The Inheritance of Pinifolic Acid in Scots Pine (Pinus sylvestris L.) needles

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(Received 9th May 1984)

Summary

The inheritance patterns of free pinifolic acid and monomethyl pinifolate in needles of 14 genotypes of Scots pine as well as their selfed progenies were investigated. It is concluded that the amount of monomethyl pinifolate is not a strongly inherited character. Free pinifolic acid seems to be under moderately strong genetic control.

Key words: Scots pine, inheritance, pinifolic acid, monomethyl pinifolate.

Zusammenfassung

Die Nadeln von 14 Kiefern-Klonen (*Pinus sylvestris* L.) und deren geselbsteten Nachkommenschaften wurden auf die Vererbungsmuster für freie Pinifolsäure und Pinifolsäure-Monomethylester hin untersucht. Es wird der Schluß gezogen, daß die Menge von Pinifolsäure-Monomethylester in Kiefernnadeln kein streng erbliches Merkmal ist. Freie Pinifolsäure scheint mäßig streng genetisch kontrolliert zu sein.

Introduction

Pinifolic acid is the main resin acid of *Pinus sylvestris* needles (Enzell and Theander 1962). It belongs to the labdane type diterpenoid acids, which are widely distributed in conifers. The major portion of pinifolic acid is present as the monomethyl ester (Bardyshev *et al.*, 1982). This observation has also been confirmed by Ekman (pers. comm.) and this laboratory.

In genetic research of conifers, isozymes (Rudin, 1975, 1977; Yazdani and Rudin 1982), phenols (Chen and van Buijtenen 1980) and terpenoids (Griggs and Squillace 1982; Hiltunen 1975, 1976; Squillace et al., 1980; von Rudloff and Rehfeldt 1980) have been used. In this laboratory isoabienol, the main diterpene alcohol in Scots pine needles has been extensively studied in the search for genetical markers (Gref 1981, Gref and Rudin 1981), Although the diterpene resin acids constitute 2—3% of needle dry weight, they have not been used in genetic studies. Pinifolic acid could be a good marker because it is very stable (compared to abietadienoic acids) and occurs in two forms (free dicarboxylic acid and as the monomethyl ester). This study was carried out to examine the variation of pinifolic acid of Scots pine needles and to determine the inheritance patterns of monomethyl pinifolate and free pinifolic acid.

Material and Methods

Needles from parental clones were collected at the seed orchard E468 Tjuttorp (58° 48' N, 15° 51' E, Alt. 60 m.) in November 1982 on grafts planted 1961. Certain of the plustree clones at Tjuttorp were selfed and crossed. A field trial was sown 1969 and planted in the autumn 1971, at Hägerdal (60° 10' N, 17° 29' E, Alt. 50 m., IDL 14911) around 40 km north of Uppsala. Needles were collected in November 1982 from progenies originating from selfing. Needles from the same plants were also used for a study of the inheritance of isoabienol. Some of the families have also been used for a study of phenolic compounds of Scots pine needles (Theander, pers. comm.). The needles were stored cool and transported as quickly as possible to - 20° C, and then analysed within 6 months. Fourteen clones (two grafts from each clone) and 10 individuals from each of the families were sampled. Approximately 2 g fresh wt of needles were taken for each sample. Before the extraction the needles were cut to pieces and immediately crushed in a mortar with liquid N_2 and freezedried for 24 h.

A 100 mg sample of the needle meal was extracted with 2 ml petroleum ether-diethyl ether (1:1) containing 1 mg of heptadecanoic acid as an internal standard in an ultrasonic bath at 5° C for 2 h. The sample was centrifuged and the extractive solution was transformed to a second screw cap test tube. The residue was washed twice with 1 ml of diethyl ether and the combined extracts were evaporated to dryness under a stream of nitrogen. To the dried extract 0.4 ml bis (trimethylsilyl) trifluoroacetamide and 0.2 ml trimethylchlorosilane were added. After standing at 70° C for 1 h, the TMS esters were analysed by gas chromatography (GC). The samples were analysed using a Varian 2700 gas chromatograph equipped with a glass capillary wall coated open tubular (WCOT) column (45 m × 0.3 mm) coated with SE-30 and operated isothermally at 240° C. Other operational conditions were: injector and detector (FID) temperature 280° C, carrier gas H_2 at 2.0 ml/min, split ratio 1:50. Absolute amount of pinifolic acid was calculated by peak area relative to the internal standard using a CDS III C data system. The identity of pinifolic acid was confirmed by comparison with pure reference compound and by gas chromatography - mass spectrometry (GC-MS). Mass spectra were recorded with an LKB-9000 GC-MS instrument equipped with a column similar to that used in the GC analyses.

Results

Isoabienol

The results are presented and discussed in another paper together with isoabienol data obtained from other plant materials. There are two distinct groups of individuals — with and without isoabienol.

Methyl pinifolate

Between tree levels of methyl pinifolate were relatively constant (data not shown). No trees without methylpinifolate were found. A weak, non-significant correlation ($r = 0.22$) was found between levels of methyl pinifolate in parents and selfed progeny. It is suggested that this is not a strongly inherited character.

Free pinifolic acid

Results are summarized in *Table 1* and *Fig. 1.*

Analysis of variance

The data on individual trees according to *Table 1* were

Table 1. — Free pinifolic acid in parents and selfed progeny (parts per 10 000).

Parental Clone	Progeny 1	2	3	4	5	6	7	8	9	10	Progeny Average	Parent
H1000*	96	25*	55	23*	56	18*	19*	35*	43	-	41	32
H1007*	32*	30*	46	12*	26	45	70	40*	16*	21*	34	22
H1010*	103*	26	33	53	33	51	27	40	51	41	46	37
H1011*	76*	65*	25	58	48*	32*	55	56*	36*	27*	48	78
E3001	19	26	26	64	16	60	46	22	18	-	33	103
E3003	33	74	22	46	19	53	70	38	30	47	43	201
E3004	0	22	29	56	21	29	15	0	0	25	28(20)	83
H1008	18	14	40	15	19	19	26	22	53	26	25	164
S3004	25	0	20	0	20	0	0	0	12	15	18(9)	61
W1015	58	17	17	17	15	27	13	21	35	10	23	57
W3051	23	23	101	31	50	143	69	64	68	40	61	257
W3059	34	43	33	12	39	14	29	12	54	14	28	69
W3123	32	32	38	58	15	34	39	72	21	29	37	141
W3124	26	28	19	52	43	38	27	27	30	31	32	75
Average											35.6	98.6

* an individual with isoabienol
( ) values including assumed segregating zeros

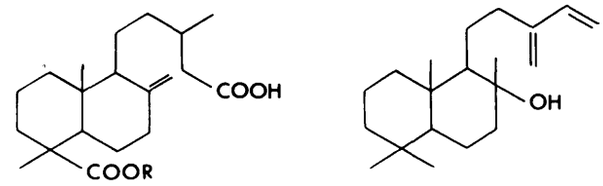
subjected to an analysis of variance. To get the within progeny variance more constant, a logarithmic transformation log (x + 1) was done before the analysis of variance (according to the scheme in Sokal and Rohlf, 1981).

ANOVA

	D.f.	M.s.	Expected mean squares
Between families	13	0.8300	$\sigma_w^2 + 9.80\,\sigma_b^2$
Within families	124	0.1080	σ_w^2

F between families/within families = 7.685, the probability to get such a high value if there is no between family variation is < 0.001, thus it can be concluded that families do differ.

Components of variance: $\sigma_w^2 = 0.1080$; $\sigma_b^2 = 0.0732$.



Pinifolic acid Isoabienol

Figure 2. — Structures of compounds mentioned in the paper.
R = H (free pinifolic acid)
R = CH_3 (monomethyl pinifolate)

An exact estimate of heritability cannot be done, but if it is assumed that all genetic variance is additive; $\sigma_b^2 = V_A$ and $\sigma_w^2 = 1/2\,V_A + V_E$.

$h^2 = V_A/(V_A + V_E) = 0.51$

Regression analysis

The regression of the "midprogeny" on the parental values is illustrated in *Fig. 1*. The average of the segregating progenies (see below) is based on values > 0 only. The progenies of parents with isoabienol seem to form a separate group, and two analysis are carried out, for all 14 parents, as well as for the 10 parents without isoabienol. In the later case a significant (r = 0.86, p < 0.01) and in the former an insignificant (r = 0.45) correlation was obtained.

Segregations

In two progenies segregations of trees without measurable amounts of free pinifolic acid was found.

	with	without
E3004	7	3
S3004	5	5

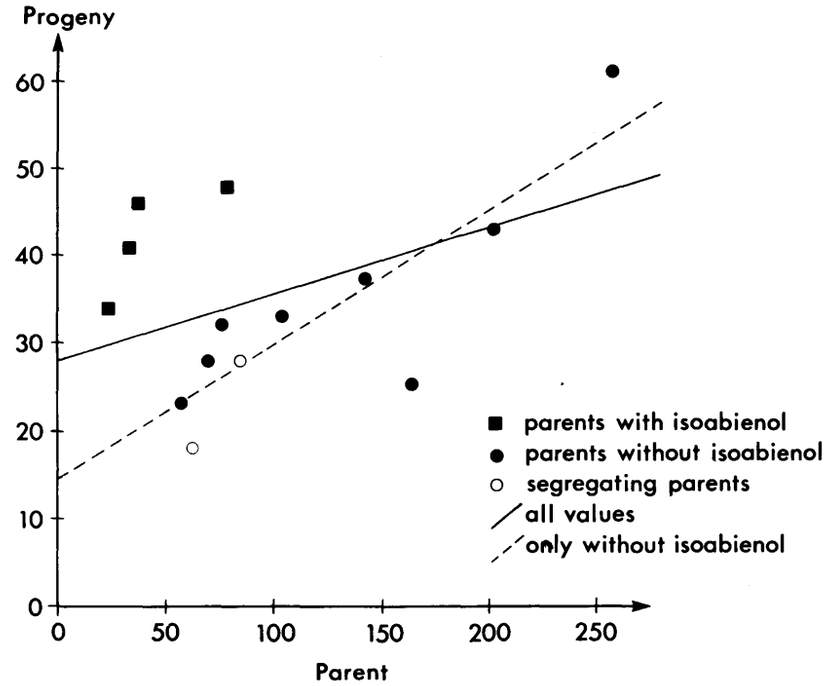


Figure 1. — Regression of selfed progeny values on the parental values. Free pinifolic acid, parts per 10 000 of the dry weight. The regression line is y = 28.1 + 0.075 x if all values are considered, and y = 14.4 + 0.153 x if only parents without isoabienol are considered.

Discussion

Connection isoabienol - free pinifolic acid?

The pathways for the biosynthesis of isoabienol and pinifolic acid has not been established but the structural similarity between these two compounds suggest that they may be formed from a common intermediate.

In *Fig. 1*, parents with isoabienol seem to be lower in free pinifolic acid. However, this trend does not seem to be supported within progenies. Thus no clear conclusion is possible from this investigation.

Difference parent - progeny

A distinct difference in the amount of free pinifolic acid between parents and progenies was found *(Table 1)*. This difference could be attributed to genetic and/or environmental effects. The genetic control of pinifolic acid biosynthesis is not known. Neither there is any information about the influence of environmental factors on pinifolic acid biosynthesis. Thus this difference remains unexplained.

Are there true segregations?

The data are in accordance with a rather rare recessive allele a (gene frequency around 0.15, giving two Aa in the sample of parents studied), aa individuals are without free pinifolic acid. On the other hand, the individuals with free pinifolic acid in the segregating families have rather low content (S3004 get the lowest family value even if the zeros are not considered), indicating that the zeros may rather be low values in a quantitative character. Even a di- or trihybrid segregation with intermediary inheritance may fit to the observations. The occurence of monohybrid mendelian segregations is indicated, but cannot be considered finally proven.

The role of heridity in the amount of free pinifolic acid

The evident variation between progenies give considerable support, and with the additional, but not in itself conclusive, support from the positive parent-progeny relationship and the suggested segregations, it is justified to conclude that the character is under at least moderately strong genetic control.

Conclusions

Screening for individuals lacking free pinifolic acid might be of interest in future investigations. Lack of free pinifolic acid might be caused by selfing. A study of segregation following controlled outcrossing rather than selfing would contribute additional clues. Many questions concerning the genetic and environmental effects on pinifolic acid remain unanswered. However, this investigation indicates that pinifolic acid of Scots pine needles might be a suitable genetic marker for population studies.

Acknowledgements

The authors are indebted to Dr. Rainer Ekman, Abo Academy, Abo, Finland, for GC-MS and for helpful discussions and to Professor Olof Theander, Swedish University of Agricultural Sciences, Uppsala, for the gift of a sample of pure pinifolic acid. Dr. Colin Matheson has made helpful comments on the manuscript and checked the language. Fonden för skogsvetenskaplig forskning has given financial support for the investigation.

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