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MORPHOMETRIC CHARACTERISTICS OF *HELICHRYSUM ITALICUM* (ROTH.) G. DON FROM NORTHWESTERN COAST OF ISTRIA

Marin Tomičić
MSc, Teaching Assistant, Polytechnic of Rijeka, Vukovarska 58, 51000 Rijeka, Croatia;
e-mail: mtomicic@veleri.hr

Marko Magdić
BSc, Student, Polytechnic of Rijeka, Vukovarska 58, 51000 Rijeka, Croatia; e-mail: marko.bato@gmail.com

Martina Peršić
PhD, Lecturer, Polytechnic of Rijeka, Vukovarska 58, 51000 Rijeka, Croatia; e-mail: mpersic1@veleri.hr

Melita Zec Vojinović
PhD, Lecturer, Polytechnic of Rijeka, Vukovarska 58, 51000 Rijeka, Croatia; e-mail: mzecvo@veleri.hr

Slavica Dudaš
PhD, College Professor, Polytechnic of Rijeka, Vukovarska 58, 51000 Rijeka, Croatia; e-mail: sdudas@veleri.hr

ABSTRACT

Helichrysum italicum *(Roth.) G. Don is an aromatic perennial shrub widespread across the Mediterranean Basin. Plants from this species show a high degree of morphological variability. This research examines morphological variation inside the* H. italicum *population growing in natural conditions and the correlation between observed morphological traits. Morphometric characteristics of shrub and synflorescence were measured on* H. italicum *plants from the wild population on the northwestern coast of Istria, at eight locations a short distance apart. Results showed significant variation in the plants' height and diameter. Pearson's correlation analysis showed a strong positive correlation between the diameter and height of the shrubs. Hierarchical cluster analysis (HCA) indicates that the observed plants are divided into two clades, based on characteristics measured. Principal component analysis (PCA) showed clear separation of the plants into two groups. Observed characteristics accounted for nearly 76% of the total variance.*

Key words: Helichrysum italicum *(Roth.) G. Don, morphometric characteristic, variability, wild population*

1. INTRODUCTION

Helichrysum italicum (Roth.) G. Don is a perennial aromatic shrub that belongs to the *Helichrysum* genus (family *Asteraceae),* which includes approximately 600 species widespread all over the world. It is the most widespread of the 25 species from the *Helichrysum* genus, native to the Mediterranean area (Guinoiseau et al., 2013). Due to the fact that it is a xerophyte plant, *H. italicum* can grow in dry, sandy, and stony areas at a wide range of altitudes, up to 2200 m (Viegas et al, 2013). The species is widespread in the central Mediterranean area. It can also be found in scattered, isolated localities in western parts of North Africa and Cyprus (Galbany-Casals et al., 2006). Two subspecies, *H. italicum* subsp. *italicum* and *H. italicum* subsp. *microphyllum*, can be found in the Croatian coastal area (Ivanković et al., 2016).

H. italicum is a strongly aromatic perennial shrub or subshrub, 30 – 70 cm high, with herbaceous parts of vegetative and flowering stems 3.2 – 36.1 (40.5) cm long. The stems are arachnoid to densely tomentose, erect to ascendent-erect, leafy all their length, sometimes bearing axillary leaf fascicles. Basal and medial leaves of the vegetative and flowering stems are 2 – 37 mm in length and 0.4 – 1.8 mm in width, alternate, linear, with obtuse to rounded tips, sessile and subdecurrent on the stem; greenish on the upper side and silvery on the lower. Basal leaves of the flowering stems are patent to erecto-patent, more densely distributed in the basal part of the stem, decreasing in size towards the synflorescence. The synflorescence is terminal, corymbose, with 2 – 120 capitula that are round to oval, cylindrical to narrowly campanulate, heterogamous, with 8 – 39 yellow florets per capitulum (1 – 12 pistillate and 7 – 29 hermaphroditic florets) (Galbany-Casals et al., 2006; Herrando Moraira et al., 2016; Ninčević et al., 2019).

Yellow flowers that blossom between May and June give the *H. italicum* its ornamental value. Besides ornamental, it also has medicinal value due to its anti-inflammatory, anti-allergic, anti-viral, anti-microbial, insecticidal and repellent properties (Dimitrova, Nacheva, 2018; Guinoiseau et al., 2013; Ninčević et al., 2019). Its flowers and leaves are traditionally used in the treatment of a variety of health disorders such as allergies, colds, coughs, skin, liver, and gallbladder disorders, inflammation, infections, and sleeplessness (Viegas et al, 2013). Essential oils, extracted from all green parts of the plant, are used for cosmetic and medicinal purposes (Dimitrova, Nacheva, 2018). In some Mediterranean countries, *H. italicum* is used for flavoring food and aromatizing spirits, as a hop plant replacement in beer production, and as a source for seashore honey production (Appendino et al., 2015).

Due to its use in the pharmaceutical and cosmetic industries, along with the high market price, the demand for *H. italicum* has greatly increased in recent years, resulting in the higher exploitation of wild populations, especially on the East Adriatic coast. Consequently, there was a significant increase in plantation farming, often accompanied by inadequate planning and a poor knowledge base (Čagalj et al., 2019; Ninčević et al., 2019).

Plants from the *Helichrysum* genus show a high degree of morphological variability and phenotypic plasticity, which has led to problems with taxonomic classification (Guliani et al., 2016). The same is true for the *H. italicum* species, where subspecies classification differs between various authors (Ninčević et al., 2019). Due to morphological variability, the *H. italicum* subspecies often overlap

and morphologically intermediate specimens between subspecies are frequently found (Galbany-Casals et al., 2006; Galbany-Casals et al., 2011; Herrando Moraira et al., 2016). Polymorphism is mainly expressed in the habitus, leaves, and capitulum of plants. It can also have an effect on the chemical composition of the plant's extracts (Kramberger et al., 2021; Schipilliti et al., 2016).

There are several reasons for the *H. italicum* variability. The species is adapted to grow in diverse habitats throughout the Mediterranean Basin that have different climate conditions. Variation in climate, especially precipitation, may lead to variation in phenotypic traits (Martin, Puech, 2001). Its variability also appears to be, in part, genetically determined (Galbany-Casals et al., 2006). *H. italicum* has a large genetic diversity (Morone-Fortunato, 2010), as is expected in cross-pollinating plants, which may lead to inter and intrapopulation diversity.

The variability of the *H. italicum* can also be seen at the intrapopulation level. Galbany-Casals et al. (2011) found a considerable intrapopulation variation of *H. italicum* across analyzed characteristics, such as the height of the plant, leaf length and percentage of leaf fascicles/total leaves. Ninčević (2020) found a high diversity among 18 wild populations of *H. italicum* from the Croatian Adriatic coast. A major part (93.08%) of genetic diversity was caused by differences between specimens within populations, while the rest of the diversity was caused by differences between populations.

Due to the described variability of *H. italicum*, the aim of this research is to analyze morphological variation in a small population of the *H. italicum* from Istria, which grows in natural conditions, and to correlate its various morphological traits.

2. MATERIALS AND METHODS

The research was conducted in June of 2016 at the Mirna River mouth in the Antenal bay near Novigrad, on the northwestern coast of Istria (45.316336, 13.591523), at the beginning of the flowering stage. On an area that is approximately 0.75 ha in size the starting point was arbitrarily selected. From there eight microlocations were chosen at a distance of approximately 25 m from each other. The microlocations were labeled with numbers one to eight. All of the sampled shrubs had reached maturity.

To determine the morphometric characteristics of the shrubs, the following characteristics were monitored on site: shrub height (cm), shrub diameter (cm), number of the flowering stems, and the length of the flowering stems per shrub (cm). The height of each shrub was measured at eight approximately evenly spread-out points. The shrubs' diameter was measured in eight directions through the shrub's center. The number of flowering stems was counted for each shrub. The length was measured for the eight flowering stems on each shrub.

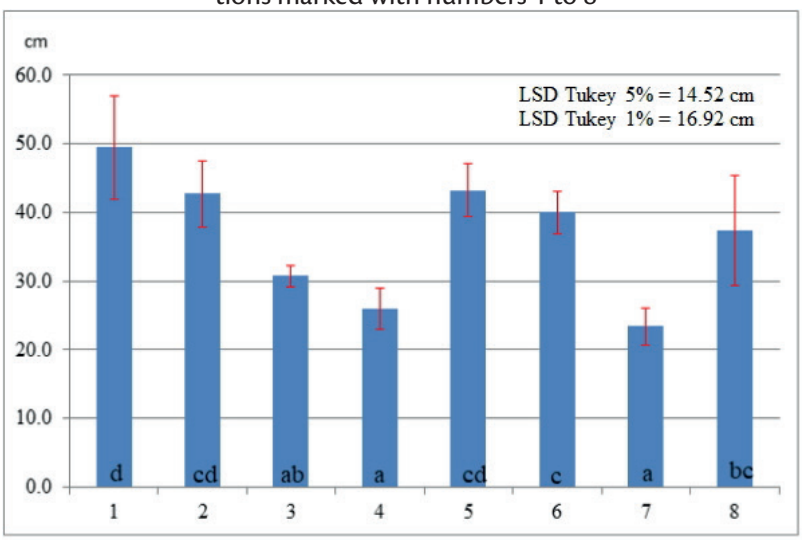
Weight of the flowering stem and synflorescence (g), number of peduncles per flowering stem, and number of capitula per peduncle were monitored to determine the morphometric characteristics of the generative stems. For these measurements, monitored specimens were harvested on 26 June 2016. After drying, the flowering stems were weighed. Afterwards, leaves and capita were removed from the stems and all three characteristics were weighed separately.

The collected data were analyzed using IBM SPSS Statistics, version 23.0. To determine if there were significant differences between the means, the one-way ANOVA was used. In post-hoc analysis, the means were separated by the least significant difference (LSD) at a 5% and 1% level of significance using Tukey's test. Monitored characteristics were correlated using Pearson's correlation coefficient. To evaluate whether the monitored morphometric characteristics could be useful in reflecting the relationships and variability between samples from eight close locations within the observed wild population, they were subjected to principal component analysis (PCA). Hierarchical cluster analysis (HCA) was applied to identify potential similarity of samples based on monitored characteristics to provide a better understanding of interrelationships between different shrubs with similar characteristics within the wild population. Squared Euclidean distance was used as a measure of dissimilarity between objects. Hierarchical clustering was performed using the Ward method in IBM SPSS Statistics.

3. RESULTS AND DISCUSSION

Shrub height results are shown in Figure 1. Shrub height varied between 23.4±2.7 and 49.5±7.6 cm, with an average of 36.6±9.1 cm. Statistical analysis with one-way ANOVA showed statistically significant differences in shrub height, with the LSD for Tukey test at a 5% significance of 14.52 cm. The obtained results are in accordance with the results of Galbany-Casals et al. (2011), who observed high variability in height of plants that grow at the same location, comparing *H. italicum* plants at 27 locations in Corsica. Ivanković et al. (2016) have shown that fertilization affects the height of *H. italicum* plants, so it is possible that soil composition varies within microlocations affecting plant height. However, height differences are more likely to be influenced by gene expression, given the high intrapopulation genetic diversity in *H. italicum* plants (Ninčević et al., 2021).

Figure 1. Height of the observed shrubs of *H. italicum* (cm) on eight different microlocations marked with numbers 1 to 8

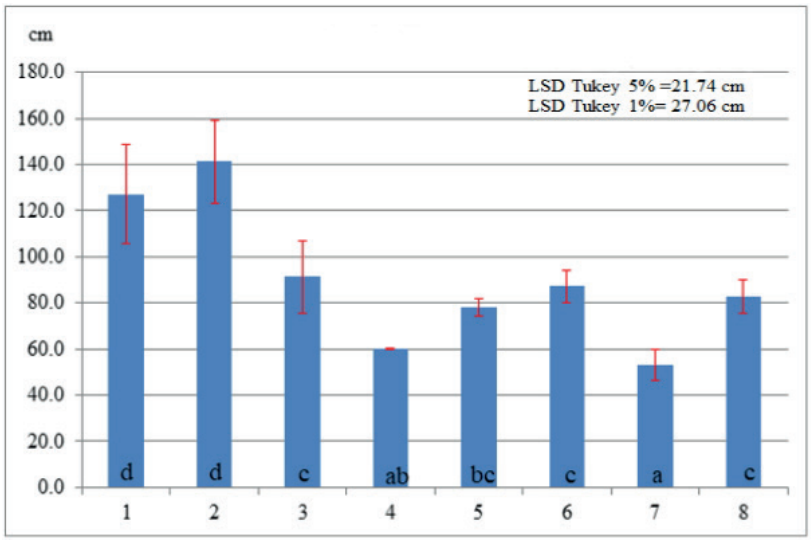


The different letters denote statistical differences according to the LSD Tukey test ($p < 0.05$). Error bars represent the standard error.

Source: Authors

Results from shrub diameter measurements are shown in Figure 2. Shrub diameter varied from 53.0±6.8 to 141.3±18.1 cm, with an average of 90.1±30.4 cm. Statistical analysis with one-way ANOVA showed statistically significant differences in shrub height, with the LSD for Tukey test at a 5% significance of 21.74 cm. These results, together with shrub height, show there is high variability in *H. italicum* shrub size in a small area with the same microclimatic conditions.

Figure 2. Diameter of the observed shrubs of *H. italicum* (cm) on eight different microlocations marked with numbers 1 to 8



The different letters denote statistical differences according to the LSD Tukey test ($p < 0.05$). Error bars represent the standard error.

Source: Authors

There was no statistically significant difference in the length of flowering stems on the samples observed even though they varied from 16.3±2.9 to 22.4±3.7 cm, with an average of 18.2±2.3 cm (data not shown).

Morphometric characteristics of flowering stems during the flowering stage are shown in Table 1. For the number of flowering stems, only one measurement was taken on each shrub. Therefore, no analysis of variance could be calculated. The number of flowering stems on shrubs varied from 119 to 261, with an average of 184.51. The number of flowering stems was not correlated with the shrubs' height or diameter. Some of the narrower shrubs had the largest number of flowering stems. As the flowering parts are an important source of *H. italicum* essential oil (Angioni et al., 2003), the number of the flowering stems may greatly affect essential oil yield. From the data obtained in this study, the number of flowering stems on *H. italicum* differs significantly between microlocations and individual plants in the natural population. This indicates that raising plantations with seeds collected from natural habitats is not recommended due to the consequent heterogeneity of plants. Therefore, *H. italicum* plantations should be raised with selected, vegetatively propagated material.

The average number of secondary peduncles was 10.25, while the average number of tertiary peduncles was 31.75. The former varied between 8 and 13, with the latter varying from 20 to 44. The average number of capitula on secondary peduncles was 21.14.

Table 1. Morphometric characteristics of flowering stems during the flowering stage

Shrub/ microlocation	Number of the flowering stems per shrub	Number of secondary peduncles	Number of tertiary peduncles	Number of capitula per secondary peduncle	Number of capitula per tertiary peduncle
1	185	10	20	15.4	7.7
2	186	8	26	27.1	8.3
3	119	10	33	22.0	6.6
4	186	12	44	23.8	6.5
5	126	11	35	20.0	6.3
6	261	9	31	20.0	5.8.
7	221	13	28	17.6	6.0
8	193	9	37	23.2	5.7
Mean	184.51	10.25	31.75	21.14	6.61
SD	46.25	1.56	6.85	3.46	0.87

Source: Authors

Dry weight of the *H. italicum* flowering stem, capitula, and leaves are shown in Table 2. The average flowering stem weight was 0.85±0.15 grams. It varied greatly, from 0.60 to 1.01 g. The largest part of the weight came from the capitula of the flowering stem, approximately 60%. The leaves and stem accounted for 14% and 26% of the flowering stem weight, respectively. This makes a flower/stem ratio of 1.5:1 at the beginning of the flowering stage. Similar results were obtained by Angioni et al. (2003) who obtained a 1:1 flower/stem ratio at the harvest before the flowering stage, 2:1 during the flowering stage, and 3:1 at the end of the flowering stage. As chemical compounds in leaves and flowers differ in content and concentration (Angioni et al., 2003; Marongiu et al., 2003), the flower/stem ratio could have an effect on the yield and composition of the *H. italicum* essential oil. The weight of the single capitulum varied between 6.6 mg and 8.7 mg, with a mean of 7.6±0.6 mg. One-way ANOVA showed there was no statistically significant difference in single capitulum weight between observed shrubs.

Table 2. Dry weight of the *H. italicum* flowering stem and flowering stem parts

Shrub/microlocation	Flowering stem weight (g)	Capitula weight (g)	Leaves weight (g)	Stem weight (g)	Single capitulum weight (mg)
1	0.84	0.49	0.12	0.23	7.9
2	0.96	0.61	0.11	0.24	7.2
3	0.98	0.62	0.11	0.24	8.7
4	1.01	0.57	0.17	0.26	7.9
5	0.60	0.36	0.09	0.15	6.6
6	0.78	0.50	0.09	0.20	8.1
7	0.96	0.54	0.15	0.26	7.4
8	0.66	0.35	0.10	0.19	7.5
Mean	0.85	0.50	0.12	0.22	7.4
SD	0.15	0.08	0.03	0.04	0.6

Source: Authors

All the observed characteristics were correlated using Pearson's correlation analysis. Because of the considerable number of analyzed characteristics, only statistically significant correlations are shown in Table 3. The analysis showed a strong positive correlation between the diameter and height of the shrubs, indicating that wider shrubs tend to have longer vegetative stems. However, there was no statistically significant correlation between the size (height and diameter) of the shrubs and the length or number of flowering stems. The weight of the leaves, stems, capitula, and single capitulum were correlated with flowering stem weight, as expected. There was also correlation between a single capitulum and total capitula weight. Stem weight was correlated with leaves and capitula weight, showing that plants with larger leaves tend to have larger stems and capitula. Larger leaves consequently lead to increased photosynthesis and carbon assimilation rate resulting in a larger source of nutrients for the growth of the generative parts of the plant (Bonan, 1993). There was no statistically significant correlation between other observed characteristics.

Table 3. Pearson correlation of the monitored characteristics of *H. italicum*

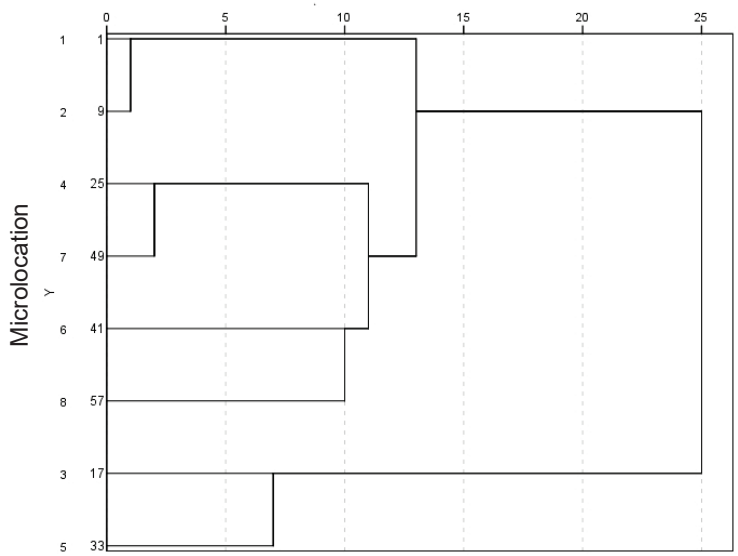
		Shrub height	No. of capitula per tertiary peduncle	Capitula weight	Leaves weight	Stem weight	Single capitulum weight
Shrub diameter	Pearson correlation Sig. (2-tailed) N	,652** ,000 64	,737* ,037 8				
Flowering stem weight	Pearson correlation Sig. (2-tailed) N			,958** ,000 8	,719* 0,44 8	,938** ,001 8	,727* ,041 8
Capitula weight	Pearson correlation Sig. (2-tailed) N					,814* ,014 8	,739* ,036 8
Leaves weight	Pearson correlation Sig. (2-tailed) N					,819* ,013 8	

* Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)

Source: Authors

The HCA resulting dendrogram shows a clustering of compared samples into two main clades (Figure 3), indicating separation of *H. italicum* shrubs at microlocations 1, 2, 4, 7, 6, 8, and 3, 5. The characteristics responsible for the differentiation of the second clade were the height of the shrub and the number of the flowering stems. All the other morphometric characteristics provided the separation of shrubs from the other microlocations to the first clade and to the differentiation inside of the first clade. Considering that microlocation could not have had a very significant effect on morphological characteristics, it is very probable that the differences in morphology were influenced by *H. italicum* genetic variability (Galbany-Casals et al., 2011; Ninčević, 2020).

Figure 3. Dendrogram depicting grouping of *H. italicum* plants based on monitored characteristics

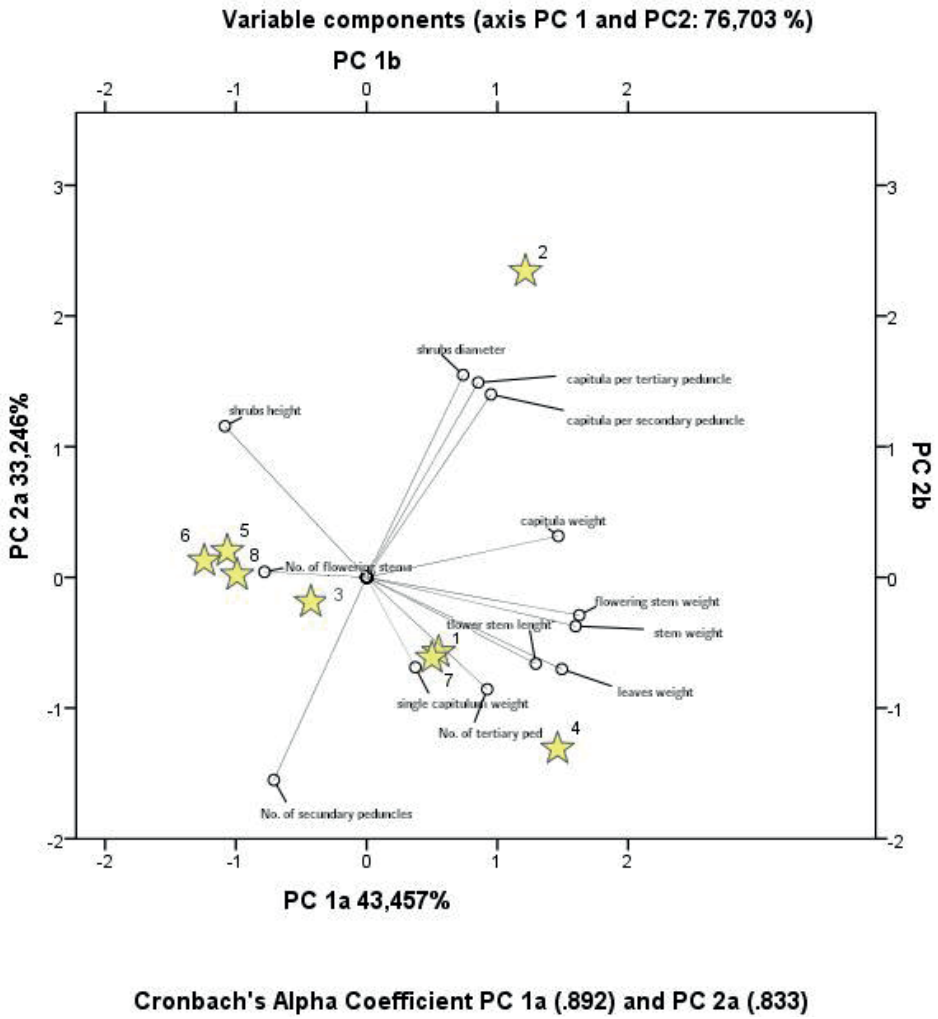


Squared Euclidean distance was used as a measure of dissimilarity between objects.

Source: Authors

The biplot constructed by two principal components showing microlocations and morphometrical traits is presented in Figure 4. Principal component 1 (PC1) and principal component 2 (PC2) provided significant indications of 75.77% of the total variance in the data and showed clear separation of the samples into two groups.

Figure 4. Principal Component Analysis of the monitored morphometric characteristics of *H. italicum* plants



Vectors indicate the direction and strength of each morphometric character relative to the overall distribution. Stars correspond to the eight microlocations.

Source: Authors

The first group consisted of samples from microlocations 6, 5, 8, 3 with morphometric characteristics the height of the shrub and number of the flowering stems, which contributed significantly to the intergroup difference. The other eleven morphometric characteristics were distinctive traits for clustering samples 1, 7, 4, and 2 in the second group. Parameters that distinguish sample 2 from the other samples in the research are the diameter of the shrub and number of capitula per secondary and tertiary peduncle. These results once more confirm high genetic variability among individual plants of *H. italicum* in the wild population. High genetic variability in wild populations is

common in cross-pollinating plants from family *Asteraceae*, such as *Tanacetum vulgare*, *Tanacetum cinerariifolium*, *Senecio vulgaris*, *Chamomilla recutita*, and *Serratula lycopifolia* (Cieslak, 2013; Ebadi, Eftekharian, 2021; Grdiša et al., 2014; Mežaka et al., Wolf et al., 2012).

4. CONCLUSION

The *H. italicum* plants show a high degree of morphological variation even in small areas, growing a few meters apart. Variation is seen in plant height, width, and the number of the flowering stems, but no variation was identified in the length of the flowering stem. Plant height and number of flowering stems contribute greatly to variation between separate groups. This can be of interest to growers and nurseries as the majority of plant material for propagation is selected from wild populations. As morphological variation may lead to differences in plant extracts, further research could be undertaken to find out how different morphological characteristics correlate with plant extracts.

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MORFOMETRIJSKA OBILJEŽJA *HELICHRYSUM ITALICUM* (ROTH.) G. DON SA SJEVEROZAPADNE OBALE ISTRE

Marin Tomičić
Mag. ing. hort., asistent, Veleučilište u Rijeci, Vukovarska 58, 51 000 Rijeka, Hrvatska;
e-mail: mtomicic@veleri.hr

Marko Magdić
Bacc. ing. agr., student, Veleučilište u Rijeci, Vukovarska 58, 51 000 Rijeka, Hrvatska;
e-mail: marko.bato@gmail.com

Martina Peršić
Dr. sc., predavačica, Veleučilište u Rijeci, Vukovarska 58, 51 000 Rijeka, Hrvatska; *e-mail:* mpersic1@veleri.hr

Melita Zec Vojinović
Dr. sc., predavačica, Veleučilište u Rijeci, Vukovarska 58, 51 000 Rijeka, Hrvatska;
e-mail: mzecvo@veleri.hr

Slavica Dudaš
Dr. sc., profesorica visoke škole, Veleučilište u Rijeci, Vukovarska 58, 51 000 Rijeka, Hrvatska;
e-mail: sdudas@veleri.hr

SAŽETAK

Helichrysum italicum *(Roth.) G. Don je višegodišnji aromatični grm rasprostranjen diljem Mediterana. Biljke iz ove vrste pokazuju visok stupanj morfološke varijabilnosti. U istraživanju se ispituju morfološke varijacije unutar prirodne populacije* H. italicum *i korelacije između promatranih morfoloških osobina. Morfometrijske karakteristike grma i cvata* H. italicum *mjerene su na osam lokacija na maloj udaljenosti u prirodnoj populaciji sa sjeverozapadne obale Istre. Rezultati su pokazali da postoje značajne varijacije u visini i promjeru grmova. Na temelju promatranih obilježja hijerarhijska klasterska analiza (HCA) je podijelila proučavane grmove u dva klastera. Analiza glavnih komponenti (PCA) pokazala je jasno razdvajanje biljaka u dvije skupine. Promatrana obilježja objasnila su gotovo 76% ukupne varijance.*

Ključne riječi: Helichrysum italicum *(Roth.) G. Don, morfometrijska obilježja, varijabilnost, prirodna populacija*