

## ASSESSMENT OF PHENOTYPIC DIVERSITY IN SOME WILD THYME POPULATIONS FROM BANAT AREA (WESTERN ROMANIA)

Rodica BEICU, Sorina POPESCU, Florin IMBREA, Giancarla VELICEVICI,  
Alina NEACȘU, Georgeta POP, Liana BUTTA, Ilinca IMBREA

University of Life Sciences "King Mihai I" from Timișoara,  
119 Aradului Street, Timișoara, Romania

Corresponding author email: sorinapopescu@usvt.ro

### Abstract

The paper aimed to analyse the morphological characters for eight spontaneous populations of thyme, including an endemic population (*Thymus comosus*), collected from different regions of the Banat area. Both interpopulation and intrapopulation variability determinations were pursued, and results were correlated with statistical interpretations. Morphological analyses were carried out, including the shape of the leaves, as this morphological character is decisive in the taxonomy of the genus *Thymus*. Based on morphological similarity, the analysed populations were hierarchically classified, by the cluster mean method, in two main clusters. In the first cluster, six of the eight analysed populations were grouped, respectively those belonging to the species *Th. praecox* (with the two subspecies *janke* and *polytrichus*) and *Th. dacicus*. In the second cluster we find the endemic population of *Th. comosus* and the population of *Th. pulegioides*, which are 97.51% morphologically similar, but different from the populations found in the first cluster. The obtained data allowed the quantification of the associative relationships between the morphological parameters studied.

**Key words:** morphological traits, wild thyme, *Thymus comosus*, endemic.

### INTRODUCTION

The *Lamiaceae* (*Labiatae*) family is a family of angiosperm plants of real economic value, intensively studied from a phytotechnical, chemical and pharmaceutical aspect (Cocan et al., 2018; Guriță et al., 2019; Karpinski, 2020). The family includes over 200 genera, the *Thymus* genus being one of the numerically well-represented genera (over 200 species), used since ancient times (Morales, 1997; Morales in Stahl-Biskup & Saez, 2002). It is recognized as a genus that poses taxonomic problems, due to the variability of the species (Ložiene, 2006; Sostarić et al., 2012; Beicu et al., 2021), but of interest for the purpose of improving and identifying new local populations of interest, for the purpose of introduction into culture (Rus et al., 2016), direct harvesting, in the case of widespread species, as well as finding measures of conservation for endemic species.

The variability of the morphological traits of the analyzed populations increases the degree of selection of the desired traits for reproduction, allowing the breeder to choose the optimal variant to produce the desired hybrid (Razaei et al., 2016; Nurzynska-Wierdak et al., 2022).

We have compared in the study, common widespread, frequently used and studied populations, such as *Th. pulegioides*, but also some populations with a reduced distribution area in rocky habitats, endemic or rare and less studied. Among these, *Th. comosus* is a Carpathian endemism (mentioned only in Romania as a distribution) and *Th. dacicus* has a small distribution area only in Romania and the former Yugoslavia (Jalas, 1972; Sârbu et al., 2013; Pavel et al., 2010). The two species of *Th. praecox* are adapted to rocky areas, skeletal soils, with morpho-genetic characters of interest to breeders. All these species are also important from a phytotherapeutic point of view (Stahl-Biskup, 1991; Ložiene et al., 2007; Petrović, 2017; Alfonso et al., 2018; Vaičulite et al., 2021; Babotă et al., 2022; Babotă et al., 2023).

### MATERIALS AND METHODS

The analyzed material was harvested during the vegetation period of the plants, at full bloom, in 2019, from several western areas of Banat, located at different altitude. Eight populations were analyzed, with individuals randomly selected within each population, at least 30

individuals. The phenological determinations (plant height, flower (inflorescence) length, leaf length, leaf width, leaf shape) were carried out in the laboratory. The accurate determination of the species was carried out based on specialized determinatory (Guşuleac, 1961; Sârbu et al., 2013). After identification, specimen samples for each population were stored in the herbarium of the Biology Department of the Banat's University of Life "King Michael I<sup>st</sup>" from Timișoara.

The data obtained as a result of the biometric measurements on the morphological traits, were statistically processed, determining the estimated values of the average standard deviation and the coefficient of variability (Ciulcă, 2006).

To determine the significance of the differences between the genotypes studied, the experimental data obtained were processed by analysis of variance and the t-test (Mantel, 1967). The UPGMA method was used for the representation of the dendrogram (Yan et al., 2000).

## RESULTS AND DISCUSSIONS

The analysed populations, as well as their location, are shown in Table 1.

Based on the results of the variance analysis regarding the height of the thyme populations, (Table 2), the existence of real and strongly statistically ensured differences between the studied populations in terms of plant height can be found.

Table 1. Thyme populations studied and their location

Code	Population	Location	Voucher specimen	GPS coordinates (degree, minutes, secondes)		
				Altitude (m)	Latitude	Longitude
Th1	<i>Th. praecox</i> ssp. <i>janke</i>	Domogled	VSNH.BUASTM: 1904	426	44. 884950	22.428793
Th2	<i>Th. dacicus</i>	Coronini	VSNH.BUASTM: 1910	110	44.665889	21.694774
Th3	<i>Th. dacicus</i>	Lescovita	VSNH.BUASTM: 1912	117	44.874057	21.539302
Th4	<i>Th. comosus</i>	Dobraia	VSNH.BUASTM: 1913	932	44.998398	22.480722
Th5	<i>Th. praecox</i> ssp. <i>polytrichus</i>	Gozna Peak	VSNH.BUASTM: 1918	1411	45.189375	22.073795
Th6	<i>Th. praecox</i> ssp. <i>polytrichus</i>	Semenic Peak	VSNH.BUASTM: 1919	1408	45.188707	22.076211
Th7	<i>Th. pulegioides</i> ssp. <i>pulegioides</i>	Semenic	VSNH.BUASTM: 1925	1004	45.224281	22.073924
Th8	<i>Th. praecox</i> ssp. <i>janke</i>	Coronini	VSNH.BUASTM: 1926	94	44.664652	21.699375

Table 2. Variance analysis for plant height and flowers length of *Thymus* populations

Variation source	Plant height			Flower length			
	GL	SP	S <sup>2</sup>	F test	SP	S <sup>2</sup>	F test
Total	79	375401			25476.80		
Population	8	349786	49969	140,45**	18481.07	2640.15	27.17**
Error	72	25615	356		6995.72	97.16	

GL - degrees of freedom; SP - sum of squares; S<sup>2</sup> - weighted sum of squares; Significance for F-test: ns p > 0.05; \*p ≤ 0.05; \*\* p ≤ 0.01; \*\*\* p ≤ 0.001.

Considering the mutual comparisons between the populations, a significantly higher height of the plants can be found in the *Th. pulegioides* (Th7), by more than 31.5 mm, compared to the rest of the populations. The endemic population of *Th. comosus* (Th4), recorded significantly higher values of this character, compared to the other six populations. The highest inter-individual homogeneity of this character (Figure 1a) was recorded in the *Th. pulegioides*, followed by the *Th. praecox* ssp. *polytrichus* (Th5).

The analysis of variance components (Table 2) shows that there are distinctly significant real differences between the populations studied in

terms of the inflorescences length in the conditions of the existence of a very high variability at the intra-population level, which, however, does not significantly influence the hierarchy of the populations.

The values of this character generally recorded a high variability and amplitude of variation, between 6.9 mm at the *Th. praecox* ssp. *polytrichus* (Th6) and 49.70 mm at the endemic population (Th4). Based on the multiple comparisons, it can be observed that the *Th. comosus* population, presented values of this character significantly higher, by more than 12 mm, compared to the other populations,

followed by the *Th. pulegioides*. Both populations of *Th. praecox* ssp. *janke* (Th1, Th8) and *Th. praecox* ssp. *polytrichus* (Th5, Th6), had values below 9 mm, correlated with their smaller waist.

The lowest intra-population variability and amplitude was determined in the populations *Th. praecox* ssp. *polytrichus* (Th5, Th6) and *Th. praecox* ssp. *janke* (Th8), whose distributions show a certain degree of symmetry (Figure 1b).

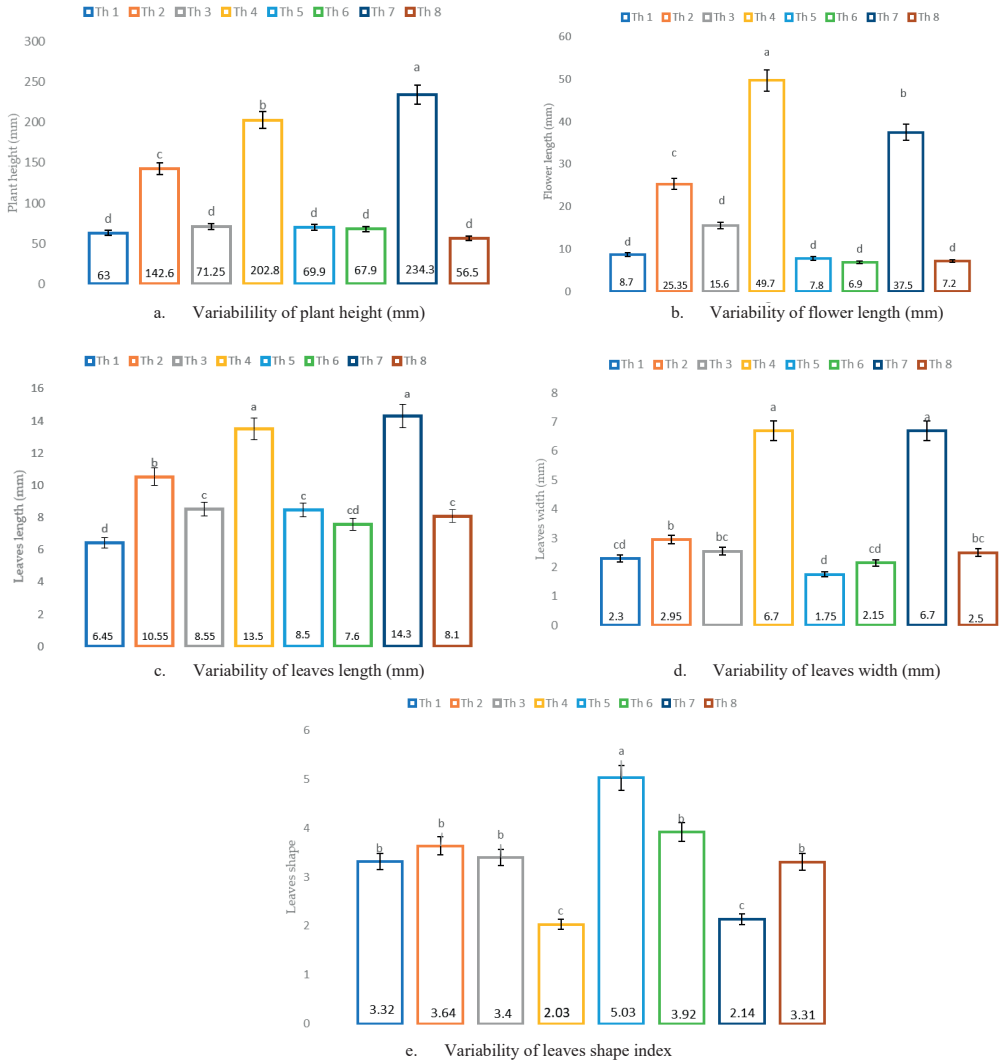


Figure 1. Phenotypic diversity based on morphological traits

The results are expressed as the average value of three determinations  $\pm$  the standard deviation (SD) indicated by the error bars. According to *t*-test, the different lower case letters (a - f) represent the significant differences ( $p < 0.05$ ) between samples obtained by the same shredding method

The analysis of the variance for the length, width and shape of the leaves, attests that there are real and distinctly significant differences between the studied populations, from the point of view of this character, given the existence of moderate variability at the intra-population level (Table 3).

Inter-individual variability for leaf length was higher in *Th. praecox* ssp. *polytrichus* (Th6), while in *Th. dacicus* (Th3), *Th. pulegioides* (Th7) and *Th. praecox* ssp. *janke* (Th8) populations, leaf uniformity was considerably higher (Figure 1c). Based on the multiple comparisons, it can be observed that the *Th.*

*pulegioides* (Th7) and *Th. comosus* (Th4) populations, presented a significantly higher

leaf length, than the other populations, with more than 2.95 mm.

Table 3. Variance analysis for length, width and shape index of leaves for *Thymus* populations

Variation source	Leaves length				Leaves width			Shape index		
	GL	SP	S <sup>2</sup>	F test	SP	S <sup>2</sup>	F	SP	S <sup>2</sup>	F test
Total	79	741.75	80.88		325.80			135.70		
Population	8	566.17	2.44	33.17**	289.90	41.41	83.06**	64.44	9.21	9.30**
Error	72	175.58			35.90	0.50		71.26	0.99	

GL - degrees of freedom, SP- sum of squares, S<sup>2</sup>- weighted sum of squares, Significance for F-test: ns p>0.05; \*p≤0.05; \*\* p≤0.01; \*\*\* p≤0.001

The width of the leaves in the eight populations, recorded values between 1.75 mm at *Th. praecox* ssp. *polytrichus* (Th5) and 6.70 mm at *Th. comosus* (Th4) and *Th. pulegioides* (Th7), respectively, with an amplitude of variation of 4.95 mm and a high inter-population variability, associated with moderate heterogeneity at the level intrapopulation (Table 3). A significantly higher width of the leaves was also found in *Th. comosus* (Th4) and *Th. pulegioides* (Th7) populations, by more than 3.75 mm compared to the rest of the populations. The other populations presented values below 3 mm and generally not statistically assured (Figure 1d).

Regarding the leaf shape index, the inter-individual variability was higher in the *Th. praecox* ssp. *janke* (Th1) and *Th. praecox* ssp. *polytrichus* (Th6) populations, respectively lower in the *Th. pulegioides* (Th7), and *Th. dacicus* (Th3) populations (Table 3). Considering the mutual comparisons between the populations, it is observed that the population *Th. praecox* ssp. *polytrichus* (Th5),

presented the highest degree of leaf elongation associated with significant differences, compared to the shape of the leaves in the rest of the analysed populations. Populations *Th. praecox* ssp. *janke* (Th1), *Th. dacicus* (Th2, Th3), *Th. praecox* ssp. *polytrichus* (Th6), *Th. praecox* ssp. *janke* (Th8), made up a class of significance, presenting a more elongated shape of the leaves compared to populations *Th. comosus* (Th4) and *Th. pulegioides* (Th7) (Figure 1e).

Regarding the data presented in the similarity matrix, the highest phenotypic similarity is manifested between the two populations of *Th. praecox* ssp. *janke* (Th1, Th8), analysed from different locations - 99.03%; *Th. praecox* ssp. *janke* (Th8) and *Th. dacicus* (Th3) - 99.01%; *Th. praecox* ssp. *janke* (Th8) and *Th. praecox* ssp. *polytrichus* (Th6) - 98.91%; *Th. praecox* ssp. *polytrichus* (Th6) and *Th. praecox* ssp. *janke* (Th1) -98.70%; *Th. praecox* ssp. *polytrichus* (Th6) and *Th. dacicus* (Th3) - 98.14% (Table 4).

Table 4. The similarities coefficients between *Thymus* populations for analysed morphological traits

Population	1	2	3	4	5	6	7	8
<i>Th. praecox</i> ssp. <i>janke</i> (Th1)	1							
<i>Th. dacicus</i> (Th2)	0.8694	1						
<i>Th. dacicus</i> (Th3)	0.9794	0.9418	1					
<i>Th. comosus</i> (Th4)	0.3365	0.7117	0.5018	1				
<i>Th. praecox</i> ssp. <i>polytrichus</i> (Th5)	0.9185	0.8646	0.9291	0.2154	1			
<i>Th. praecox</i> ssp. <i>polytrichus</i> (Th6)	0.9870	0.8923	0.9814	0.3235	0.9686	1		
<i>Th. pulegioides</i> ssp. <i>pulegioides</i> (Th7)	0.3348	0.7203	0.4963	0.9751	0.2379	0.3375	1	
<i>Th. praecox</i> ssp. <i>janke</i> (Th8)	0.9903	0.8936	0.9901	0.3923	0.9280	0.9891	0.4006	1

The dendrogram (Figure 2), based on morphological similarity, groups the analysed populations into two main clusters. The first cluster has a complex structure, being made up of six populations, between which there is an average similarity of approximately 90%. Only two thyme populations were grouped in the second cluster *Th. comosus* (Th4) and *Th.*

*pulegioides* (Th7), which are morphologically similar to a degree of 97.51%, at the same time differing by approximately 68% compared to the populations of the first cluster.

Regarding the variance analysis for the analysed morphological characters (Table 5), it is found that the width of the leaves recorded higher and significant values of the variance, thus

contributing to a greater extent (23.50%) to the diversity between the populations. The shape of the leaves had a smaller influence (16.05%) on the morphological differentiation of the populations, while the rest of the characters had close contributions (19.79-20.44%). Within the clusters, morphological diversity was generated to a higher extent (38.53%) by leaf shape. Based on the variance analysis related to the hierarchical classification of the populations (Table 6), it is observed that *Th. praecox* ssp. *polytrichus* (Th5, Th6), generate the greatest

differences between the studied morphological characters, having high contributions to the total variability. The *Th. praecox* ssp. *polytrichus* (Th5) population, registers the highest effect (47.34%), on the diversity at the level of the first cluster, while the *Th. dacicus* (Th2, Th3) populations, influence the diversity to an extremely low extent (0.33%). In the case of the second cluster, the *Th. pulegioides* (Th7) population, has a higher contribution (56.34%) than the *Th. comosus* (Th4) population (43.66%).

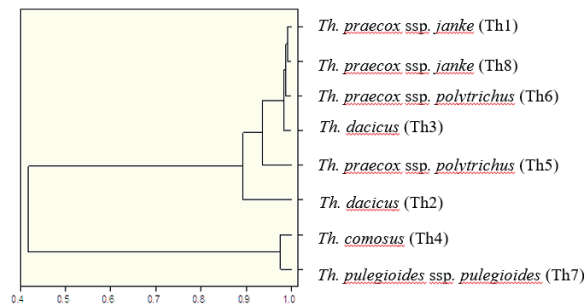


Figure 2. Cluster analysis based on morphological traits

Table 5. Variance analysis for the morphological traits for *Thymus* populations

Character	Between		Within		F test
	SP	GL	SP	GL	
Plant height	5.886	1	1.114	6	31.69**
Flower length	5.699	1	1.299	6	26.31**
Leaves length	5.850	1	1.170	6	30.00**
Leaves width	6.768	1	0.198	6	205.26**
Index shape	4.621	1	2.370	6	11.70**

GL - degrees of freedom, SP - sum of squares, Significance for F-test: ns  $p > 0.05$ ; \* $p \leq 0.05$ ; \*\* $p \leq 0.01$ ; \*\*\* $p \leq 0.001$

Table 6. Variance analysis for *Thymus* populations based on morphological traits

Population	Between		Within		F test
	SP	GL	SP	GL	
<i>Th. praecox</i> ssp. <i>janke</i> (Th1)	4.864	1	0.882	3	16.54*
<i>Th. dacicus</i> (Th2)	5.186	1	0.186	3	83.76**
<i>Th. dacicus</i> (Th3)	5.826	1	0.189	3	92.30**
<i>Th. comosus</i> (Th4)	0.159	1	3.569	3	0.13
<i>Th. praecox</i> ssp. <i>polytrichus</i> (Th5)	13.392	1	2.663	3	15.09*
<i>Th. praecox</i> ssp. <i>polytrichus</i> (Th6)	7.905	1	1.221	3	19.43*
<i>Th. pulegioides</i> ssp. <i>pulegioides</i> (Th7)	0.528	1	4.593	3	0.34
<i>Th. praecox</i> ssp. <i>janke</i> (Th8)	6.500	1	0.484	3	40.26**

GL - degrees of freedom, SP - sum of squares, Significance for F-test: ns  $p > 0.05$ ; \* $p \leq 0.05$ ; \*\* $p \leq 0.01$ ; \*\*\* $p \leq 0.001$

The multivariate analysis (Figure 3) based on the first two main components expresses 99.98% of the variability of the five morphological characters in the analysed populations. In the case of the populations of second cluster, the plants had a higher height,

associated with a larger size of the inflorescences and leaves.

In Table 7, it is observed that 90.63% (Th1) and respectively 83.34% (Th8), of the variability of the length of the inflorescence in the populations of *Th. praecox* ssp. *janke*, can be explained as

the result of the influence of the four characters. Plant height, for *Th. praecox ssp. janke* from Domogled (Th1), has a major (82.82 %) and distinctly significant contribution to the realization of the size of the inflorescence, followed by the width of the leaves which influences to an extent of approximately 7.3% the variability of this character, while the length and leaf shape had a very low influence of about 0.3%. The variability of inflorescence length in this population was caused to a degree of 9.4% by environmental conditions or other factors. For *Th. praecox ssp. janke* from Coronini (Th8), the length of the leaves has a distinctly significant major influence of approximately 64.71% on the realization of that character, being followed by the shape of the leaves which influences the size of the inflorescence to a degree of 13.48%. Based on the data presented in Table 8, it is observed that the length of the inflorescence for *Th. praecox ssp. janke* (Th1), shows positive and strongly statistically assured correlations with respect to plant height and leaf width, except for

leaf length where the correlation coefficient is positive but does not reach the level of significance. The shape of the leaf shows a negative correlation, as such the elongation of the shape of the leaves is associated with a smaller size of the inflorescences. Plant height shows a significantly positive correlation with leaf width and, respectively, a very high negative correlation with leaf shape. Thus, in the case of this population, the short stature of the plants was associated with the elongated shape of the leaves.

Analysis of the correlation coefficients between the five morphological characters studied in the population of *Th. praecox ssp. janke* (Th8) (Table 8), shows the existence of low and statistically uncertain correlations between the length of the inflorescence and the other characters, in contradiction with the situation presented previously. The differences between the character structure of the two populations are due to the different ecological conditions in Domogled (Th1) and Coronini (Th8).

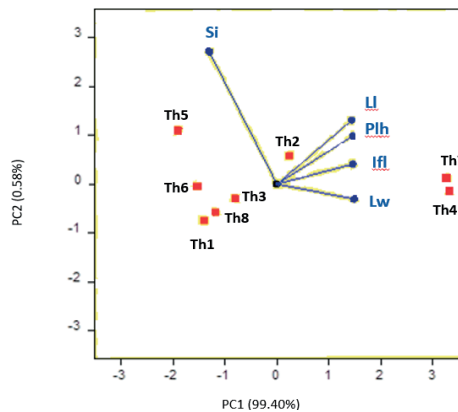


Figure 3. Biplot diagram for morphological traits  
Plh-plant height; Ifl-Inflorescence length; LI-leaves length; Lw-leaves width; Si-Shape index

Table 7. Variance components of multiple regression between flowers length and other morphological traits for *Th. praecox ssp. janke*

Source of variability	<i>Th. praecox ssp. janke</i> (Th1)				<i>Th. praecox ssp. janke</i> (Th2)			
	SP	GL	S <sup>2</sup>	F test	SP	GL	S <sup>2</sup>	F test
Regression	119.40 (90.63 %)	4	29.85	36.26**	8.28 (83.34 %)	4	2.07	18.71**
Plant height (x <sub>1</sub> )	109.12 (82.82 %)	1	109.12	132.53**	0.03 (0.28 %)	1	0.03	0.25
Leaves length (x <sub>2</sub> )	0.30 (0.22 %)	1	0.30	0.36	6.43 (64.71 %)	1	6.43	58.12**
Leaves width (x <sub>3</sub> )	9.64 (7.32 %)	1	9.64	11.71**	0.48 (4.86 %)	1	0.48	4.37
Index shape (x <sub>4</sub> )	0.35 (0.27 %)	1	0.35	0.43	1.34 (13.48 %)	1	1.34	12.11**
Other sources	12.35 (9.37 %)	15	0.82		1.66 (16.70 %)	15	0.11	
Total	131.75	19			9.94	19		

Th1:  $y = -1.2 + 0.29x_1 - 0.14x_2 + 0.82x_3 + 0.13x_4$ ;  $R^2 = 0.9063$ ; DW = 2.2; SDE = 2.22 mm;  
Th8:  $y = -6.22 + 0.09x_1 - 5.97x_2 + 11.92x_3 + 8.07x_4$ ;  $R^2 = 0.8334$ ; DW = 2.12; SDE = 0.81 mm

Table 8. Correlation coefficients values between studied morphological traits for *Th. praecox* ssp. *janke*

Character	<i>Th. praecox</i> ssp. <i>janke</i> (Th1)					<i>Th. praecox</i> ssp. <i>janke</i> (Th2)				
	Plant height	Leaves length	Leaves width	Index shape	Flower length	Plant height	Leaves length	Leaves width	Index shape	Flower length
Plant height	<b>1.000</b>	0.337	0.892***	-0.820 <sup>000</sup>	0.870***	<b>1.000</b>	0.783***	0.634**	-0.384	0.049
Leaves length		<b>1.000</b>	0.535*	-0.442	0.336		<b>1.000</b>	0.799***	-0.447	-0.424
Leaves width			<b>1.000</b>	-0.923 <sup>000</sup>	0.885***			<b>1.000</b>	-0.891 <sup>000</sup>	-0.208
Index shape				<b>1.000</b>	-0.805 <sup>000</sup>				<b>1.000</b>	0.012
Flower length					<b>1.000</b>					<b>1.000</b>

Th1: r<sub>5%</sub> = 0.444; r<sub>1%</sub> = 0.561; r<sub>0.1%</sub> = 0.679; Th8: r<sub>5%</sub> = 0.444; r<sub>1%</sub> = 0.561; r<sub>0.1%</sub> = 0.679

According to the data in Table 9, it is observed that the direct effects of plant height, length and shape of leaves on the correlation with the length of inflorescences, for *Th. praecox* ssp. *janke* from Domogled (Th1), are reduced (10.98-24.75%), so the indirect effects of other characters must also be taken into account. The relationship between the length of the inflorescence and the width of the leaf is mostly due to the direct effect of the respective character (64.14%), against the background of reduced indirect effects from the other characters. Also, the width of the leaves has major indirect effects of 59.35-63.57% on the

connection of the other characters with the length of the inflorescence. In the case of *Th. praecox* ssp. *janke* from Coronini (Th8), the analysis of the path coefficients (Table 9) confirms that the relationship between the length of the inflorescence and the width of the leaves shows the highest stability (42.80%), while the relationship between the length of the inflorescence and the height of the plants has the lowest stability (13.48%). The width of the leaves also showed a high indirect influence in this population (36.81-45.40%) on the length of the inflorescence through the other morphological characters.

Table 9. Path coefficients values for flowers length for *Th. praecox* ssp. *janke* and *Th dacicus*

Correlative links	Th1		Th8		Th2		Th3	
	Value	%	Value	%	Value	%	Value	%
<b>Flower length - Plant height</b>								
The direct effect of plant height	0.293	24.75	1.149	13.48	-0.734	24.70	0.310	9.09
The indirect. through the leaves length	-0.049	4.12	-3.038	35.63	-0.540	18.16	-0.460	13.50
through the leaves width	0.734	62.03	3.139	36.81	1.354	45.56	1.721	50.50
through the index shape	-0.108	9.10	-1.200	14.08	-0.344	11.59	-0.918	26.92
Total correlation	0.870	100.00	0.049	100.00	-0.264	100.00	0.653	100.00
<b>Flower length - Leaves length</b>								
The direct effect of leaves length	-0.145	19.50	-3.879	38.29	-1.192	38.08	-1.166	44.11
The indirect. through the plant height	0.099	13.32	0.900	8.88	-0.332	10.61	0.122	4.63
through the leaves width	0.440	59.35	3.953	39.02	1.238	39.53	1.273	48.18
through the index shape	-0.058	7.82	-1.398	13.80	0.369	11.77	0.082	3.09
Total correlation	0.336	100.00	-0.424	100.00	0.082	100.00	0.312	100.00
<b>Index shape - Leaves width</b>								
The direct effect of width leaves	0.823	64.14	4.949	42.80	2.690	52.31	2.501	53.97
The indirect. through plant height	0.261	20.37	0.729	6.30	-0.370	7.19	0.213	4.60
through leaves length	-0.077	6.03	-3.098	26.79	-0.549	10.67	-0.594	12.81
through index shape	-0.121	9.45	-2.788	24.11	-1.534	29.83	-1.326	28.62
Total correlation	0.885	100.00	-0.208	100.00	0.238	100.00	0.794	100.00
<b>Flower length - Index shape</b>								
The direct effect of index shape	0.131	10.98	3.130	32.22	1.981	44.90	1.607	41.14
The indirect. prin plant height	-0.240	20.10	-0.441	4.54	0.128	2.89	-0.177	4.53
through leaves length	0.064	5.35	1.733	17.84	-0.222	5.03	-0.059	1.51
through leaves width	-0.760	63.57	-4.409	45.40	-2.082	47.18	-2.063	52.82
Total correlation	-0.805	100.00	0.012	100.00	-0.195	100.00	-0.692	100.00

The analysis of the variance of the multiple regression, regarding the influence of different morphological characters on the size of the

inflorescence, in the two populations of *Th. dacicus*, indicates that, the variability of the length of the inflorescence is due to the



influence of the four characters, to a significant extent of 51.89% for the species from Coronini (Th2) and 83.27% for the one from Lescovița (Th3) (Table 10). The length of the

inflorescences in the population of *Th. dacicus* from Lescovița showed a higher stability compared to the population from Coronini.

Table 10. Variance components of multiple regression between flowers length and other morphological traits for *Th. dacicus* (Th2, Th3)

Source of variability	<i>Th. dacicus</i> (Th2)				<i>Th. dacicus</i> (Th3)			
	SP	GL	S <sup>2</sup>	F test	SP	GL	S <sup>2</sup>	F test
Regression	598.20 (51.86 %)	4	149.55	4.04*	69.85 (83.27 %)	4	17.46	18.68**
Plant height (x <sub>1</sub> )	119.20 (10.33 %)	1	119.20	3.22	41.81 (49.84 %)	1	41.81	44.73**
Leaves length (x <sub>2</sub> )	87.10 (7.55 %)	1	87.10	2.35	0.33 (0.40 %)	1	0.33	0.36
Leaves width (x <sub>3</sub> )	245.40 (21.27 %)	1	245.40	6.63*	23.15 (27.59 %)	1	23.15	24.76**
Index shape (x <sub>4</sub> )	146.50 (12.70 %)	1	146.50	3.96	4.57 (5.44 %)	1	4.57	4.89*
Other sources	555.45 (48.15 %)	15	37.03		14.02 (16.71 %)	15	0.93	
Total	1153.55	19			83.88	19		

Th2:  $y = y = -162 - 0.53x_1 - 14.7x_2 + 84.7x_3 + 46.4x_4$ ;  $R^2 = 0.5186$ ; DW = 2.03; SDE = 4.9 mm;

Th3:  $y = y = -40.8 + 0.05x_1 - 5.31x_2 + 22.4x_3 + 12.1x_4$ ;  $R^2 = 0.8327$ ; DW = 1.75; SDE = 2.37 mm

The study of the correlations indicates, the existence of very low and statistically uncertain links between the length of the inflorescences and the rest of the morphological characters, for the population from Coronini (Th2). The closest positive and significant correlations are observed between the height of the plants and the dimensions of the leaves, while the width of the leaves shows a high negative correlation

with their shape. In the case of the population from Lescovița (Th3), the length of the inflorescence shows positive and strongly statistically assured correlations with respect to the height of the plants and the width of the leaves, while the correlation coefficient with respect to the length of the leaf is positive but does not reach the level of significance (Table 11).

Table 11. Correlation coefficients values between studied morphological traits for *Th. dacicus* (Th2, Th3)

Character	<i>Th. dacicus</i> (Th2)					<i>Th. dacicus</i> (Th3)				
	Plant height	Leaves length	Leaves width	Index shape	Flower length	Plant height	Leaves length	Leaves width	Index shape	Flower length
Plant height	<b>1.000</b>	0.453*	0.503*	-0.174	-0.264	<b>1.000</b>	0.395	0.688***	-0.571 <sup>00</sup>	0.653**
Leaves length		<b>1.000</b>	0.460*	0.186	0.082		<b>1.000</b>	0.509*	0.051	0.312
Leaves width			<b>1.000</b>	-0.774 <sup>000</sup>	0.238			<b>1.000</b>	-0.825 <sup>000</sup>	0.794***
Index shape				<b>1.000</b>	-0.195				<b>1.000</b>	-0.692 <sup>000</sup>
Flower length					<b>1.000</b>					<b>1.000</b>

Th2:  $r_{5\%} = 0.444$ ;  $r_{1\%} = 0.561$ ;  $r_{0.1\%} = 0.679$ ; Th3:  $r_{5\%} = 0.444$ ;  $r_{1\%} = 0.561$ ;  $r_{0.1\%} = 0.679$

In the case of both populations, the relationship between the length of the inflorescence and the width of the leaf is mostly due to its direct effect (50%), against the background of smaller indirect effects from the other characters. Also, the shape of the leaf has important direct effects on the connection with the size of the inflorescence (Table 9).

The variability of the length of the inflorescence in the two populations of *Th. praecox* ssp. *polytrichus*, is due to a distinctly significant extent to the influence of the four characters included in the regression model. The shape of the leaves has an important and significant

contribution to the inflorescence, while the width of the leaves, the length of the leaves and the height of the plant have smaller and insignificant influences on the size of the inflorescences. The size of the inflorescence in the population from the Semenic Peak area (Th6) was influenced to a degree of 38.12% by environmental conditions or other factors, showing a higher stability compared to the population belonging to the same species, harvested from the Gozna Peak, where the influence on some external factors was 49.33% (Table 12).



Table 12. Variance components of multiple regression between flowers length and other morphological traits for *Th. praecox* ssp. *polytrichus*

Source of variability	<i>Th. praecox</i> ssp. <i>polytrichus</i> (Th5)				<i>Th. praecox</i> ssp. <i>polytrichus</i> (Th6)			
	SP	GL	S <sup>2</sup>	F test	SP	GL	S <sup>2</sup>	F test
Regression	10.61 (50.66%)	4	2.65	3.85*	14.74 (61.89%)	4	3.69	6.09**
Plant height (x <sub>1</sub> )	0.07 (0.33%)	1	0.07	0.10	0.26 (1.09%)	1	0.26	0.43
Leaves length (x <sub>2</sub> )	0.35 (1.65%)	1	0.35	0.50	0.17 (0.69%)	1	0.17	0.27
Leaves width (x <sub>3</sub> )	2.05 (9.77%)	1	2.05	2.97	0.49 (2.06%)	1	0.49	0.81
Index shape (x <sub>4</sub> )	8.15 (38.91%)	1	8.15	11.83**	13.83 (58.05%)	1	13.83	22.84**
Other sources	10.33 (49.33%)	15	0.69		9.08 (38.12%)	15	0.61	
Total	20.94	19			23.82	19		

Th5:  $y = 48.76 + 0.01x_1 + 3.39x_2 - 21.2x_3 - 6.63x_4$ ;  $R^2 = 0.5066$ ; DW = 3.28; SDE = 2.49 mm

Th6:  $y = 25.53 - 0.06x_1 + 1.38x_2 - 6.40x_3 - 2.90x_4$ ;  $R^2 = 0.6189$ ; DW = 1.91; SDE = 1.89 mm

The analysis of the correlation coefficients between the morphological characters studied in these two populations, prove the existence of some low and statistically uncertain correlations between the length of the inflorescence and the other characters. The height of the plants showed a significantly positive correlation with the width of the leaves, respectively a significantly negative

relationship with the shape of the leaves. There is an increase in the height of the plants, associated with a rounder shape of the leaves, respectively a higher value of their width. In the case of the shape of the leaves, close positive correlations are observed with respect to the length and negative with respect to the width of the leaves, for both analyzed populations (Table 13).

Table 13. Correlation coefficients values between studied morphological traits for *Th. praecox* ssp. *polytrichus*

Character	<i>Th. praecox</i> ssp. <i>polytrichus</i> (Th5)					<i>Th. praecox</i> ssp. <i>polytrichus</i> (Th6)				
	Plant height	Leaves length	Leaves width	Index shape	Flower length	Plant height	Leaves length	Leaves width	Index shape	Flower length
Plant height	<b>1.000</b>	0.046	0.673**	-0.451 <sup>0</sup>	-0.041	<b>1.000</b>	0.194	0.066	-0.052	0.089
Leaves length		<b>1.000</b>	0.169	0.640**	0.089		<b>1.000</b>	-0.140	0.667**	0.087
Leaves width			<b>1.000</b>	-0.642 <sup>00</sup>	0.146			<b>1.000</b>	-0.786 <sup>000</sup>	-0.125
Index shape				<b>1.000</b>	-0.095				<b>1.000</b>	-0.021
Flower length					<b>1.000</b>					<b>1.000</b>

Th5:  $r_{5\%} = 0.444$ ;  $r_{1\%} = 0.561$ ;  $r_{0.1\%} = 0.679$ ; Th6:  $r_{5\%} = 0.444$ ;  $r_{1\%} = 0.561$ ;  $r_{0.1\%} = 0.679$

The study of the path coefficients confirms that the link between the length of the inflorescence and the dimensions of the leaves shows close levels of stability for both populations (50%), while the link between the length of the inflorescence and the height of the plants has the lowest stability (below 1%). Only in the case of the shape of the leaves, close positive correlations are observed with respect to the length and negative with respect to the width of the leaves, so the relationships between these characters of the leaves are similar in the two populations (Table 14). Analysis of the variance of the multiple regression regarding the influence of different morphological characters on the size of the inflorescence in the population of *Th. comosus* (Th4), shows that 78.78% of the variability of the length of the inflorescence is due to the influence of the four characters. The shape of the leaves has a major (43.85%) and distinctly

significant contribution to the realization of the size of the inflorescence, followed by the size of the plants, which influences to an extent of approximately 22.03% the variability of this character. The variability of the length of the inflorescence depends to an extent of 21.22% on environmental conditions or other factors (Table 15).

The length of the inflorescence shows low correlations, mainly negative and not statistically assured compared to the other four characters. The length of the leaves shows significantly positive correlations with respect to the width and shape of the leaves, indicating that the increase in the size of the leaves is associated with an elongation of their shape. (Table 16). In this endemic population, the direct effects of plant height on the correlation with the length of inflorescences are lower (17.11%), so the connection between these two characters is mainly influenced by the indirect effects of the other

characters. The height of the plants also shows low indirect effects, so the change in the height of the plants of this population was not associated with

significant variations of the other characters (Table 14).

Table 14. Path coefficients value for flowers length for *Th. praecox* ssp. *polytrichus*, *Th. comosus*, *Th. pulegioides*

Correlative links	Th5		Th6		Th4		Th7	
	Value	%	Value	%	Value	%	Value	%
<b>Flower length - Plant height</b>								
The direct effect of plant height	0.039	0.83	-0.258	29.24	-0.480	17.11	0.255	9.05
The indirect. through the leaves length	0.168	3.61	0.337	38.20	1.188	42.39	-0.333	11.80
through the leaves width	-2.346	50.44	-0.139	15.72	-0.848	30.25	-0.740	26.27
through the index shape	2.099	45.12	0.149	16.84	-0.287	10.24	1.490	52.88
Total correlation	<b>-0.041</b>	<b>100.00</b>	<b>0.089</b>	<b>100.00</b>	<b>-0.426</b>	<b>100.00</b>	<b>0.672</b>	<b>100.00</b>
<b>Flower length - Leaves length</b>								
The direct effect of leaves length	3.656	50.59	1.735	43.72	-5.490	50.64	-4.480	48.26
The indirect. through the plant height	0.002	0.02	-0.050	1.26	0.104	0.96	0.019	0.20
through the leaves width	-0.590	8.16	0.293	7.37	2.628	24.24	2.620	28.23
through the index shape	-2.979	41.22	-1.891	47.64	2.620	24.17	2.163	23.31
Total correlation	<b>0.089</b>	<b>100.00</b>	<b>0.087</b>	<b>100.00</b>	<b>-0.138</b>	<b>100.00</b>	<b>0.323</b>	<b>100.00</b>
<b>Index shape - Leaves width</b>								
The direct effect of width leaves	-3.487	48.97	-2.093	45.70	4.738	47.94	5.108	50.48
The indirect. through plant height	0.026	0.36	-0.017	0.37	0.086	0.87	-0.037	0.37
through leaves length	0.619	8.69	-0.243	5.30	-3.045	30.81	-2.298	22.71
through index shape	2.988	41.97	2.228	48.63	-2.014	20.38	-2.676	26.44
Total correlation	<b>0.146</b>	<b>100.00</b>	<b>-0.125</b>	<b>100.00</b>	<b>-0.236</b>	<b>100.00</b>	<b>0.098</b>	<b>100.00</b>
<b>Flower length - Index shape</b>								
The direct effect of index shape	-4.655	50.32	-2.835	50.18	4.959	50.54	4.936	50.66
The indirect. prin plant height	-0.017	0.19	0.014	0.24	0.028	0.28	0.077	0.79
through leaves length	2.340	25.29	1.157	20.47	-2.901	29.56	-1.963	20.14
through leaves width	2.238	24.20	1.645	29.11	-1.924	19.61	-2.769	28.41
Total correlation	<b>-0.095</b>	<b>100.00</b>	<b>-0.021</b>	<b>100.00</b>	<b>0.162</b>	<b>100.00</b>	<b>0.282</b>	<b>100.00</b>

Table 15. Variance components of multiple regression between flowers length and other morphological traits for *Th. comosus* and *Th. pulegioides* ssp. *pulegioides*

Source of variability	<i>Th. comosus</i> (Th4)				<i>Th. pulegioides</i> ssp. <i>pulegioides</i> (Th7)			
	SP	GL	S <sup>2</sup>	F test	SP	GL	S <sup>2</sup>	F test
Regression	631.82 (78.78 %)	4	157.96	13.93**	2455.4 (76.23 %)	4	613.85	12.03**
Plant height (x <sub>1</sub> )	176.68 (22.03 %)	1	176.68	15.58**	1801.4 (55.93 %)	1	1801.40	35.29**
Leaves length (x <sub>2</sub> )	54.00 (6.73 %)	1	54.00	4.76**	298.3 (9.26 %)	1	298.30	5.84*
Leaves width (x <sub>3</sub> )	49.50 (6.17 %)	1	49.50	4.36	15.0 (0.47 %)	1	15.00	0.29
Index shape (x <sub>4</sub> )	351.64 (43.85 %)	1	351.64	31.00**	340.7 (10.58 %)	1	340.70	6.68*
Other sources	170.14 (21.22 %)	15	11.34		765.6 (23.77 %)	15	51.04	
Total	801.96	19	157.96	13.93	3220.9	19		

Th4:  $y = 248 - 0.15x_1 - 30x_2 + 51.9x_3 + 189.7x_4$ ;  $R^2 = 0.7878$ ; DW = 2.41; SDE = 8.25 mm

Th7:  $y = 248 - 0.15x_1 - 30x_2 + 51.9x_3 + 189.7x_4$ ;  $R^2 = 0.7623$ ; DW = 1.95; SDE = 7.5 mm

Table 16. Correlation coefficients values between studied morphological traits for *Th. comosus* and *Th. pulegioides* ssp. *pulegioides*

Character	<i>Th. comosus</i> (Th4)					<i>Th. pulegioides</i> ssp. <i>pulegioides</i> (Th7)				
	Plant height	Leaves length	Leaves width	Index shape	Flower length	Plant height	Leaves length	Leaves width	Index shape	Flower length
Plant height	<b>1.000</b>	-0.216	-0.179	-0.058	-0.426	<b>1.000</b>	0.074	-0.145	0.302	0.672**
Leaves length		<b>1.000</b>	0.555*	0.528*	-0.138		<b>1.000</b>	0.513*	0.438	0.323
Leaves width			<b>1.000</b>	-0.406	-0.236			<b>1.000</b>	-0.542 <sup>9</sup>	0.098
Index shape				<b>1.000</b>	0.162				<b>1.000</b>	0.282
Flower length					<b>1.000</b>					<b>1.000</b>

Th4:  $r_{5\%} = 0.444$ ;  $r_{1\%} = 0.561$ ;  $r_{0.1\%} = 0.679$ ; Th7:  $r_{5\%} = 0.444$ ;  $r_{1\%} = 0.561$ ;  $r_{0.1\%} = 0.679$

Regarding the population of *Th. pulegioides* ssp. *pulegioides* (Th7), 76.23% of the variability of the length of the inflorescence, can be explained as being the result of the influence of the four characters. The height of the plants has a distinctly significant major influence (55.93%), when achieving the respective character, followed by the shape of the leaves (10.58%). Changing the width of the leaves (0.47%) had smaller and insignificant influences on the size of the inflorescences (Table 15). The analysis of the correlation coefficients shows the existence of small positive correlations and not statistically ensured between the length of the inflorescence and the other characters. The height of the plants showed a significantly positive correlation with the length of the inflorescences, thus indicating that an increase in the height of the plants of this population is associated with an increase in the size of the inflorescences against the background of small and insignificant variations in the characters of the leaves (Table 16). The study of the path coefficients presented in Table 14, proves that the relationship between the length of the inflorescence and the dimensions of the leaves shows close levels of stability (48.26-50.66%), while the link between the length of the inflorescence and the height of the plants has the lowest stability (below 10%). The smaller direct effects of the leaf sizes (around 50%) on their links with the length of the inflorescence, explain the insignificant values of the related correlation coefficients.

## CONCLUSIONS

The quantitative morphological characterization based on biometric measurements, as well as the quantification of the associative relationships between the morphological parameters studied, allowed the quantification of the degree of phenotypic similarity between the analysed populations. Morphological differences are confirmed between the species of the same genera harvested from different areas, which explains the difficulty of precise taxonomic determination, at the subspecies level by classical methods.

The results of the research are extremely useful to the breeders, knowing the continuous interest in finding valuable local populations, more

resistant to diseases and pests but also better adapted to the biotope conditions. Biochemical studies are recommended to recommend these populations also in terms of volatile oil quality.

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