Analysis of the variability of the quantitative traits of the generative shoot of two white-flowered carnation species (*Dianthus awaricus, Dianthus fragrans*) under the conditions of Dagestan

Ruslan Osmanov*, *Ziyarat* Guseynova, and *Dzhalaludin* Anatov

Mountain Botanical Garden DFRS RAS, 367000, str. 75 Yaragskogo, Makhachkala, Russian Federation

Abstract. The paper presents the results of assessing the quantitative traits of generative shoots of two white-flowered carnation species from four geographically isolated points: *Dianthus awaricus* (Oboda, Khvarada) and *Dianthus fragrans* (Khindakh, Charoda). Comparative analysis showed that, in general, the average values of most morphological characters are higher in *D. fragrans*, and the width of the petal and the number of lobe denticles are higher in *D. awaricus*. According to the results of two-way analysis of variance, the greatest contribution to the differentiation of species according to the relative components of variance (h^2) is made by the traits "number of serrature on a petal" (73.2%), "cup length" (64.7%) and "petal index" (45.8%); unreliable traits (0.0–1.2%) – "flower length", "flower diameter", "flower mass" and "internode index". Growing conditions significantly affect the characteristics of the apical flower: length (53.6%), diameter (40.1%) and its mass (46.3%). Squares of Mahalanobis distances showed the similarity of *D. fragrans* cenopopulations and significant isolation in *D. awaricus*, which is possibly associated with significant differences in the complex of ecological-cenotic conditions and possible microevolutionary processes occurring in highly isolated populations. Between the two species, there is a clear separation in space of the two roots of canonical analysis and complete self-identification according to the classification matrix. Such studies contribute to the identification of similarities and differences between closely related species in contrasting environmental conditions.

1 Introduction

Currently, for the Russian part of the Caucasus, 32 species of the genus *Dianthus* L. are listed, belonging to five sections. Among them, 10 endemics with different types of habitats were noted. The *Dianthus* section is dominant in this study area in terms of the number of species, which includes 12 species (37.5%) [1].

*Corresponding author: ru.osmanov@mail.ru

In the North Caucasus, the genus *Dianthus* is widespread in the high mountain areas of the Main and Side Ridges, especially in the subnival zone, to a lesser extent in the Rocky Ridge area along the southern slopes, which are a series of rocky terraces [2]. Anna Lukyanovna Kharadze was engaged in the study of highly specialized problems of florogenesis of the highlands of the Greater Caucasus, as well as the description of the Caryophyllaceae family, including the genus *Dianthus* [3, 4].

Based on the study of the complex of morphological traits of the annual shoot, the peculiarities of the life cycle of plants, the similarities and differences between closely related species in contrasting environmental conditions, as well as the ability to self-renewal in natural populations can be revealed [5–7].

The study of morphological traits helps to determine not only the identification of systematic characters, but also the selection procedure, which is necessary to improve the trait in a given situation. On the example of *Dianthus caryophyllus*, it was shown that higher average values clearly indicate a high degree of genotypic variability of quantitative traits, which characterizes the overall relative variability [8, 9].

In our previous works, we studied population and ontogenetic aspects, morphological features, and seed productivity of *D. awaricus*, both in natural conditions and during introduction [10–12].

In this work, we carried out a comparative assessment of the variability of the quantitative traits of the generative shoot of two species of white-flowered carnations (*Dianthus awaricus* Kharadze, *Dianthus fragrans* Adam) under the conditions of Dagestan.

2 Methods

We give a brief description of the studied species. *Dianthus awaricus* is a herbaceous perennial 10–30 cm high; single stems or several of them, woody at the base; leaves are linear-lanceolate, 2–4 cm length and about 1 mm wide, sharp at the edges, rough, at the base welded into the vagina with a length of 2–3 mm; flowers are single at the top of the stems; the cup is oblong-cylindrical, about 20 mm length; bracts, 6–8 in number, ovate, drawn into a short acumen, slightly longer than the middle of the cup; petals are white, deeply incised along the edge; flowering occurs in July-August, blooms in culture from the second year of life; the fruit is represented by a dry capsule. *D. awaricus* is an endemic species that grows in stony places in the middle mountain belt of Dagestan [13–15].

Dianthus fragrans is a herbaceous perennial that forms dense bushes 30–50 cm in height. Stems are numerous, erect, simple or slightly branched. Leaves, mainly in a basal rosette, numerous, straight, narrow-linear, sharp, three-five-veined, rough. Stem leaves are not numerous. Flowers are solitary, rarely 2–3, at the top of the stem and branches, very large, up to 5 cm in diameter, white, sometimes with a pinkish tinge or pale pink, yellowish-green outside. The bend of the petals is up to 2.5 cm in length, obovate, deeply incised up to a third of the length into sharp lobes. The petals are less fringed than those of *D. awaricus*. The cup is 2.5–3.5 cm length, cylindrical, with short, lanceolate-linear, pointed serrature. *D. fragrans* is a xerophilic species that grows on dry slopes in the lower and middle mountain belts of Dagestan [14].

Material for research was collected in four geographically isolated locations: *D. awaricus* (Oboda, Khvarada) and *D. fragrans* (Khindakh, Charoda). 17 quantitative and 3 index traits were taken into account (Table 1).

Table 1. Morphological traits of generative shoot, indices, and their note.

No	Traits	Note
1	Generative shoot length, mm	GSL
2	Number of flowers, pcs.	NF
3	Number of internodes, pcs.	NI
4	Length from flower to 1st internode, mm	LF1I
5	Flower length, mm	FL
6	Flower diameter	FD
7	Cup length, mm	CL
8	Cup diameter, mm	CD
9	Petal length, mm	PL
10	Petal width, mm	PW
11	Number of serrature on a petal, pcs.	NSP
12	Sheet length, mm	SL
13	Generative shoot mass, mg	GSM
14	Apical flower mass, mg	AFM
15	Mass of generative structures, mg	MGS
16	Leaf mass, mg	LM
17	Weight of stems, mg	WS
18	Petal shape index, %	PShI
19	Petal serration index, %	PSeI
20	Internode index, %	II

The characteristics of the collection points for field material are given in Table 2 [16].

Table 2. Comparative characteristics of collection points for field material for study.

Collection point for study material, coordinates	Exposure and steepness of the slope, height a. s. l.	Soil name	Plant community
Dianthus awaricus			
The village of Khvarada (Gergebil'skiy district) N 42°28′16″ E 46°59′53″	south-east 25–35° 1320	mountain meadow	forbs-cereal, with dominance *Salvia canencens* C. A. Mey.
The village of Oboda (Khunzakhskiy district) N 42°34′60″ E 46°40′00″	north-west 5–10° 1850	mountain meadow chernozem	forbs-cereal
Dianthus fragrans			
The village of Khindakh (Shamil'skiy district) N 42°26′24″ E 46°35′09″	south-west 30–35° 1220	mountain meadow-steppe	herb-cereal, with dominance *Botriochloa ischaemum* (L.) Keng
The village of Charoda (Charodinskiy district) N 42°16′48″ E 46°48′09″	south 20–25° 1480	mountain meadow-steppe	forbs-cereal, with dominance *Salvia verticillata* L.

Methods of descriptive statistics, variance and discriminant analyzes were used for mathematical processing of the data obtained. Statistical processing of the results was carried out using the Statistica v. 13.

3 Results

As a result of the studies carried out, intra- and interpopulation variability was revealed in two species of the genus *Dianthus.* Table 3 shows the mean values and their coefficients of variation. They reflect the degree of intrapopulation variability of species traits.

Table 3. Average values of morphological traits of *Dianthus* species.

Ttaits	***Dianthus fragrans***				***Dianthus awaricus***			
	Charoda – 1480		**Khindakh – 1220**		**Khvarada – 1320**		**Oboda – 1850**	
	$\bar{x} \pm s_{\bar{x}}$	**CV, %**	$\bar{x} \pm s_{\bar{x}}$	**CV, %**	$\bar{x} \pm s_{\bar{x}}$	**CV, %**	$\bar{x} \pm s_{\bar{x}}$	**CV, %**
NF	3,8±0,25	35,7	2,4±0,27	60,5	3,5±0,40	62,5	1,3±0,09	36,0
NI	8,5±0,25	16,0	7,2±0,24	18,0	7,3±0,21	16,1	6,8±0,14	11,3
GSL	427,2±11,66	15,0	425,7±15,39	19,8	402,2±13,26	18,0	283,6±6,69	12,9
LF1I	36,8±2,12	31,6	25,2±2,10	45,4	22,8±2,62	62,8	20,3±0,85	22,8
SL	17,3±0,93	29,4	12,3±0,56	25,0	11,9±0,82	37,5	10,6±0,20	10,3
FL	29,8±0,78	14,3	34,4±0,82	13,0	36,3±0,71	10,7	29,36±0,31	5,8
FD	14,6±0,26	9,7	16,4±0,57	19,0	18,3±0,60	17,8	14,0±0,30	11,6
CL	24,3±0,49	11,0	27,0±0,56	11,2	13,8±0,51	20,1	21,6±0,39	9,8
CD	4,0±0	0	3,8±0,11	16,0	4,0±0,15	20,3	4,4±0,09	11,3
PL	24,3±0,18	4,1	22,9±0,38	9,08	19,0±0,64	18,5	22,6±0,29	6,9
PW	3,3±0,08	13,8	3,2±0,07	12,7	4,7±0,14	16,2	3,7±0,10	15,0
NSP	8,7±0,12	7,5	8,2±0,21	14,1	12,0±0,47	21,6	15,1±0,27	10,0
GSM	457,2±32,39	38,8	492,7±42,14	46,8	575,5±59,65	56,7	189,8±8,32	23,9
AFM	38,6±1,42	20,1	56,0±2,49	24,3	51,5±1,85	19,6	42,7±0,91	11,6
MGS	123,6±9,90	43,8	113,0±11,85	57,4	143,6±18,86	71,8	27,6±7,30	144,8
LM	26,4±1,52	31,4	35,3±3,77	58,5	63,9±6,50	55,7	23,8±0,81	18,7
WS	307,1±28,83	51,4	344,4±29,86	47,4	367,9±37,49	55,8	138,4±7,42	29,3
PShI	13,5±0,40	16,0	14,0±0,29	11,3	26,0±1,37	28,6	16,7±0,50	16,4
PSeI	2,7±0,06	11,7	2,6±0,08	16,3	2,6±0,15	31,1	4,1±0,12	16,5
II	9,3±0,51	30,1	6,7±0,63	51,1	6,4±0,75	64,2	8,2±0,49	32,4

Thus, in *D. fragrans*, numerical traits (“the number of flowers”, “the number of internodes” and “the number of serrature on a petal”) are higher in plants in the cenopopulation from the vicinity of the village of Charoda. The values of most of the linear traits under study are also maximal (“length of the generative shoot”, “length from flower to first internode”, “cup diameter”, “petal width”), with the exception of the length and diameter of the flower and the length of the petiole. As for the weight characteristics, their values are higher in the cenopopulation in the vicinity of the village of Khindakh. Of the index traits, the index of internodes is slightly higher in the Khindakh cenopopulation, the other two are comparatively the same.

In *D. awaricus*, numerical traits (“number of flowers”, “number of internodes”) are higher in plants in the cenopopulation from the vicinity of the village of Khvarada, with the exception of the trait “number of serrature on a petal”. The values of most of the accounted linear traits in Khvarada are also maximal, with the exception of the linear cup traits. Accordingly, all weight traits are also significantly higher in plants from the Khvarada cenopopulation.

Analysis of the variability of *D. fragrans* traits by the coefficient of variation (CV) at the intrapopulation level showed that in the Charoda cenopopulation the traits “cup diameter”, “petal length” and “number of serrature on a petal” are characterized by relative determinism, i.e. a very low level of variation on the Mamaev scale [17]. Flower traits (“flower diameter”, “cup length” and serration index) were characterized by low variation. The number of characters with average values of the coefficient of variation includes the

number of internodes and the length of the generative shoot, the length of the flower, the width of the leaf, and the index of the shape of the petal. The rest of the characters are attributed to high and very high levels of variability: "number of flowers", "petal length" and others.

In the Khindakh cenopopulation, the traits "petal length", "cup length", "petal width" and the index of petal shape are characterized by relative determinism, average variation was characterized by the traits "number of internodes", "length of generative shoot", "flower length" and others. Most of the features are attributed to high and very high levels of variation.

In *D. awaricus* in the Khvarada cenopopulation, most of the studied characters are attributed to a high level of variation, with the exception of one – "flower length", which is attributed to a low one.

In Oboda, characters with an average level of variation prevail ("number of internodes" and "length of generative shoot", "flower length", "petal width" and petal shape index). The "number of flowers", "leaf length" and others have a high and very high level of variability, and a low and very low level of variability has the characteristics of "flower length", "petal length", "cup length", etc.).

Comparative characteristics of two white-flowered carnations of the genus *Dianthus* showed that the differences in systematic characters correspond to the literature data. For example, the length of the generative shoot and the length of the cup are greater in *D. fragrans*, while the width of the petal and the number of serrature are greater in *D. awaricus*. In general, the average values of most of the studied morphological characters are higher in *D. fragrans* (Table 4).

Table 4. Comparative characteristics of white-flowered carnations of the genus *Dianthus.*

Traits	***Dianthus awaricus***				***Dianthus fragrans***			
	$\bar{x} \pm s_{\bar{x}}$	**Range**		**CV, %**	$\bar{x} \pm s_{\bar{x}}$	**Range**		**CV, %**
		min	max			min	max	
GSL	2,4±0,24	1,0	10,0	79,5	3,1±0,20	1,0	6,0	50,5
NF	7,0±0,12	5,0	10,0	14,2	7,8±0,18	5,0	12,0	18,6
NI	342,9±10,66	225,0	575,0	24,0	426,4±9,57	310,0	600,0	17,3
LF1I	21,5±1,37	9,0	52,0	49,3	31,0±1,65	9,0	60,0	41,3
FL	11,2±0,42	6,0	30,0	29,2	14,8±0,62	5,0	30,0	32,7
FD	32,8±0,59	26,0	42,0	14,0	32,1±0,63	22,0	32,1	46,0
CL	16,1±0,43	10,0	25,0	20,5	15,4±0,33	11,0	24,0	16,6
CD	17,7±0,59	10,0	26,0	26,0	25,6±0,40	18,0	31,0	12,3
PL	4,2±0,09	3,0	6,0	16,6	3,9±0,05	3,0	5,0	11,2
PW	20,8±0,41	10,0	25,0	15,6	23,6±0,22	19,0	28,0	7,47
NSP	4,2±0,10	3,0	6,0	19,7	3,2±0,05	3,0	4,0	13,2
SL	13,5±0,34	8,0	18,0	19,4	8,4±0,12	6,0	12,0	11,4
GSM	382,6±39,00	136,0	1359,0	79,0	474,9±26,4	43,1	1028,0	26,4
AFM	47,1±1,16	32,0	70,0	19,2	47,2±1,8	23,0	99,0	29,7
MGS	85,6±12,55	0,0	382,0	113,5	118,3±7,68	0,0	280,0	50,3
LM	43,8±4,16	13,0	145,0	73,6	30,8±2,09	10,0	85,0	52,5
WS	253,1±24,12	65,0	849,0	73,8	325,7±20,71	104,0	776,0	49,2
PShI	21,4±0,94	12,5	50,0	34,1	13,7±0,24	11,5	19,0	13,7
PSeI	3,3±0,13	1,6	6,0	31,6	2,6±0,04	1,5	3,3	14,1
II	7,3±0,46	2,1	18,9	48,6	8,0±0,43	2,1	18,0	42,0

Analysis of variability in terms of the coefficient of variation (CV) of the two species without taking into account coenotic differences showed that the greatest variation is observed in terms of weight characteristics and the number of flowers, the least in terms of linear characteristics of a flower and the number of internodes. Of the three indices taken

into account, the petal index is more variable, as in *D. awaricus* the coefficient of variation is 34.1%, and in *D. fragrans* – 13.7%.

Based on the results of the two-way analysis of variance (Figure 1), species differences were revealed in most of the considered traits of generative shoot, which were significant at the significance level of $p<0.05$. The greatest differences between species in terms of the relative components of dispersion (h^2) are due to the traits "number of serrature on a petal" (73.2%), "cup length" (64.7%) and "petal index" (45.8%), differences in "flower length" and "flower diameter", "flower mass" and "internode index". The influence of the factor "habitat", is significant in terms of the apical flower: length (53.6%), diameter (40.1%) and its mass (46.3%).

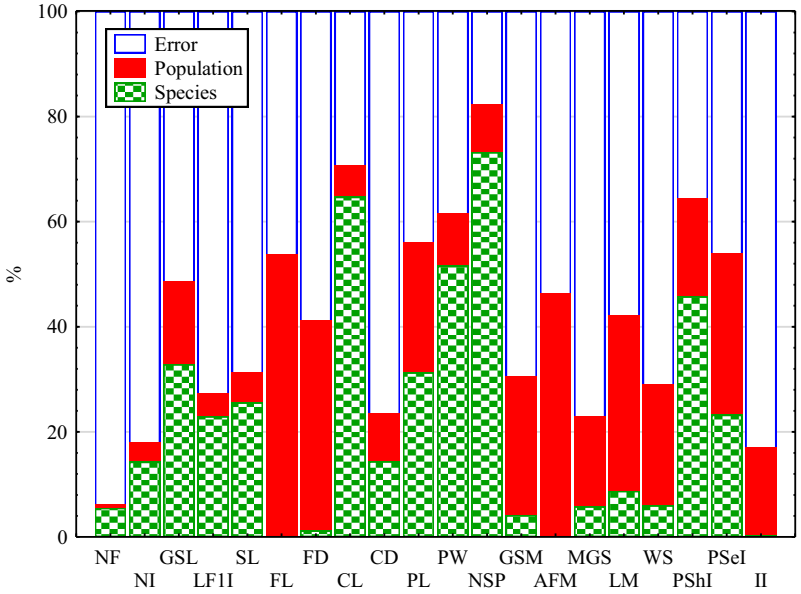

Fig. 1. Results of two-way analysis of variance of two types of carnation for the grouping variable "population" and "species".

To identify the features with the highest discriminating function, a discriminant analysis was carried out with the step-by-step exclusion of features that insignificantly affect the variability. The results of the analysis showed that the largest discriminating features are: the number of internodes, cup length, leaf length and width, flower mass and serration index (Table 5).

Table 5. Results of discriminant analysis with step-by-step exclusion of morphological traits of generative shoot of all cenopopulations of white-flowered carnations.

Traits	**F**	**p**	**Traits**	**F**	**p**
In model			**Not in model**		
NF	11,786	0,000001	SL	0,972	0,408938
FL	12,295	0,000001	GSL	4,386	0,005908
CD	75,387	0,000000	NI	10,938	0,000002
PW	15,272	0,000000	LM	3,693	0,014101
NSP	52,784	0,000000	CL	3,413	0,020069
AFM	21,906	0,000000	PShI	4,129	0,008156
PSeI	80,357	0,000000	PL	5,397	0,001681
			LF1I	2,734	0,047165

Traits	F	p	Traits	F	p
In model			**Not in model**		
			II	3,789	0,012494
			GSM	4,045	0,009057
			MGS	4,753	0,003740
			FD	8,237	0,000055

Note: Variables in model: 7; not in model: 12; grouping: Population (4 groups); F (21,316) = 58,956, p<0,0000.

In this work, the method of squares of Mahalanobis distances is applied. In mathematical statistics, Mahalanobis distance is a measure of the distance between vectors of random variables, generalizing the concept of Euclidean distance. It differs from it in that it takes into account the correlations between variables and is scale invariant. This metric is effective for analyzing quantitative data. When using it, the dependence of the object's attributes is taken into account. The Mahalanobis distance can be used to determine the similarity between the unknown and the known sample [18].

The squares of the Mahalanobis distances showed the similarity of the *D. fragrans* cenopopulations and a significant isolation of the cenopopulations in *D. awaricus*, which is possibly associated with significant differences in the complex of ecological-cenotic conditions and possible microevolutionary processes occurring in highly isolated populations, to which the “Oboda” (1850 m) on the Khunzakh plateau (Table 6).

Table 6. Results of discriminant analysis for the studied features and their indices (squares of Mahalanobis distances).

Population	Khindakh	Charoda	Oboda
Charoda	10,49		
Oboda	31,70	29,24	
Khvarada	65,15	59,57	29,12

This is graphically shown in Figure 2 in the space of two roots of canonical analysis and complete self-identification according to the classification matrix.

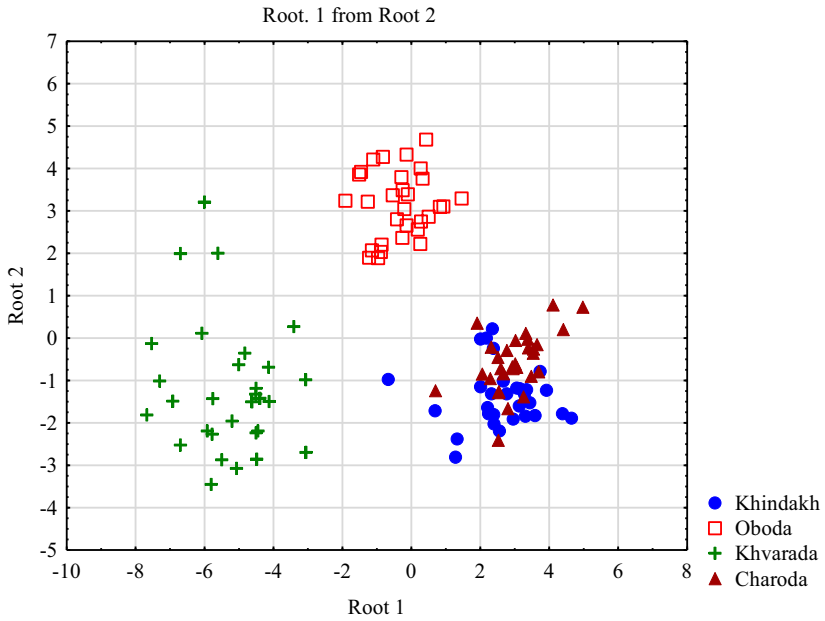

Fig. 2. Results of discriminant analysis based on morphological characteristics of generative shoot of carnation (two species).

The Figure clearly shows how closely the coenopopulations of *D. fragrans* are located in the plane and how differentiated are the coenopopulations in *D. awaricus*. That is, the use of this method revealed the similarities and differences between closely related species in contrasting natural conditions.

4 Discussion

Comparative characteristics of two white-flowered carnations of the genus *Dianthus* showed that the differences in systematic characters correspond to the literature data [13].

But as a result of our studies, intra- and interpopulation variability was revealed in many traits, which may be associated with the place of their growth at different altitude levels with ecological and climatic conditions characteristic of each level.

The factor “habitat” at the intraspecific level significantly affects the linear features of a flower – length (53.6%) and diameter (40.1%), as well as one weight parameter – mass (46.3%).

The two-way analysis of variance revealed the traits that make the greatest differences between species, such as the number of serrature petal (73.2%), cup length (64.7%) and petal index (45.8%).

A number of authors Yoshioka Y, Iwata H. et al. [19] argue that the change in the shape of both symmetric and asymmetric elements in the environment is possibly associated with developmental instability. The contribution of the genotype to the petal area is also great. However, the area of the petal is more dependent on the environment than the shape of the petal. However, the area of the petal as such an index should be used with caution, because the influence of the environment on the area of the petal is much greater than on the shape of the petal. Among the features we took into account are the length, width and index of the petal. Their variability in our case is not high either by species or by population.

The applied method of squares of Mahalanobis distances showed the closeness of the

cenopopulations of *D. fragrans* and significant isolation in *D. awaricus*, which may be explained by various microevolutionary processes. There is a clear separation in space between the two roots of canonical analysis and complete self-identification according to the classification matrix between the two species.

5 Conclusion

An exploratory analysis of the variability of two white-flowered carnation species (*Dianthus awaricus, Dianthus fragrans*) based on the quantitative traits of the generative shoot showed a different degree of inter-specific differentiation relative to the intra-specific one, which is of scientific interest for clarifying and identifying some systematic traits of closely related species of the genus *Dianthus* L. Isolated populations of *Dianthus awaricus* significantly more distinctly separated than *Dianthus fragrans*. This will allow planning the tactics for further study of micro-evolutionary processes in these species.

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