Use of Cortical Terpenes to Discriminate *Pinus brutia* (TEN.), *Pinus halepensis* (MILL.) and Their Hybrids[1])

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Abstract

Terpene composition of cortical oleoresin was analyzed by gas chromatography in 283 individuals from F1 and F2 *Pinus brutia* x *Pinus halepensis* hybrids, back – crosses and parental species. The objective was to explore the utility of terpenes in hybrid identification between the 2 species. Fifteen compounds were detected in the cortical oleoresin of all trees, 14 of which were identified. No qualitative differences were found among species and hybrids. Aleppo pine oleoresin composition differs significantly in the amounts of several major terpenes from that of *Pinus brutia* whereas the composition of hybrids was more or less intermediate between the 2 species. A cluster analysis of the data revealed the classification of all trees into 7 major chemotypes. Occurrence of various chemotypes within parental species and hybrids in different rates has the result to separate the taxa and distinguish the hybrids.

Key words: Pinus halepensis, Pinus brutia, hybridization, terpenes, chemotypes, gas chromatography, oleoresin.

FDC: 165.7; 165.53; 160.2; 174.7 *Pinus brutia* x *Pinus halepensis;* 174.7 *Pinus brutia;* 174.7 *Pinus halepensis;* (495).

Introduction

Pinus halepensis (MILL.) and *Pinus brutia* (TEN.), belong to the section *Halepensis* of the genus *Pinus* and are 2 important forest tree species with extensive distribution in the Mediterranean region.

In Greece, *Pinus halepensis* occurs in Peloponessos, the Ionian islands, central Greece, Euboia, the islands of Sporades and in the peninsula of Chalkidiki where are the northern limits of its range. On the other hand, the range of *Pinus brutia* extents from Thrace and the island of Thassos to the islands of North Aegean sea as well as to Crete.

Extensive studies (NAHAL, 1962, 1984; PANETSOS, 1981, 1986b) showed that Aleppo and *brutia* pines should be considered as 2 well established pine species with common origin from a primitive pine population which existed in north Europe during Tertiary which have evolved independently.

The species have developed several kinds of barriers such as spatial, elogical, seasonal, partial embryo and F1 sterility (PANETSOS, 1981, 1986b). Despite these isolation mechanisms when they come in contact they form natural hybrids (PANETSOS, 1975). In Greece, natural hybrids were first reported by PAPAIOANNOU (1936), in the area of NE Chalkidiki where the natural distributions of 2 the species overlap. Since that time, several studies reported natural hybrids in the areas where in some way the species come in contact (PAPAIOANNOU, 1954; PANETSOS, 1975, 1981, 1989).

With respect to artificial hybridization the first crossing between species was performed in 1948 by MOULOPOULOS and BASSIOTIS and the results obtained were presented in 1961. Afterwards, many controlled crosses of different combinations performed by several researchers produced a large number of artificial hybrids (BASSIOTIS, 1972; MOULALIS and MITSOPOULOS, 1975; MOULALIS et al., 1976).

Evaluation of F1 and advanced generation hybrids showed that F1 hybrids possess impressive hybrid vigor over parent tree species in growth and adaptation. Their superiority in growth varied from 5% to 190%. They can also resist freezing temperatures much better than parental species. Advanced generations were always inferior in growth compared to F1 hybrids (PANETSOS et al., 1983; PANETSOS, 1986b, 1989, 1990).

Hybrid identification and description can be possible using morphological and anatomical characteristics as suggested by several researchers in the past (PAPAIOANNOU, 1936; MOULALIS and MITSOPOULOS, 1975; PANETSOS, 1981, 1986a).

Besides this possibility, biochemical characters such as terpenes can be used. Monoterpene and other terpene compounds have received a great deal of attention in taxonomic studies of coniferous species since many of them are under strong genetic control and are not greatly influenced by environmental factors (HANOVER, 1966a and b, 1990; SQUILLACE, 1976; VON RUDLOFF, 1975). As a result of rapid improvements in gas-chromatography (GLC) they are easily analyzed and quantified (BIRKS and KANOWSKI, 1988).

Terpenes of *Pinus halepensis* as well as of *Pinus brutia* have been analyzed by several researchers in the past (ICONOMOU et al., 1964; MIROV et al., 1966; MITSOPOULOS, 1987; SCHILLER and GRUNWALD, 1986, 1987a and b; SCHILLER and GENIZI, 1993). In addition MITSOPOULOS (1986) analyzed the cortical oleoresin of artificial F1 hybrids grown in Greece.

The objectives of this study were: a) to determine the cortical oleoresin terpene composition in F1 and advanced generations (F2 and BCs) hybrids between *Pinus brutia* x *Pinus halepensis* and in parental species b) to examine whether terpenes could be used to differentiate the 2 species and identify hybrids between them.

Materials and Methods

Plant Material

All samples were taken from 2 plantations near Thessaloniki in North Greece. Both plantations were established in the years 1970 to 1972 and were planted with seedlings produced in the years 1966 to 1969 (MOULALIS et al., 1976). Samples included provenances of both species, F1 and F2 generation hybrids and back-crosses (*Table 1*). Provenances were originated from open pollination seeds (bulk seeds). The F1 generation hybrids have produced under the following mating design: 10 *P. brutia* trees (used as female parents) were pollinated with a

[1]) The paper is based in parts on a Doctoral dissertation defended by the senior author in the Department of Forestry, University of Thessaloniki, Greece.

Table 1. – Species provenances, hybrids, back-crosses and number of sampled trees of each type.

Species/Hybrids	Area	Latitude	Longitude	Elevation(m)	number of sampled trees
P.brutia					
P.brutia (phitoriou)	Univ.Forest nursery (Vassilika)	40° 35' 41"	22° 59' 17"	9	11
P.brutia (thassou)	Thassos island	40° 37' 00"	24° 14' 00"	30 -150	20
P.halepensis					
P.halepensis (killinis)	killinis (Peloponessos)	37° 55' 00"	22° 38' 00"	700	18
P.halep.(killinis x kriopigis)	killinis (Peloponessos)	37° 55' 00"	22° 38' 00"	700	21
P.halep.(killinis x patras)	killinis (Peloponessos)	37° 55' 00"	22° 38' 00"	700	19
P.halepensis (kriopigis)	Chalkidiki	40° 00' 30"	23° 33' 40"	150-200	24
P.hallepensis (phitoriqu)	Univ.Forest nursery (Vassilika)	40° 35' 41"	22° 59' 17"	9	20
Hybrids[1]					
F 1 hybrid	Chalkidiki	40° 00' 30"	23° 33' 40"	700	22
F 1 P.brut.(thas.) x P.halep.(kriop.)r[2]	Univ.Forest nursery (Vassilika)	40° 35' 41"	22° 59' 17"	9	16
F 1 P.brut (thas.) x P.halep. (kriop.)	Univ.Forest nursery (Vassilika)	40° 35' 41"	22° 59' 17"	9	25
F 1 P.brut.(phitor.) x P.halep.(phitor.)	Univ.Forest nursery (Vassilika)	40° 35' 41"	22° 59' 17"	9	5
F1 generation P.halepensis x P.brutia	Univ.Forest nursery (Vassilika)	40° 35' 41"	22° 59' 17"	9	19
F 2 generation (F 1 x F 1)	Univ.Forest nursery (Vassilika)	40° 35' 41"	22° 59' 17"	9	23
Back - cross					
F1 x P.brutia (phitoriou)	Univ.Forest nursery (Vassilika)	40° 35' 41"	22° 59' 17"	9	25
P.halepenis(phitoriou) x F1	Univ.Forest nursery (Vassilika)	40° 35' 41"	22° 59' 17"	9	19
F 1 x P.halepensis	Univ.Forest nursery (Vassilika)	40° 35' 41"	22° 59' 17"	9	6

[1]) The area given for hybrids and back-crosses is the area of hybrization center
[2]) Irradiated pollen

pollen mixture obtained from 5 *P. halepensis* trees. Besides natural also irradiated pollen used, in one case, to produce F1 hybrids. In the case of the reciprocal cross (where Aleppo pine used as female parent) 10 *P. halepensis* trees were pollinated with pollen mixture of 5 *brutia* pine trees. For the production of F2 generation, 2 F1 hybrids trees were pollinated with pollen mixture from 2 F1 generation trees (used as male parents). The mating desing for back-crosses always included pollination of 3 trees, with a pollen mixture obtained from also 3 male trees (see *Table 3*).

Within plantations, the resin was collected from trees of approximately the same age and similar in development. A total of 283 trees was sampled in February 1991. The number of trees per provenance or hybrid cross varied from 5 to 25 with an average of 17 trees, since in some cases only a limited number of trees was available (*Table 1*). In view of the findings by SQUILLACE (1976) we assumed that plantation, age and seasonal effects on terpene composition were negligible.

Oleoresin Sample Collection and Analysis

Oleoresin samples were obtained from cortical tissue by excising 1 year old branches at approximately 1 cm below the base of buds. The exuding oleoresin was placed in screw – cap vials, sealed and stored in a refrigerator at –20 °C until analyzed. Each sample was diluted with n – pentane (proanalysis) prior to analysis in proportion 2 solvent: 1 oleoresin. 1 μl of pentane-resin mixture was injected into gas chromatography. Sample analysis was carried out by means of a Hewlett Packard 5890 A II gas chromatograph equipped with a F.I.D.. Individual terpenes were separated on a capillary column of

WCOT fused silica 25 m x 0.22 mm i.d coating CPtm-Wax-58 CB. The sample was run isothermally at 65 °C for 8.5 minutes and then programmed to 170 °C increasing at a rate of 10 °C/ min. The injection and detector temperatures were set at 240 °C and 280 °C respectively. Nitrogen was used as carrier gas at a flow rate of 25 mil/min.

The identification of terpene compounds was achieved by comparison of their retention times with those of the pure standards. Enhancement of the unknown peak, after mixing it with standards was also used. Terpenes were quantified as percentage contribution of each peak to total terpenes present. For statistical evaluation of data, SPSS/PC+ was used. A cluster analysis with the WARD method and squared euclidian distance (seuclid) was used to determine the number of chemotypes on the base of percentages of α-pinene, β-pinene, myrcene, 3-d-carene, a-terpinene caryophyllene after Z-standardization (SPSS/PC, 1988). With the cluster analysis procedure all individuals could be assigned to the chemotypes. For every provenance or hybrid cross the frequency of each chemotype was calculated (*Table 3*).

Results

Cortical oleoresin analysis of all samples produced 15 peaks (i.e resin compounds) on the chromatography 14 of wich could be identified (*Fig. 1*). Besides the monoterpenes, sesquiterpenes were also identified due to the technique used. The identified terpenes were: α-pinene, β-pinene, 3-δ-carene, myrcene, limonene, camphene, sabinene, santene, α-terpinene, bornyl acetate, terpinolen, humulene, longifolene and caryophyllene. The 5 terpenes α-pinene, β-pinene, 3-δ-carene, myrcene, caryophyllene were found to be the major components since they occurred in all or most of the samples in relatively large amounts. The remaining components occurred in traces or in relatively small amounts and no one represented more than 5% of total terpenes. Therefore the evaluation included the 5 major components mentioned above and also the monoterpene α-terpinene due to its high variation within all samples.

Table 2. – Mean cortical resin terpene composition (%) of Aleppo and brutia pine species and hybrids.

Components / Species	% mean					
	α - pinene	β - pinene	3 - δ- carene	myrcene	α - terpinene	caryophyllene
Pinus brutia	14,006	28,081	15,838	10,598	1,202	21,239
Pinus halepensis	25,652	2,12	10,04	18,112	4,367	28,362
F1 generation hybrids (P.brutia x P.halepensis)	10,981	12,26	19,171	14,264	3,901	28,854
F2 generation hybrids (F1xF1)	20,867	10,915	17,086	13,987	10,238	18,933
F1 generation hybrids (P.halepensis x P.brutia) *	21,473	23,73	19,986	15,754	1,596	27,763

*) This is a reciprocal cross using as female parent *P. halepensis.*

No qualitative differences were found in cortical oleoresin terpene composition among species and hybrids. Nevertheless the amounts of most terpenes selected varied between species and hybrids. *Pinus halepensis* resin contained much more α-pinene (25.65%), myrcene (18.11%) and caryophyllene (28.36%) while *Pinus brutia* contained higher levels β-pinene (28.08%) and 3-δ-carene (15.83%). F1 generation hybrids oleoresin exhibited intermediate levels of most terpenes except for the level of 3-δ-carene (19.17%) which was higher than the maximum (15.83%) observed in parental species. Higher levels of α-terpinene, α-pinene and lower levels of caryophyllene were observed in F2 generation hybrids oleoresin in comparison to F1 generation (*Table 2*).

Numerical analysis by ward cluster analysis (SPSS/PC, 1988) based on relative quantity (%) of 6 selected terpenes was carried out. The dendrogramm obtained (*Fig. 2*) revealed the

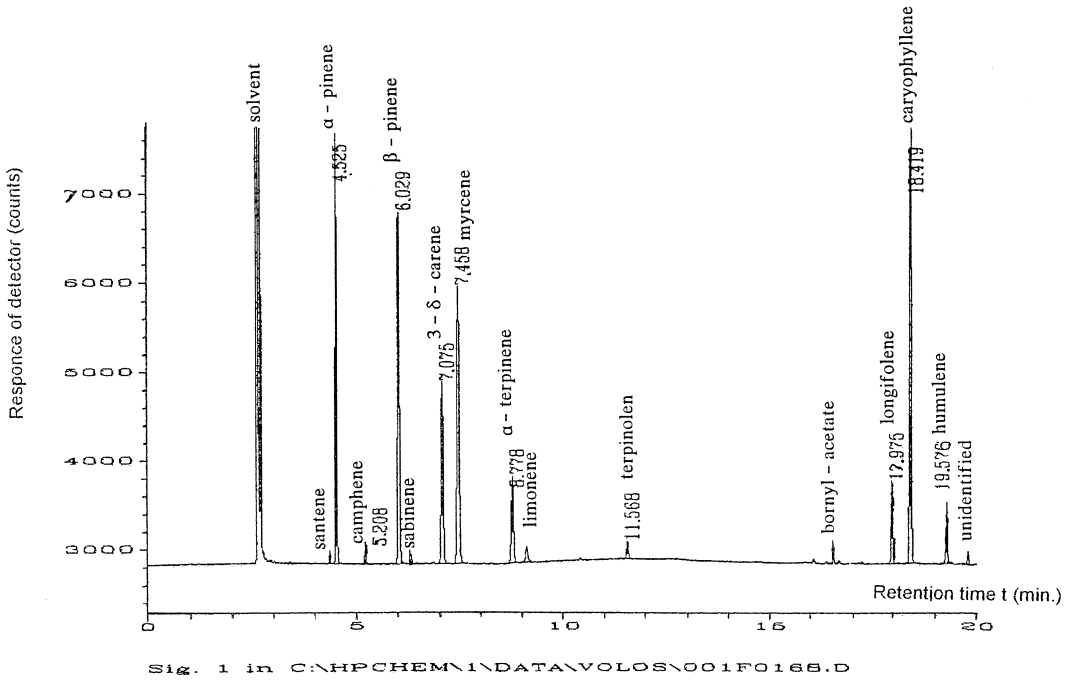

Figure 1. – Chromatogram of the cortical oleoresin.
(Numbers on the peaks represent the retention time of each component).

Table 3. – Chemotype frequencies (%) within Aleppo and brutia pine provenances and hybrids.

Species	Chemotypes						
	A	B	C	D	E	F	G
P.brutia							
P.brutia (phitoriou) (open pollinated - bulk seeds)	9,09	–	–	45,45	45,45	–	–
P.brutia (thassou) (open pollinated - bulk seeds)	–	–	–	50,00	50,00	–	–
P.halepensis							
P.halepensis (killinis) (open pollinated - bulk seeds)	33,33	5,56	5,56	–	–	38,89	16,67
P.halep.(killin.x kriopigis) (open pollinated - bulk seeds)	14,29	80,95	–	–	–	4,76	–
P.halep.(killinis x patras) (open pollinated - bulk seeds)	10,53	–	–	5,26	–	5,26	78,95
P.halepensis (kriopigis) (open pollinated - bulk seeds)	12,5	41,67	33,33	–	–	8,33	4,17
P.halepensis (phitoriou) (open pollinated - bulk seeds)	5,00	20,00	–	–	10,00	50,00	15,00
Hybrids *							
F 1 hybrid 10 P.brutia x 5 P.halepensis(pollen mixture)	45,45	9,09	9,09	9,09	9,09	18,18	–
F 1 hybrid P.brut.(thas.) x P.halep.(kriop.)r 10 P.brutia x 5 P.halepensis(pollen mixture)	43,75	–	–	56,25	–	–	–
F 1hybrid P.brut.(thas.) x P.halep.(kriop.) 10 P.brutia x 5 P.halepensis(pollen mixture)	16,00	–	–	84,00	–	–	–
F 1hybrid P.brut.(phitor.) x P.halep.(phitor.) 10 P.brutia x 5 P.halepensis(pollen mixture)	20,00	20,00	–	20,00	–	40,00	–
F1 generation P.halepensis x P.brutia 10 P.halepensis x 5 P.brutia (pollen mixture)	36,84	10,53	36,84	–	–	5,26	10,53
F 2 generation (F 1 x F 1) 2 F1 x 2 F1 (pollen mixture)	4,35	–	4,35	17,39	30,43	43,48	–
Back - cross							
F1 x P.brutia (phitoriou) 3 F1 x 3 P.brutia (pollen mixture)	–	–	12,00	28,00	56,00	4,00	–
P.halepenis(phitoriou) x F1 3 P.halepensis x 3 F1(pollen mixture)	–	–	22,22	11,11	22,22	44,44	–
F 1 x P.halepensis 3 F1 x 3 P.halepensis(pollen mixture)	33,33	50,00	–	16,67	–	–	–

*) Always in the crosses the first parent is the female parent.

classification of all trees into 7 major chemotypes which occurred at different frequencies. Chemotype A occurs in 16.96% of the trees and its frequency ranges from zero to 45.45%. Chemotype B occurs in 14.13% of trees and its frequency rises in some case to 80.95%. Chemotypes C and G occur in the same frequency 8.49% (24 trees). Chemotypes D and E occur in 21.9% (62 trees) and 14.84% (42 trees) respectively. Finally chemotype F includes 43 trees (15.19%) and its frequency ranges from zero to 50% (see *Fig. 2*).

Each one of the chemotypes has a special terpene composition based on resin composition of the trees included. *Table 4* shows the mean terpene composition (%) of each chemotype. Chemotype A is characterized by a high amount of caryophyllene. Chemotype B contains a very low amount of β-pinene and a very high amount of myrcene. A low amount of β-pinene is also contained in chemotype C which is characterized by a very high amount of 3-δ-carene. The same terpene exists in relatively large amount in chemotype D which also contains a high amount of β-pinene. Chemotype E is characterized by a very high amount of β-pinene. Both chemotypes (D&E) contain much more amounts of this character in comparison to all the rest chemotypes. Chemotype F is characterized by a high amount of α-terpinene. Finally, chemotype G contains an extremely high amount of α-pinene.

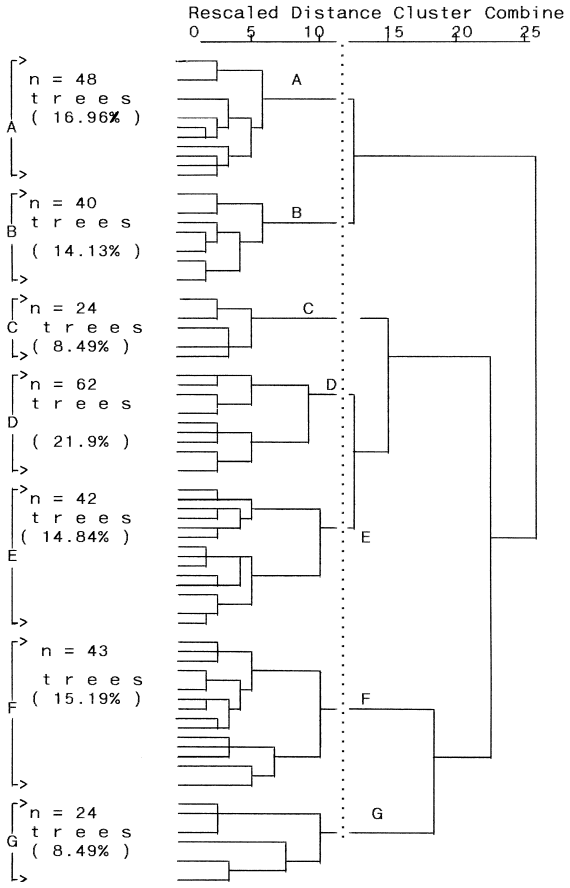

Figure 2. – Dendrogram of the cluster analysis of the six terpenes (α-pinene, β-pinene, 3-δ-carene, α-terpinene, myrcene, caryophyllene). The distance is measured by squared euclidian and the agglomeration shedule is WARD method. Seven chemotypes found as follows: A (48 trees, 16.96%), B (40 trees, 14.13%), C (24 Trees, 8.49%), D (42 trees, 21.9%), E (42 trees, 14.84%), F (43 trees, 15.19%) and G (24 trees, 8.49%).

Table 4. – Mean terpene composition (%) of the 7 chemotypes present in Aleppo and brutia pine provenances and hybrids.

Chemotypes	% Components					
	α - pinene	β - pinene	3 - δ - carene	myrcene	α - terpinene	caryophyllene
A	12,76	7,10	14,74	17,77	2,28	45,31
B	12,97	1,84	7,47	41,58	2,46	33,65
C	29,4	2,02	33,79	13,87	0,88	20,05
D	13,02	21,3	21,19	17,39	1,00	26,06
E	23,39	30,74	13,33	10,61	4,65	17,25
F	27,41	3,62	19,89	7,12	17,05	24,88
G	50,79	2,92	9,67	11,31	0,3	24,99

Discussion and Conclusion

The results of the present study are consistent with the findings of SCHILLER and GRUNWALD (1987a) who analyzed cortical oleoresin in *Pinus brutia* subspecies as well as in Aleppo pine provenances. The above mentioned authors did not report several terpenes detected in the present study. On the other hand we did not find phellandrene in our analyses as this terpene was found to be present in the terpene composition determined by them. These differences in terpene composition could be the result of analysis factors such as column, temperature program e.t.c..

MITSOPOULOS (1986) analyzed the cortical oleoresin of F1 generation hybrids and parental species. Many of the peaks detected in our study were not reported by the previous author, probably as a result of the greater sensitivity of capillary column compared to packed column and of his isothermal chromatographic analysis.

Turpentine of Aleppo and brutia pines was analyzed by ICONOMOU et al. (1964), MIROV et al. (1966) and MITSOPOULOS (1987). Xylem oleoresin of *Pinus halepensis* was analyzed by SCHILLER and GRUNWALD (1986, 1987b) and needle resin of *Pinus brutia* was analyzed by SCHILLER and GENIZI (1993). The terpene pattern determined in the present study differs significantly from the terpene composition determined by the previous authors because they have used different kind of tissue (xylem, needle) or resin (distillate).

In our work we did not find qualitative differences between the species and their hybrids. On the other hand, quantitative differences in several major terpenes could be used to discriminate the species and distinguish the hybrids. SCHILLER and GRUNWALD (1987a) did not report qualitative differences between the taxa. MITSOPOULOS (1986) also found only quantitative differences between parental species and F1 generation hybrids.

It should be mentioned here that the provenances of both species analyzed by SCHILLER and GRUNWALD (1986, 1987a and b) also by SCHILLER and GENIZI (1993) were of quite different origin (grown more or less in Israel and in Minor Asia) than those (Greek provenances) studied in our investigation.

In recent taxonomic and genetic studies, the evaluation of the data obtained from resin chromatographic analysis is carried out with a variety of standard techniques such as principal components, cluster analysis e.t.c.. These multiple variance analyses are more appropriate than mean of individual terpenes because they can yield more information about the extent to which resin phenotype reflects genotype (BIRKS and KANOWSKI, 1988, 1993).

Cluster analysis for the determination of chemotypes has been applied in the genus *Pinus* (SCHILLER and GRUNWALD, 1986, 1987a; VON RUDLOFF and LAPP, 1987; ZAVARIN et al., 1990;

SCHILLER and GENIZI, 1993) and also in other conifer species (SCHILLER, 1990; CHANG and HANOVER, 1991; LANG, 1994).

In our analysis, 7 chemotypes were identified in all trees but only 2 (D&E) occur in the *Pinus brutia* provenances. Indeed chemotypes D and E together occur in 96.77% of the *Pinus brutia* trees under investigation.

On the other hand, in Aleppo pine, at least 3 chemotypes were detected occurring in different rates. Some of them are very common (A&F) while some others are very rare (chemotypes C, D&E). Chemotypes A and F occurred in all provenances, chemotypes B and G in almost all of them (except one). The chemotypes A, B, F and G together occur in 88.23% of the *Pinus halepensis* trees. It is very interesting to note here the complete absence of chemotypes B, F, G in *Pinus brutia* and also the extremely rare occurrence of chemotype A. Versa the very rare occurrence of chemotypes D&E in *Pinus halepensis* trees (see *Table 3*).

As mentioned earlier, one objective in this investigation was to determine how the 2 species are discriminated. The comparison of chemotypes distribution occurring in *brutia* pine provenances with those in Aleppo pine proved to be very useful in the distinction of the 2 species. *Pinus halepensis* oleoresin contained high level amount of α-pinene, myrcene and caryophyllene and *Pinus brutia* of β-pinene and 3-δ-carene. Quantitative chemotype composition (*Table 4*) shows that typical *brutia* pine chemotypes D&E are very rich in 3-δ-carene and β-pinene respectively. Also all typical Aleppo pine chemotypes contain a low amount of β-pinene. In addition, chemotypes A and B contain a very high amount of caryophyllene and myrcene respectively and chemotype G has an extremely high amount of α-pinene. This fact indicates that the chemotype distribution among species reflects in the best way the quantitative differences between them. Furthermore, it indicates the great ability of cluster analysis to distinguish between *brutia* and Aleppo pines.

Two chemotypes (A&D) are common in F1 generation (*P. brutia* x *P. halepensis*) hybrids and also occur in other generations (see *Table 3*). This distribution reflects the intermediate terpene composition of F1 generation since chemotypes A and D are typical of *P. halepensis* and *P. brutia* respectively. Chemotype G is completely absent and chemotypes C and E are very rare. Chemotype B occurs in 2 of the 4 F1 generation hybrids investigated in our work but in relatively low frequencies. In addition, chemotype F occurs in 2 of the 4 F1 hybrids – in one in high frequency (40%) – it also occurs in F2 generation hybrids in high frequency (43.48%).

In conclusion, it appears that the 3 chemotypes A, D&F are reliable enough for the identification of F1 generation hybrids.

With regard to F2 generation hybrids, the occurrence of chemotype E in high rate 30.43% could be used to differentiate F1 and F2 generation hybrids.

In F1 putative hybrids of the reciprocal combination (*P. halepensis* x *P. brutia*), the chemotypes distribution seems to be more or less similar to that of *P. halepensis.* Indeed, chemotype D which is common in all F1 and F2 hybrids is completely absent in these hybrids, while chemotype G is present. It should be mentioned here that chemotype G is typical of Aleppo pine provenances (see *Table 3*). Furthermore, comparison of mean values between Aleppo pine and these hybrids clearly show their similarity (see *Table 2*). These findings come in contrast with BASSIOTIS (1972) who considerated these trees as real hybrids. Probably, they have originated from pollination of *P. halepensis* pollen due to contamination for various reasons (PANETSOS, 1989).

In conclusion, the results of the present study showed that the cortical terpene characters of *P. halepensis* and *P. brutia* offer a very valuable tool for hybrid identification and species characterization.

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Leaf Peroxidase Types in *Acacia karroo* HAYNE (Acacieae, Leguminosae): a Range-Wide Study

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Summary

Peroxidase variation has been assessed in 63 populations (4322 individuals) of *Acacia karroo* HAYNE (Acaciae: Mimosoideae) from across its entire southern African range. The 5 bands detected in the fast zone by starch gel electrophoresis combine to form 27 different phenotypes, with between 2 and 22 phenotypes found per population. SHANNON's measure of phenotype diversity varied from 0.15 to 4.11 (mean 2.71), whilst apportionment of the diversity showed that 81% of the diversity occurred within populations. Six coastal dune populations of *A. karroo* from Zululand/Mozambique clustered together and had a low phenotypic diversity. Band M is common throughout the range of the species, but it is virtually fixed to the exclusion of the other four bands in the coastal dune populations. Band K, on the other hand, is restricted to the Karoo region of the Cape Province in South Africa with lower frequencies in the adjacent highveld region of the central and western Transvaal Province to the North; it is completely absent to the east of the Drakensberg mountains and very rare in the west and north of its range. The implications of these data for seed sampling strategies in *A. karroo* are that although the high proportion of within-population variation makes it appropriate to sample large numbers of individuals within fewer populations to sample maximum genetic diversity, it is equally important to sample populations where bands are at high frequency because of the adaptive advantage that this may represent.

Key words: Acacia karroo, phenotypic variation, peroxidase, Leguminosae.

FDC: 165.3; 165.53; 161; 176.1 *Acacia karroo.*

Introduction

Acacia karroo HAYNE is one of the most widely distributed trees in southern Africa (BARNES *et al.,* 1996). It occurs from the Cape Peninsula, at the southern extremity of the continent, northwards to the southern parts of Angola, Zambia and Malawi. It is adapted to a wide range of climatic and edaphic conditions (ROSS, 1979). It will grow under summer maximum to winter maximum rainfall patterns where annual precipitation varies from 100 mm to greater than 1500 mm, and mean annual temperatures from 24 °C down to 12 °C, where daily temperatures may rise to greater than 40 °C and drop to less than – 10 °C. Over its inland range it tends to be restricted to the heavier soils and it is one of the few species that thrives on heavy black vertisols with high pH, but it will grow on deep alluvial clay-loam soils, in river valleys and even on acid soils and shales. At the other extreme, it thrives on the unconsolidated sands of the coastal dunes in Zululand and it is tolerant of extremely saline conditions (BARNES *et al*., 1996).

Acacia karroo has many desirable attributes and products, such as fuelwood, an edible gum, an ability to ameliorate the environment through fixing atmospheric nitrogen and utilising water and nutrients from great depths. It is resistant to drought, frost, fire and salinity. However, such traits are offset by its invasive tendency (WELLS *et al.,* 1986). As a result of the extensive geographical and ecological range of the species and its economic potential there has been considerable interest in assembling a collection of the genetic resources of *A. karroo* from across the species' natural range.

The traditional approach to the assessment of forest genetic resources has been to examine a combination of morphological and agronomic traits that, in the main, exhibit continuous variation. However, the effectiveness of this approach has been questioned by several authors (BROWN, 1979; GOTTLIEB, 1977). The application of isozyme markers has provided a powerful tool for the study of genetic diversity within and among plant populations (SOLTIS and SOLTIS, 1990).

BRAIN (1985; 1989) in a study of leaf peroxidase variation in South African populations of *Acacia karroo* revealed interesting patterns of variation among them, suggesting the existence of distinct geographic races and the correlation of isozyme phenotypes with environmental factors such as low temperature and rainfall. A similar correlation between allele distribution and environmental factors has also been shown for shikimate dehydrogenase and alcohol dehydrogenase loci (OBALLA, unpubl.).

Leaf peroxidase is a valuable and practical means of assessing genetic variation in *Acacia* since it can be measured

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