# Genetic Variation in Morphological and Anatomical Needle Characteristics in Pinus brutia Ten.

By R. CAIAMASSI<sup>1</sup>), S. R. Puglisi<sup>2</sup>) and G. G. Vendramin<sup>2</sup>)

(Received 4th August 1987)

#### Summary

In a Pinus brutia Ten. plantation, including 14 populations, from different regions of its natural range, 11 morphological and anatomical needle traits (needle length, sheath length, number of rows of stomata on the dorsal face, number of rows of stomata on the ventral face, total number of rows of stomata, needle width, needle thickness, number of resin ducts, number of stomata per cm of row length, number of hypodermal layers in the dorsal face, total number of stomata per 1 cm segment) were studied. The univariate and multivariate analyses showed that:

- 1) significative differences exist between populations for all considered traits, even if most of the variation is due to differences between and within trees;
- 2) the populations of the Mediterranean sector of Turkey tend to group, while the populations of Iran ( $Pinus\ eldarica$  Medw.) and of Cyprus are quite different from the others;
- 3) number of rows of stomata, needle width and thickness show a clinal trend with respect to longitude;
- 4) our result show that it is possible to discriminate the populations of *P. brutia* considering less characters: total number of rows of stomata, total number of stomata, needle width and number of hypodermal layers.

Key words: Pinus brutia Ten., Pinus eldarica Medw., genetic variation, morphological variation, anatomical variation, multivariate analysis.

#### Zusammenfassung

In einer Pinus brutia Ten. Plantage mit 14 Populationen aus dem natürlichen Verbreitungsgebiet der Art sind 11 morphologische und anatomische Nadelmerkmale untersucht worden, und zwar: Länge, Breite und Dicke der Nadeln, Länge der Scheide, Anzahl der Stomata-Reihen an der dorsalen und ventralen Seite, Gesamtanzahl der Stomata-Reihen, Anzahl der Harzkanäle, Anzahl der Stomata pro cm Stomata-Reihen-Länge, Anzahl der Hypoderm-Schichten an der dorsalen Seite sowie die Gesamtanzahl der Stomata bezogen auf ein 1 cm langes Segment. Die univariaten und multivariaten Varianzanalysen beweisen,

- daß signifikante Unterschiede zwischen den Populationen für alle betrachteten Merkmale bestehen, auch wenn ein Großteil der Variabilität auf die Unterschiede zwischen und innerhalb der Einzelbäume zurückzuführen ist;
- daß die Populationen des mediterranen Gebietes der Türkei zu einer Gruppe tendieren, während sich die Populationen aus Iran (Pinus eldarica Medw.) und von Zypern von den anderen stark unterscheiden;
- daß die Anzahl der Stomata-Reihen, sowie Breite und Dicke der Nadeln bezogen auf die geographische Breite einen klinalen Trend zeigen.
- 4.) Unsere Ergebnisse zeigen, daß die Populationen von Pinus brutia Ten. anhand weniger Merkmale unterschieden werden können, und zwar anhand der Gesamtzahl der Stomata-Reihen, der Gesamtzahl der Stomata

¹) Dipartimento di Biologia Vegetale, Laboratori di Botanica Agraria e Forestale, Universita' di Firenze, P. le delle Cascine, 28, 50144 Firenze, Italy. bezogen auf ein 1 cm langes Segment, sowie anhand der Breite der Nadeln sowie der Anzahl der Hypoderm-Schichten.

#### Introduction

The actual distribution of *Pinus brutia* Ten., which is determined both by climatic factors as well as by the geological history of the Mediterranean basin, is quite large (Panetsos, 1981).

Because of the ancient isolation of many of its populations, the variability of *P. brutia* should be high; this is the reason why it is considered interesting for improvement programs. In Turkey, the widest region in its natural range, *P. brutia* grows from sea level up to 1400 m, on much different geological formations and in areas with quite different rain density and climatic variations (Arbez, 1971; Panetsos, 1981). This suggests a certain plasticity and adaptability of *P. brutia* but also the existence of ecotypes adapted to different environments.

Characterizing the provenances is one of the objectives of the FAO 4 bis Project "International experiences upon provenances of *Pinus halepensis* and *Pinus brutia*".

In this connection some researches have been carried out during the last years, particularly into seeds and seedlings characteristics (Pelizzo and Tocci, 1978), germination (Falusi, 1982), resistance of seeds and trees against environmental stress (Calamassi *et al.*, 1980; Falusi and Calamassi, 1982; Falusi *et al.*, 1984) and growth pattern of seedlings (Calamassi *et al.*, 1987).

Needle morphology was used to study the variability existing in some *Pinus* species, both in trees grown in their natural environment (Stover, 1944; Vidakovic, 1957; Bassiotis, 1967; Gellini, 1968; Schoenike, 1976; Salazar, 1983; Eguiluz Piedra, 1984), and in trees raised in one or more plantations (Mergen, 1963; Lee, 1968; Arbez and Millier, 1971; Lamontagne, 1971; Burley and Burrows, 1972; Matziris, 1984; Calamassi, 1986).

The objectives of this research, that was carried out into 13 populations of  $P.\ brutia$  Ten. and 1 of  $P.\ eldarica$  Medw. (Table 1) are: to study the genetic variation pattern in needle anatomical and morphological characters, to determine the relationships among the characteristics measured, and furthermore, to help in enlarging the knowledge of needle morphology of  $P.\ brutia$ .

#### **Materials and Methods**

Needle samples were collected, during the end of the vegetative season 1984, from 8-year-old trees raised in a plantation of the Experimental Institute of Forestry of Arezzo, using a completely random design. The plantation was established using seeds collected in the species natural range according to the recomendations given by Arbez (1971). 10 1-year-old fascicles belonging to two different branches in the upper third of the crown from 10 trees of each population were collected.

All the fascicles were picked on the same day, and kept at 4°C until the time of the testing. The test was carried

<sup>2)</sup> Istituto Miglioramento Genetico Piante Forestali, Consiglio Nazionale delle Ricerche, via S. Bonaventura, 13, 50145 Firenze Italy.

Table 1. — Details of population origin.

Рорі	ulations	Country	Latitude Nord	Longitude East	Altitude (m)	Mean annual Rainfall (mm)	Mean Temperature (°C)
В2	Kavala	Greece	40°48'	24°42'	20-180	450	18.0
B5	Cyprus	Cyprus	35°08'	33°17'	100-200	439	16.9
В6	Marmaris	Turkey	37°00'	28°18'	100-250	1187	18.5
В7	Isparta	Turkey	38°04'	29°32'	1100	628	12.1
B8	Düzlerçani	Turkey	37°03'	30°25'	100-250	1031	18.7
В9	Pämuçak	Turkey	37°40'	30°41'	850	758	15.6
B10	Bozburum	Turkey	37°21'	30°45'	520	N.A.	N.A.
B11	Bakara	Turkey	36°09'	32°43'	300	601	18.6
B13	Cämgölu	Turkey	41°50'	35°20'	70	719	14.3
B14	Baspinar	Turkey	37°48'	35°15'	860	1031	N.A.
B15	Kizildag	Turkey	36°21'	35°58'	400	1153	18.2
B16	Zawita	Irak	36°35'	44°20'	414	536	22.5
B17	Lebanon	Lebanon	N.A.	N.A.	N.A.	N.A.	N.A.
E	Karaj	Iran	35°36'	51°00'	1300	238	13.9

The labels of populations are the accession codes of the F.A.O., Committe for Mediterranean Forest Research. N.A. information was not available.

out on one needle chosen at random for each fascicle (100 needles per population). The following characters were observed:

V 1 -- Needle length (mm)

V 2 - Sheath length (mm)

V 3 — Number of rows of stomata on the dorsal face

V 4 — Number of rows of stomata on the ventral face

V 5 - Total number of rows of stomata

V 6 - Needle width (mm)

V 7 — Needle thickness (mm)

V 8 - Number of resin ducts

V 9 - Number of stomata per cm of row length

V10 - Number of hypodermal layers in the dorsal face

V11 — Total number of stomata per 1 cm segment (V5  $\times$  V9)

Needle and sheath length were measured to the nearest millimetre; rows of stomata (on the dorsal and ventral faces) were counted in midsection, while stomata were counted on a section 1 cm long, as average value on two rows of stomata; in cross section needle width and thickness were measured with a micrometric eye-piece, and number and position of resin ducts and number of hypodermal layers in the dorsal face were noticed.

Each of the 11 traits was submitted to a hierarchical analysis of variance, using the following model:

 $\mathrm{Yijk} \, = \, \mu \, + \, \mathrm{Pi} \, + \, \mathrm{Tj/i} \, + \, \mathrm{Eijk}$ 

where

Yijk = mean value of the trait Y in the kth needle of the jth tree in the ith population;

Table 2. — Analysis of variance and expected mean squares.

Entry number	Sources of variation	d.f.	Test against entry	Expected mean squares
1	Populations (P)	(P-1)	2	σ <sup>2</sup> +nσ <sup>2</sup> N T/P+ntσ <sup>2</sup>
2	Trees (T) in P	T(P-1)	3	$\sigma_{N}^{2}+n\sigma_{T/P}^{2}$
3	Needles (N) in			
	T in P	PT(N-1)		σ² N

 $\mu = \text{true mean effect};$ 

Pi = effect of the ith population; i = 1,2,...14;

Tj/i = effect of the jth tree in the ith population; j = 1,2,...10;

Eijk = error specified by the effect of the kth needle of the jth tree in the ith population;  $k = 1, 2, \dots 10$ .

The analysis of variance and the expectation mean squares, are shown in *Table 2*.

F value relative to trait V9 was not calculated as this characteristic does not offer a normal distribution; consequently, for this trait, the analysis of variance was used only as a numerical method to calculate the variance components.

The correlations between needle traits and 5 geoclimatic traits (latitude, longitude, altitude, mean annual rainfall, mean annual temperature) were also calculated.

The following multivariate analysis methods were also used: principal component analysis; factor analysis with both orthogonal ("Varimax") and oblique ("Quartimax") principal component axes rotation; discriminant analysis; cluster analysis (Sadocchi, 1980).

All the statistical analyses were carried out using the Genstat 4.04 statistics package (Anonymous, 1981), implemented on a VAX/VMS computer by Digital.

#### **Results and Discussion**

 ${\it Hierarchical\ univariate\ analysis\ of\ variance}$ 

The results of the analysis of variance (*Table 3*) show that all the morphological and anatomical characteristics offer highly significant differences between the 14 populations examined.

Number of hypodermal layers, needle width and total number of rows of stomata are the traits that show the highest population components (24.73, 22.05 and 20.45% of the total variance, respectively). Generally, although there are significant differences between populations, for all the characters the highest rate of variability is due to the differences between trees within populations (for 6 characters the component value given from such source of variation

 $\it Table~3.-Summary~of~analysis~of~variance~for~morphological~and~anatomical~traits~of~the~needles.$ 

Entry N°	Sources of variation	D.F.	Test against entry	V1	<b>V</b> 2	V3	V4	V5	V6	<b>V</b> 7	V8	v9	V10	V11
								MEAN	SQUARE					
1	Populations (P)	13	2	3219.54	40.18	48.66	14.20	108.57	0.38	0.17	29.66	861.08	5.18	211.40
2	Trees (T) in P	126	3	1420.27	15.09	10.89	3.54	21.31	0.09	0.06	11.32	526.09	0.66	48.11
3	Needles (N) in T in P	1260		49.24	0.52	1.03	0.35	1.40	0.002	0.001	0.64	35.76	0.08	3.71
								F	test					
1	P			••	••	••	••	••	••	••	••		••	**
2	T in P			••	••	••	••	••	••	••	••		••	**
							Va	ariance C	omponent	ts (%)				
1	P			8.81	11.27	15.76	13.77	20.45	22.05	14.37	9.68	3.80	24.73	16.69
2	T in P			67.10	65.44	41.12	41.23	46.66	61.41	72.12	56.32	55.63	31.48	45.39
3	N in T in P			24.10	23.29	43.11	45.00	32.88	16.54	13.50	34.01	40.57	43.79	37.92

<sup>\*\*</sup> significant at P < 0.01

is greater than 50%) and between needles within trees and populations.

Number of hypodermal layers, needle width and total in the hypodermis are characteristics helpful to some  $% \left\{ 1,2,...,n\right\}$ 

authors to differentiate specific and intraspecific entities or hybrids (Vidakovic, 1958; Gellini, 1968; Lee, 1968), while in other researches (Schutt and Hattemer, 1959; Kriebel and Fowler, 1965), such characteristics were not useful di-

Table 4. — Descriptive statistics of morphological and anatomical characteristics of the needles. Simbology: S.E., standard error; STD DEVN, standard deviation; C.V., coefficient of variation.

					Va	riables					
Populations	V1	V2	VЗ	V4	<u>V5</u>	<b>V</b> 6	V7	V8	<u>v9</u>	V10	V11
x x	116.6	10.20	9.94	4.39	14.33	1.16	0.73	2.67	95.94	2.35	1381.4
B2 S.E.	1.2	.10	.14	.07	.18	.01	.01	.13	.90	.04	22.0
5.5	108.0	10.30	9.90	3.84	13.74	1.26	0.81	3.00	99.68	2.91	1366.5
В5	1.4	.10	.15	.09	.19	.01	.01	.15	.32	.04	19.5
В6	116.9	11.67	8.51	3.82	12.33	1.19	0.74	2.63	98.26	2.69	1209.6
1 00	1.8	.17	.10	.08	.15	.01	.01	.13	.91	.04	17.1
В7	116.8	11.34	9.36	4.09	13.45	1.14	0.71	2.20	95.36	2.25	1294.2
, °,	1.1	.13	.12	.08	.17	.01	.01	.17	.84	.03	18.2
B8	117.9	11.00	8.73	3.63	12.36	1.15	0.72	1.71	96.90	2.36	1194.6
1 00	1.3	.14	.13	.06	.14	.01	.01	.12	.98	.03	15.8
В9	114.8	10.33	8.97	4.05	13.02	1.13	0.71	1.81	98.10	2.26	1278.3
B9	.7	.20	.10	.07	.13	.01	.01	.05	.89	.03	18.1
P.10	114.3	11.22	8.75	3.84	12.59	1.11	0.70	1.88	96.28	2.32	1209.1
B10	1.4	.14	.11	.77	.14	.01	.01	.10	1.09	.03	17.4
	107.2	10.54	8.18	3.87	12.05	1.09	0.70	1.31	96.34	2.25	1159.3
B11	1.0	.12	.18	.08	.19	.01	.01	.09	.72	.03	18.8
D12	126.5	11.61	9.92	4.31	14.22	1.20	0.76	2.10	104.78	2.31	1485.0
B13	1.7	.11	.11	.07	.18	.01	.01	.06	.82	.04	19.3
	120.3	11.36	9.11	4.20	13.31	1.11	0.72	1.49	102.27	2.09	1361.8
B14	1.3	.11	.11	.08	.16	.01	.01	.10	.78	.03	18.8
22.5	125.0	11.87	9.56	4.46	14.02	1.22	0.77	2.35	96.38	2.34	1350.4
B15	1.4	.14	.12	.07	.16	.01	.01	.14	.73	.04	18.0
, n. c	109.5	10.21	9.77	4.08	13.85	1.17	0.76	2.06	101.50	2.50	1403.7
B16	1.4	.13	.21	.10	.28	.01	.01	.14	1.18	.05	30.4
B17	119.5	11.50	9.59	4.14	13.73	1.20	0.76	2.73	97.06	2.68	1331.2
B17	1.2	.10	.15	.09	.21	.01	.01	.19	1.04	.04	24.1
	118.6	10.19	10.89	5.15	16.04	1.31	0.84	1.63	94.08	2.72	1508.7
E	1.1	.14	.12	.09	.16	.01	.01	.12	.68	.04	18.9
	1										
Χ̈́	116.6	10.95	9.38	4.13	13.51		0.75		98.14 .25	2.43	1323.8
S.E.	14.2	.04 1.48	.04 1.54	.02 .87	.06 2.05	.003	.002			.41	6.3 226.4
STD. DEVN.	12.20	13.54	16.39	21.16	15.16		11.73			17.22	17.20

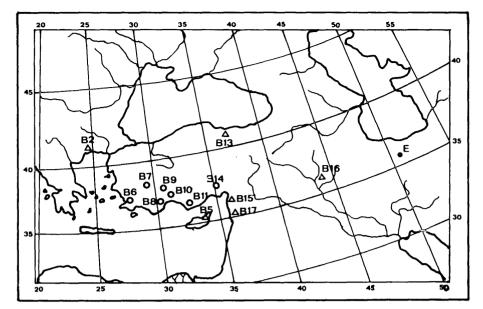


Figure 1. — Location of studied populations. The labels indicate the groups resulting by cluster analysis.

agnostic parameters. Number of hypodermal layers did not help Calamassi (1986) either to discriminate the provenances of *P. halepensis*. In our case, instead, it shows the highest variation between populations, and Duncan's studentized range test, which allow us to calculate variable significative differences dependent on the number of population mean values involved at any stage, separates sharply populations B5, E, B6 and B17 from all the others (*Fig. 2.*).

As regards needle width and the total number of rows of stomata, we observe a sharp discrimination of population E from the others (Fig. 2). Mergen (1958) found out that the number of stomata per mm of row-length may be used to distinguish putative hybrids of Pinus elliottii Engelm.

and he suggests that such a characteristic is relatively independent of environmental conditions. Burley and Burrows (1972) pointed out significant differences between provenances of *Pinus kesiya* Royle and Gordon, both in the number of rows of stomata and in the number of stomata per 5 mm of row length, and they came to the conclusion that the stomata traits allow the best discrimination of provenances when working on a univariate basis. Arbez and Miller (1971) also consider the total number of rows of stomata to be a good diagnostic character to discriminate the subspecies in *Pinus nigra* Arn. Matziris (1984) found a very sharp increase in the number of stomata per cm of row length in southern provenances of *P. nigra* 

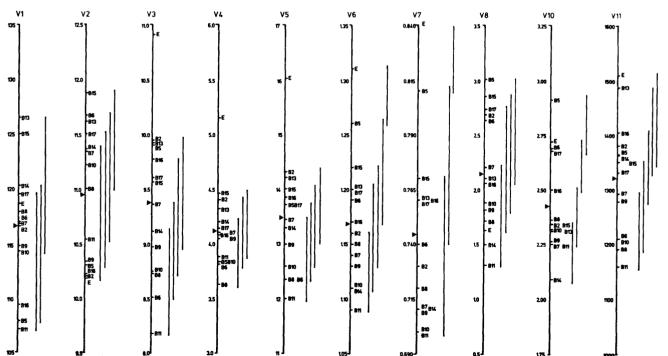


Figure 2. — Duncan's studentized range test for morphological and anatomical traits of the needles (P < 0.05). ▶ indicates the mean value.

from Greece, to confirm what Bassiotis (1967) had already discovered, suggesting that the highest number of stomata in southern populations could be considered to be an adaptation to xeric conditions. Calamassi (1986) pointed out in *P. halepensis* (species similar to *P. brutia*) an increase in the number of stomata per cm of row length in provenances with a higher drought tolerance.

Thomes (Squillace, 1966) and Salazar (1983) assert that a low stomatal frequency may form an adaptation to conditions of aridity. This seeming contradictory condition may be explained by Kozlowski (1971), who asserts that drought tolerance is strictly related to the capacity for rapid stomatal closure under stress conditions.

The high number of stomata per cm of row length found in each population of *P. brutia*, which is similar to the number found in populations of *P. halepensis* (Calamassi, 1986) with a stronger drought tolerance, confirms the substantial drought tolerance of this species.

Contrary to what already was discovered about *P. hale-pensis*, there does not seem to be in *P. brutia* any relation between the number of stomata and the reactions to moisture stress during germination and the first stages of root growth; in fact populations with similar moisture stress tolerance thresholds have a very different number of stomata (E, B2, B5, B8, and B10) (*Table 3*; Calamassi *et al.*, 1980; Falusi and Calamassi, 1982; Calamassi, 1986).

From the analysis of Duncan's studentized range test (Fig. 2) we can see a clear discrimination of the population of *P. eldarica* (E) from the others, for many of the traits observed; moreover the populations of *P. brutia* of Turkey Mediterranean coasting and inland area (Arbez, 1971) (B6, B7, B8, B9, B10, B11 and B14), besides to show similar mean values for nearly all the characters, are generally positioned below the species mean values, unlike the ones of others sectors of natural range (*Table 3* and *Fig. 2*).

The subdivision of the populations of these two groups becomes less marked about needle length and resin ducts number; Harlow (1931) and Doi and Morikawa (Lee, 1968) consider, for this latter character, the position to be more important than the number; Schutt and Hattemer (1959)

noticed that both characters are influenced by environmental conditions, and show a high variability between and within needles, but the position appears most constant. A considerable variability in the number of resin ducts within each population (*Table 3*) resulted from our research, while the position always turned out to be bordery.

#### Correlation coefficient

The correlation coefficients between needle traits, and between these and the geoclimatic traits are shown in Table 5

Needle width and thickness appeared to be positively and significantly related to the number of rows of stomata on the dorsal and ventral faces, and to the total number of rows of stomata, according to what Lee (1968) and Calamassi (1986) discovered respectively about *P. nigra* and *P. halepensis*. The number of stomata per cm of row length, on the contrary, is unrelated to any size trait of the needles, and this may suggest that the formation of stomata is genetically predetermined; in fact, an increase in needle length due to favorable microenvironments, does not cause an increasing number.

Besides, any significant relation was not found between the number of stomata per cm of row length and the number of rows of stomata on the dorsal and ventral faces, according to what was shown by Matziris (1984) in different clones of P. nigra and by CALAMASSI (1986) in populations of P. halepensis, Burley and Burrows (1972) instead found in P. kesiya a negative correlation between these characters. A positive correlation of longitude both in needle width and thickness as well as in the number of rows of stomata was evidenced. CALAMASSI (1986) found about P. halepensis, a negative correlation for the same traits. Moreover, a negative correlation between rainfall and number of rows of stomata was found, but this does not allow us to give a decisive opinion about drought tolerance of the several populations, both because not only abundance of rainfalls but also their distribution is important, and also because the climatic data that are avail-

٧2 VЗ ٧4 ٧5 ٧6 ٧7 ٧8 ٧9 ٧10 V11 V12 V13 V14 V15 V16 .69\*\* V١ . 26 . 35 .23 . 26 .05 .37 -.10 -.30 .23 -.14 -.26 -.10 -.22 .20 -.20 -.16 .15 -.02 -.17 .70\* .19 VЗ 79\*\*\* 97\*\*\* 79\*\*\* .82\*\*\* .29 .02 . 32 .93\*\*\* .08 .60\* .30 -.63 -.36 .58\* .61\* -.04 -.20 -.01 .77\*\* .07 .64 .51 -.43 -.36 v5 .75\*\* .78\*\* .19 -.06 .21 .92\*\*\* .11 .62\* .36 -.59\* -.36 lv6 .97\*\*\* .45 .70\* .67\*\* .62\* .08 -.37 ~.13 lv7 .37 -.00 .67\* .73\*\* -.32 .69\* .13 -.13 - . 44 งล -.01 .46 .19 .01 -.15 -.44 .02 .30 v9 -.18 .33 .47 -.11 -.31 .29 -.15 V10 .13 -.53 . 38 -.13 .24 V11 .28 . 55 .24 -.46 -.41 V12 Latitude -.41 -.28 -.28 V13 -,40 Longitude -.40 .01 V14 Altitude -.57 Mean annual rainfall .25 Mean annual temperature

Table 5. — Matrix of correlation calculated on the mean of 11 variables.

<sup>\*</sup> significant at P < 0.05 \*\* significant at P < 0.01 \*\*\* significant at P < 0.001

Table 6. — Results of factor analysis performed on the correlation matrix of 11 variables.

		ROTATED FACT	OR PATTERNS:	"Vari	max	" and	9'' £	uart	timax"	rotation	s respectively*
				F	A	СТ	0	R	S		
		1	2			3				4	5
Latent roots		4.84	1.62			1.4	12			1.11	0.60
% total	simple	44.02	14.76			12.9	91			10.06	5.45
variance	cumulative		58.78			71.6	9			81.75	87.20
Variables											
V1			-0.83(-0.82	) '							
V2			-0.91(-0.90								
٧3		-0.90(-0.92)									
V4		-0.87(-0.85)									
<b>V</b> 5		-0.97(-0.98)									
V6		-0.70(-0.78)									
<b>V</b> 7		-0.57(-0.66)									
V8											+0.96(+0.95)
٧9									-0.9	8(-0.98)	
V10					+0.	87(+0	.86	)			
V11		-0.88(+0.90)									

<sup>\*)</sup> The Quartimax values are in parentheses. These values are correlations of each variable to each factor or principal component (correlation above or below ca. ± 0.55 were not included here).

able (Walter and Lieth, 1967; Arbez, 1971) are only largely indicative for the stands of seed provenance.

# Principal component and factor analysis

The principal component analysis established that 87.20% of total variation is contained in the first 5 components ( $Table\ 6$ ). The first component accounts for 44.02% of total variation and is strictly related to the number of rows of stomata (total, on the dorsal and ventral faces), to the total number of stomata, and to the needle width and thickness. The second accounts for 14.76% and is related to needle and sheath length. The remaining 3 components explain on the whole 28.42% of total variation and they are related respectively to the number of hypodermal layers, to the number of stomata per cm of row length, and to the number of resin ducts ( $Table\ 6$ ).

May be that the high weight of several characters within the same factor is the result of pleiotropy or linkage effects, whenever, as in our case, the environmental influence might be disregarded.

In Fig. 3, in the plain defined by the projection of the first 2 principal components, the separation of population

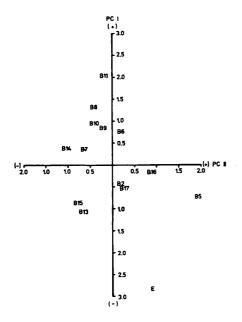


Figure 3. — Projection of the components I and II.

E (for the first) and B5 (for the second) from the others is quite clear. We furthermore can notice the group of populations of the Mediterranean sector of Turkey.

#### Discriminant analysis

The discriminant analysis confirmed the results of the principal component analysis on the whole, moreover it allowed us to allocate each tree to the population to which it has the highest probability of belonging. The data concerning the correctly classified cases are shown in *Table 6*: the larger the proportion of trees allocated to the actual population, the better the population is assumed to be discriminated from the others. *Table 6* shows the high discrimination of B5, B13, B14 and E population, as well as the low discrimination of others (B7, B8, B9, B10, B15, B16 and B17). It is quite interesting to notice how the most distinct and homogenous populations are those situated on the periphery of the range (*Fig. 1*). The lower discrimination of the others could be attributed to an intense gene flow among neighbouring populations.

#### Cluster analysis

The phenogram resulting from cluster analysis shows the existence of 3 well defined groups (Fig. 4). The populations of the Mediterranean sector of Turkey (B8, B10, B7, B9, B11 and B14) belong to the first group, while the remaining of other sectors in which the natural range of P. brutia is devided (B15, B17, B13, B2, B16 and B5) belong to the second group. Only population E stands out quite clearly from these groups, particularly because of stomata traits and needle width and thickness.

#### Conclusions

Although most of the variation is concentrated in trees within populations, *P. brutia* and *P. eldarica* populations show significant differences in each morphological and anatomical needle trait examined (*Fig. 2*), thus confirming what has already been discovered about other traits (Pelizzo and Tocci, 1978; Falusi and Calamassi, 1982; Calamassi *et al.*, 1987).

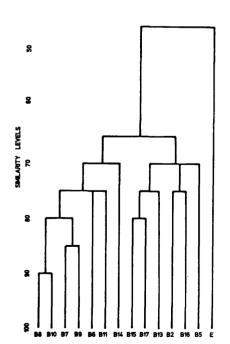
The given results in the geographic subdivision of the Turkish range show the existence of variations between the Mediterranean sector populations (B6, B7, B8, B9, B10 and B11) and the population of Pontus (B13). They also reflect the differences among populations of different regions in *P. brutia* natural range (Arbez, 1971).

Table 7. — Classification results by discrimi	nating analysis (in narcent)

ACTUAL GROUP					PREI	OICTED	GROUP	MEMBE	RSHIP					
	В2	В5	В6	В7	88	В9	B10	B11	B13	B14	B15	B16	B17	E
B2	50					30					10	10		
B5		80									10	10		
В6		10	60		10		10						10	
В7	10			10	10	10			20	20			20	
В8		10	10	20	30	10	10			10				
В9	10			10	10	10	30			20				10
В10		10		10		20	30		~-	10			10	10
B11	10				10	10	20	50						
B13									<b>®</b>		10	10		
B14						10	10			80				
B15				10		10	20		10	10	30		10	
B16		10		10	10	20			20			30		
B17		10	20	10	10		10				10		20	10
E	10												10	89
Percent	of g	roupe	d cas	es co	rrect	ly cla	ssifie	d: 45	.71					

Besides the Cyprus population (B5, geographically belonging to the Mediterranean sector) is sharply separated from the others. This situation can be explained by supposing the existence of an independent evolution, due to the isolation state caused to this population by geological events that happened in the Aegean region between the end of Pliocene and the Quaternary (Panersos, 1981). This behaviour of B5 was also observed in previous researches carried out into germination and tolerance against environmental stress (Calamassi et al., 1980; Falusi, 1982; Falusi and Calamassi, 1982). The sharp separation of population E, because of its geographic isolation, can be easily ex-

plained; moreover the systematic position of P. eldarica



Phenogram showing the grouping pattern of popu-Figure 4. lations, calculated from the 11 needle traits.

has not yet been univocally made clear; in fact for GAUSSEN (1960), NAHAL (1962), DEBAZAC and TOMASSONE (1965) it is a geographic variety of P. brutia, while, according to Kolesniкоv (1963) it is a separate species.

It is useful to remember that genetic differences between populations can be inferred only for the considered environment; at this moment it is not possible to estimate the environmental stability of these genetical differences.

Our results show for a good deal of characters (number of rows of stomata on the dorsal and ventral face, total number of rows of stomata, needle widh and thickness) a clinal trend with respect to longitude, as already found out about P. halepensis (Calamassi, 1986), a species morphologically similar to P. brutia.

We furthermore think it is possible to discriminate the populations of P. brutia considering less characters, using, among the ones more strictly related with the first 3 principal components, those with a larger variability between populations: total number of rows of stomata, total number of stomata, needle width and number of hypodermal layers (Tables 3 and 5). This statement confirms the results of a preliminary research carried out into the same 4-yearold trees, which stated that the characters that better discriminated the populations were only rows of stomata, needle width and thicknees (CALAMASSI, unpublished data).

## Acknowledgements

We thank Prof. H. R. Gregorius (Institut für Forstgenetik der Universität Göttingen) for critical reading of the manuscript. We are also indebted to Dr. F. MIGLIETTA (Istituto di Analisi Ambientale e Telerilevamento applicato all'Agricoltura - C.N.R. Firenze) for help in use of Genstat statistics package. The technical assistance of F. Bandini is also gratefully acknowledged.

#### References

Anonymous: Genstat — A general statistical program. Rothamsted Experimental Station (1980). — Arbez, M.: Repartition, ecologie et variabilité de Pinus brutia en Turquie. Recommandations pour la recolte des graines de l'experience internationale de provenances. Rapport de mission F.A.O. (29 aout — 9 octobre 1971). FAO - Arbez, M. and Millier, C.: Contribution Paris et Rome (1971). a l'étude de la variabilité geographique de Pinus nigra Arn. Ann. - Bassiotis, C.: Variation in charac-Sci. For. 28: 23-49 (1971). -

teristics of black pine in Greece. Scientific Annals of Agricolture and Forestry Faculty Thessaloniki 11: 79-133 (1967). -J. and Burrow, P. M.: Multivariate analysis of variation in needles among provenances of Pinus kesiya Royle et Gordon (Syn. P. khasya Royle; P. insularis Endlicher). Silvae Gen. 21: 69-77 (1972). CALAMASSI. R: Caracterisation de quelques provenances de Pinus halepensis Mill. sur la base de la structure anatomique et morphologique des aiguilles. Ann. Sci. For. 43: 281-298 (1986). CALAMASSI, R., FALUSI, M. and MUGNAI, L.: Shoot morphology and growth pattern in seedlings of Pinus brutia provenances. Can. J. For. Res. 18, 188—194 (1988). - CALAMASSI, R., FALUSI, M. and Tocci, A.: Variazione geografica e resistenza a stress idrici in semi die Pinus halepensis Mill., Pinus brutia Ten. e Pinus eldarica Medw. Ann. Ist. Sper. Selv. Arezzo 11: 195-230 (1980). -E. and Tomassone, R.: Contribution a une etude comparée de Pins Mediterraneen de la Section Halepensis. Ann. Eco. Natio. des Eaux et For. 27: 215-256 (1965). - EGUILUZ PIEDRA, T.: Geographic variation in needles, cones and seeds of Pinus tecumani in Guatemala. Silvae Gen. 33: 72-79 (1984). - FALUSI, M.: Variazione geografica e germinazione in semi di Pinus brutia Ten. Ann. Accad. It. Sci. For. 31: 157-178 (1982). - FALUSI, M. and CALA-MASSI, R.: Effetti degli stress idrici su germinazione e crescita della radice in provenienze di Pinus brutia Ten. Ann. Accad. It. Sci. For. 31: 99-118 (1982). - FALUSI, M., CALAMASSI, R. and Tocci, A.: Resistenza al freddo in Pinus halepensis Mill., Pinus brutia Ten. e Pinus eldarica Medw. Atti Soc. Tosc. Sci. Nat., Mem. Serie B 91: 1-23 (1984). - GAUSSEN, H.: Les gymnosperms actuelles et fossiles. Toulose, 6: 14-29 (1960). -- Gellini, R.: Posizione sistematica del Pino Nero di Villetta Barrea in base ai caratteri anatomici e morfologici degli aghi. Ann. Accad. It. Sci. For. 17: 101-122 (1968). HARLOW, W. M.: The identification of the pines of the United States native and introduced by needle structure. Tech. Publ. New York Sta. Coll. For. Syracuse Univ. N. 32: 19 pp. plus 19 plates - Kolesnikov, A. I.: Pinus pityusa and similar species. Moscow, 175 pp. (1963). — Kozlowski, T. T.: Growth and development of trees. Vol. II. Acad. Press, New York and London (1971). KRIEBEL, H. B. and Fowler, D. P.: Variability in needle cha-

racteristics of soft pines species and hybrids. Silvae Gen. 14: 73-76 (1965). — LAMONTAGNE, Y.: Variation in bud characteristics, needle morphology and needle anatomy among white pine populations of the southern Appalachians. M. S. Thesis. North Carolina State Univ.. Raleigh N.C. (1971). — Lee, C. H.: Geographic variation in European black pine. Silvae Gen. 17: 165—172 (1968). — MATZIRIS, D. I.: Genetic variation in morphological and anatomical needle characteristics in the blackpine of Peloponnesos. Silvae Gen. 33: 164-169 (1984). -- Mergen, F.: Genetic variation in needle characteristics of slash pine and some of the hybrids. Silvae Gen. 7: 1-9 (1958). -- Mergen, F.: Ecotyhic variation in Pinus strobus L. Ecology 44: 716—727 (1963). — NAHAL, J.: Le Pin d'Alep (Pinus halepensis Mill.). Etude taxonomique, phytogeographique, ecologique et sylvicole. Ann. Eco. Natio. des Eaux et For. 19: 208 pp. (1962). — PANETSOS, K. C. P.: Monograph of Pinus halepensis (Mill.) and Pinus brutia (Ten.). Ann. Forest 9 (2): 39-77 - Pelizzo, A. and Tocci, A.: Indagini preliminari sui semi e i semenzali di Pinus halepensis e Pinus brutia — eldarica. Ann. Ist. Sper. Selv. Arezzo 9: 111-130 (1978). - SADOCCHI, S.: Manuale di analisi statistica multivariata. F. Angeli, Milano (1980). SALAZAR, R.: Genetic variation in needles of Pinus caribaea var. hondurensis BARR. et Golf. from natural stands. Silvae Gen. 32: 52-59 (1983). - Schoenike, R. E.: Geographical variation in jack pine (Pinus banksiana). Tech. Bull. Agric. Exp. Sta. Univ. of Minnesota N. 304: 47 pp. (1976). — Schütt, P. and Hattemer, H. H.: Die Eignung von Merkmalen des Nadelquerschnitts für Kiefern-Bastarddiagnose. Silvae Gen. 8: 93-99 (1959). -LACE, A.: Geographic variation in slash pine. For. Sci. Monograph 10: 56 pp. (1966). — Stover, E. L.: Varying structure of conifers leaves in different habitats. Bot. Gaz. 106: 12-25 (1944). VIDAKOVIC, M.: Forms of Pinus nigra in Jugoslavia on the basis of anatomical structure of the needles. Glase Sunske Pakuse Zagreb 13: 111-148 (1957). - Vidakovic, M.: Investigations of the intermediate type between the Austrian and Scots pine. Silvae Gen. 7: 12-19 (1958). -- WALTER, H. and LIETH, H.: Klimadiagramm -Weltatlas. G. Fischer, Jena (1967).

# Provenance Hybrids in Jack Pine: 15-Year Results in Eastern Canada

By S. Magnussen<sup>1</sup>) and C. W. Yeatman<sup>2</sup>)

(Received 4th August 1987)

#### Summary

Results of height growth, stem form, injury, and branch angle at age 15 (16 in one case) are reported for 26 jack pine provenance hybrids and 16 control provenances on six sites in eastern Canada. Variations in stem form, tree status, and branch angle were comparable in hybrids and provenances, with greater differences among provenances and hybrids than between the two groups. Hybrid height growth adjusted for the effects of damage was, as a rule, intermediate to that of its control provenances; hybrids captured on an average 68% of the difference between control provenances. No hybrid exceeded significantly the best control provenance. Examples of hybrid or provenance tree heights in excess of results obtained with local unimproved seed sources were very rare. A strong genotype  $\times$  environment interaction of tree height on five sites could be isolated to 11 of the 37 tested entries. These entries had below average rank stability and above average regression coefficients of height versus local height. Hybrids had environmental stability intermediate to that of the control provenances.

Key words: Pinus banksiana, provenance, population-hybrids, stem, form, branch angle, height adjustment, genotype × environment interactions, stability analysis.

### Zusammenfassung

Es wird über das Höhenwachstum, die Stammform, Schäden und den Astwinkel im Alter von 15 (in einem Fall 16) Jahren von 26 Hybriden der Bankskiefer und 16 Kontrollprovenienzen, die auf sechs Orten in Ostkanada stocken, berichtet. Die festgestellte Variation bei der Stammform, dem Baumstatus und dem Astwinkel war bei Hybriden und Provenienzen vergleichbar, wobei aber größere Unterschiede zwischen Provenienzen und Hybriden auftraten als zwischen den beiden Gruppen. Nach Adjustierung des Höhenwachstums der Hybriden in bezug auf Schadeffekte war dieses in der Regel intermediär zu den Kontrollprovenienzen. Die Hybriden lagen im Durchschnitt bei 68% der Differenz zwischen den Kontrollprovenienzen. Kein Hybrid war der besten Kontrollprovenienz signifikant überlegen. Beispiele, in denen die Höhe von Hybriden oder Provenienzen über der der lokalen, züchterisch nicht bearbeiteten Saatherkunft lag, waren sehr selten. Eine starke Genotyp-Umwelt-Interaktion der Baumhöhe auf fünf Standorten konnte überwiegend auf 11 der 37 Versuchsglieder zurückgeführt werden. Diese Versuchsglieder hatten eine unterdurchschnittliche Rangstabilität und einen über dem Durchschnitt liegenden Regressions-Koeffizienten der Höhe gegenüber der lokalen Höhe. Hybriden hatten eine gegenüber den Kontrollprovenienzen intermediäre Umweltstabilität.

Research Fellow, Canadian Forestry Service, Petawawa National Forestry Institute, Chalk River, Ontario, K0J 1J0, Canada

<sup>2)</sup> Project leader, Tree Breeding and Genetics, Petawawa National Forestry Institute, Chalk River, Ontario, K0J 1J0, Canada