isoabienol has not been found together with manool or manoyl oxid acid in Scots pine needles.

It is tempting to consider manool as an intermediate in the biosynthesis of manoyl oxid acid as proposed by the hypothetical route in *figure 1*. This is further supported by the fact that trace concentrations of manoyl oxid and 2 unknown diterpenes with a hydroxyl and a carbonyl group respectively, most probably at the carbon 19, were detected by MS.

The frequency distribution for occurrence of manoyl oxid acid in the crosses is presented in *table 1*.

AC 5045 does not segregate + progeny in any of 3 progenies with — parents, but segregates in a progeny with a + parent. BD 5056 segregates + in all four progenies tested, including 3 with-parents. Thus it seems as the gene is differently inherited in the carrier clones. It is more dominant in BD 5056 than in AC 5045.

For a dominant gene, +x— crosses are expected to segregate 1:1 or 1:0. This was not found, thus it is not a simple dominant inheritance.

For a recessive gene, +x— crosses are expected to segregate 0:1 or 1:1, +x+ crosses are expected to segregate 1:0. Segregation ratios are lower than that. Thus it is no simple Mendelian monogenic segregation.

The character still seems to be inherited, + individuals are much more common in progeny from BD 5056, than in the general population, and AC 5045 as parent seems to increase the incidence. BD 5027 gives rise to high frequency of empty seeds. Probably the pollen is good enough for triggering seed formation, but too bad to produce a high frequency of vital embryos. Crosses +x+ give the fewest empty seeds of all seed lots. Thus genes causing embryo lethality may not be involved.

Possible conclusions from the inheritance study is that (1) there is a hereditary component, (2) the genetic control is not simple Mendelian monogenic, (3) the control is different for the 2 genotypes studied.

A comparison with the segregation patterns of the structurally related isoabienol is interesting. Although there were some irregularities, trees with or without isoabienol often segregated in agreement with a monohybrid pattern (Gref et al., 1985). Trees with isoabienol also occur in high frequency compared to trees with manoyl oxid acid, at least in Scandinavia. The reason for this is unknown, but a possible explanation could be the stereospecificity of the cyclication enzymes. Manool could be easily cyclized to manoyl oxid, whereas isoabienol is unaffected by the enzymes. This could account for the accumulation of isoabienol in some pine trees.

The cyclization of manool to manoyl oxid and the further oxidation of this compound to manoyl oxid acid is certainly under enzymatic control but the cyclization could also be influenced by e.g. phytochemical or other environmental factors. Such secondary reactions have been observed among terpenes (Newman, 1972). Such non-enzymatic reactions will negatively affect the possibility to use a compound as a marker in genetical studies.

Many questions considering the biosynthesis and genetics of manoyl oxid acid remain unanswered. However, this compound could be useful as a marker in population studies as it is rare and distinct.

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Allozyme Variation in Four Populations of Taiwania cryptomerioides in Taiwan

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Summary

Genetic diversity within and genetic differentiation among 4 populations of Taiwania *(Taiwania cryptomerioides* HAY.) in Taiwan were investigated using 327 offspring growing in a 10-year-old provenance/progeny test plantation. Eight of the 15 loci examined were polymorphic. The average proportion of polymorphic loci per population was 50.2% (99% criterion for polymorphism). Mean expected heterozygosity was 0.145, ranging from 0.126 to 0.173 in the different populations. On average, the percent heterozygous loci/individual ranged from 12.3 to 19.4, the number of alleles/locus from 1.53 to 1.67, and the effective number of alleles/locus from 1.16 to 1.26. The slightly lower number of alleles/locus compared to other coniferous species probobly reflects the insular nature of Taiwania's distribution.

Partitioning the genetic variability into within- and among-population components with F-statistics led to an estimate of within-population variation amounting to 94.7% of total variation. This suggests a lack of barriers to gene flow among populations. Geographic distance and NEI's genetic distance appear to be positively related, except the Ta-Jiann population differs from the others in terms of genetic distance to a greater degree than they do among themselves. This may result from topographic isolation of the Ta-Jiann population.

Key words: Taiwania cryptomerioides, allozyme variation.

Introduction

A high proportion of the vascular plants of Taiwan overlap with the flora of mainland China (KENG, 1956). The presence of many endemic species, however, suggests a long evolutionary history since the isolation of Taiwan from the mainland. A landbridge may have existed during the late Miocene and early Pliocene periods (HUANG, 1988). Taiwania (*Taiwania cryptomerioides* HAY.) appeared in the Miocene or Tertiary, as deduced from pollen analyses (HUANG, 1988).

Numerous steep mountains occupy 4/5's of the island and raise the question of the apportionment of genetic variation within and among the populations which extend more or less continuously from north to south at an elevation of 1800m to 2600m (LIU, 1966).

In general, Taiwania grows in ultisol and aceptisol soils, with a pH in the range of 3.5 to 5.0. Stands occur on all exposures (N, E, S, W) when annual precipitation is around 3000mm. Taiwania is usually found in valleys or on hillslopes, but not on the ridge tops, and is mixed with varying proportions of broadleaved trees and other conifers (i. e., *Chamaecyparis, Tsuga, Picea,* and *Cunninghamia).*

Taiwania produces a valuable timber and has been widely used in reforestation in Taiwan over the past 10 years. Six natural populations have been compared in a provenance test. After 10 years, there was no difference in growth among provenances even though they differed in the first 1 to 2 years (LIU et al., 1985). Taking advantage of another younger provenance/progeny test plantation of Taiwania, we were able to quantitatively characterize the genetic structure within and among major populations using isozymes.

Materials and Methods

1. Sampling

Young leaf tissue of 327 offspring belonging to 34 families in 4 populations were collected from a provenance/progeny test plantation. The 4 populations were among the major seed sources of Taiwania collected for reforestation in Taiwan. The location of the original 34 seed trees is given in *Figure 1.* DBH varied from 120 cm to 380 cm, corresponding to an age roughly between 500 years to 1500 years (estimated from unpublished observations). Data on the seed trees is given in *table 1.* The plantation was planted in 1981 at Lien-Hwa-Chi branch station of the Taiwan Forestry Research Institute.

2. Electrophoresis methods

Horizontal starch gel electrophoresis was used to separate isozymes in 8 enzyme systems; namely, EST, F-EST, GOT, MDH, 6PGD, PGI, PGM, SKDH (GOT = glutamate oxalacetate transaminase, E.C.2.6.1.1; 6PGD = 6-phospho-