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VARIABILITY OF MORPHOLOGICAL TRAITS IN ROMANIAN WILD THYME POPULATIONS

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Abstract

The wild species of the genus Thymus reveal a great taxonomic diversity, indicating a natural evolution that has allowed the adaptation to different habitats and has created the premises of a divergent evolution based on a great genetic variability. The study aims at quantitative morphological characterization by quantifying the associative relationships between the morphological parameters studied, for thirteen thyme populations growing wild in western Romania. Five morphological parameters were used to evaluate the quantitative morphological characters to establish the degree of phenotypic similarity between the studied populations. Depending on the phenotypic similarity, the populations were hierarchically classified into three main clusters using the UPGMA multiparametric cluster analysis. Concerning the analysis of variance for the morphological traits studied in the thyme populations, high and significant values of variance were recorded in the case of leaf length which shows a high capacity for differentiation between the three groups. The lowest diversity between populations of different clusters was observed for leaf width. The overall results revealed high intraspecific and interspecific variability in wild populations of thyme and provided significant information for plant breeding.

Key words: *polymorphism, Thymus pannonicus, Th. dacicus, Th. glabrescens, Th. pulegioides.*

INTRODUCTION

Family *Lamiaceae* (*Labiatae*), is a well-known angiosperm family, comprising of more than 200 genera, many of them being of medicinal (Nieto, 2017; Gurita et al., 2019; Sitarek et al., 2020) aromatic (Karpinski, 2020; Raja, 2021; Butta et al., 2023) and nutritive interest (Carović-Stanko et al., 2016). Within the *Lamiaceae* family, the *Thymus* genus is distinguished, characterized by a great ecological plasticity, including about 250 taxa (species and subspecies), spread all over the world in varied biotopes, the centre of origin being considered the West Mediterranean Region (Jalas, 1971; Hartvig, 1987; Morales, 2002). In the Romanian flora, 18 species of the genus *Thymus* are mentioned, 17 spontaneous and one cultivated species (Sârbu et al., 2013). The spontaneous species in Romania belong with one exception (*T. zygioides* - Section *Hypodromi*, subsection *Serpyllastrum)*, section *Serpyllum*, being mostly classified in the last 4 subsections (Beicu et al., 2023). The taxa of these subsections pose the greatest taxonomic problems, as the species here evolved from the species of the Mediterranean basin centre of origin after the last glaciation, being therefore considered of newer origin and still in a dynamic evolutionary process (Pitarokili et al., 2014; Bălășoiu et al., 2024).

The species of the genus *Thymus* reveal a high variability, both from a morphological (Ložiene, 2006; Dajic-Stevanovic et al., 2008; Baczek et al., 2019), genetical (Thompson, 2002; Sostaric et al., 2012; Soorni et al., 2019; Kim et al., 2020; Alqahtani et al., 2020) and biochemical point of view (Hernández et al., 1987; Imbrea et al., 2016; Ložiene & Venskutonis, 2005; Hadian et al., 2014; Rus et al., 2016; Taghouti et al., 2019), resulting from the influence of abiotic factors. Nomenclature of the genus is in a continuous dynamic, has undergone profound changes, and is constantly subject to critical revisions (El-Gazzar & Watson, 1970; Rosello, 1981; Mártonfi, 1997; Bartolucci et al., 2013).

MATERIALS AND METHODS

Thirteen samples were collected for morphological and molecular analyses, representing wild populations of thyme from different areas in south-western Romania. For the thirteen samples, thirty individuals were collected from each sample. Plants were harvested at full maturity during the flowering period, between May and July 2018. Samples were chosen from locations at a distance from each other to avoid harvesting individuals from the same population and were coded according to the locations from which they were collected (Table 1).

Table 1. *Thymus* populations studied

Population	Abbreviation	Location	Voucher	GPS coordinates (degree, minutes, seconds)		
				Altitude (m)	Latitude	Longitude
Th. pannonicus ssp. *auctus*	Th1	Silagiu	VSNH.BUASTM: 1838	192	45. 60703	21.60335
Th. Glabrescens	Th2	Silagiu	VSNH.BUASTM: 1837	191	45.61382	21.61459
Th. pannonicus ssp. *auctus*	Th3	Pojejena	VSNH.BUASTM: 1821	172	44.78258	21.58887
Th. pulegioides ssp. *chamaedrys*	Th4	Pojejena	VSNH.BUASTM: 1822	194	44.78452	21.59027
Th. pulegioides ssp. *montanus*	Th5	Pojejena	VSNH.BUASTM: 1823	170	44.78465	21.58705
Th. pulegioides ssp. *montanus*	Th6	Pojejena	VSNH.BUASTM: 1824	190	44.78640	21.58897
Th. Dacicus	Th7	Ostrov	VSNH.BUASTM: 1826	70	44.70163	21.62073
Th. Dacicus	Th8	Ostrov	VSNH.BUASTM: 1827	71	44.40145	21.62113
Th. pulegioides ssp. *chamaedrys*	Th9	Carașova	VSNH.BUASTM: 1832	593	45.15665	21.88147
Th. pulegioides ssp. *pulegioides*	Th10	Carașova	VSNH.BUASTM: 1831	582	45.15468	21.88160
Th. pulegioides ssp. *montanus*	Th11	Carașova	VSNH.BUASTM: 1829	511	45.15465	21.87672
Th. pulegioides ssp. *montanus*	Th12	Iabalcea	VSNH.BUASTM: 1833	417	45.22255	21.89523
Th. pulegioides ssp. *pulegioides*	Th13	Nermet	VSNH.BUASTM: 1830	389	45.24185	21.88790

The taxa of the genus *Thymus* were identified based on specialized plant identification books (Sârbu et al., 2013). The voucher specimens (Table 1) were deposited in the herbarium of the Biology Department, University of Life Sciences "King Mihai I" from Timisoara, Romania.

To determine the significance of the differences between the studied genotypes, the processing of the experimental data obtained was done by analyzing the variance and the t-test (two-sample assuming equal variances). Differences were considered significant when p-values < 0.05. Principal component analysis (PCA) was performed using the Euclidean distance, according to Statistica 10.0 (StatSoft Inc., Tulsa, USA).

RESULTS AND DISCUSSIONS

Quantitative morphological characteristics, namely plant height, flower length, leaf length, leaf width, and leaf shape, were recorded and statistically analyzed. Thyme flowers are grouped into inflorescences (racemes); however, for ease of interpretation, the term "flower" will be used.

Plant height in the studied populations exhibited moderate variability (s% = 23.20), with a variation range of 95.2 mm, from 175.90 mm in population Th13 to 80.70 mm in population Th2. High interindividual variability for plant height was observed in populations Th1, Th2, Th3, Th6, Th12, and Th13. Multiple comparisons indicate that populations Th11 and Th13 had significantly greater plant height than the other populations, with differences exceeding 26.1 mm (Table 2).

Regarding flower length, recorded values ranged from 18.05 mm in Th2 to 66.10 mm in Th13, with a variation range of 48.05 mm. Compared to plant height, flower length displayed higher interpopulation variability (40.92%) and pronounced intrapopulation heterogeneity. Populations Th11 and Th13 exhibited significantly longer flowers, exceeding those of the other populations by more than 21.80 mm. The highest interindividual homogeneity for flower length was observed in population Th5, followed by Th4, both from the Pojejena area (Table 2).

Leaf length in the studied populations displayed moderate variability (s% = 16.80), with a range of 6.75 mm, from 8.9 mm in Th6 to 12.65 mm in Th13. As illustrated in Figure 1c, the highest variability and amplitude for this trait were recorded in population Th1 from Silagiu, along with populations from Ostrov (Th7, Th8), whose distributions exhibited a high level of symmetry. Conversely, the lowest intrapopulation variability was observed in population Th9 from Carașova.

For leaf width, statistically significant differences were observed among the studied populations. Interindividual heterogeneity had a relatively minor impact on the overall results compared to other traits analyzed (Table 3). Values ranged from 1.82 mm in Th1 to 5.85 mm in Th11, with a variation range of 4.03 mm and very high interpopulation variability (41.75%), accompanied by moderate intrapopulation heterogeneity. Pairwise comparisons revealed significantly greater leaf width in populations Th10, Th11, and Th13, with differences exceeding 0.8 mm compared to other populations. Low leaf width values (below 2.5 mm) were observed in populations from Silagiu (Th1, Th2) and Ostrov (Th7, Th8). The highest interindividual homogeneity for leaf width was found in populations Th4, Th6, and Th2, while populations Th10 and Th11 exhibited the greatest intrapopulation variability and amplitude. In contrast, Th9 had moderate variability but a higher amplitude (Table 3).

According to Table 3, leaf shape exhibited the highest variability and amplitude in Th1 and Th2 (Silagiu) and Th8 (Ostrov) populations. In contrast, the lowest intrapopulation variability and amplitude were recorded in Th3, Th4, Th5, and Th6 (Pojejena) populations.

Table 2. Average plants height and flowers length

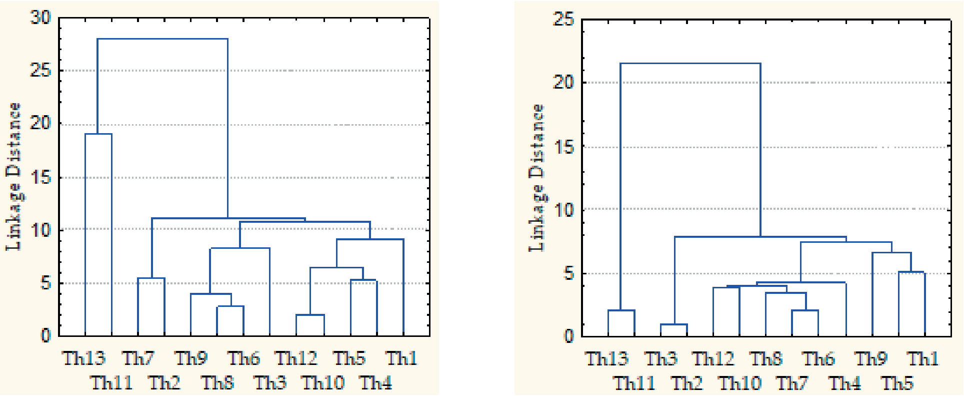
Population	Plant height (mm)		Flower length (mm)	
	$\bar{x} \pm s_{\bar{x}}$	s %	$\bar{x} \pm s_{\bar{x}}$	s %
Th 1	130,80±13,52 b	32,70	42,30±3,40 bc	25,39
Th 2	80,70±6,49 f	25,42	18,05±3,68 f	64,42
Th 3	92,85±6,52 e	22,21	23,85±1,96 e	26,04
Th 4	117,40±5,06 bcd	13,62	31,40±2,50 cde	25,13
Th 5	122,40±5,65 bc	14,59	39,00±1,74 bcd	14,15
Th 6	101,40±6,71 cdef	20,92	26,80±2,58 ef	30,41
Th 7	82,80±4,81 ef	18,37	24,90±3,29 ef	41,76
Th 8	99,50±4,78 def	15,19	29,60±3,19 def	34,09
Th 9	104,00±4,65 cde	14,15	44,30±3,55 b	25,36
Th 10	114,90±6,70 bcd	18,44	33,60±4,81 bcde	45,27
Th 11	156,90±8,29 a	16,71	65,90±9,20 a	44,14
Th 12	115,10±7,64 bcd	20,99	30,10±3,52 de	36,98
Th 13	175,90±13,56 a	32,70	66,10±5,69 a	25,39
	DL$_{5\%}$=21,11 mm		DL$_{5\%}$=11,80 mm	

$\bar{x}$ -mean; $\pm s_{\bar{x}}$ the standard deviation of the mean; s%- coefficient of variability; data sharing different superscripts (a-f) in the same row show statistically significant differences ($p < 0.05$)

Table 3. Average length, width and shape index of leaves

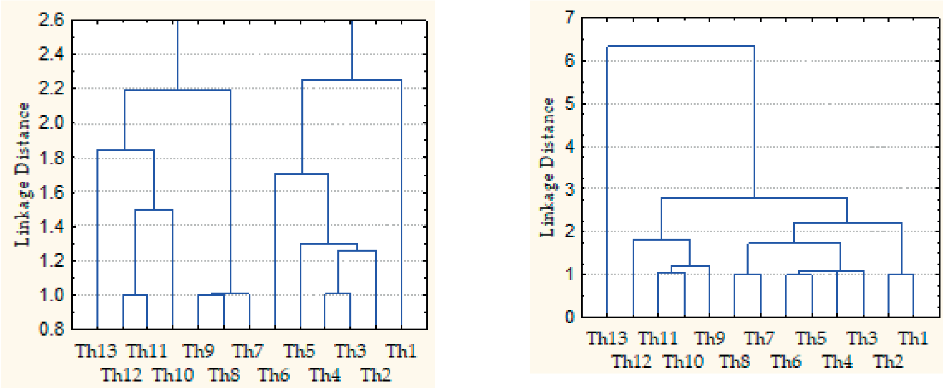
Population	Leaves length (mm)		Leaves width (mm)		Shape index	
	$\bar{x} \pm s_{\bar{x}}$	s %	$\bar{x} \pm s_{\bar{x}}$	s %	$\bar{x} \pm s_{\bar{x}}$	s %
Th 1	12,15±0,80 ab	20.72	1,82±0,15 ef	26,64	6,08±0,40 ab	20,72
Th 2	10,25±0,57 cde	17.70	1,85±0,08 ef	13,06	5,13±0,29 cde	17,70
Th 3	9,45±0,43 de	14.43	4,12±0,23 bc	17,72	4,73±0,22 de	14,43
Th 4	9,60±0,46 de	15.29	3,35±0,11 d	10,07	4,80±0,23 de	15,29
Th 5	10,25±0,37 cde	11.56	4,00±0,24 cd	18,63	5,13±0,19 cde	11,56
Th 6	8,90±0,43 e	15.40	3,80±0,15 cd	12,71	4,45±0,22 e	15,40
Th 7	12,10±0,95 ab	24.78	2,30±0,17 e	23,37	6,05±0,47 ab	24,78
Th 8	11,80±0,79 abc	21.06	2,15±0,18 e	26,97	5,90±0,39 abc	21,06
Th 9	11,90±0,35 ab	9.25	4,70±0,24 b	16,02	5,95±0,17 ab	9,25
Th 10	9,80±0,61 de	19.72	5,50±0,37 a	20,99	4,90±0,31 de	19,72
Th 11	11,00±0,58 bcd	16.60	5,85±0,49 a	26,74	5,50±0,29 bcd	16,60
Th 12	11,00±0,54 bcd	15.45	4,25±0,29 bc	21,66	5,50±0,27 bcd	15,45
Th 13	12,65±0,47 a	20.72	5,70±0,20 a	26,64	6,33±0,24 a	20,72
	DL$_{5\%}$=1,61 mm		DL$_{5\%}$=0,67 mm		DL$_{5\%}$=0,81	

$\bar{x}$-mean; $\pm s_{\bar{x}}$ the standard deviation of the mean; s%- coefficient of variability; data sharing different superscripts (a-f) in the same row show statistically significant differences ($p < 0.05$)



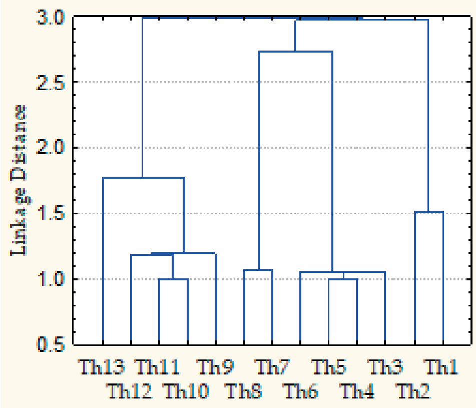
a. Cluster analysis of plant height

b. Cluster analysis of flower length



c. Cluster analysis of leaf length

d. Cluster analysis of leaf width



e. Cluster analysis of leaf shape index

Figure 1. Cluster analysis based on morphological characters

Based on both morphological similarity for plant height and flower length, all populations were classified into two main clusters. The first cluster groups the two populations with the highest values of these characters, populations Th11 and Th13. The second cluster is more complex, grouping several sub-clusters in which the other populations analysed are included (Figure 1a, 1b).

Hierarchical classification of populations based on similarity of leaf length and width groups them into two main clusters, each with several sub-clusters. A medium variability is observed, with the exception of the Th13 population, which for both characters show the highest values and is differentially represented (Figure 1c, 1d). Regarding the cluster analysis of populations based on similarity of leaf shape, three main clusters can be noticed, as can be seen in the dendrogram shown in Figure 1e.

In terms of the five morphological characters studied (plant height, inflorescence length, leaf length, width and shape), the highest phenotypic similarity exists between Th7 and Th8 populations belonging to the species *Th. dacicus* from Ostrov, at 98.91%. An increased phenotypic similarity can also be observed between the following populations: Th3 - Th6 (98.76%); Th3 - Th4 (97.39%); Th4 - Th5 (97.71%); Th5 - Th12 (97.54%). A pronounced morphological similarity of 91.72% was also observed between populations Th9, Th10, 11 of *Th. pulegioides* collected from Carașova. A high degree of phenotypic differentiation was observed among the populations: Th2 - Th13 (74.59%); Th6 -Th13 (70.07%); Th3 -Th13 (62.81%); Th4 - Th13 (51.26%). As such, it is found that overall, the Th13 population of *Th. pulegioides* from Nermet was morphologically differentiated to a considerable extent of 42.34% compared to the other populations included in the study. A significant phenotypic diversity of 53.92% was also recorded between Th2 and Th11 populations, *Th. panonnicus* (Th1) and *Th. glabrescens* (Th2) populations from Silagiu showed a morphological similarity of 79.12%.

Based on the morphological similarity of the five characters, the populations were hierarchically classified into three main clusters between which there is an average diversity of about 37%. The first cluster consists of the *Th. dacicus* populations from Ostrov, Th 7 and Th8, which are phenotypically similar to approximately 95% of the Th1 population of *Th. pannonicus* from Silagiu and to approximately 89% of the Th9 (*Th. pulegioides*) population from Carașova. The second group consists of Th3, Th4, Th5, Th6 populations belonging to *Th. pulegioides* collected from Pojejena which show a morphological similarity of about 94% to Th10 and Th12 populations of *Th. pulegioides* collected from Carașova. Also included in this group is the Th2 population of *Th. glabrescens* from Silagiu, which show a similarity of about 8% to the other six populations. The Th11 and Th13 populations of *Th. pulegioides*, between which there is a morphological similarity of 91.41%, make up the third cluster, differing by about 20% from the populations in the other clusters (Figure 2).

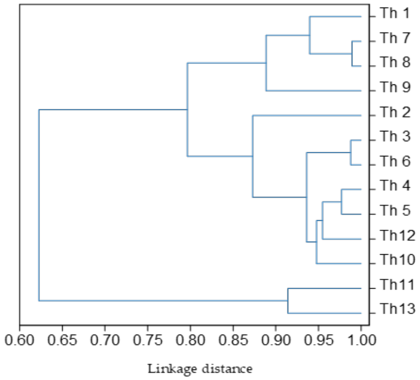


Figure 2. Dendrogram by UPGMA method for morphological traits

Concerning the analysis of variance for the morphological characters studied in the thyme wild populations, it can be observed that high and significant values of variance were recorded in the case of leaf length which shows a high capacity of differentiation between the three clusters. The lowest diversity between populations of different clusters was observed for leaf width (Table 4).

Table 4. Variance analysis for all morphological traits analysed

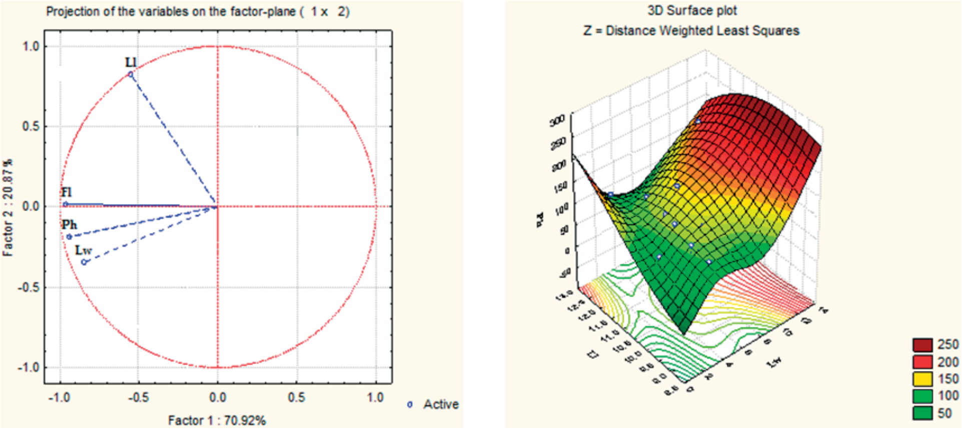
No.	**Character**	**Between**		**Within**		**F test**
		SP	**GL**	**SP**	**GL**	
1.	Plant height	1,491	1	10,512	11	1,56
2.	Flower length	3,040	1	8,962	11	3,73
3.	Length leaves	9,654	1	2,426	11	43,77**
4.	Width leaves	0,005	1	11,934	11	0,01

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

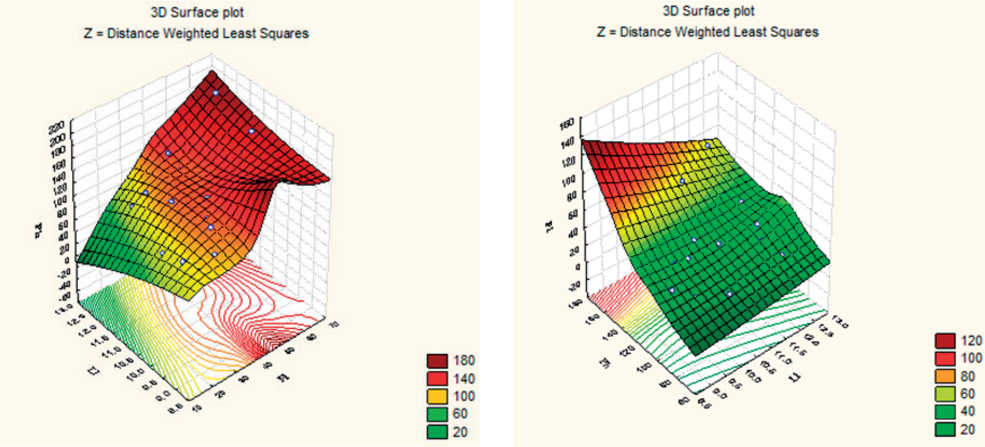
Table 5. Analysis of variance for each population regarding morphological traits

Population	Between		Within		F test
	SP	GL	SP	GL	
Th 1	59.67	1	6.17	3	28.99*
Th 2	52.74	1	1.93	3	82.07**
Th 3	32.06	1	1.70	3	56.47**
Th 4	30.00	1	2.89	3	31.19*
Th 5	31.94	1	2.11	3	45.33**
Th 6	25.58	1	1.83	3	41.90**
Th 7	74.25	1	1.29	3	172.20**
Th 8	64.76	1	2.51	3	77.34**
Th 9	50.02	1	0.36	3	416.18**
Th 10	25.93	1	2.14	3	36.34**
Th 11	22.45	1	1.54	3	43.68**
Th 12	43.21	1	2.41	3	53.73**
Th 13	36.13	1	3.42	3	31.69*

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



a. CP1 and CP2 b. Length leaves vs width leaves vs. plant height



c. Flowers length vs. length leaves vs. plant height d. Plant height vs. length leaves vs. flowers length

Figure 3. Projection of the variables for the two main components CP1 and CP2

Regarding the results presented in Table 5, it is observed that the Th7 and Th8 populations belonging to the *Th. dacicus* from Ostrov, generate the highest morphological differences, with high and distinctly significant contributions to the total variability recorded on the basis of the dendrogram. The lowest contribution to the total variability among the three clusters was observed for Th10 and Th11 populations belonging to *Th. pulegioides* from Carașova.

At the level of the first cluster the Th1 population has the highest contribution to interpopulation diversity, while at the level of the second cluster the Th4 population stands out.

Based on a linear correlation matrix, PCA (Principal Component Analysis) was applied to the mean values of the measured traits to study which parameters contributed the most to total data variation. The PCA produced 4 components. The first two principal components accounted for 70.92% and 20.87% of the variance respectively, for a total of 91.79% (Figure 3 a-d). Component 1 (CP1) includes the variables with the highest correlation coefficients: plant height, flower length, leaf width and leaf length, and component 2 (CP2) includes the correlation coefficient with the highest value, represented by leaf length, as shown in Table 6. The most important variable integrated into the first component was flower length, which was negatively correlated with this component.

The two-dimensional plot based on the two main components CP1 (70.92%) and CP2 (20.87%), expresses 91.79% of the variability of morphological characters. Based on the position of the populations to the vectors of the different characters, the dimensions of the characters were described. It can be seen that Th13 population shows the highest values of all characters (Table 6). Thyme populations show significant morphological variability, with some clustering closely while others are dispersed, indicating indicating phenotypic differences relevant for selection.

Table 6. Matrix of main components

Variable	Factor 1	Factor 2	Factor 3	Factor 4
Plant height	**-0.943068**	-0.189173	-0.219034	0.163889
Flower height	**-0.962191**	0.016169	-0.211304	-0.171106
Length leaves	**-0.550645**	**0.824935**	0.122341	0.036114
Width leaves	**-0.847552**	-0.343815	0.404119	-0.011572

CONCLUSIONS

Distinctive morphological features of thyme populations belonging to the four species found in the wild flora of Banat were identified, showing significant variability for the morphological parameters studied. These differences indicate a major influence of environmental factors in the phenotypic expression of the populations of this species. Statistical analyses allowed the identification of distinct groups with improvement potential. The analysis of plant height and inflorescence are the most influential traits, as taller plants also have longer inflorescences. Populations Th13, Th11, and Th10 can be considered for breeding programs when biomass production is the main objective. The analysis of leaf length indicates that leaf variation is not closely correlated with plant and flower height. This suggests that leaf size can evolve independently, a trait that may be valuable for cultivation. Populations Th9 and Th13, which have large leaves, are ideal for selecting high-yield varieties for biomaterial and essential oil production. The analysis of leaf width is important but does not determine the main variation. Although leaf width contributes significantly, it does not dominate variation in the same way as plant and inflorescence height. Populations Th5 and Th6, which have wider leaves and high genetic stability, can be considered for specific selection. The research results provide a solid scientific basis for breeding programs, optimizing the selection of the most valuable genetic lines. Correlating these results with genetic and chemical analyses is necessary to determine the most valuable spontaneous populations.

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