distinguished from the parents. This may be a consequence of the fact that RAPD-based markers are dominant. Alternatively, it is possible that the hybrids are genetically contaminated as a result of back-crossing to one of the parents in the wild. Finally, it is possible that some individuals may have been phenotypically mis-typed as hybrids. Nevertheless, the reliability of markers based on the OPN06 primer is demonstrated by the fact that clones of the same species from different geographic areas have a common RAPD banding pattern.

Figure 5 shows the banding pattern for each species. The products of the PCR reactions from 5 members of each species were pooled before electrophoresis. The exception is *P. trichocarpa*, since only one clone was available.

Figure 6 is series of software derived schematics (ALDEA et al., 1989). *Figure 6A* is a representation of *figure 5. Figure 6B* shows a scheme of the banding pattern characterising each species, so that these bands are common to all clones of the same species. *Figure 6C* shows a scheme of identificative bands for each species, that is to say, bands that are present in a species but absent in all others. In *P. tremula* there are no identificative bands because the two characteristic bands signalled in *figure 6B,* lane 5 are present in some clones of *P. alba,* for instance in "Raket" and "Bolleana". However, *P. tremula* was identified by the absence of the *P. alba* identifiative bands (*Fig. 6B,* lane 4). *Figure 6D* shows 3 different groups of *P. alba;* all three groups have the identificative band of *P. alba,* but at the same time have other bands that identify the group.

Previous analyses of RAPD in *Populus* (CASTIGLIONE et al., 1993; SIGURDSSON et al., 1995; LIN et al., 1994; RANI et al., 1995) have identified many different clones. In this work, the discrimination has been made between species. The different species identified are *P. deltoides, P. nigra, P. alba, P. tremula* and *P. trichocarpa.* This one must be considered a previous study in the case of *P. trichocarpa,* because this hypothesis must be confirmed with more clones.

A deeper knowledge of species and clones of *Populus* fingerprints through molecular markers not only can help to clarify the genetic variability for improvement programs, but also will be useful for the recognition of patent rights.

Acknowledgements

The authors would like to thank Ms. N. ALBA and Mr. F. G. ANTOÑANZAS for their help in collecting tree material, and to Ms. IRENA TRNKOVA-FARRELL for the linguistic revision of the manuscript. This study was funded by project SC 94-138 of the National Institute of Agricultural Research and Technology (INIA). N. SÁNCHEZ was a recipient of an INIA Postdoctoral grant during the tenure of this study.

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Bud Monoterpene Composition in *Pinus brutia* (TEN.), *Pinus halepensis* (MILL.) and their Hybrids

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(Received 11th March 1997)

Abstract

Bud terpene composition was determined by headspace gas chromatography in *Pinus halepensis* (MILL.), *Pinus brutia* (TEN.) and F1 hybrids between the two species (59 trees). The object was to explore the utility of bud monoterpenes in studying hybridization between the two species.

Sixteen components were detected in the bud resin of all the trees, twelve of which identified. No qualitative differences were found in bud terpene composition between the species and the hybrids. *Pinus halepensis* trees had much more α-pinene, myrcene and α-phellandrene whereas *Pinus brutia* had higher amounts of α-pinene and 3-δ-carene. In buds of F1 hybrids the composition was more or less intermediate for most of the

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components except the 3-δ-carene which was found to occur in higher amounts than in parental species. A comparison between bud and cortical oleoresin of the same trees revealed several qualitative and quantitative differences.

Key words: Pinus halepensis, Pinus brutia, hybrids, bud monoterpenes, headspace chromatography.

FDC: 160.22; 164.4; 165.7; 174.7 *Pinus brutia;* 174.7 *Pinus halepensis.*

Zusammenfassung

Monoterpene in Knospen von Pinus brutia TEN. Pinus halepensis MILL. und deren Hybriden.

Die Zusammensetzung der Monoterpene und Monoterpenmuster im Knospenharz von *Pinus halepensis, Pinus brutia* und F1 Hybriden dieser beiden Arten wurde mit der Methode der headspace-GC an insgesamt 59 Bäumen untersucht. Von den 16 Komponenten, die bei allen untersuchten Individuen vorhanden waren, konnten 12 identifiziert werden. Zwischen den Arten und den Hybriden traten keine qualitativen Unterschiede auf *Pinus halepensis* wies i.d.R. die höheren α-Pinen-, Myrcen- und α-Phellandren-Anteile auf, *Pinus brutia* die höheren β-Pinen- und 3 δ-Caren-Anteile. In den Knospen der F1 Hybriden war die Monoterpenzusammensetzung für die meisten Komponenten ± intermediär. Das 3-δ-Caren trat hingegen in größeren Anteilen auf als bei den beiden Elternarten.

Zwischen Knospenharz und Rindenharz derselben Bäume traten einige qualitative und quantitative Unterschiede auf.

Schlagwörter: Pinus halepensis, Pinus brutia, Hybriden, Knospen, Monoterme, headspace-GC.

Introduction

In mediterranean region *Pinus brutia* and *Pinus halepensis* are two well established and very important forest tree species. They are distinguished from each other by a number of morphological and anatomical characters (PANETSOS, 1981). Quantitative differences in the terpene composition can also be used to separate the two species (MIROV et al., 1966; SCHILLER and GRUNWALD, 1987a; GALLIS and PANETSOS, 1997).

Pinus brutia hybridizes naturally with *Pinus halepensis* and several researchers have reported natural hybrids between the two species (PAPAIOANNOU, 1936, 1954; PANETSOS, 1975). Hybrid identification and description can be possible using morphological and anatomical characteristics (PAPAIOANNOU, 1936; PANETSOS, 1981, 1986). Cortical terpene characters can also be used successfully to identify hybrids between *Pinus brutia* and *Pinus halepensis* (GALLIS and PANETSOS, 1997).

This paper presents data of bud terpene analysis by headspace chromatography in F1 hybrid trees between Aleppo and brutia pine and in trees of the parental species. The cortical terpene composition of the same trees which were analyzed in the present study have been previously determined by GALLIS and PANETSOS (1997).

The objectives were: a) to gain knowledge of bud monoterpene composition of *Pinus brutia, Pinus halepensis* and artificial F1 hybrids between them and b) to evaluate if bud terpene composition could be used as an additional approach to separate the two species and to identify hybrids between them.

Materials and Methods

The buds were collected from two plantations near Thessaloniki in Northern Greece. Both plantations were established in 1970 to 1972 with seedlings produced in the years 1966 to 1969 (MOULALIS et al., 1976). Samples included trees of different provenances of the parental species (*Pinus halepensis:* Kriopigis, Phitoriou Killinis, Patras; *Pinus brutia:* Thassou) and artificially produced F1 hybrids. Details about the plant material have been described by GALLIS and PANETSOS (1997). The buds were collected from trees of approximately the same age and similar development. Buds of 59 trees were sampled during February 1991. The number of trees investigated per provenance or hybrid is shown in *table 1.*

Table 1. – Mean (%), standard deviation and range of *bud* monoterpenes in *Pinus halepensis* provenances, *Pinus brutia* and hybrids.

c o m p o n e n t s *

plant material	statist.	Tricyc	thuj	α-pin	β-pin	myrc	care	α-phell
1) Pinus halepensis	mean	15,71	0,25	24,68	1,56	43,21	11,53	0,52
(prov. Kriopigis)	std.dev.	4,13	0,51	17,27	0,61	35,11	11,56	0,55
n = 5	min.	12,23	0,00	11,91	1,20	2,68	3,50	0,20
	max.	20,53	1,29	44,33	2,26	64,21	24,80	1,15
2) Pinus halepensis	mean	37,81	1,34	32,25	1,81	12,33	7,10	4,56
(prov. Phitoriou)	std.dev.	32,54	3,26	19,40	1,59	13,23	4,55	4,02
n = 10	min.	2,00	0,00	0,10	0,00	0,00	0,06	0,02
	max.	99,66	11,04	65,04	5,83	38,74	13,73	12,72
3) Pinus halepensis	mean	24,30	7,72	48,57	1,60	7,38	3,34	3,15
(prov. Killinis)	std.dev.	8,83	16,63	21,46	0,65	6,94	4,43	3,38
n = 8	min.	5,29	0,00	13,01	0,45	2,06	0,23	0,32
	max.	30,90	34,70	73,80	2,73	19,84	11,33	9,74
4) Pinus halepensis	mean	26,93	4,60	41,73	0,81	17,87	4,42	1,34
(Killinis x Kriopigis)	std.dev.	31,08	7,97	23,52	0,21	13,18	1,57	0,47
n = 3	min.	5,48	0,00	20,09	0,63	6,84	3,00	0,80
	max.	62,57	13,80	66,53	1,04	32,46	6,11	1,68
5) Pinus halepensis	mean	7,36	4,85	67,26	2,82	7,69	4,14	0,82
(Killinis x Patras)	std.dev.	2,97	3,65	11,34	1,15	7,91	1,60	0,48
n = 6	min.	4,10	0,00	51,85	1,65	1,02	1,62	0,40
	max.	11,77	10,09	79,48	4,46	19,99	5,77	1,50
6) Pinus brutia	mean	36,22	5,02	11,97	23,66	9,44	9,10	0,53
(prov. Thassou)	std.dev.	20,50	8,77	4,45	8,45	2,80	3,47	0,33
n = 11	min.	3,62	0,00	5,42	9,12	5,07	4,01	0,19
	max.	74,45	22,32	18,46	41,13	13,91	14,01	1,41
7) F1 hybrids	mean	12,70	3,28	32,74	8,43	16,36	19,20	2,73
P. halep. x. P. brutia	std.dev.	11,31	4,28	10,10	5,60	16,48	8,36	3,14
n = 10	min.	0,00	0,00	19,08	1,78	1,63	3,92	0,32
	max.	34,50	10,95	46,67	16,69	48,19	29,13	9,88
8) F1 hybrids	mean	25,85	8,30	35,54	1,64	9,42	12,85	1,40
P. brutia x P. halep.	std.dev.	17,67	19,50	16,16	0,68	9,60	10,38	2,43
n = 6	min.	0,00	0,00	20,65	0,80	1,02	2,28	0,19
	max.	54,97	48,08	58,85	2,65	24,97	31,48	9,35

*) tricyc = tricyclene, thuj = thujene, α-pin = α-pinene, β-pin = β-pinene, myrc = myrcene, carene = 3-δ-carene, α-phell = α-phellandrene

Samples (one bud per tree) were obtained by excising 1 year old branches at the base of buds. Until the analysis the material was stored at –20 °C. For headspace chromatography each bud was cut into small pieces and placed into a vial of 5 ml volume, which was closed with an elastic silicone-Teflon membrane. After heating for 5 minutes at 100 °C, a sample of 1 ml was taken from the vial with a gas syringe and injected into the gas chromatograph. Sample analysis was carried out with a Packard 427 gas chromatograph equipped with a FID. The components were separated on a 25 m column packed with 10% OV-17 on Chromosorb W/AW 60/80. Temperatures were set: injector 120 °C, oven 80 °C, detector 120 °C. Nitrogen was used as carrier gas at a flow rate of 20 ml/min.

Peak identification was achieved by comparison of the retention times with those of pure standards. The standards were kindly offered by DRAGOCO, Holzminden, Germany. Peak areas were calculated with an Shimadzu C-R5A integrator. Percentages of the components α-pinene, β-pinene, 3-δ-carene and myrcene found to be common in bud and cortical resin were transformed into arcsin-square root functions (KUNG, 1988) for statistical analysis (t-test). As recommended by BIRKS and KANOWSKI (1988, 1993) the resin data evaluation it should be carried out with a variety of standard techniques, such cluster analysis, principal components e.t.c. In our study the number of "chemotypes" was determined by cluster analysis

(method: Ward, squared euclidian distance) on the basis of the values of the most important components (LANG, 1992, 1994).

Results

Bud terpene analysis by headspace gas chromatography revealed that sixteen components were present in all samples. Twelve of them could be identified: tricyclene, thujene, α-pinene, camphene, β-pinene, myrcene, 3-δ-carene, α-phellandrene, p-cymene, limonene, β-phellandrene, γ-terpinene. Tricyclene, thujene, α-pinene, β-pinene, myrcene, 3-δ-carene and α-phellandrene were the major components (*Table 1*). The remaining components occurred in very small amounts or only in traces and were not evaluated. No qualitative but only quantitative differences were found in bud monoterpene composition among parental species and hybrids.

Quantitative differences existed between *Pinus brutia* and *Pinus halepensis* mainly with α-pinene and β-pinene. Within the plant material of *P. halepensis* (*Table 1, 1* to *5*) a great variability of the different components could be found. The mean values must not be overestimated because the number of individual trees included is very small. Nevertheless, a comparison of the mean values of α-pinene, β-pinene, myrcene and 3-δ-carene between the two pine species and their hybrids (*Table 2*) showed, that the two species were clearly to distinguish by means of bud terpenes (and also cortical terpenes). Compared with the species, the hybrids showed as well intermediate as increased as unchanged mean values. Also between the two groups of hybrids (*P. brutia* x *P. halepensis* and *P. halepensis* x *P. brutia*) differences existed.

Table 2. – Mean values (%) for the most important components common in cortical and bud monoterpenes.

		Mean values of components							
		α-pinene		β-pinene		myrcene		δ-3-carene	
plant material	n	cortical	bud	cortical	bud	cortical	bud	cortical	bud
Pinus halepensis	32	26,36*	42,89	2,15	1,72	15,40	17,69	9,27*	6,10
Pinus brutia	11	21,89*	11,97	35,70*	23,66	17,69*	9,44	19,41*	9,10
F1 brutia x halep.	10	22,31*	32,74	17,64*	8,43	5,93	16,36	22,54	19,20
F1 halep. x brutia	6	23,45*	35,54	0,99	1,64	15,00	9,42	21,40	12,85

*) = cortical terpene different from bud terpene at a = 0.05

If the individual trees are attached to different "chemotypes" as described by LANG (1992, 1994) for *Abies alba* and *Larix decidua,* it is obvious that the individuals of the two species can be distinguished from each other in nearly all cases and also more than 40% of the hybrids can be identified because they belong to a chemotype, which is not present in *Pinus brutia* or in *Pinus halepensis* (*Table 3*). Comparison between bud and cortical monoterpenes of the same trees showed, that some qualitative differences existed (*Table 1*). Also the amount of the four main monoterpenes varied significantly (a = 0.05) between bud and cortex (* *Table 2*).

Discussion

During the last 30 years some studies dealing with the terpenes of *Pinus halepensis* and/or *Pinus brutia* have been published. Turpentine of Aleppo- and brutia pine were analyzed by ICONOMOU et al. (1964), MIROV et al. (1966). Xylem and cortex resin of Aleppo pine provenances were analyzed by SCHILLER and GRUNWALD (1986, 1987b). In provenances of *Pinus brutia* the composition of cortex and needle resin was investigated SCHILLER and GRUNWALD (1987a) and SCHILLER and GENICI (1993) respectively. Relatively little information is available on the terpenes of hybrids between Aleppo- and brutia pine. Recently GALLIS and PANETSOS (1997) identified F1 and F2 generation hybrids grown in Greece using the chemotype patterns of cortical terpenes.

No qualitative differences have been reported in all these studies either between species and hybrids or between the taxa themselves. Considerable quantitative differences were detected within and between the species and between the species and the hybrids. Our results confirm these previous reports because in the bud terpene composition there are no qualitative but some quantitative differences.

Tricyclene and thujene were not reported in previous studies about *Pinus halepensis* and *Pinus brutia.* Although there is considerable variability in tricyclene and thujene amounts, these two components seem not to be suitable to distinguish species or hybrids.

In the *P. brutia* x *P. halepensis* hybrid trees the monoterpene composition for most of the components was more or less intermediate, whilst 3-δ-carene was present in higher amounts compared with the parental species. The data of the cortical terpenes of the same trees (GALLIS and PANETSOS, 1997) also showed increased 3-δ-carene in F1 hybrids. These findings suggest, that the high content of 3-δ-carene in most of these cases could be an indicator for F1 generation hybrids.

Table 3. – Amounts (%) of different chemotypes in the 2 species and in the hybrids.

	"Chemotypes"				
	1	2	3	4	5
Pinus halepensis	45%	36%	19%	0%	0%
Pinus brutia	9%	0%	0%	0%	91%
hybrids total	13%	6%	37%	44%	0%
P.halepensis x P.*brutia*	0%	0%	40%	60%	0%
P.brutia x P. *halepensis*	33%	17%	33%	17%	0%

In F1 hybrids of the reciprocal combination (*P. halepensis* x *P. brutia*) the terpene composition was quite different than those of *P. brutia* x *P. halepensis* hybrids. For example the amount of α-pinene was more or less on the same level as in the *Pinus halepensis* trees and the amount of myrcene was similar to that of *Pinus brutia.* Cortical oleoresin of the same hybrid trees indicated also similarity to *Pinus halepensis* (GALLIS and PANETSOS, 1997) whilst on the basis of the bud terpenes chemotype analysis had 60% of these trees belonging to a chemotype not present in the parental species (*Table 3*) and therefore indicating hybrid character. It would be interesting to apply also other methods e.g. isoenzyme analysis to get more informations about the hybrid status of this material.

The clear differences between the two groups of hybrids (*Table 2, 3*) may be explained by the influence of the parental trees, used in these combinations. GAUDLITZ (1983) reports a strong male influence on monoterpenes with *Abies* hybrids of known parental trees. The data in *table 3* also suggest greater male than female influence.

From our results we conclude that bud terpene analysis by headspace chromatography seems to be a valuable tool to separate the taxa and to help identify hybrids of *Pinus halepensis* and *Pinus brutia.*

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Analysis of Half Diallel Mating Designs

I – A Practical Analysis Procedure for ANOVA Approximation

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(Received 23rd June 1997)

Abstract

Procedures to analyze half-diallel mating designs using the SAS statistical package are presented. The procedure requires two runs of PROC VARCOMP and results in estimates of additive and non-additive genetic variation. The procedures described can be modified to work on most statistical software packages which can compute variance component estimates. The procedure is relatively simple and provides unbiased estimates for balanced designs and gives good approximations for unbalanced data.

Key words: diallel matings, variance estimates, GCA, SCA.

FDC: 165.3; 165.41; 174.7 *Pinus radiata;* (931).

Introduction

Diallel mating designs are widely used in the genetic improvement programs of many tree species (YEH and HEAMAN, 1987; SNYDER and NAMKOONG, 1978; TALBERT, 1979). Besides their practicality as a dual function mating design that provides both a pedigreed breeding population for selection and a progeny test of parents, they are also highly useful designs for estimating genetic parameters. Estimates of genetic variances and other population parameters provide essential information for the development of breeding strategies. The diallel mating design is of interest, in that the analysis of variance uses the concepts of general combining ability (GCA) and specific combining ability (SCA) to distinguish between the average performance of parents in crosses (GCA) and the deviation of individual crosses from the average of the parents (SCA). In the population improvement strategy using recurrent selection for general combining ability (GCA), we would naturally wish to know the relative amount of the genetic variation caused by additive gene effects (GCA) and whether non-additive gene action is important.

A drawback to the diallel mating design is that it is relatively complex to analyze. Because the genetic effects are not readily separated, they cannot easily be analyzed in a single execution of a linear model procedure in standard statistical packages. The computation of the appropriate sum-of-squares and expected mean squares have been derived in the literature for balanced (GRIFFING, 1956) and unbalanced data (e.g., GARRETSEN and KEULS, 1977: KEULS and GARRETSEN, 1978: BARADAT and DESPREZ-LOUSTAU, 1997). Estimation of the GCA and SCA effects are demonstrated by HUBER *et al.* 1992. Because standard statistical packages cannot handle diallel analyses, the breeder must either program the procedures or use special packages, such as the DIALL program of SCHAFFER and USANIS (1969). These specialty programs lack the convenience and ease associated with large data handling packages and can limit one's options in data analysis. Limita-

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