tertiary period (BERTSCH, 1940; MIROV, 1967) and they could gain and maintain the unique alleles in particular populations, in contrast to other populations in Europe, where the relatively recent reforestation is a result of the migration of Scots pine from several refugia after the last glaciation. An intensive genes exchange from different sources was possible.

As the pattern of geographic variability reflects the migration of Scots pine after the glaciation one can presume that the populations from the Iberian peninsula are relics and did not take part in reforestation of Europe after the last glaciation. This hypothesis is supported by our findings of the unique alleles not present in populations from eastern and northern Europe. Also data of PARDOS et al. (1990) from monoterpene analysis confirm this opinion.

Acknowledgements

We are grateful to JOLANTA LUKASZEWICZ, M. Sc., and DOROTA WYSOCKA, M. Sc., for their technical help. We have to thank also Prof. JOSÉ PARDOS and Dr. RICARDO ALIA, both Madrid, for supplying the seed samples from Spain. The study was conducted in the frame of the German-Spanish cooperation program and financially supported by the Ministry of Food, Agriculture and Forestry (BML), Bonn, and the Instituto Nacionale de Investigaciones Agrarias (INIA), Madrid. The work was also partly financed by the grant CPBP 09.02.04 from the Polish Academy of Sciences. We are grateful to Mr. H. WOHLTORF for preparing the drawings.

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Interspecific Hybridization of Swiss Stone Pine (Pinus cembra L.)

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(Received 22nd February 1993)

Summary

Many crosses between *P. cembra* and other white pines have been attempted but only *P. cembra* x *P. wallichiana* A. B. JACKS. and *P. cembra* x *P. monticola* DOUGL. were successful. After 11 years of testing, the following important results were observed: (1) Significant genetic differences were found between hybrids and their parents for most traits; (2) Both hybrid crosses resulted in individuals that have some important traits outside the range of the parents; (3) *P. cembra* x *P. wallichiana* was intermediate between parents in both blister rust resistance and growth traits; this hybrid incorporated blister rust resistance genes from the female parent and fast grvwth from the male parent; (4) *P. cembra* x *P. monticola* displayed heterosis in 6 of the 9 tested traits, including all growth traits; it inherited outstanding form and growth from the male parent; (5) Both hybrids represent a potentially important planting stock for middle to high altitude sites.

Key words: Pinus cembra, P. wallichiana, P. monticola, hybrid, heterosis, blister rust resistance, growth traits.

Introduction

The natural distribution of Swiss stone pine (*Pinus cembra* L.) is at high elevations (1200 m to 2600 m) in the Alps and Carpatian Mountains (GEORGESCU and IONESCU-BARLAD, 1932; BELDIE, 1941; CRITCHFIELD and LITTLE, 1966; HOLZER, 1972, 1975).

Because of its tolerance to low temperature and other unfavourable climatic factors, this species is widely used for reforestation of the subalpine zone. It is also important on watersheds, for stabilizing avalanche areas, and for reducing the impacts of flash floods in this zone (HOLZER, 1972). In addition, stone pine is a potential source of genetic resistance to blister rust caused by *Cronartium ribicola* FISCH. ex RABENH. (BINGHAM, 1972a; HOFF and McDONALD, 1972; HOLZER, 1975; HOFF et al., 1980; BLADA, 1982, 1987). Unfortunately earlier trials indicated that stone pine would not successfully cross with many of the other 5-needle pine species (RIGHTER and DUFFIELD, 1951; WRIGHT, 1959; PATTON, 1964; HEIMBURGER, 1972; BINGHAM, 1972a; HOLZER, 1975). BINGHAM et al. (1971) obtained 3 seedlings of *P. monticola* x *P. cembra* but according to HOFF (1992, personal communication) these seedlings did not survive. LEANDRU (1982) obtained 4 and 7 hybrid seedlings, respectively, of *P. cembra* x *P. strobus* L. and *P. cembra* x *P. koraiensis* SIEB. All of these seedlings died before they could be outplanted in the field (LEANDRU, 1990, personal communication).

Extensive research on crossability barriers in white pines by several authors showed that the main reasons for crossing failure were as follows: (1) Seed failure in pines can occur before, during or after fertilization (BUCHOLZ, 1944); (2) Fertilization took place in ovules of *P. peuce* GRISEB. x *P. cembra* but degeneration occured inside the archegonia at the proembryo stage (HAGMAN and MIKKOLA, 1963; DOYLE, 1963); (3) Embryo inviability, rather than gametic incompatibility, was the critical factor limiting crossability in *P. strobus* x *P. cembra* and other white pine species (KRIEBEL, 1967; 1968, 1972); (4) Development of the gametophytes during the first year after pollination is normal, regardless of species combination (UEDA et al., 1961).

The experiments reported here were initiated to test the following hypotheses: (1) can stone pine be crossed with would the blister rust resistance of stone pine and the other white pine species? (2) if hybrids can be produced, fast growth of other pines be evident in the hybrids? and (3) would hybrid performance for economically important traits be equal to, or better than, the parent species? With these goals in mind, a number of interspecific crosses between stone pine and other white pines were attempted in 1979. Only two hybrid combinations were successful: *P. cembra* x *P. wallichiana* and *P. cembra* x *P. monticola* (BLADA, 1987).

The main economically important traits of the 3 crossed species are, as follows:

— *P. cembra* is very slow growing (HOLZER, 1972) but as earlier stated, it is highly resistant to blister rust;

— P. *wallichiana* or blue pine, naturally distributed in the Himalayas, is moderate to fast growing species (AHSAN and KHAN, 1972) and has a moderate to relatively high level of resistance to blister rust (PATTON, 1964; BINGHAM, 1972a; HEIMBURGER, 1972; HOFF et al., 1980);

— *P. monticola* or western white pine, from the western North America, is fast growing but highly susceptible to blister rust (BINGHAM et al., 1971; BINGHAM, 1972a);

The performance of *P. cembra* x *P. wallichiana* and *P. cembra* x *P. monticola* hybrids at age 11 is reported in this paper.

Materials and Methods

Parents and mating design

Controlled pollinations were made during July 1979 in a *P. cembra* natural population from the Retezat Mountains (Southern Carpathians). A simple A x B mating design was used with 10 female strobili for each combination. One female tree of *P. cembra* was pollinated by 4 male trees (of unknown origin), 2 of *P. wallichiana* and 2 of *P. monticola*. Both hybrid seeds and open pollinated seeds of the parents were collected in October 1980. The seeds for the interspecific crosses were pooled separately for *P. cembra* x *P. wallichiana* and for *P. cembra* x *P. monticola*. Thus, further comparisons refer to a mixture of 2 progenies of the same species cross, quite independently of the other species cross; these are compared with open pollinated progeny of the mother tree and a mixture of 2 open pollinated progenies of the 2 father trees. Before crossing, the parental trees were not tested.

Seeds were stratified according to KRIEBEL's (1973) procedure, and were planted in March 1981, in individual polyethylene pots (22cm x 18 cm) using a mixture of 70% spruce humus and 30% sand. The seedlings remained in these pots for the first 6 years.

Inoculation and experimental design

During the inoculation phase, the hybrids and open pollinated progenies of their parents were placed in a

Table 1. — Measured traits.

Traits	Symbol	Units
1. Blister-rust resistance	BR	Index 1...10
2. Total height growth	H	dm
3. Annual growth in 1991	h	dm
4. Diameter at 1/2 H	D	cm
5. Basal area at 1/2 H	BA	dm^2
6. Stem volume	V	dm^3
7. Stem straightness	SS	Index 1...4
8. Branch thickness	BT	Index 1...4
9. Crown width	CW	dm
10. Number of leaders	NL	Index 1...4
11. Needle length	Nl	cm
12. Number of stomata/mm^2	S	No.

Table 2. — Variance analysis of the hybrid and parent traits.

Source of variation	DF	Mean squares for the traits											
		BR	H	h	D	BA	V	SS	BT	CW	NL	Nl	S
		P. cembra x *P. walliachiana*											
Blocks	2	0.32	2.17	0.18	0.10	0.05	1.64	0.02	0.10	0.32	0.02	75.50	3.30
Genotypes	2	20.69++	42.91+++	5.45+++	56.40++	5.00++	144.76++	0.92	1.42+	29.16++	0.52+	567.80++	460.30+
Error	4	0.45	0.50	0.04	1.80	0.14	3.60	0.31	0.12	0.55	0.05	10.10	60.20
		P. cembra x *P. monticola*											
Blocks	2	-	0.19	0.56	0.01	0.13	0.88	0.02	0.67	5.98	0.004	-	-
Genotypes	2	-	177.30+++	21.81+++	0.56++	4.46+	414.70+++	1.83+	0.34	67.17++	0.018	-	-
Error	4	-	0.28	0.33	0.01	0.27	0.60	0.21	0.18	1.94	0.004	-	-

Mean squares of some traits were multiplied, as follows: D by 100; BA by 10000; V by 1000. + = $p < 0.05$; ++ = $p < 0.01$; +++ = $p < 0.001$

tent using a complete block design; each family being represented by a 10-seedling plot in each of 3 replications. The progenies were artificially inoculated 3 times, between August 20 and 25, in 1982, 1983 and 1984 when the seedlings were 2, 3 and 4 years old. The inoculum was collected from heavily infected leaves of *Ribes nigrum* L. Inoculation methods were similar to those described by BINGHAM (1972b). Due to unforseen problems, only the *P. cembra* x *P. wallichiana* hybrid and its parents were successfully inoculated.

At age 6, the seedlings were outplanted in the hybrid collection area of the Forest Research Station at Timisoara, using the same experimental design that was used in the inoculation test. A spacing of 3 m x 3 m was used within and between rows of trees. This trial was laid out within a 'Blister Rust Area'.

Measurements and statistical analysis

Twelve traits for *P. cembra* x *P. wallichiana* and 9 traits for *P. cembra* x *P. monticola* hybrids were measured at age 11 (*Table 1*).

Blister rust resistance was measured using a 1 to 10 scale that reflects the economic and biological impact, as well as the incidence of the disease; the scale considers both number and severity of lesions. Numerical values were assigned, as follows: 1 = tree dead or total susceptibility; 2 = 4 or more severe stem lesions; 3 = 3 severe lesions; 4 = 3 more or less severe stem lesions; 5 = 2 severe stem lesions; 6 = 2 more or less severe stem lesions; 7 = 1 severe stem lesion; 8 = 1 more or less severe stem lesion; 9 = branch or very light stem lesions; 10 = no lesions or total resistance.

Stem straightness was assessed by a 1 to 4 scale, where 1 = crocked stem and 4 = straight stem.

Branch thickness was assessed using a 4 point visual score with 1 = thin branches and 4 = thick branches. Also, the number of leaders was counted and assigned a score 1 = 4 or more leaders; 2 = 3 leaders; 3 = 2 leaders: 4 = 1 leader: the other traits are listed in *table 1* and do not require explanation.

The plot means were the initial data for statistical analysis (SNEDECOR, 1956).

Heterosis calculation

Hybrid vigor or heterosis was calculated taking into account ZOBEL and TALBERT's (1984) definition of heterosis which is in agreement with HALAUER and MIRANDA's (1981) formula (1) for heterosis:

$$He = \frac{Hy - P}{P} 100 \quad (1)$$

where: He = heterosis; Hy and P = the hybrid and the best parent performance, respectively.

Results

P. cembra x *P. wallichiana*

Significant ($p<0.05$) and highly significant ($p<0.01$; $p<0.001$) differeces were found among hybrid and parents for all traits except stem straightness (*Table 2*).

According to DUNCAN's multiple range test (*Table 3*), *P. cembra* x *P. wallichiana* hybrid cross resulted in individuals that had several traits outside the range of the parents. These traits ,that are intermediate otherwise, are: blister rust resistance (BR), annual height growth (h), diameter at 1/2 height (D), basal area at 1/2 height (BA), and

Table 3. — Distribution of the genotypes[1]) for the main traits according to DUNCAN test ($p < 0.05$).

BR			H			h			D		
Genotypes	Means	DUNCAN test	Genotypes	Means	DUNCAN test	Genotypes	Means	DUNCAN test	Genotypes	Means	DUNCAN test
P. cembra × *P. wallichiana*											
P.c	9,7	I	P.w	13,1	I	P.w	4,0	I	P.w	2,3	I
H	6,7	I	Hy	12,3	I	Hy	2,3	I	Hy	1,9	I
P.w	4,5	I	P.c	6,2	I	P.c	1,3	I	P.c	1.4	I
P. cembra × *P. monticola*											
			Hy	21,5	I	Hy	6.4	I	Hy	2.2	I
			P.m	16.0	I	P.m	5.4	I	P.m	2,0	I
			P.c	6.2	I	P.c	1.3	I	P.c	1.4	I

BA			V			SS			BT			CW		
Genotypes	Means	DUNCAN test	Genotypes	Means	DUNCAN test	Genotypes	Means	DUNCAN test	Genotypes	Means	DUNCAN test	Genotypes	Means	DUNCAN test
P. cembra × *P. wallichiana*														
P.w	0,041	I	P.w	0,532	I	P.w	3,1	I	P.w	2,3	I	P.w	9,0	I
Hy	0,027	I	Hy	0,334	I	P.c	2,3	I	Hy	2,0	I	Hy	9,0	I
P.c	0.015	I	P.c	0,096	I	H.y	2,1	I	P.c	1,0	I	P.c	3,6	I
P. cembra × *P. monticola*														
Hy	0,039	I	Hy	0,837	I	P.m	3,7	I	P.m	1,6	I	Hy	12,1	I
P.m	0,030	I	P.m	0,518	I	Hy	3.6	I	Hy	1,6	I	P.m	11,3	I
P.c	0.015	I	P.c	0,096	I	P.c	2.3	I	P.c	1.0	I	P.c	3.6	I

P.c = *P. cembra*; P.w = *P. wallichiana*; P.m = *P. monticola*; Hy = hybrid

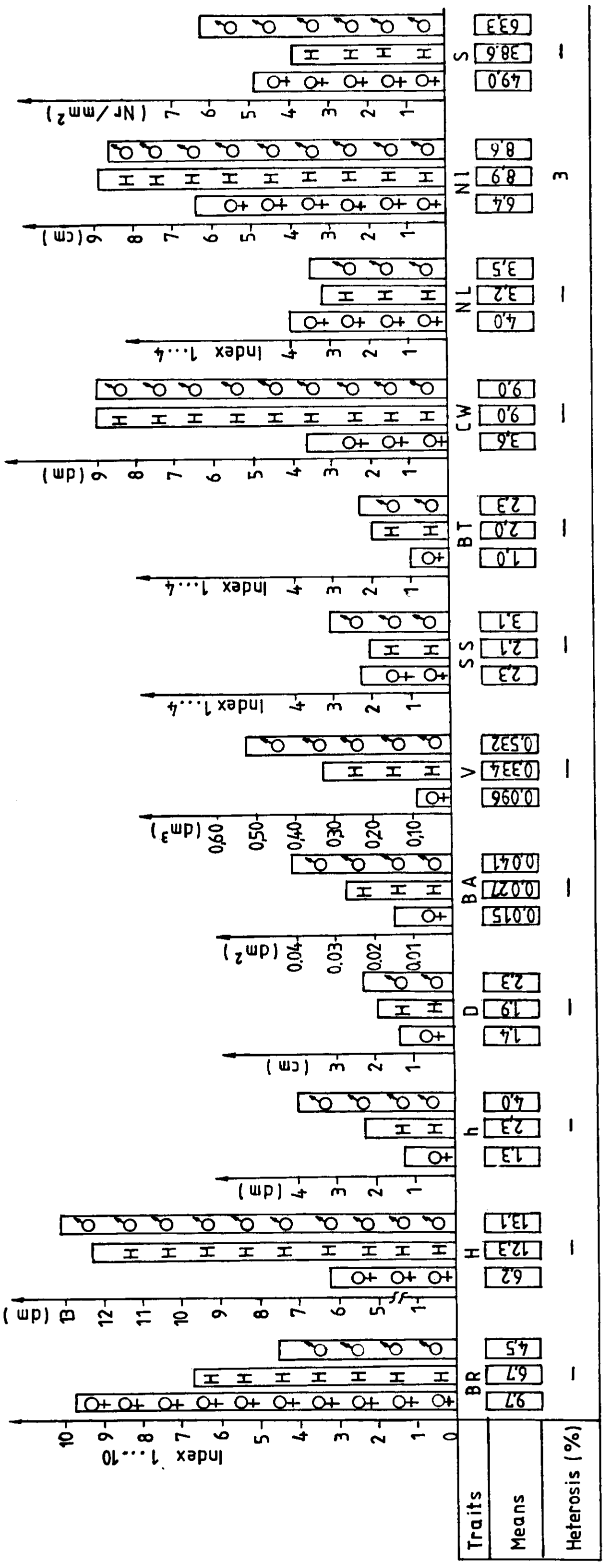


Figure 1. — *P. cembra* x *P. wallichiana* hybrid performance and heterosis compared to the best parent.

stem volume (V). For other 3 traits, such as: total height gromth (H), branch thickness (BT) and crown width (CW), the hybrid fall into the range of the blue pine male parent, but outside the range of the stone pine female parent. Therefore, the hybrid carries genes from the blue pine that controlled H, BT, and CW.

Hybrid performance and presence or absence of heterosis are shown in *figure 1,* as follows:

The *P. cembra* x *P. wallichiana* hybrid exceeded female parent in H, h, D, BA, V, BT, CW and Nl. For example, the hybrid had almost twice as much total height growth and 3 times as much volume growth as the *P. cembra* female parent.

The hybrid scored lower than the female parent in blister rust resistance (BR), stem straightness (SS), number of leaders (NL) and the number of stomata / mm² (S). It should be emphasized that though blister rust resistance of the hybrid is lower than that of the stone pine female parent, the difference is not great. On the other hand, the lower scores for SS and NL for the hybrid are considered negative features.

The hybrid outgrew the *P. wallichiana* male parent only in BR and Nl. It is important to note that the hybrid exhibited a higher rust resistance than *P. wallichiana.*

The hybrid scores lower than the male parent in H, h, D, BA, V, SS, BT, NL and S.

Hybrid displayed heterosis (3%) only in Nl, while it was lower than the both parents in SS, NL and S.

It is obvious that the hybrid, for most traits, was intermediate between the 2 parents. Combining stone pine with blue pine produced hybrids with economically desirable characteristics from both parents, such as, blister rust resistance from female parent and fast growth from the male parent. These traits make the hybrid a potentially valuable tree for further testing in the subalpine zone. More work is still needed to improve stem straightness and reduce multiple-leader formation in the hybrid.

P. cembra x *P. monticola*

Differences among hybrid and parents were significant ($p<0.05$) and highly significant ($p<0.01$; $p<0.001$) for all traits except BT and NL (*Table 2*).

DUNCAN's multiple range test (*Table 3*) demonstrated that the hybrid had some traits outside the range of the parents; these traits are H, D and V. For other 4 traits, such as h, BA, SS and CW, the hybrid fall into the range of the *P. monticola* male parent, but outside the range of the *P. cembra* female parent. This suggests that these 4 traits were inherited from the male parent.

Hybrid vigor or heterosis and its performance compared to the parents are given in figure 2, as follows:

Hybrid exceeded the female parent in H, h, D, BA, V, SS, BT and CW. The hybrid also exceeded the male parent in H, h, D, BA, V and CW.

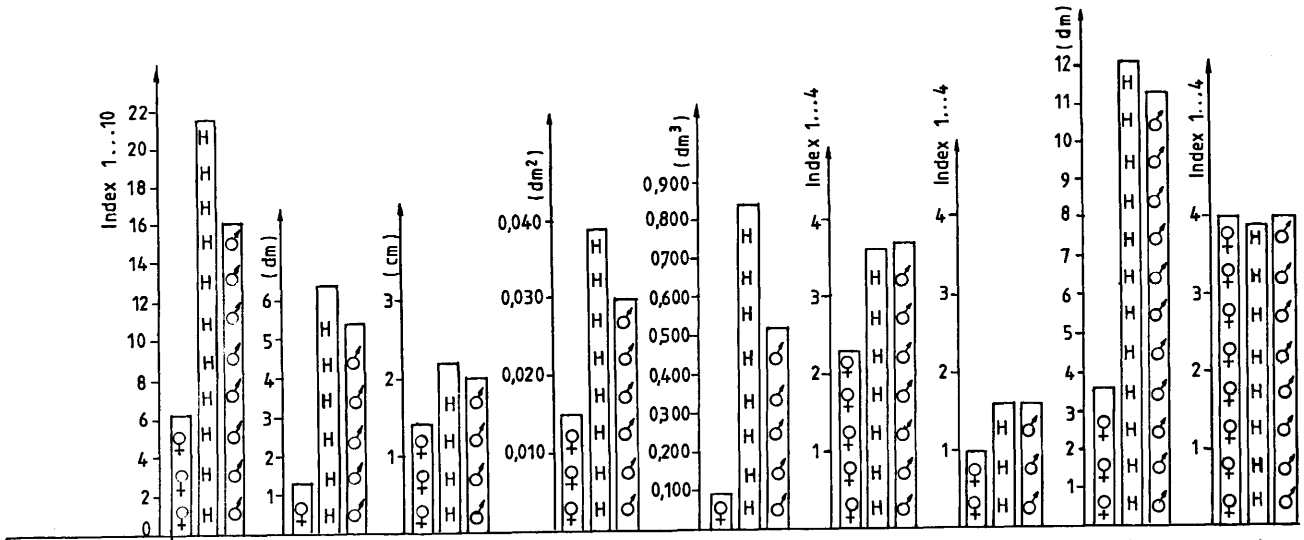
Hybrid displayed definite heterosis, exceeding the best parent in H, h, D, BA, V and CW by 34%, 19%, 10%, 30%, 62% and 7%, respectively. It inherited outstanding form and desirable growth traits from western white pine and, perhaps also blister rust resistance from stone pine. The heterotic increase by 62% in volume growth over the western white pine is a remarkable performance.

The hybrid showed no intermediacy for any trait with the possible exception of stem straightness.

As previously mentioned, this hybrid was not successfully inoculated with blister rust. However, it demonstrated total phenotypic resistance over 11 years of heavy natural exposure to this pathogen. These observations suggest a possible blister rust resistant genes transfer from the stone pine to the hybrid.

This hybrid will continue to be monitored to see if the advantages in blister rust resistance and growth rates persist.

Based on these observations, the *P. cembra* x *P. monticola* hybrid should be a good candidate for operational regeneration programs. The first step is to produce large



Traits	H	h	D	BA	V	SS	BT	CW	NL
Means	6.2 / 21.5 / 16.0	1.3 / 6.4 / 5.4	1.4 / 2.2 / 2.0	0.015 / 0.039 / 0.030	0.096 / 0.837 / 0.518	2.3 / 3.6 / 3.7	1.0 / 1.6 / 1.6	3.6 / 12.1 / 11.3	4.0 / 3.9 / 4.0
Heterosis (%)	34	19	10	30	62	-	-	7	-

Figure 2. — *P. cembra* x *P. monticola* **hybrid performance and heterosis compared to the best parent.**

quantities of hybrid seeds using a suitable mating design and parents selected for their general combining ability. The next step would be to test for blister rust resistance and plant the hybrids in a number of different environments.

Discussion

As mentioned in the Introduction, the blue pine was found with a moderate to relatively high level of resistance to blister rust. In the present test, the two pooled progenies of blue pine male parents proved, under artificial inoculation, to have low resistance. In the same conditions, the progeny of stone pine female parent was highly resistant. This suggest that the *P. cembra* x *P. wallichiana* hybrid inherited both genes of resistance from the female parent and genes of susceptibility from the male parents, resulting in a hybrid with an intermediate blister rust resistance.

P. cembra x *P. monticola* hybrid was not successfully artificially inoculated. But, before and after outplanting, trials have been placed within a 'Blister Rust Area' with high incidence of blister rust. Therefore, the progenies have been growing under a heavy natural exposure. During this 11 year period of exposure, the hybrid and stone pine female parent open pollinated offsprings remained disease-free whereas 47% of western white pine male parents offsprings were killed by blister rust. Although this test is not significant, it does suggest that the hybrid phenotypic resistance may be attributed to the resistance genes passed on from the stone pine parent.

Conclusions

1. *P. cembra* was successfully crossed with both *P. wallichiana* and *P. monticola*.

2. The *P. cembra* x *P. wallichiana* hybrid was intermediate between parent species for most traits. It probably incorporated blister rust resistance from the female parent and some susceptibility to blister rust and fast growth from the male parents.

3. *P. cembra* x *P. monticola* hybrid displayed heterosis for all growth traits, and inherited outstanding form and fast growth from the male parents.

4. Both hybrids would probably be useful alternatives for planting on middle to high altitude sites.

5. The key to success in the future use of either hybrid will be the degree to which both generative and vegetative propagation is used operationally.

6. The detection of parents with a good general combining ability is of promise for further breeding work.

Acknowledgements

I wish to thank Dr. Peter Garrett from the Northeastern Forest Experiment Station, Durham, New Hampshire, U.S.A., who generously improved the English version of the paper. Also, I express my gratitude to the (unknown for me) official reviewer for his/her helpflul criticism.

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Allozyme Frequency Distributions in Five European Populations of Black Pine (Pinus nigra Arnold)[1][2]

I) Estimation of Genetic Variation Within and Among Populations
II) Contribution of Isozyme Analysis to the Taxonomic Status of the Species

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(Received 19th April 1993)

Summary

European black pine (*Pinus nigra* ARNOLD) is generally considered a collective species with many taxonomic problems.

Genetic variation in 5 natural populations of *Pinus nigra* (Austria, Bulgaria, Greece, Corsica, Calabria) was investigated, at the enzyme level, by using the technique of starch gel electrophoresis.

Isozyme patterns of 10 enzyme systems (MDH, 6PGD, MR, IDH, PGM, DIA, AAT, LAP, PGI, GDH) were studied. Analysis, using haploid megagametophyte tissue, demonstrated that the allozyme variants in the above enzyme systems were coded by a total of 42 structural genes in the 16 readable loci. In one of these loci (IDH), no variants were found.

The results of this study showed the following:

a) The loci (isozymes) of DIA appeared in MR enzyme system as well.
b) On the average, 70.0 % of the analyzed loci were polymorphic, while the number of alleles detected per locus (A/L) ranged from 1.87 (Calabria, Greece) to 2.31 (Bulgaria) with a mean value for all studied populations of 2.025 allleles per locus.
c) Heterozygosity ranged from 0.180 (Corsica) to 0.257 (Bulgaria).
d) 94% of the total variation of the species was due to intrapopulation gene diversity.
e) Isozymes contributed differently in the total variation of the species.
f) Isozyme variation is a useful tool for the taxonomy of *Pinus nigra*, since it can discriminate either different subspecies or different populations within a given subspecies.
g) The most appropriate enzyme systems for classification of the subspecies [ssp. *laricio*, *nigra (austriaca)*, *pallasiana*] were MDH, DIA, 6PGD and AAT.
h) There was a clear distinction between *laricio* group (Calabria, Corsica) and *austriaca* group (Austria, Bulgaria).
i) The Greek provenance was closer to the *austriaca* group.

Key words: Pinus nigra, isozyme variation, taxonomy.

[1]) Part of this research was conducted by Dr. A. SCALTSOYIANNES in his work under the title "Genetic variation of malate dehydrogenase isozymes in subspecies of European black pine (*Pinus nigra* ARNOLD)" granted by the Commision of the European Communities (Division for the Coordination of Agricultural Research) in the framework of the "Competitiveness of Agriculture and Management of Agricultural Resources" Programme. The work was conducted in the Lab. Interaction Plantes-Champignons et Micropropagation, Lyon, France.

[2]) Another part of the present research was submitted as a partial fullfilment of the "Diplome d'Associe aux Recherches — Diplome d'Etudes Superieures" of Ms M. TSAKTSIRA in the Lab. Interaction Plantes-Champignons et Micropropagation, University Claude Bernard, Lyon I, Lyon, France.

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Introduction

European black pine (*Pinus nigra* ARNOLD) is one of the most valuable species in the Mediterranean region and it is distributed in a discontinuous area which includes Southern Europe, Minor Asia, Cyprus and North-Western Africa (CRITCHFIELD and LITTLE, 1966). According to STOJANOFF and STEFANOFF (1929) and MIROV (1967), *P. nigra* is a