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VARIABILITY STUDIES OF LOCAL POPULATIONS OF *ANACAMPTIS LAXIFLORA* SSP. *ELEGANCE* LAM. SSP. *ELEGANS* (HEUFFEL) SOÓ FROM THE OLTENIA REGION, ROMANIA

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Abstract

Orchids are some of the most popular flowers. They are appreciated for their beauty and the varied range of colors and shapes Anacamptis laxiflora ssp. elegans is a "gem of the Romanian landscape" that grows in meadows, forest edges and clearings. The unique characteristics of this orchid species are to be appreciated because of its morphological complexity. Comparative analysis of plant morphological traits indicates that there are significant differences between natural populations of this terrestrial orchid species. A special priority should be given to this type of orchid because they are very common in the habitats of the Oltenia region and have large populations. Their monitoring in the context of global warming is also necessary, and one of the conservation priorities concerns the protection of the habitats in which they occur. Regarding the analysis of character variability according to plant density, it influences the average plant length, stem diameter and leaf number/pl., so the values recorded for these characters have significant differences compared to the values recorded by plants at lower densities of individuals.

Key words*: Anacamptis laxiflora ssp. elegans, morphological characters, population, variability.*

INTRODUCTION

Orchids are among the most beautiful wonders of nature and are considered splendors of the plant world, growing in the warm areas of the planet, with very varied shapes and colors, as well as strongly scented.

Family *Orchidaceae* Juss. is the second largest family of the Plant Kingdom, after the *Asteraceae* Family (Bercht. & J. Presl), with about 750-850 spontaneous and cultivated genera, respectively 20,000-35,000 species and over 120,000 hybrids (Tomescu, 2018). The same author claims that orchids are found on all continents, except Antarctica, and the area of growth and distribution stretches between 54^0 south latitudes and 68^0 north latitudes, from the low plains, at altitudes of 2,000 and even 4,000 m, in territories from America, Asia, Africa, Australia, Oceania and Europe.

In our country there are orchids with smaller and more mysterious inflorescences and with very varied shapes. According to Ciocarlan (2009), the *Orchidaceae* family in Romania is represented by 58 species while Sârbu (2013), says that there are 25 genera of orchids, with 60 species, which are sometimes divided into subspecies according to the color of the corolla, the sizes of the various organs, etc. The best represented genera are: *Orchis, Dactylorhiza, Epipactis* with over 5 species each. Even within a genus, the shape of the flower can vary quite a bit, making it easy to differentiate the species. We observe this in the case of the genus *Orchis* (*Orchis militaris, Orchis purpurea, Anacamptis coriophora, Anacamptis laxiflora* ssp*. elegans, Orchis tridentata, Orchis ustulata*).

Wild orchids in Romania can be found on all landforms, from the highest rocky peaks with an altitude of 2,400 meters, to the sand dunes on the Black Sea coast. They grow on any type of substrate, covering the whole range of humidity, from the driest to the wettest, marshes, swamps or periodically flooded areas. Some orchids are sun-loving species, growing in full sun in short grass and hay meadows or meadows, while others are shade-loving and can grow hidden in the darkest woods.

Compared to other European countries where some species are very rare or have very low populations, in Romania most species are still well represented by numerous and relatively constant populations. They are quite special from a botanical point of view.

Due to the lack of education, in terms of environmental protection, many locations with native orchids have been destroyed, thus becoming one of the most endangered plant groups, both in our country and even worldwide. That why most of the orchid species from the Romania's flora are included in "The Red List of superior plants in Romania" as rare or in danger (Oltean et al., 1994). Orchids are mostly found in tropical areas, but Romania is also a favorable area for the development of orchids.

Long-term monitoring programs of orchid populations have shown that their dynamics are highly dependent on prevailing weather conditions, suggesting that changes in climatic conditions can have far-reaching effects on population dynamics and thus on orchid distribution (Evans et al., 2020).

Thus, there are limited studies related to the effects of site factors on the morphological and anatomical characters of the *Orchidaceae* family. The present study was carried out to bring new data on the variability of *Anacamptis laxiflora* ssp. *elegans* identified in the Băile Govora resort area. The areal is placed between Govora-Village and Bunești, Vâlcea county, Oltenia region, Romania. This is a sporadically encountered species and currently is charted only in a few sites in Oltenia, being subject to threats and anthropogenic pressures.

The species was identified in swampy meadows, in the meadow area of Govora River, between Bunești and Govora-Village. Following the studies, it was found that populations with numerous and very vigorous individuals were identified in the floristic composition of two plant communities: ***Poëtum trivialis*** Soó 1940 (Syn. *Agrosteto-Poëtum trivialis* Soó 1938, *Trifolio- Poëtum trivialis* Soran 1962) (Resmeriţă et al., 1971) and ***Anthoxantho-Agrostetum capillaris*** (Sillinger 1933, Jurko 1969).

1. *Poëtum trivialis* Soó 1940 (Syn. *Agrosteto-Poëtum trivialis* Soó 1938, *Trifolio- Poëtum trivialis* (Soran 1962; Resmeriţă et al., 1971).

Phytocoenoses of mountain sedge grow on lands with excess moisture in stream meadows, swampy or temporarily flooded places. It is a plant community that is less widespread in the Govora basin, being found in the hilly floor, within the radius of the Bunești locality. In the floristic composition of the phytocoenoses of this plant community, the analyzed species was found only in two.

In the floristic composition of the observed phytocoenoses, the following species enjoy a high constancy: *Festuca pratensis*, *Galium palustre*, *Oenanthe banatica*, *Ranunculus repens*, *Agrostis stolonifera*, *Filipendula vulgaris*, *Lythrum salicaria*, *Juncus inflexus*, *Symphytum officinale*, *Equisetum palustre*, *Poa pratensis*, *Anacamptis laxiflora* ssp. *elegans* etc.

2. *Anthoxantho-Agrostetum capillaris* Sillinger 1933, Jurko 1969.

Field grass meadows with sedge usually establish themselves on cleared, flat or gently sloping land in the hilly and montane floor, preferring typical eutricambosols or luvosols. In the Govora basin, such phytocoenoses were analyzed near the town of Govora-Village, at the border of the meadow forest habitats built by *Alnus gutinosa*. Phytocoenological observations for the knowledge of the vegetation in the Pesceana river basin, Vâlcea county, carried out by Măceșeanu & Făgăraș (2023) allowed the identification of 9 types of habitats of community interest, including the one referring to alluvial forests of *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion, Alnion incanae, Salicion albae*).

In the floristic composition of the phytocoenoses analyzed, along with the edifying species of the plant community, there are also numerous species belonging to the class *Molinio-Arrhenatheretea* such as: *Briza media*, *Trifolium repens*, *T. pratense*, *Luzula campestris*, *Vicia cracca*, *Poa pratensis*, *Holcus lanatus, Anacamptis laxiflora* ssp. *elegans* (Figure 1), etc. Following the analyzes we carried out in the field, the species from the floristic composition of the phytocoenoses in the studied area are presented in Table 1.

Figure 1. *Anacamptis laxiflora* ssp. *elegans* in the Govora Stream basin (foto. M. Niculescu)

Table 1. The floristic structure of *Anthoxantho-Agrostetum capillaris* Sillinger 1933, Jurko 1969 plant community

No. of relevée	1	2	3	4	5	6	7	8	K
Altitude (x 10 m.s.m)	35	35	38	38	40	38	40	35	
Coverage of herbacaeous layer (%)	100	95	100	100	100	100	95	95	
Area (m^2)	100	100	100	100	100	100	100	100	
Character species of plant community									
Anthoxanthum odoratum	4	4	3-4	4	4	4-5	4	5	V
Agrostis capillaris	1	1	2	1	2	+	1	+	V
Molinietalia									
Agrostis stolonifera	+	+	-	-	-	-	+	+	III
Trifolium hybridum	-	-	+	-	+	+	-	-	II
Alopecurus pratensis	+	-	-	+	-	+	-	-	II
Dactylorhiza maculata	+	+	-	-	-	-	+	+	III
Myosotis scorpioides	-	-	+	-	+	+	-	-	II
Juncus articulatus	+	-	-	+	-	+	-	-	II
Juncus conglomeratus	+	+	-	-	-	-	+	+	III
Anacamptis laxiflora ssp. *elegans*	1	1	+-1	1-2	1	1-2	+-1	+-1	V
Molinio-Arrhenatheretea									
Briza media	+	+	+	+	+	+	+	+	V
Holcus lanatus	-	+	+	+	+	+	+	-	IV
Poa pratensis	-	+	-	+	-	-	+	+	III
Trifolium pratense	+	-	+	+	-	+	-	-	III
Trifolium repens	+	+	-	+	+	+	+	+	IV
Vicia cracca	-	-	+	-	+	-	-	-	II
Festuca pratensis	-	+	+	-	-	-	+	+	III
Centaurea austriaca	+	-	-	+	-	+	-	-	II
Ranunculus acris	-	+	+	-	+	+	+	+	IV
Dactylis glomerata	+	+	-	+	-	+	+	+	IV
Poa trivialis	-	+	+	-	-	-	+	+	III
Stellaria graminea	-	+	-	+	-	-	+	+	III
Luzula campestris	+	+	-	+	+	+	+	+	IV
Ononis arvensis	+	-	+	-	-	-	+	-	II
Polygala vulgaris	+	+	+	+	+	+	-	-	IV
Lychnis flos-cuculi	+	-	+	+	-	+	-	-	III
Achillea millefolium	+	-	+	-	-	-	+	-	II
Leontodon autumnalis	+	-	-	+	-	+	-	+	III
Leucanthemum vulgare	+	+	+-1	1	1	+	+	+	V
Lychnis flos-cuculi	+	-	+	+	-	+	-	-	III
Arrhenatheretalia									
Cynosurus cristatus	1	+	+	+	+	1	+	+	V
Rhinanthus rumelicus	+	-	+	+	+	+	+	-	IV
Carum carvi	+	-	-	+	-	+	-	-	II
Campanula patula ssp. patula	+	+	-	+	+	+	+	+	IV
Festuco-Brometea									
Carex caryophyllea	-	+	-	-	+	-	+	+	III
Galium verum	+	+	+	+	+	+	+	+	V
Danthonia provincialis	+	-	+	-	+	+	+	+	IV
Dianthus carthusianorum	+	+	+	+	+	+	+	+	V
Thymus pulegioides	-	+	-	-	+	-	+	+	III
Potentilla argentea	-	-	-	-	+	-	-	+	II
Prunella laciniata	-	-	-	-	+	-	-	+	II
Coronilla varia	+	+	+	+	+	+	+	+	V
Variae Syntaxa									
Vicia tetrasperma	-	-	-	-	+	-	-	+	II
Veronica chamaedrys	-	-	-	-	+	-	-	+	II
Vicia sativa	+	+	-	-	-	+	+	+	III
Rorippa sylvestris	+	+	-	-	-	-	+	+	III
Lychnis viscaria	-	+	-	-	-	-	+	+	II
Prunella vulgaris	+	+	-	-	-	-	-	+	II
Viola collina	+	+	-	-	-	+	+	+	III
Rumex crispus	+	+	-	-	+	-	+	+	III
Equisetum arvense	+	-	-	-	-	+	-	+	II
Cruciata laevipes	+	+	-	-	-	+	+	+	III

Place and data of the relevés: Govora Valley, Govora-Sat, 2.V.2022; 25.IV.2022
Source: performed by the authors based on the own research

MATERIALS AND METHODS

An investigation was carried out to study the variability of the morphological characteristics of *Anacamptis laxiflora* ssp. *elegans* population identified in Băile Govora area. The climate of the experimental site is mild, semi-Mediterranean. The air temperature shows small seasonal and diurnal variations, the lowest being in the cold season, when average monthly temperatures are $+0.2^0$C in December, -2.8^0C in January, $+9^0$C in February.

The heaviest winter days occur in the first half of January and 28 days of genuine winter are recorded annually. The record minimum temperature in the cold season was recorded on 11.02.1929, when it reached the value of -27.4^0C.

The warm season lasts more than 6 months, from April to October; the days with the maximum temperature above 25^0C start from April (9 days) and end in October (3 days) so that during the summer there are 88 days with a temperature of 25^0C. Tropical days with temperatures of 30^0C and rarely above, they meet in June, July and August, totaling 23 days. Characteristics are the absence of heat during the night, and autumns are warmer than springs. In this sense, the measurements showed values of the insolation coefficient that vary from 0.20 in the cold season to 0.54 in the warm season.

Atmospheric precipitation falls within normal annual limits, determining a pluviometric regime favorable to the development of vegetation. The most days with precipitation were recorded in the months of May - June (13 days) and in August - September, 5 days each. Massive downpours, followed by local floods, were recorded at long time intervals (15-25 years), these phenomenon being closely related to the global ones. The electrical state of the atmosphere has a potential of 42 V/m, the electrical gradient having values equal to or greater than 100 V, only when the weather is unstable and with electrical manifestations (https://www.primaria-govora.ro/clima).

In this study, the morphological characters were: plant height, stem diameter, leaf number, leaf width, leaf length, ratio of leaf width/length, inflorescence length, flower number, flower width, flower length and ratio of flower length/width. The analyzed plants were kept under careful observation during the determinations. Plants were verified using the classical determination method. For the identification were used Ciocârlan (2009) and Sârbu et al. (2013).

The obtained results were calculated using analyses of variance method. Statistical analyses were executed in Excel program. The significance of the difference was evaluated by the least significance difference (LSD 5%).

RESULTS AND DISCUSSIONS

The present study was conducted during the period from March to September 2022. The Băile Govora Resort area appears to be a suitable habitat where the distribution of orchid populations increases under conditions of mild climate change. Ilie et al. (2023) reported a decrease in the number of dryland loving species, largely represented by annual plants growing in open areas.

In Tables 2 and 5 the variability of the morphological characters of plants, respectively flowers, is presented for *Anacamptis laxiflora* ssp. *elegans*, depending on the plants density.

The obtained results show that the average height of the plant varied from 23.54 cm at 4 pl./m^2 to 73.99 cm at densities higher than 35 pl./m^2. The stem diameter varied from 3.72 mm at 25 pl./m^2 to 6.25 mm at densities higher than 35 pl./m^2. The average number of leaves presented smaller limits, between 6.67 at 4 pl./m^2 and 7.63 at densities higher than 35 pl./m^2. The length and width of the leaves varied from 15.8 mm at more than 35 pl./m^2 to 29.4 mm at 4 pl./m^2, respectively 2.6 mm at 25 pl./m^2 to 3.6 mm at 4 pl./m^2.

Intermediate values to those obtained in this research for plant height and stem diameter were reported by Anghelescu et al. (2020) for a population of 7 hybrids between two highly divergent species, *Anacamptis coriophora* (from *Anacamptis coriophora* group) and *Anacamptis palustris* subssp. *elegans* (from *Anacamptis palustris* group), species which are taking part from the same family as *Anacamptis laxiflora* ssp. *elegans*. Also, Niculescu et al. (2023) concluded that at *Anacamptis coriophora*, plant height is a character influenced by humidity, light and location.

For the character length and width of the leaf, values of 20 and 2.0 cm, respectively, were mentioned by Şeker (2022) for the taxon *Anacamptis laxiflora* (Lam.) R.M. Bateman, Pridgeon & M.W. Chase identified in a habitat at the edges of coniferous forests and meadows. Related to the analysis of character variability according to plant density, it influences the characters plant length, stem diameter and leaf number, so the values recorded for these characters record significant differences compared to the values recorded by plants at lower densities of individuals.

For the character leaf number and leaf width, the highest values are recorded for plants at low densities, these values recording significant differences compared to the values recorded for these characters at plants at high densities.

As variability coefficient point of view, the most variable character was leaf length followed by leaf width.

Table 2. Variability of *Orchis laxiflora* ssp. *elegans* morphological characters depending on plants density

Character / Statistical Index	Plant length (cm)		Stem diameter (mm)		Leaf number		Leaf length (mm)		Leaf width (mm)	
	Value	C%	Value	C%	Value	C%	Value	C%	Value	C%
4 pl./m^2	23.54±3.24^c	13.78	4.36±0.56^b	12.75	6.67±0.41^b	6.14	29.4±5.03^a	25.93	3.6±0.55^a	15.21
15 pl./m^2	34.30±1.14^b	3.31	4.09±0.78^b	18.97	6.96±0.49^b	7.04	27.0±7.00^a	24.89	3.2±0.84^a	21.07
25 pl./m^2	39.73±2.83^b	7.13	3.72±0.62^b	16.74	7.52±0.27^a	3.55	20.6±5.13^b	23.43	2.6±0.55^b	29.88
>35 pl./m^2	73.99±15.59^a	21.08	6.25±1.37^a	21.92	7.63±0.18^a	2.39	15.8±3.70^c	17.11	2.8±0.84^b	26.15
LSD 5%	7.11		1.08		0.351		4.22		0.56	

Related to the analysis of the trend model between the character average plant length and plant density, a coefficient of correlation of 0.862 was calculated, a value that demonstrates a very strong link between these two indices. In other words, the increase in plant density brings with it an increase in value for this character. The coefficient of determination had a value of 0.743, which implies that the trend model calculated based on the simple linear equation is a valid one. Thus, the value of the coefficient of regression calculated was equal to 1.513, which implies that when the plant density increases by individual/m^2, the average plant height/m^2 increases by 1.5 cm (Figure 2).

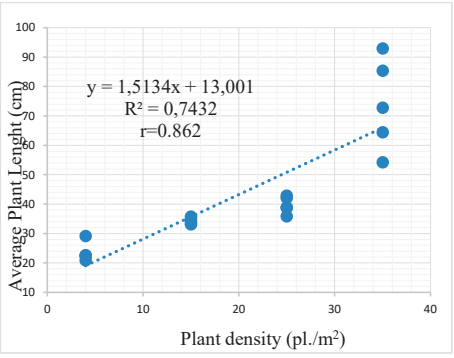

Figure 2. Correlation and trend pattern analysis between average plant length and plant density

As concern the analysis between the average stem diameter and plant density, the correlation coefficient was of 0.461 which indicates that there is no strong connection between these two indices (Figure 3). Between the character average leaf number/pl. and plant density, the coefficient of correlation of 0.755 demonstrates a strong link between these two indices. In other words, the increase in plant density brings with it an increase in value for this character. The coefficient of determination had a value of 0.5706, which means that the trend model calculated based on the simple linear equation is not a valid one (Figure 4). For the character leaf length and plant density, the coefficient of correlation of 0.732 demonstrates a strong link between these two indices, which indicate that the increase in plant density brings with it an increase in value for this character. The coefficient of determination had a value of 0.536, which implies that the trend model calculated based on the simple linear equation is not a valid one (Figure 5).

Related to the analysis of the trend model between the character average leaf width and plant density, a coefficient was calculated. of correlation of -0.456, a value that proves that there is no strong connection between these two indices (Figure 6).

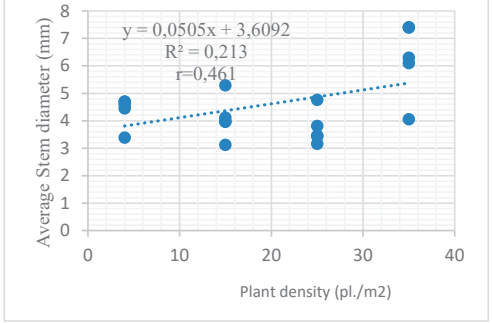

Figure 3. Correlation and trend pattern analysis between average stem diameter and plant density

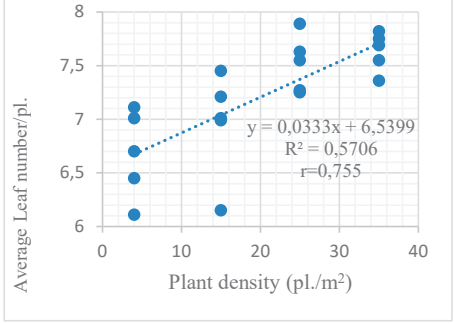

Figure 4. Correlation and trend pattern analysis between average leaf number/pl. and plant density

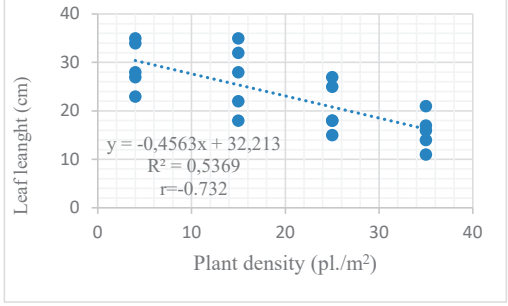

Figure 5. Correlation and trend pattern analysis between leaf length and plant density

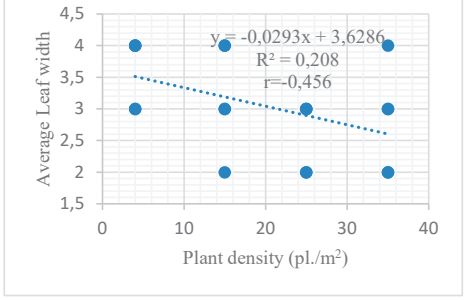

Figure 6. Correlation and trend pattern analysis between leaf length and plant density

Regarding the Principal Components Analysis, for the plant's morphological characteristics the first two components explain 93.918 % of total variance, the first component registering 56.007 % and the second component 37.911% (Table 3).

Table 3. Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.800	56.007	56.007	2.800	56.007	56.007
2	1.896	37.911	93.918	1.896	37.911	93.918
3	0.304	6.082	100.000			
4	4.491E-016	8.983E-015	100.000			
5	-3.281E-017	-6.562E-016	100.000			
Extraction Method: Principal Component Analysis.						

Related to the first component, the studied characters that obtain high positive value are average leaf length, average stem diameter and average leaf length. The first component can also be named plant capacity to have well developed stem on which long leaves can be inserted. For the second component, high positive values are obtained by average leaf width. The second component can be named the ability of plants to develop leaves with large width (Table 4).

Table 4. Component Matrix-Extraction Method: Principal Component Analysis, 2 components extracted

Character \ Comp. value	Component	
	1	2
Average plant length	0.879	-0.476
Average stem diameter	0.989	0.019
Average leaf number	0.523	-0.800
Average leaf length	0.781	0.598
Average leaf width	0.409	0.819

The four groups based on the values of the two components were the following (Figure 7):
- the variant with the first component positive and the second one negative is the one with more than 35 pl./m^2 density. This variant has high values for the first component;
- the variants with both negative components are the ones with 15 and 25 pl./m^2 density;
- the variant with the first component negative and the second one positive is the one with 4 pl./m^2 density.

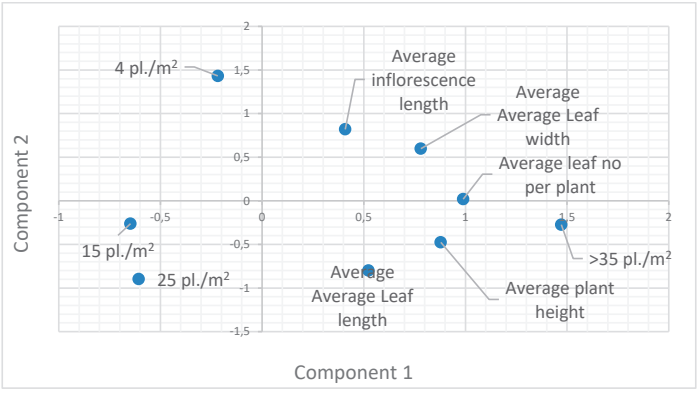

Figure 7. Classification of variants according to the score of the two components

Average length of the inflorescence varied from 98 mm at 25 pl./m^2 to 182 mm at 4 pl./m^2. The number of flowers varied from 15.6 at 4 pl./m^2 to 47.8 at over 35 pl./m^2. The length of the flowers varied more than their width (Table 5). Lower values for flower length and width than in this research were reported by Erzurumlu et al. (2018) in a phylogenetic relationship among fourteen different tuber-producing orchid species and after analyzing phenotypic and genetic variation within and among the natural population through fifteen morphometric traits and ten random amplified polymorphic DNA (RAPD) primer combinations.

The development of orchid populations is high in some sunny places and low in others. However, we appreciate this species as showing adaptive variation. These results indicate the direct and indirect effects of climate change on population viability. They are consistent with the data obtained by Sletvold et al. (2013). Therefore, it is recommended that the conservation studies of this orchid species be carried out on a limited geographical unit.

A. laxiflora is known as having a dense inflorescence and deeply lobed lip. The occasional hybrids between other members of the subgroup are often very robust, sometimes attaining 135 cm in height, with many-flowered inflorescences (Wood & Ramsay (2004). In another type of orchid, *Spiranthes spiralis,* average length of the inflorescences is strongly influenced by the height of the plant, meaning that tall plants usually have longer inflorescences (Niculescu et al., 2024).

Table 5. Variability of *Anacamptis laxiflora* ssp. *elegans* morphological flower traits depending on density

Character / Statistical Index	Inflorescence length (mm)		Flower number		Flower length (mm)		Flower width (mm)	
	Value	C%	Value	C%	Value	C%	Value	C%
4 pl./m^2	182±37.4^a	20.55	15.6±2.22^d	14.23	37.6±5.29^a	14.07	4.4±0.62^a	14.09
15 pl./m^2	135±22.5^b	16.67	26.8±4.56^c	17.01	29.2±4.55^b	15.58	3.8±0.59^b	15.53
25 pl./m^2	98±15.8^c	16.12	33.17±5.11^b	15.41	18.67±3.22^c	17.25	2.63±0.33^c	12.55
>35 pl./m^2	70±10.21^d	14.59	47.8±8.25^a	17.26	10.2±1.59^d	15.59	2.52±0.29^c	11.51
LSD 5%	11.78		7.81		6.81		0.43	

In this study, the dendrogram of the plants was made according to the density of the plants, based on the analyzed characters of the flower. Thus, the plants at the highest densities are the closest in terms of flower characters, which in turn are most similar to the plants at a density of 15 pl./m^2. The plants with the lowest density, in terms of flower characters, are the most different from the other plants (Figure 8).

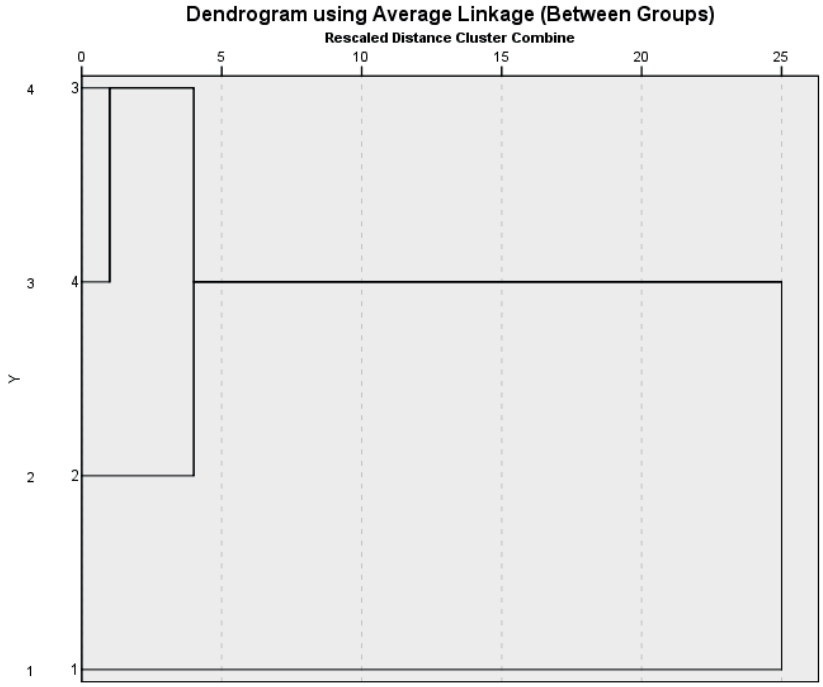

Figure 8. Dendrogram of the plants according to density

CONCLUSIONS

Anacamptis laxiflora ssp. *elegans* is a rare species and is recommended to be admired without being collected. We must let wild orchids grow in their natural habitats, otherwise we will only have artificially grown orchids.

From a cenotaxonomic point of view, this taxon was identified in the floristic composition of two plant communities: ***Poëtum trivialis*** Soó 1940 (Syn. *Agrosteto-Poëtum trivialis* Soó 1938, *Trifolio- Poëtum trivialis* (Soran 1962, Resmeriţă et al., 1971) and ***Anthoxantho-Agrostetum capillaris*** Sillinger 1933, Jurko 1969.

The abundance-dominance of the taxon within the analyzed phytocoenoses varies mainly according to the soil moisture level, being quite high, and the populations with increased density and vigor being found in the meadows of the Govora basin in the plant community ***Anthoxantho-Agrostetum capillaris*** Sillinger 1933, Jurko 1969.

The density of plants per square meter is a determining factor in terms of their evolution and development. Thus, plants at high density are the tallest compared to plants at lower densities, a situation that is also found in the analysis of other characters such as stem diameter or leaf no./pl. Also, low density positively influences average leaf length and leaf width.

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