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Variability of *Pinus uncinata* Ramond ex DC as expressed in needle traits

Abstract: Two-year needles were collected from 42 trees from 5 localities in Spanish Pyrenees. The needles were analysed in respect to 15 morphological and anatomical traits. Data obtained were subject of multivariate statistical analyses. The most stable traits appear to be width of epidermis cells, width and thickness of the needles and ratio of the last two characters. Number of the resin canals and the vascular bundles distance were the most variable characters. The differences among the 42 investigated trees are not evident, considerably small and not significant statistically.

Additional key words: pine, Spain, morphoanatomy, statistical analysis

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Introduction

Pinus uncinata Ramond occurs in the Pyrenees and the western and central part of the Alps. The species is met also in single, isolated localities in the central Iberian mountainous system in Spain, in the Massif Central, Vosges and Jura in France and in the Ligurian Apennines in Italy (Fig. 1). It forms a forest belt at elevations between (850) 1400 and 2400 (2700) m (Jalas and Souminen 1973; Amaral Franco 1986; Carrillo and Ninot 1992; Villar et al. 1997). The species was reported also from the mountains of central Europe, from western Czech, eastern Germany and south Poland (Szafer et al. 1967, Krzakowa et al. 1984, Christensen 1987). These latter records do not concern *P. uncinata* sensu stricto, but closely related, not completely taxonomically determined *Pinus uliginosa* Neumann or introgressive hybrids *Pinus mugo* Turra and *P. sylvestris* L. Ranges of *Pinus uncinata* and *P. mugo* are overlapping in the Alps. Some intermediate forms between the species mentioned were observed there,

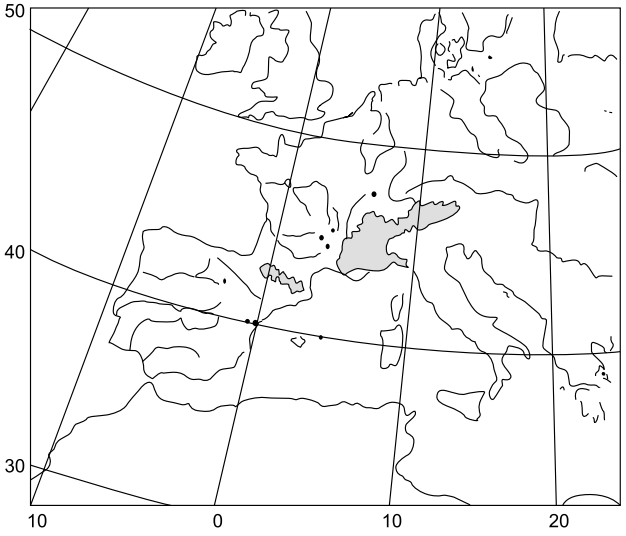

Fig. 1. Distribution of *Pinus uncinata* Ramond (after Jalas, Suominen 1973, Franco Amaral 1986, Christensen 1987)

most frequently known as *P. mugo* var *rotundata* (Link) Ant. (Christensen 1987, Minghetti 1997).

The aim of the present work is to describe variability of *Pinus uncinata* on the basis of morphologic and anatomic characters of needles.

Material and methods

Needles for the present study were collected in October 1994 in the 5 natural localities of *Pinus uncinata* in the Central and Eastern Pyrenees:

1. Vallibierna in Massif of Maladeta – Pico de Aneto, 2150–2200 m. *P. uncinata* forms there more or less dense forest with *Rhododendron ferrugineum* (*Rhododendro-Vacinion* alliance), on humid, developed from granite, substratum on the slopes above the stream. Trees were 18–20 m tall. The needles were gathered from 7 trees.
2. Barranco de Vallibierna, below Maladeta, at the elevation of 2200–2350 m at the forest upper limit zone. *P. uncinata* forms groups or grows singly on the slopes among the *Rhododendron ferrugineum* thicket. The trees attain 12–18 m. The material originated from 10 trees.
3. Parque Nacional de of Aigües y Tortes y de Sant Maurici in the Eastern Pyrenees. Samples were collected from 10 trees, which form forest along the stream between Lake San Maurici and Portarro Pass, at the elevations of 2050–2300 m. The forest is formed on the substratum developed from granite or schist rocks. The trees were 15–22 m tall.
4. North slopes of Tossal de l'Orri de Rubio near Sort, at the elevations of about 2200 m. *P. uncinata* forms there dense forests on the substratum developed from schist rocks. The trees are 18–22 m tall. The material was gathered from 10 trees.
5. Sierra del Cadi, northern slopes of Aguiló above Prat de Aguiló, at the altitude of 2150–2200 m. *P. uncinata* forms fragments of the forests among mountain pastures on the substratum developed from calcareous rocks. The material originated from 5 trees.

Ten two year-old dwarf shoots were gathered from the sunny side of a crown of 42 trees. Totally 420 needles were analysed, 10 for everyone tree. Each specimen of *P. uncinata* was analysed separately on the basis of 15 characters of the needles (Table 1).

The lengths of the needles were measured in the field on the fresh material then central parts of the needles (about 2 cm long) were fixed in 70% alcohol and preserved in cold temperatures for the study. All other characters were studied on material fixed. Stomatal rows (traits 2 and 3) and numbers of stomata (traits 4 and 5) on 2 mm long central fragment of the needles were counted under binocular at magnification of 40×. Other characters were examined on the needle cross sections. Slides for study were made in hand on the needle put into styrofoam, then immersed in polyvinyl alcohol and covered with microscopic cover glass. Microscopic observations and measurements were done on such subpersistent slides under Jenamed 2 light microscope. Traits 7 and 8 were examined under magnification of 50×, trait 6 under 160×, and traits 9, 10 and 11 under 320×.

The character of sclerenchyma cells around resin canals and cells between vascular bundles were also estimated. Seven types of specific composition were distinguished in composition of the sclerenchyma tis-

Table 1. The characteristics of needle traits of *Pinus uncinata* (average for 42 trees, 10 needles/tree)

Traits	Minimum	Maximum	Arithmetic mean	Standard deviation	Variability coefficient
1. Needle length (mm)	49.00	82.00	66.36	0.73	11.11
2. Number of stomatal rows on convex (abaxial) side of the needle	8.10	13.20	10.15	1.10	10.84
3. Number of stomatal rows on flat (adaxial) side of the needle	6.20	10.40	7.47	0.93	12.39
4. Number of stomata on 2 mm long section of the needle, on convex (abaxial) side	15.63	21.50	18.51	1.49	8.03
5. Number of stomata on 2 mm long section on the needle, on flat (adaxial) side	15.80	21.30	18.44	1.51	8.17
6. Number of resin canals	1.00	5.70	3.57	1.03	28.72
7. Width of the needle (m)	1324.25	1993.88	1600.97	125.08	7.81
8. Thickness of the needle (m)	770.75	1058.38	911.02	68.47	7.51
9. Distance between vascular bundles (m)	60.27	211.46	126.02	34.71	27.55
10. Thickness of epidermis cells (m)	27.97	38.30	32.39	2.61	8.06
11. Width of epidermis cells (m)	13.07	17.21	15.71	0.98	6.26
12. Marcet's coefficient (=traits 9×7/8)	94.39	384.51	224.39	68.53	30.54
13. Stomatal rows ratio (=traits 2/3)	1.09	1.65	1.37	0.13	9.39
14. Needle thickness/width ratio (=traits 8/7)	0.48	0.68	0.57	0.03	5.47
15. Cell of epidermis width/thickness ratio (=traits 11/10)	0.42	0.61	0.49	0.04	8.92

Table 2. Discriminant power testing for the traits of *Pinus uncinata* (** – significant at the level α=0.01)

Traits	F statistics
1. Needle length (mm)	29.669**
2. Number of stomatal rows on convex (abaxial) side of the needle	1.473
3. Number of stomatal rows on flat (adaxial) side of the needle	1.785**
4. Number of stomata on 2 mm long section of the needle, on convex (abaxial) side	4.226**
5. Number of stomata on 2 mm long section on the needle, on flat (adaxial) side	3.986**
6. Number of resin canals	15.800**
7. Width of the needle (m)	2.321**
8. Thickness of the needle (m)	2.391**
9. Distance between vascular bundles (m)	4.468**
10. Thickness of epidermis cells (m)	1.695**
11. Width of epidermis cell (m)	1.442
12. Marcet's coefficient (=traits 9×7/8)	4.288**
13. Stomatal rows ratio (=traits 2/3)	1.726**
14. Needle thickness/width ratio (=traits 8/7)	2.957**
15. Cell of epidermis width/thickness ratio (=traits 11/10)	1.671**

Critical value $F_{0.01}$=1.641

Table 3. Differentiation of *Pinus uncinata* population in 15 traits of the needles calculated using Student's – t test at significance level α=0.01 α=0.05

Traits	Significant difference between trees			
	α=0.01		α=0.05	
	Number differences	%	Number differences	%
1. Needle length (mm)	602	69.92	658	76.42
2. Number of stomatal rows on convex (abaxial) side of the needle	325	37.75	426	49.48
3. Number of stomatal rows on flat (adaxial) side of the needle	270	31.36	396	45.88
4. Number of stomata on 2 mm long section of the needle, on convex (abaxial) side	407	47.27	518	60.16
5. Number of stomata on 2 mm long section on the needle, on flat (adaxial) side	404	46.92	514	59.70
6. Number of resin canals	448	52.03	549	63.76
7. Width of the needle (m)	441	51.22	545	63.30
8. Thickness of the needle (m)	438	50.87	537	62.37
9. Distance between vascular bundles (m)	487	56.56	589	68.41
10. Thickness of epidermis cells (m)	237	27.53	362	42.04
11. Width of epidermis cell (m)	126	14.63	236	27.41
12. Marcet's coefficient (=traits 9×7/8)	486	56.45	594	68.99
13. Stomatal rows ratio (=traits 2/3)	110	12.78	238	27.64
14. Needle thickness/width ratio (=traits 8/7)	277	32.17	399	46.34
15. Cell of epidermis width/thickness ratio (=traits 11/10)	137	15.91	261	30.31

sues, following Bobowicz 1990 after Szweykowski's method (1969).

The data obtained were analysed statistically. Arithmetic means, standard deviations, variability coefficients were calculated for particular characters (Table 1). Discriminant power was calculated for each trait. Differentiation of particular trees was examined with t-Student's test, than correlation coefficients between traits were examined. Discriminant analysis was examined to determine character of species variability. To check the variability within the 42 trees the ordination methods were used. Minimum spanning tree (dendrite) was constructed using the shortest Mahalanobis distances and Hottelings T^2 statistic was used to check the significance of Mahalanobis distances. Dendrogram was also constructed on the basis of Euclidean distances to show the hierarchic conjunction of trees studied (cluster analysis) (Marek 1989, Morrison 1990).

Results

It can be stated on the basis of the test of discrimination power of the analysed traits, that most of the traits have significant discrimination power ($F_{calc} > F_{0.01}$). Only number of stomata rows on the convex (abaxial) needle side (trait 2) and the width of epidermal cell (trait 11) do no fulfil this condition. The needle length (trait 1) and number of resin canals (trait 6) have the greatest discriminating power (Table 2).

Variability coefficient, calculated as average for particular traits of all studied trees, is rather small and varies between 5.47 and 30.54%. The number of resin canals (trait 6) and the distance between the vascular bundles (trait 9) are the most variable traits. The most stable are width of epidermis cells (trait 11), thickness of epidermis cells (trait 10), needle width and thickness (traits 7 and 8) and ratio of these characters (trait 14). Number of stomata on adaxial and abaxial side of the needle calculated on the distance of 2 mm of its central part (traits 4 and 5) were also not very variable (Table 1).

On the basis of t-Student's distribution of the mean values of analysed traits of particular trees it can be said that length of the needle highly differentiate trees (Table 3).

Significant correlations between particular traits are generaly rare. Only traits 7 (width of the needle) and 8 (thickness of the needle) were significantly correlated in 6.67% of all trees analysed (Table 4).

Table 4. Significant correlations between studied traits of 42 trees of *Pinus uncinata:* number of significant correlations (straight number) and percent participation correlations (italic number), α=0.01

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1															
2	1 *0.95*														
3	1 *0.95*	0													
4	1 *0.95*	0	2 *1.90*												
5	0	0	2 *1.90*	4 *3.81*											
6	0	0	2 *1.90*	1 *0.95*	0										
7	0	0	1 *0.95*	0	2 *1.90*	1 *0.95*									
8	0	0	2 *1.90*	1 *0.95*	1 *0.95*	3 *2.86*	7 *6.67*								
9	0	0	2 *1.90*	0	0	3 *2.86*	2 *1.90*	0							
10	0	0	0	1 *0.95*	1 *0.95*	0	1 *0.95*	2 *1.90*	0						
11	0	1 *0.95*	0	1 *0.95*	1 *0.95*	1 *0.95*	0	1 *0.95*	2 *1.90*	0					
12	0	0	1 *0.95*	1 *0.95*	0	2 *1.90*	2 *1.90*	2 *1.90*	37 *35.24*	0	0				
13	0	7 *6.67*	17 *16.19*	2 *1.90*	1 *0.95*	0	1 *0.95*	0	0	0	0	0			
14	0	0	0	1 *0.95*	1 *0.95*	0	10 *9.52*	8 *7.62*	4 *3.81*	0	0	7 *6.67*	1 *0.95*		
15	0	0	0	1 *0.95*	0	0	0	0	0	14 *13.33*	9 *8.57*	0	0	0	

Table 5. Significantly correlation between studied trees of *Pinus uncinata* for 15 traits at the level of significance α=0.01 and α=0.05

Number of tree	Correlation			
	α=0.01		α=0.05	
	Number	%	Number	%
1	5	4.76	12	11.43
2	5	4.76	17	16.19
3	4	3.81	12	11.43
4	6	5.71	14	13.33
5	5	4.76	12	11.43
6	3	2.86	7	6.67
7	5	4.76	12	11.43
8	4	3.81	6	5.71
9	3	2.86	10	9.52
10	11	10.48	21	20.00
11	3	2.86	9	8.57
12	3	2.86	13	12.38
13	5	4.76	13	12.38
14	4	3.81	8	7.62
15	2	1.90	10	9.52
16	6	5.71	14	13.33
17	7	6.67	16	15.24
18	6	5.71	9	8.57
19	5	4.76	16	15.24
20	10	9.52	16	15.24
21	6	5.71	13	12.38
22	2	1.90	9	8.57
23	6	5.71	7	6.67
24	7	6.67	10	9.52
25	2	1.90	10	9.52
26	1	0.95	8	7.62
27	4	3.81	11	10.48
28	2	1.90	6	5.71
29	2	1.90	9	8.57
30	3	2.86	11	10.48
31	4	3.81	8	7.62
32	4	3.81	10	9.52
33	5	4.76	13	12.38
34	3	2.86	10	9.52
35	5	4.76	14	13.33
36	4	3.81	8	7.62
37	3	2.86	12	11.43
38	4	3.81	8	7.62
39	4	3.81	16	15.24
40	2	1.90	15	14.29
41	4	3.81	13	12.38
42	4	3.81	9	8.57

Table 6. The determination coefficients between discriminant variables U_1, U_2, and 15 traits of needles of *Pinus uncinata*

Traits	U_1 (37.35%)	U_2 (14.55%)
1. Needle length (mm)	19.4369	0.4263
2. Number of stomatal rows on convex (abaxial) side of the needle	1.5463	0.1091
3. Number of stomatal rows on flat (adaxial) side of the needle	1.7891	0.1775
4. Number of stomata on 2 mm long section of the needle, on convex (abaxial) side	1.9011	2.9391
5. Number of stomata on 2 mm long section on the needle, on flat (adaxial) side	1.5015	3.2825
6. Number of resin canals	0.0374	5.2496
7. Width of the needle (m)	7.7725	0.2619
8. Thickness of the needle (m)	4.4110	0.5704
9. Distance between vascular bundles (m)	5.4008	0.2823
10. Thickness of epidermis cells (m)	0.4631	0.2517
11. Width of epidermis cell (m)	0.0112	0.3667
12. Marcet's coefficient (=traits 9×7/8)	4.8443	0.2619
13. Stomatal rows ratio (=traits 2/3)	0.1083	0.3099
14. Needle thickness/width ratio (=traits 8/7)	0.1905	0.1135
15. Cell of epidermis width/thickness ratio (=traits 11/10)	0.3586	0.0073

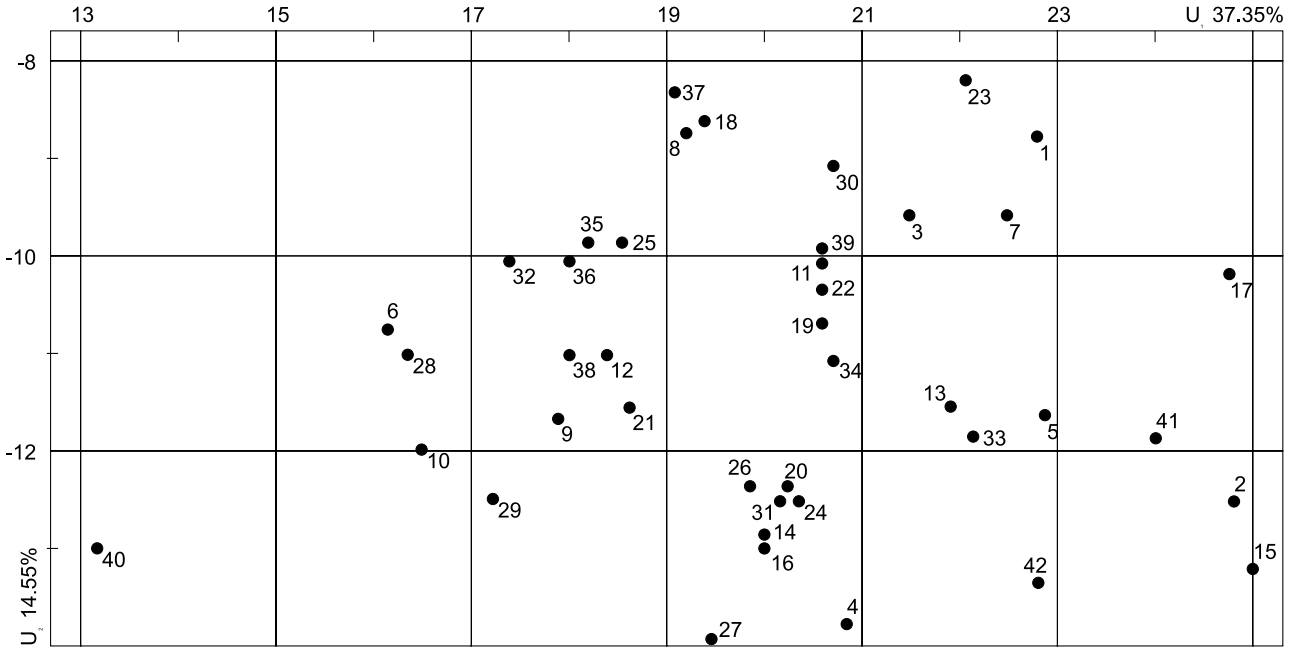

Fig. 2. Result of the discriminant analysis for 42 trees of *Pinus uncinata* on the plane of the first two discriminant variables U_1 and U_2 (number of trees 1–7 from Vallibierna, 8–17 from Barranco de Vallibierna, 18–27 from St. Maurici, 28–37 from Tossal de l'Orri de Rubio, 38–42 from Sierra del Cadi)

Correlations between all specimens of *P. uncinata* were also calculated. Trees 8 and 28 have the smallest participation of correlation at significance level of α=0.05, which attain only 5.71%. On the level of significance at α=0.01 the participation of significant correlation coefficient was the lowest in tree 26 and amounts only to 0.95%. Tree number 10 has most closely correlated traits at both significance levels (Table 5).

The variability of 42 trees on the basis of two first canonical discriminant variables U_1 and U_2, which contain almost 52% of information, do not correspond with localities of trees and do not form agglomerations (Fig. 2). On the basis of coefficients determination between 15 studied traits and discriminant variables it can be stated, that variability of all population of *P. uncinata* is formed mostly by such traits as the length of needle (trait 1), the width and thickness of the needle (traits 7 and 8), the distance between vascular bundles (trait 9), the number of resin canals (trait 6) and the number of

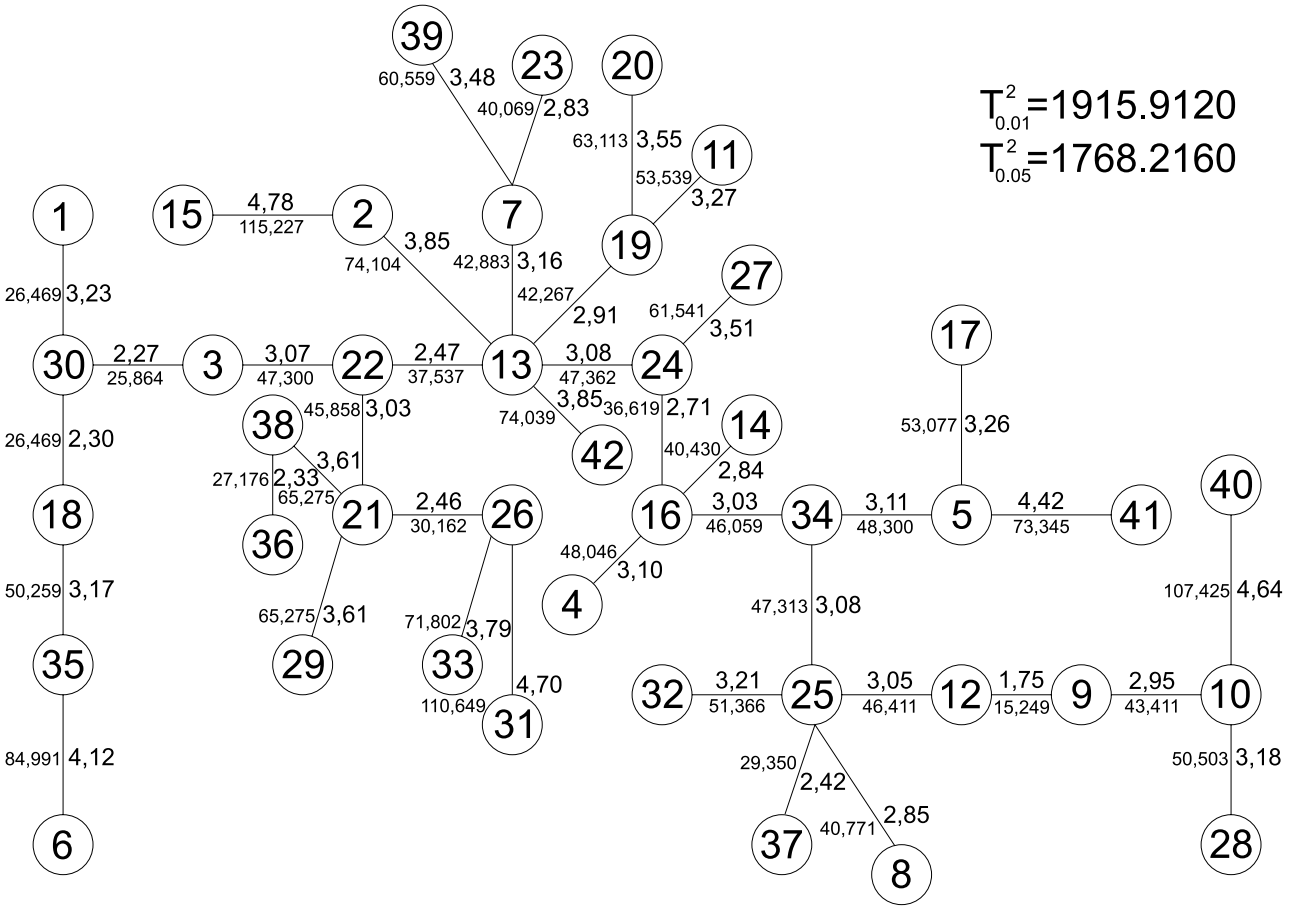

Fig. 3. Dendrite of 42 trees of *Pinus uncinata* – for each of the shortest Mahalanobis distances (large numerals) counted the values of Hotelling's T^2 statistics (small numerals). $T^2_{0.01}$ and $T^2_{0.05}$ – critical values of Hotelling's T^2 statistics (number of trees 1–7 from Vallibierna, 8–17 from Barranco de Vallibierna, 18–27 from St. Maurici, 28–37 from Tossal de l'Orri de Rubio, 38–42 from Sierra del Cadi)

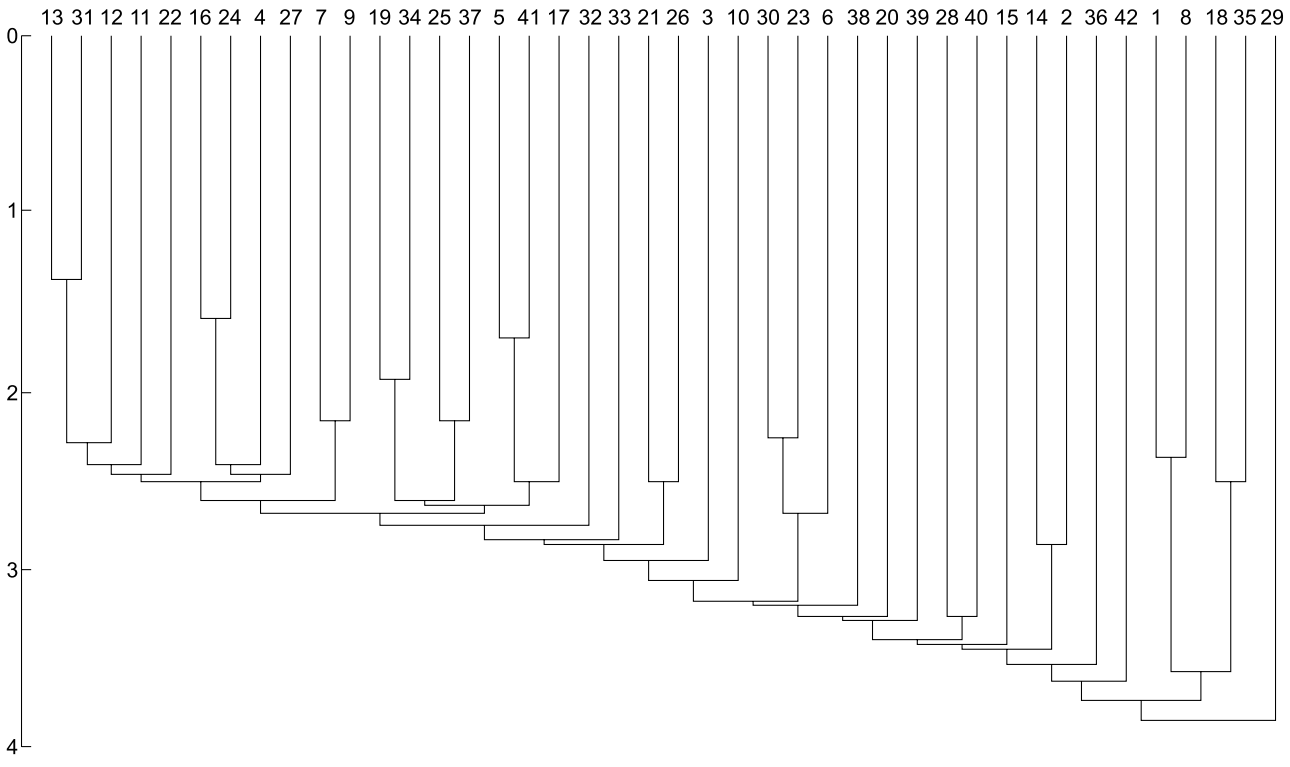

Fig. 4. Dendrogram of 42 trees of *Pinus uncinata* (number of trees 1–7 from Vallibierna, 8–17 from Barranco de Vallibierna, 18–27 from St. Maurici, 28–37 from Tossal de l'Orri de Rubio, 38–42 from Sierra del Cadi)

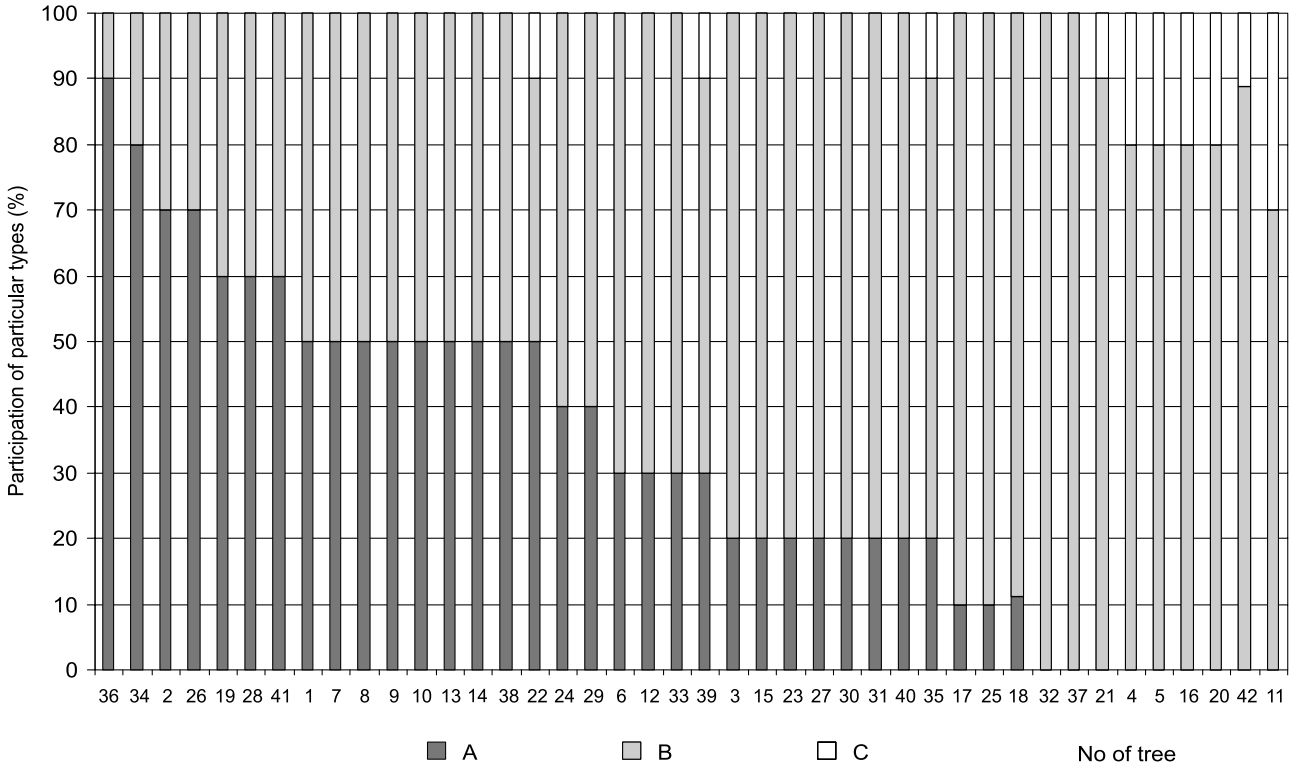

Fig. 5. Character of sclerenchyma cells around the resin canals for 42 trees of *P. uncinata*: A – fibre-like cells with very thick cell walls, lumen small, B – cells intermediate, C – no fibre like cells, cell walls medium thick, lumen distinct (number of trees 1–7 from Vallibierna, 8–17 from Barranco de Vallibierna, 18–27 from St. Maurici, 28–37 from Tossal de l'Orri de Rubio, 38–42 from Sierra del Cadi)

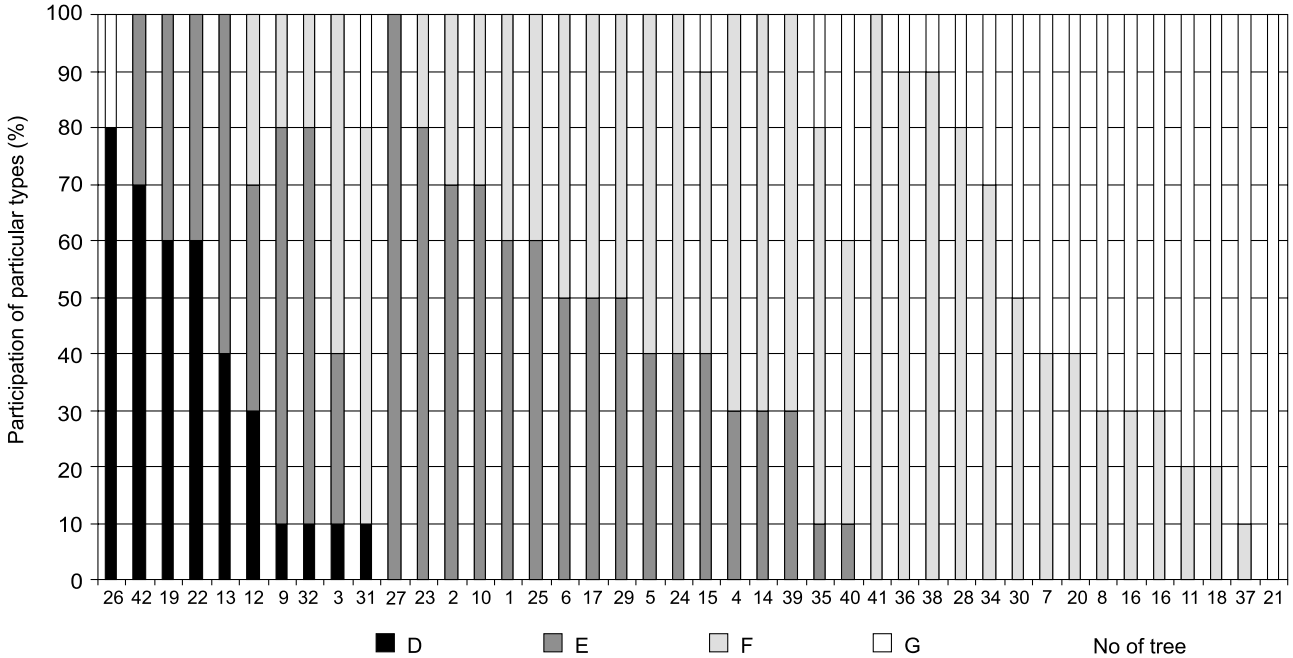

Fig. 6. Character of cells between vascular bundles: D – fibre like elements (lumen very restricted, cell walls very thick) in compact group, E – fibre like elements singularly scattered or only small groups, F – fibre like elements lacking, cell walls of medium thickness, lumen distinct, G – fibre like elements lacking, cell walls thin, lumen very distinct (number of trees 1–7 from Vallibierna, 8–17 from Barranco de Vallibierna, 18–27 from St. Maurici, 28–37 from Tossal de l'Orri de Rubio, 38–42 from Sierra del Cadi)

stomata on both sides of the needle (traits 4 and 5) (Table 6).

The calculated Mahalanobis distances between trees confirm their very small differentiation within the analysed samples of *P. uncinata*. Dendrite constructed on the shortest distances shows it precisely. According to Hotelling's T^2 ratio it can be stated that no tree pair in the dendrite differs significantly (Fig. 3). The differences between trees do not depend on the localities. The analysed population of trees also does not show large differentiation on dendrogram, constructed on Euclidean distances of the closest neighbourhood (Fig. 4).

The character of sclerenchyma surrounding the resin canals and between vascular bundles were estimated. The fibre-like cells with thick walls and small cell lumen (A-type) were frequent, in some trees even up to 40%, while cells with medium thick walls and distinct lumens (C-type) were rare ones. Cells with intermediate thickness of walls and well visible lumens (B-type) were the most common. Cells surrounding the resin canals were more similar to those of *P. sylvestris* than of *P. mugo*. (Fig. 5).

Cells with medium thickness of the walls and distinct lumen (F-type) were prevailing between vascular bundles. The fibre-like cells (E-type) occured singly or in small groups dispersed among other types of cells. Needles of a dozen of trees contain also cells with thin walls and very distinct lumens (G-type). That last type of sclerenchyma cells was found predominating in tree no 21. The fibre-like elements with very restricted lumens (D-type) were considerably less frequent. Only trees no 19, 22, 26 and 42 have this type of sclerenchyma cells as dominant in the space between vascular bundles. Cells typical for *P. sylvestris* and *P. mugo* were sporadically observed between vascular bundles. Intermediate cells predominate in the needles (Fig. 6).

The distance between vascular bundles in the needles most frequently are the same or slightly smaller than the width of the bundles. The exception of this are trees no 26, 29, 31 and 40, which have distances between vascular bundles equal or shorter than half of the bundle width, and trees no 7, 8 and 41, which have distances estimated as larger than the bundle width.

Conclusions:

1. Detailed analysis of the variability of *Pinus uncinata* needles from 42 trees of 5 natural localities in the Spanish Pyrenees are presented.
2. The most stable traits of the needles appear to be width, thickness of epidermis cells and ratio of these characters, width, thickness the needles and ratio of these characters and number of stomata on both sides of the needle. The variability coefficients of the mentioned characters do not exceed 9%.
3. The majority of variability is caused by a few traits only, mostly by the number of the resin canals and the vascular bundles distance.
4. The differences between the 42 investigated trees are not evident and considerably small.
5. The differences between the 5 studied trials are also small and statistically not significant.

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Zmienność igieł *Pinus uncinata* Ramond ex DC

Streszczenie

Praca zawiera szczegółową analizę zmienności igieł *P. uncinata*. Materiał pochodził z 5 różnych naturalnych stanowisk z 42 drzew z hiszpańskich Pirenejów. W badaniach uwzględniono 15 cech morfologicznych i anatomicznych a uzyskane wyniki z pomiarów poddano szczegółowej analizie statystycznej. Ustalono, że zmienność między drzewami w badanej populacji *P. uncinata* jest stosunkowo niewielka i nie istotna statystycznie. Najbardziej stabilnymi cechami okazały się iloraz grubości i szerokości igły (cecha 14), szerokość komórek epidermy (cecha 11), szerokość i grubość igły (cechy 7 i 8) a także grubość komórek epidermy (cecha 10) i liczba szparek po obydwu stronach igły (cechy 4 i 5). Współczynniki zmienności dla wspomnianych cech nie przekraczają 9%. Największe różnice, jednak statystycznie nie istotne, obserwowano w liczbie kanałów żywicznych (cecha 6) i w odległościach między wiązkami (cecha 9) oraz we współczynniku Marceta (cecha 12).