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Evaluation of Morphological and Anatomical Features of Iranian *Galanthus transcaucasicus*

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ABSTRACT

Galanthus transcaucasicus is a less frequently known species with a high distribution in southern regions of the Caspian Sea in Iran. So far, no precise study has been done on *Galanthus transcaucasicus* in the literatu re. This study aimed to collect and introduce the morphological and anat omical features of endemic *Galanthus transcaucasicus* in detail. Herewit h, 50 *Galanthus transcaucasicus* accessions of five populations (10 rand omly selected plants from each population) were collected from four pr ovinces, including Mazandaran, Guilan, Golestan, and Ardabil, Iran. Twel ve morphological traits and ten anatomical parameters were measured. The largest flowers were observed in Shirgah population (22.66 and 11. 97mm for outer perianth-segments length and width). The tallest stems were recorded in the Shirgah population (243.89 mm). The largest bulb size belonged to the Neka population (19.92 and 14.08 mm of bulb lengt h and diameter, respectively). Anatomical analysis showed that the cells on the lower (abaxial) epidermis were spherical and short. On the upper (adaxial) epidermis, however, they were oblong and strongly elongated. *G. transcaucasicus* had amphistomatous leaves. The number of stomata on the upper surface was less (0.16 to 0.6 per mm^2) than that on the low er surface (3.77 to 9.51 per mm^2). Anatomically, there was a significant difference between populations from different regions. The results of thi s study revealed low variability among *G. transcaucasicus* accessions, re presentative of one species' clone-population structure. Therefore, it see ms that the range of environmental conditions in which the species are d istributed can play a role in the morphological and anatomical features o f Iranian *Galanthus transcaucasicus*.

Introduction

Snowdrops (*Galanthus* spp.) are small winter plants (15-35 cm in height) that produce one small (2.5 cm or less) white flower, which hangs down off its stalk like a "drop". The genus *Galanthus* is comprised of perennial bulbous plants belonging to the Amaryllidaceae. There are 20 species currently known. They have two leaves and pendant white flowers with six perianth segments (i.e. three outer perianth segments and three inner perianth segments). The inner petals have green markings (Larsen et al., 2010; Newton et al., 2013). They prefer to grow in fertile and humus-rich soils, with a high proportion of organic matter. Snowdrop is an ornamental plant, with great popularity in Europe because of its first flowers that bloom at the end of autumn and

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winter (Rønsted et al., 2013). These plants also have many medicinal properties. There have been numerous studies on the pharmacological activities of *Galanthus* plant proteins and alkaloids (Berkov et al., 2012; Karimi, 2018). Millions of *Galanthus* bulbs are sold annually. Most of them are from the *G. nivalis*, *G. elwesii*, and *G. woronowii* species (Zonneveld et al., 2003). Despite the popularity of these flowers in European countries, they are relatively unknown in Iran and are not cultivated commercially. The northern provinces of Iran, located in the vicinity of the Caspian Sea, are potential regions for the cultivation of this flower. Its distribution region is from the western Pyrenees (France, Spain) to the eastern Caucasus, Talesh and Alborz (Iran), south to Sicily, the Peloponnese, the Aegean (Greece), southern Turkey, Lebanon, and Syria (Zubov and Davis, 2012; Rønsted et al., 2013).

Genetic diversity of wild plants and species of under-utilized germplasms can provide essential, new gene pools for breeders (Jiménez et al., 2017; von Kohn et al., 2018; Mafakheri et al., 2020). Morphological markers are one of the first methods used to study the genetic diversity of plants for breeding work (Shao et al., 2010; Rocha et al., 2020). Ornamental plant breeders usually use morphological traits to select valuable genetic resources in the first stage of breeding programs (Azimi et al., 2016; Pitoyo et al., 2018; Azimi and Alavijeh, 2020). Taxonomic patterns in the genus *Galanthus* have been based on morphological and anatomical characteristics of the leaves (Yüzbaşıoğlu, 2012; Chkhaidze et al., 2014; Tan et al., 2014; Semerdjieva et al., 2019). *Galanthus* transcaucasicus is native to Armenia, Azerbaijan, and north of Iran, although the first specimens of *Galanthus* transcaucasicus were introduced in 1909 from the Talesh mountains of Azerbaijan. Nonetheless, this species remains relatively unknown in the region (Cox, 2019). According to the distribution map of snowdrop (*Galanthus*) in Europe and Western Asia and "Flora Iranica" (Wendelbo 1971), *G. transcaucasicus* species originates from the northern regions of Iran, and the most distribution of *G. transcaucasicus* is the southern regions of the Caspian Sea in Iran. No precise study has been done on *Galanthus* transcaucasicus in the literature, so far, and more research on this unknown species is necessary (Davis et al., 2001; Rønsted et al., 2013).

This study aimed to collect and introduce the morphological and anatomical features of endemic *Galanthus transcaucasicus* in detail. In this study, important morphological and anatomical features were evaluated to identify *Galanthus transcaucasicus* accessions, with high potentials for *Galanthus* breeding. This is the first report on the variability of *Galanthus transcaucasicus* and its diversity in the north of Iran.

Material and Methods

Plant materials

In Iran, the distribution of wild *Galanthus transcaucasicus* occurs in the north of the country, especially in four provinces, Mazandaran, Gilan, Golestan and Ardabil. Fifty *Galanthus transcaucasicus* accessions, belonging to 5 populations (10 randomly selected plants from each population) were collected to explore the variability of *Galanthus transcaucasicus*. The distribution map and the detailed information for each population are shown in Figure 1 and Table 1, respectively. Sample collections were done during 2018-2019 between October and January based on the average flowering time. All plants were transferred to the Department of Horticulture Sciences, Agricultural Sciences and Natural Resources, Gorgan University. They were planted in plastic pots and were maintained outdoors. The identification and authentication of the plants were made by Dr. Dolatyari, and voucher specimens were deposited at the Herbarium of Iranian Biological Resource Center (IBRC), Tehran, Iran.

Morphological analysis

Twelve quantitative parameters were measured (Fig. 2). Measurements were done when the bulbs had mature leaves and open flowers. The measurements were aimed at considering the outer perianth segments length (mm), outer perianth segments width (mm), inner perianth segments length (mm), inner perianth segments width (mm), spatha length (mm), spatha width (mm), stem diameter (mm), stem length (mm), leaf length (mm), leaf width (mm), bulb diameter (mm) and bulb length (mm). All parameters (width, height, and diameter) were measured with a digital Vernier caliper (Aerospace, China). The measurements were taken with an accuracy of 0.01 mm (Semerdjieva et al., 2019).

Anatomical analysis

The anatomical study included the length, width, and density of the stomata, as well as the width and height of guard cells, and leaf thickness (µm). Eleven quantitative parameters were measured. In the case of leaf thickness, the widest part of transverse sections of the leaf (middle of the leaf) was measured. The imprint method with clear nail varnish was used to measure stomatal characteristics (Zhu et al., 2018).

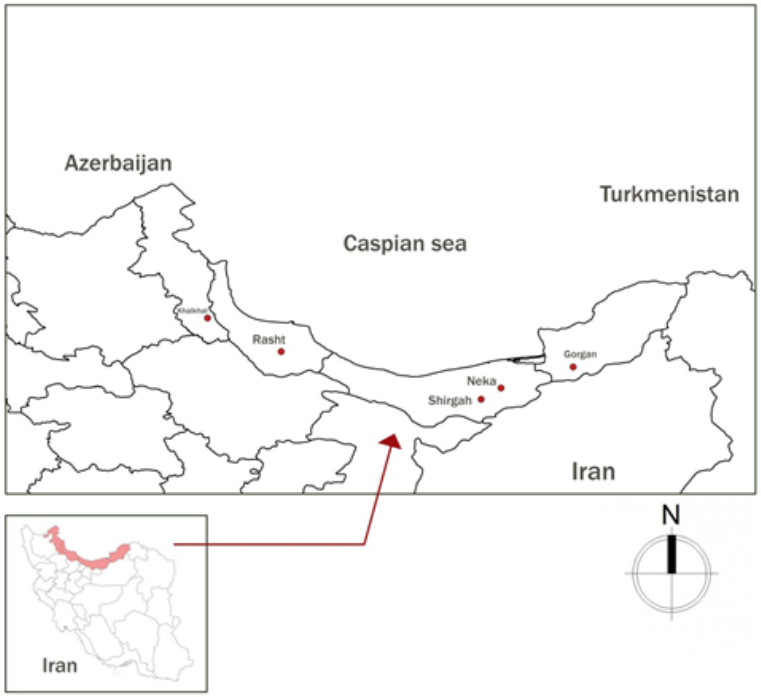

Fig. 1. Distribution map of *Galanthus transcaucasicus* populations in four northerly provinces of Iran (Mazandaran, Gilan, Golestan, and Ardabil).

Table 1. Details of 5 populations of *Galanthus transcaucasicus* in four northerly provinces of Iran

Origin	Population code	Sample size (n)	Latitude	Longitude	Elevation (m)
Gorgan	G1-G10	10	36°45'55.20"N	54°29'3.77"E	552
Shirgah	S1-S10	10	36°18'2.53"N	52°52'30.82"E	350
Neka	N1-N10	10	36°18'29.06"N	52° 7'22.62"E	510
Rasht	R1-10	10	37° 8'7.04"N	49°38'47.28"E	143
Khal khal	K1-K10	10	37°38'45.82"N	48°34'46.38"E	2050

A thin layer of clear nail polish was spread on the central part of the upper and lower epidermis of the leaf. After drying, they were peeled off with sticky tape and transferred into the slide. Transverse sections of leaves were taken with a razor blade. The cells were stained with Safranin. Zeiss Axiowert 200 M Inverted Microscope (Carl Zeiss Jena, Germany)(Kolbert et al., 2012) was used for observing leaf anatomy at 4x, 10x, and 40x magnification and photos were taken by a digital camera.

Statistical analysis

Dimensions, number, and density of stomata and guard cells were evaluated with Image J software. Average ($\bar{X}$), minimum, maximum, and standard deviation (SD) of all morphological and anatomical features were calculated by Microsoft Excel (2010). Data were evaluated by one-way analysis of variance. Significant differences were identified using Duncan's multiple range test ($P \leq 0.05$) using SPSS (version 16.0). Cluster analyses and Pearson's correlation coefficients were performed using SPSS software (version 16.0).

Results

Twelve morphological features were measured in 50 accessions of *G. transcaucasicus* collected in this study (Table 2). Minimum, maximum, and mean values, as well as standard deviations and coefficients of variation for morphological features, were calculated (Table 3). The lowest variation was documented for inner perianth segment length (8.59 %) and the highest was documented for stem length (14.98 %).

Fig. 2. Morphological variability of *Galanthus transcaucasicus* populations from the north of Iran

Table 2. Details of morphological characteristics of *Galanthus transcaucasicus* accessions

code	Outer perianth segments length (mm)	Outer perianth segments width (mm)	Inner perianth segments length (mm)	Inner perianth segments width (mm)	Spatha length (mm)	Spatha width (mm)	Leaf length (mm)	Leaf width (mm)	Stem length (mm)	Stem diameter (mm)	Bulb length (mm)	Bulb diameter (mm)
G1	22.8	8.42	10.45	5.82	32.73	5.43	205	12.09	235	2.39	18.28	11.31
G2	19.94	9.79	10.8	7.37	28.5	6.2	178	11.41	220	2.84	16.22	12.24
G3	21.78	9.73	10.19	5.48	37.4	4.41	180	12.74	236	2.82	18.24	11.79
G4	23.2	10.75	10.13	6.68	35.44	5.04	196	11.8	234	2.94	19.64	13.17
G5	24.07	12.08	10.72	7.11	36.07	4.87	208	13.28	230	2.85	20.28	14.97
G6	22.36	12.29	11.74	7.31	42.78	5.42	173	15.02	190.68	2.82	16.22	12.24
G7	23.53	12.67	11.14	7.41	33.02	5.77	170	12.67	204.57	2.84	23.24	18.27
G8	23.6	12.11	10.9	7.4	35.12	5.75	165	12.51	200.02	3.08	14.97	15.28
G9	21.62	11.14	10.67	8.23	35.03	6.5	150	12.59	196.31	3.17	13.79	15.24
G10	23.76	12.43	12	7.56	37.17	5.88	154	13.5	200.91	3.05	16.54	12.64
S1	21.55	11.62	10.66	8.73	31.86	6.52	163	10.16	250	3.65	17.68	12.52
S2	21.6	12.59	10.74	8.79	31.48	6.42	158	15.02	252	3.62	17.82	12.45
S3	22.84	12.36	10.83	8.71	30.4	5.38	163	14.71	246	3.61	18.4	14.7
S4	21.34	11.78	10.27	8.75	29.62	5.66	150	13.61	238	3.36	19.72	12.98
S5	21.72	11.89	10.45	7.93	28.06	5.59	150	13.41	248	3.26	18.16	12.03
S6	22.79	11.51	10.85	7.77	29.36	5.25	161	14.41	245.51	2.88	17.49	12.78
S7	22.36	12.42	10.32	8.6	28.22	5.42	168	14.65	245.13	2.85	19.48	12.49
S8	21.28	12.05	10.31	7.94	31.53	5.69	167	13.18	240.09	2.61	18.88	13.11
S9	22.84	12.26	10.82	8.4	30.93	5.68	170	12.54	235	2.83	19.6	13.6
S10	21.84	11.26	10.62	7.4	31.93	5.68	171	11.54	239.17	2.83	17.6	16.6
N1	23.84	12.26	11.62	8.4	37.93	5.68	176	12.63	229.17	2.23	20.05	16.87
N2	23.21	12.03	11.3	8.94	36.66	5.14	177	12.54	227.61	2.19	20.77	14.16
N3	19.89	10.66	11.5	7.16	24.68	5.96	180.2	12.62	227.66	3.07	18.6	13.6
N4	20.57	11.15	10.84	8.17	33.63	6.1	185	13	203	3.78	20.62	16.27
N5	19.76	10.5	10.77	6.24	25.56	5.43	215	14.94	228	3	21.13	15.07
N6	21.33	11.33	10.61	8.89	28.86	5.89	158	12.31	194.51	2.77	18.93	12.21
N7	23.59	11.77	10.23	7.4	27.56	5.87	162	12.62	195.53	2.98	21.05	13.87
N8	19.89	10.66	11.5	7.16	24.68	5.96	180.2	13.45	204	3.07	18.12	13.42
N9	20.57	11.15	10.84	8.17	33.63	5.4	185	10.54	223	3.71	21.13	13.07
N10	19.76	10.5	10.77	6.24	25.56	5.03	215	14.98	218	2.65	18.83	12.31
R1	21.52	10.71	10.31	5.49	28.95	4.81	186	9.25	174.71	3.29	19.62	12.16
R2	21.62	10.81	10.41	8.01	29.95	4.51	175	9.5	164.71	3.19	19.82	13.16
R3	21.63	10.45	10.46	5.67	28.93	4.43	180	9.44	163.93	3.25	19.54	13.76
R4	15.59	7.29	9.75	6.26	25.87	4.75	191	9.9	162.73	2.23	18.36	13.36
R5	17.07	11.14	11.38	8.25	27.25	5.02	185	10.12	175.64	3.05	18.7	12.81
R6	18.07	11.04	11.58	8.55	27.15	5.12	180	10.12	165.64	3.05	18.66	13.81
R7	18.65	11.67	11.75	7.38	26.37	5.17	188	10.11	178.61	3.09	17.96	13.63
R8	16.67	10.29	10.88	7.39	27.43	5.14	190	10.63	160	2.94	13.46	11.31
R9	23.58	13.43	9.29	5.39	30.98	5.78	202	13.4	160.68	3.38	22.62	15.09
R10	21.04	10.56	11.93	6.09	29.19	4.97	190	10.56	174.36	2.4	18.98	14
K1	17.33	10.58	10.34	7.39	26.09	5.03	160	10.5	173.66	2.46	15.98	12.22
K2	17.32	10.42	10.59	7.67	25.86	4.57	162	11.05	170.08	2.98	16.28	14.14
K3	17.23	10.96	11.56	7.77	26.87	4.77	162	10.76	168.5	2.17	18.12	15.44
K4	17.28	10.8	11.91	7.62	27.71	4.67	163	10.61	165.8	3.04	18.86	14.26
K5	23.2	11.51	13.04	7.3	32.71	4.33	162	12.14	175.42	2.91	18.48	12.49
K6	23.98	11.22	13.76	7.51	33.36	4.28	162	12.76	178.5	3.17	18.53	13.39
K7	24.22	11.13	14.26	8.68	32.8	4.19	160	12.66	176.36	3.12	18.68	13.68
K8	21.73	10.86	9.39	6.63	30.94	4.56	158	12.59	178.31	2.57	14.34	12.11
K9	21.72	10.81	9.69	6.73	30.98	4.9	165	12.4	180.59	2.58	14.85	12.51
K10	22.72	10.92	10.12	7.71	36.01	5.29	148	10.15	166.05	2.95	15.23	13.57

The comparison of means showed twelve morphological features using one-way analysis of variance, following Duncan's multiple range test. The minimum, maximum, and standard deviation for each *G. transcaucasicus* population is shown in Table 4. The analysis of variance for morphological characteristics revealed a significant difference ($p<0.05$) among the populations regarding all features except the inner perianth segment length, bulb diameter, and stem diameter. The largest flowers were observed in Shirgah population, with the highest mean values of outer perianth-segment length (22.66 mm), outer perianth-segment width (11.97 mm), and inner perianth segment width (8.03 mm). The tallest stems were recorded in Shirgah population (243.89 mm) as the stem length. The largest bulb size belonged to the Neka population, with 19.92 mm of bulb length and 14.08 mm of bulb diameter.

Table 4. Morphological characteristics of *Galanthus transcaucasicus* populations from the northerly provinces of Iran

	Population code	Mean $\bar{X}$	Max	Min	Standard deviation (SD)
Outer perianth segment length (mm)	Gorgan (G1- G10)	22.06[b]	21.28	22.84	0.63
	Shirgah (S1- S10)	22.66[b]	19.94	24.07	1.26
	Neka (N1- N10)	21.24[ab]	19.76	23.84	1.66
	Rasht (R1- R10)	19.54[a]	15.59	23.58	2.66
	Khal khal (K1- K10)	20.67[ab]	17.23	24.22	3.02
Outer perianth segment width (mm)	Gorgan (G1- G10)	11.14[ab]	8.42	12.67	1.43
	Shirgah (S1- S10)	11.97[b]	11.26	12.59	0.43
	Neka (N1- N10)	11.20[ab]	10.5	12.26	0.64
	Rasht (R1- R10)	10.50[a]	7.29	11.67	1.2
	Khal khal (K1- K10)	11.15[ab]	10.42	13.43	0.85
Inner perianth segment length (mm)	Gorgan (G1- G10)	10.87[a]	10.13	12	0.6
	Shirgah (S1- S10)	10.58[a]	10.27	10.85	0.23
	Neka (N1- N10)	10.99[a]	10.23	11.62	0.45
	Rasht (R1- R10)	10.46[a]	9.29	11.93	0.88
	Khal khal (K1- K10)	10.79[a]	9.39	14.26	1.7
Inner perianth segment width (mm)	Gorgan (G1- G10)	7.07[a]	5.48	8.23	0.82
	Shirgah (S1- S10)	8.03[b]	7.4	8.79	0.5
	Neka (N1- N10)	7.67[ab]	6.24	8.94	0.98
	Rasht (R1- R10)	6.84[a]	5.39	8.55	1.2
	Khal khal (K1- K10)	7.50[ab]	6.63	8.68	0.57
Spatha length (mm)	Gorgan (G1- G10)	35.32[b]	28.5	42.78	3.6
	Shirgah (S1- S10)	30.33[a]	28.06	31.93	1.45
	Neka (N1- N10)	30.33[a]	24.68	37.93	2.18
	Rasht (R1- R10)	29.87[a]	25.87	30.98	1.49
	Khal khal (K1- K10)	28.20[a]	25.86	36.01	3.5
Spatha width (mm)	Gorgan (G1- G10)	5.52[b]	4.41	6.5	0.62
	Shirgah (S1- S10)	5.72[b]	5.25	6.52	0.41
	Neka (N1- N10)	5.464[b]	5.03	6.1	0.37
	Rasht (R1- R10)	4.97[a]	4.43	5.78	0.38
	Khal khal (K1- K10)	4.65[a]	4.19	5.29	0.34
Leaf length (mm)	Gorgan (G1- G10)	177.9[b]	150	208	19.93
	Shirgah (S1- S10)	162.1[a]	150	171	7.5
	Neka (N1- N10)	183.34[b]	158	215	18.91
	Rasht (R1- R10)	186.7[b]	175	202	704
	Khal khal (K1- K10)	160.2[a]	148	165	4.6

Means followed by the same letter within columns are not significantly different at $p \leq 0.05$ level according to Duncan multiple range test.

Correlation analyses of *G. transcaucasicus* morphological traits (Table 5) showed that the outer perianth-segment length had a significant, positive correlation with the outer perianth-segment width (r = 0.54), spatha length (r = 0.66), stem length (r = 0.38) and leaf width (r = 0.45). The outer perianth segment width showed a significant, positive correlation with the inner perianth-segment length (r = 0.35), inner perianth-segment width (r = 0.59), spatha length (r = 0.31), leaf width (r = 0.42), stem length (r = 0.28), stem diameter (r = 0.25), bulb length (r = 0.31) and bulb diameter (r = 0.28). The inner perianth-segment width had a significant, positive correlation with spatha width (r = 0.51) and stem length (r = 0.28). The spatha width revealed a significant, positive correlation with leaf width (r = 0.39), stem length (r = 0.50), and stem diameter (r = 0.29). Also, bulb length and bulb diameter had a significant, positive correlation (r = 0.39). Leaf length showed a negative correlation with the outer perianth-segment width (r = -0.42) and inner-perianth segment width (r = -0.45). The weakest correlation was seen between stem diameter and bulb diameter (r = 0.006). The outer perianth-segment length and the outer perianth segment width showed the highest correlation (r = 0.54).

Table 4. Morphological features details of *Galanthus transcaucasicus* populations from the northern provinces of Iran.

	Population code	Average $\bar{X}$	Max	Min	Standard deviation (SD)
Leaf width (mm)	Gorgan (G1- G10)	12.76^{c}	11.41	15.02	1.01
	Shirgah (S1- S10)	13.32^{c}	10.16	15.02	1.55
	Neka (N1- N10)	12.96^{c}	10.54	14.98	1.29
	Rasht (R1- R10)	10.30^{a}	9.25	13.4	1.17
	Khal khal (K1- K10)	11.56^{b}	10.15	12.76	1.03
Stem length (mm)	Gorgan (G1- G10)	214.75^{b}	190.68	236	18.02
	Shirgah (S1- S10)	243.89^{c}	235	252	5.56
	Neka (N1- N10)	215.05^{b}	194.51	229.17	14.24
	Rasht (R1- R10)	168.10^{a}	160	178.61	6.9
	Khal khal (K1- K10)	173.33^{a}	165.8	180.59	5.39
Stem diameter (mm)	Gorgan (G1- G10)	2.88^{a}	2.39	3.17	0.21
	Shirgah (S1- S10)	3.15^{a}	2.61	3.65	0.39
	Neka (N1- N10)	2.94^{a}	2.19	3.78	0.52
	Rasht (R1- R10)	2.98^{a}	2.23	3.38	0.37
	Khal khal (K1- K10)	2.88^{a}	2.17	3.17	0.32
Bulb length (mm)	Gorgan (G1- G10)	17.74^{a}	13.79	23.24	2.7
	Shirgah (S1- S10)	18.48^{ab}	17.49	19.72	0.8
	Neka (N1- N10)	19.92^{c}	18.12	21.13	1.18
	Rasht (R1- R10)	18.77^{ab}	13.46	22.62	2.2
	Khal khal (K1- K10)	16.93^{a}	14.34	18.84	1.77
Bulb diameter (mm)	Gorgan (G1- G10)	13.71^{a}	11.31	18.27	2.17
	Shirgah (S1- S10)	13.32^{a}	12.03	16.06	1.37
	Neka (N1- N10)	14.08^{a}	12.21	16.87	1.56
	Rasht (R1- R10)	13.30^{a}	11.31	15.09	1.04
	Khal khal (K1- K10)	13.38^{a}	12.11	15.44	1.06

Means followed by the same letter within columns are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test.

Table 5. Pearson's correlation coefficient (r) among morphological parameters

Traits	OPSL	OPSW	IPSL	IPSW	SL	SW	LL	LW	STL	STD	BL	BD
OPSL	1											
OPSW	0.549**	1										
IPSL	0.111	0.359*	1									
IPSW	0.104	0 .59**	0.133	1								
SL	0.664**	0.310*	0.124	0.118	1							
SW	0.139	0.232	-0.253	0.518**	0.073	1						
LL	-0.167	-0.428*	-0.133	-0.451**	-0.146	-0.125	1					
LW	0.449**	0.427**	0.031	0.261	0.229	0.328*	-0.022	1				
STL	0.385**	0.285*	-0.167	0.282*	0.207	0.505**	0.006	0.582**	1			
STD	0.134	0.253	0.028	0.214	0.001	0.294*	0.127	0.071	0.134	1		
BL	0.241	0.310*	0.045	0.038	-0.39	0.07	0.395*	0.105	0.202	0.137	1	
BD	0.163	0.285*	0.114	0.122	0.149	0.185	0.44	0.049	0.07	0.006	0.395**	1

OPSL: outer perianth segments length (mm); OPSW: outer perianth segments width (mm); IPSL: inner perianth segments length (mm); IPSW: inner perianth segments width (mm); SL: spatha length (mm); SW: spatha width (mm); LL: leaf length (mm); LW: leaf width (mm); STL: stem length (mm); STD: stem diameter (mm); BL: bulb length (mm); BD: bulb diameter (mm).

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

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

A dendrogram of cluster analysis was based on morphological features, showing two main clusters in *G. transcaucasicus* accessions, named A and B (Fig. 3). These clusters eventually ended up in three subclusters, i.e. AI, AII, and AIII, as well as BI, BII, and BIII. *Galanthus* accessions from Shirgah, Rasht, and Khalkhal populations were completely separated and placed in clusters AI, BI, and BIII. The *Galanthus* accessions from Neka and Gorgan populations were not separated. They were placed in clusters AII, AIII, and BII.

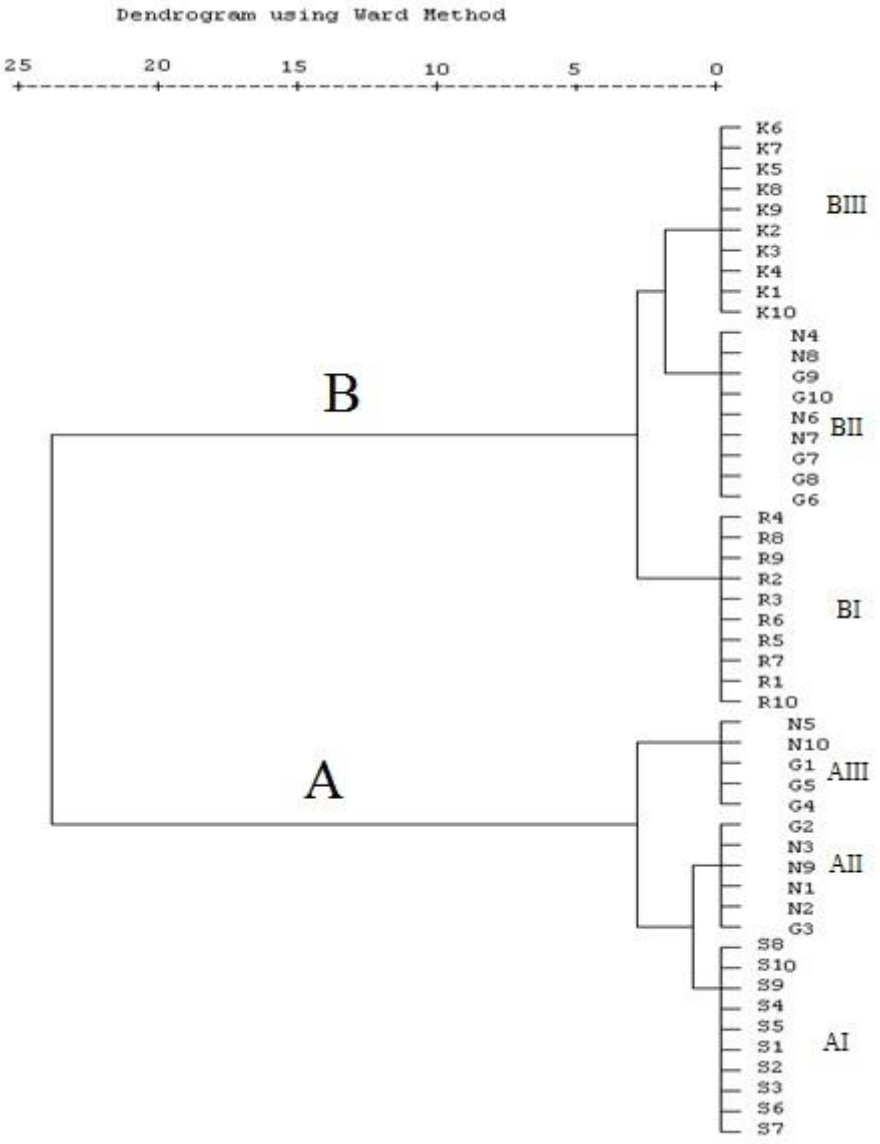

Fig. 3. Cluster analysis of *Galanthus transcaucasicus* accessions based on morphological features

An anatomical study on the leaves of *G. transcaucasicus* accessions showed that the shape and size of epidermal cells were different on the upper and lower surfaces of the leaves. The upper (adaxial) and lower (abaxial) epidermis of *Galanthus transcaucasicus* are shown in Figure 4. In this study, significant differences ($p \leq 0.05$) were observed in almost all anatomical traits of *G. transcaucasicus* populations, except for the width of the upper (ad) and lower surface (ab) guard cell, and leaf thickness. Based on the leaf thickness (Fig. 5), the most significant leaf thickness was found in the Gorgan population (775 µm). The comparison of mean values of eleven anatomical features, using one-way analysis of variance, followed by Duncan's multiple range test, showed minimum, maximum, and standard deviation for each *G.*

transcaucasicus population (Table 6). The stomata were observed on both adaxial and abaxial surfaces of the leaves (i.e. amphistomatous leaves) (Fig. 6).

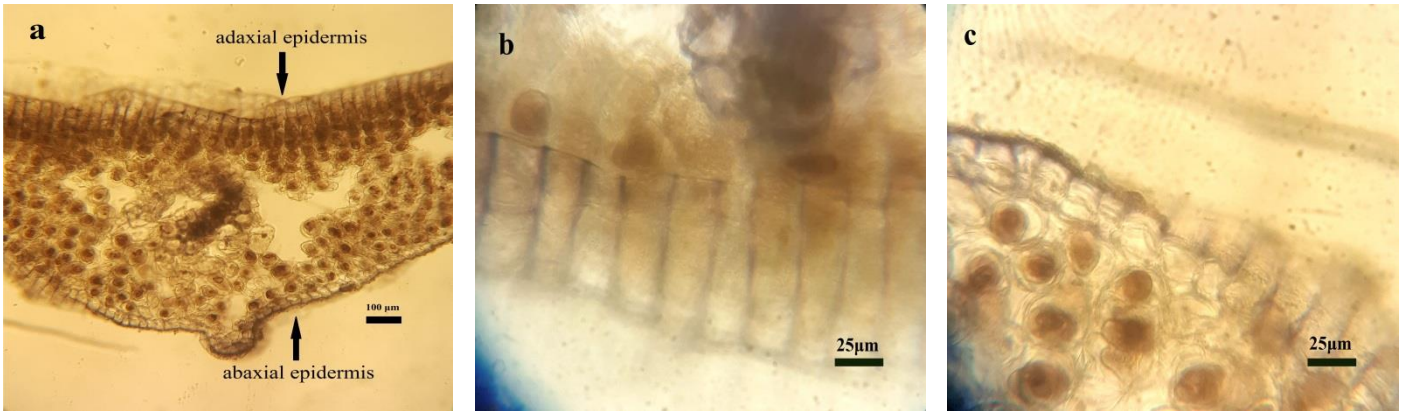

Fig. 4. Leaf anatomical features of *Galanthus transcaucasicus* (a), upper (adaxial) epidermis (b), and lower (abaxial) epidermis (c)

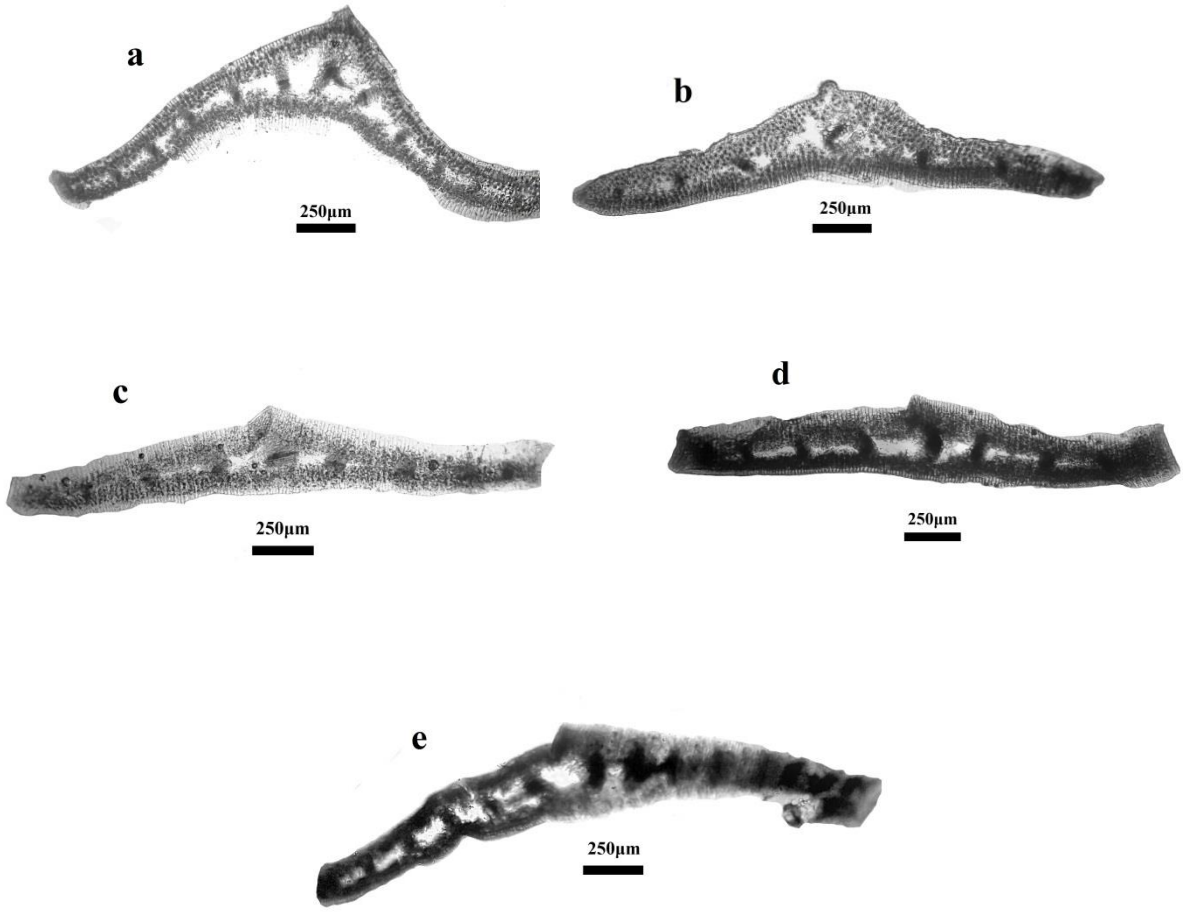

Fig. 5. Leaf cross sections of *Galanthus transcaucasicus* populations; (a) G8 from the Gorgan population, (b) S3 from the Shirgah population, (c) N3 from the Neka population, (d) R1 from the Rasht population, (e) K1 from the Khalkhal population.

Table 6. Anatomical features of *Galanthus transcaucasicus* populations from northerly provinces of Iran

	Population code	mean $\bar{X}$	Max	Min	Standard deviation (SD)
Leaf thickness, µm	Gorgan (G1- G10)	775[a]	778	768	5.13
	Shirgah (S1- S10)	718.75[a]	719	712.5	3.68
	Neka (N1- N10)	554.68[a]	560	551.62	4.24
	Rasht (R1- R10)	656.25[a]	663.7	648.5	7.60
	Khal khal (K1- K10)	583.3[a]	588	575	6.58
Upper surface (ad) stomata length, µm	Gorgan (G1- G10)	36.13[ab]	40.3	29.8	3.31
	Shirgah (S1- S10)	35.13[a]	37.2	31	3.57
	Neka (N1- N10)	40.26[b]	43.4	37.2	3.05
	Rasht (R1- R10)	38.23[ab]	40.3	37.2	1.78
	Khal khal (K1- K10)	39.26[ab]	40.3	37.2	1.60
Upper surface (ad) stomata width, µm	Gorgan (G1- G10)	13.26[ab]	15.5	9.31	2.33
	Shirgah (S1- S10)	13.43[ab]	15.5	9.31	3.57
	Neka (N1- N10)	14.88[b]	15.5	12.4	1.38
	Rasht (R1- R10)	11.37[a]	12.4	9.31	1.78
	Khal khal (K1- K10)	12.40[ab]	15.5	9.31	1.95
Lower surface (ab) stomata length, µm	Gorgan (G1- G10)	34.41[b]	37.2	27.9	3.41
	Shirgah (S1- S10)	35.65[bc]	40.3	24.8	4.44
	Neka (N1- N10)	37.88[c]	43.4	34.1	3.01
	Rasht (R1- R10)	42.47[d]	46.5	37.2	3.28
	Khal khal (K1- K10)	29.96[a]	34.1	27.9	2.68
Lower surface (ab) stomata width, µm	Gorgan (G1- G10)	10.54[ab]	15.5	6.2	2.61
	Shirgah (S1- S10)	9.30[a]	12.4	6.2	1.46
	Neka (N1- N10)	11.37[b]	15.5	9.31	2.18
	Rasht (R1- R10)	14.57[c]	15.5	12.4	1.49
	Khal khal (K1- K10)	9.99[ab]	12.4	9.31	1.36
Upper surface (ad) guard cell length, µm	Gorgan (G1- G10)	64.18[b]	68.2	58.7	3.71
	Shirgah (S1- S10)	64.06[b]	68.2	61.3	3.64
	Neka (N1- N10)	73.78[c]	77.5	68.2	4.04
	Rasht (R1- R10)	51.67[a]	52.7	49.6	1.78
	Khal khal (K1- K10)	64.18[b]	71.3	62	3.68
Upper surface (ad) guard cell width, µm	Gorgan (G1- G10)	12.40[a]	15.5	9.31	2.68
	Shirgah (S1- S10)	14.47[a]	18.6	9.31	4.72
	Neka (N1- N10)	14.83[a]	15.5	9.31	2.76
	Rasht (R1- R10)	11.16[a]	15.5	14.5	0.57
	Khal khal (K1- K10)	14.46[a]	15.5	12.4	1.60

Means followed by the same letter within columns are not significantly different at $p \leq 0.05$, according to Duncan's multiple range test.

Table 6. Anatomical features of *Galanthus transcaucasicus* populations from the northerly provinces of Iran

	Population code	mean X̄	Max	Min	Standard deviation (SD)
Lower surface (ab) guard cell length, µm	Gorgan (G1- G10)	68.22^{c}	74.4	62	5.48
	Shirgah (S1- S10)	64.99^{bc}	68.2	62	2.76
	Neka (N1- N10)	67.51^{c}	74.4	62	4.03
	Rasht (R1- R10)	63.34^{b}	66.2	61.9	1.54
	Khal khal (K1- K10)	57.86^{a}	68.2	52.7	4.90
Lower surface (ab) guard cell width, µm	Gorgan (G1- G10)	14.16^{a}	16.6	10.31	2.26
	Shirgah (S1- S10)	13.64^{a}	15.5	9.31	2.16
	Neka (N1- N10)	12.40^{a}	15.5	9.31	2.18
	Rasht (R1- R10)	13.64^{a}	15.5	9.31	2.16
	Khal khal (K1- K10)	13.08^{a}	15.5	12.4	1.36
Upper surface (ad) number of stomata, per mm^2	Gorgan (G1- G10)	0.19^{a}	0.2	0.18	0.01
	Shirgah (S1- S10)	0.23^{a}	0.25	0.2	0.02
	Neka (N1- N10)	0.16^{a}	0.18	0.15	0.01
	Rasht (R1- R10)	0.37^{b}	0.4	0.35	0.02
	Khal khal (K1- K10)	0.6^{c}	0.67	0.5	0.07
Lower surface (ab) number of stomata, per mm^2	Gorgan (G1- G10)	6.41^{c}	7	6	0.39
	Shirgah (S1- S10)	9.48^{d}	10	9	0.29
	Neka (N1- N10)	3.77^{a}	4.3	3	0.55
	Rasht (R1- R10)	5.58^{b}	6	5	0.44
	Khal khal (K1- K10)	9.51^{d}	10.5	8.7	0.69

Means followed by the same letter within columns are not significantly different at $p \leq 0.05$, according to Duncan's multiple range test.

On the upper surface, they were located around the central vein and the middle of the leaf. The largest stomatal cell on the upper surface of leaves was observed in the Neka population, with 40.26 µm length and 14.88 µm width. On the lower surface of the leaves, the largest stomatal cell was observed in the Rasht population, with 42.47 µm length and 14.57 µm width. The shortest stomatal cells occurred on the upper and lower surface of the leaves in the Shirgah population, with 35.13 µm, and in the Khalkhal population, with 29.96 µm.

The length and width of the guard cells on the upper (ad) and lower (ab) surfaces of *G. transcaucasicus* populations were calculated (Table 6). The largest guard cell on the upper surface of leaves was observed in the Neka population, with 73.10 µm length and 14.83 µm width, and on the lower surface of the leaves in the Gorgan population, with 68.22 µm length and 14.16 µm width.

Discussion

The comparative morphological analysis of *G. transcaucasicus* in this research showed small degrees of variability, with coefficients of variation ranging from 8.59 to 14.98%. Traits with a high coefficient of variation (CV) potentially create a wide range of options for breeding programs (Heydari et al., 2019). This study revealed that the average value of bulb size in *G. transcaucasicus* was larger than in *G. elwesii*, *G. gracilis* and *G. nivalis* populations collected from Bulgaria (Semerdjieva et al., 2019). Also, according to a morphological study on *G. elwesii G. nivalis* collected from Serbia, *G. transcaucasicus* had greater leaf length and width (Jovanovi et al., 2018). *G. alpinus* had greater inner perianth segments, outer perianth segments, spatha length, and leaf width than *G. transcaucasicus*. The widths of the inner perianth segments and outer perianth of *Galanthus transcaucasicus* were more significant than previously reported regarding *G. elwesii* (Sidjimova, 2006).

The correlation analysis of morphological traits of *G. transcaucasicus* in this research showed 18 significant positive correlations. In a study on six populations of *G. nivalis*, from Western Ukraine, there were six populations, each of which showed correlations between the length of the assimilating leaves and height of the flower stalk, width of the assimilating leaves and height of the pedicel, length of the outer perianth segments and length of the inner perianth segments, as well as the height of the pedicel and length of the spathe (Budnikov, 2011).

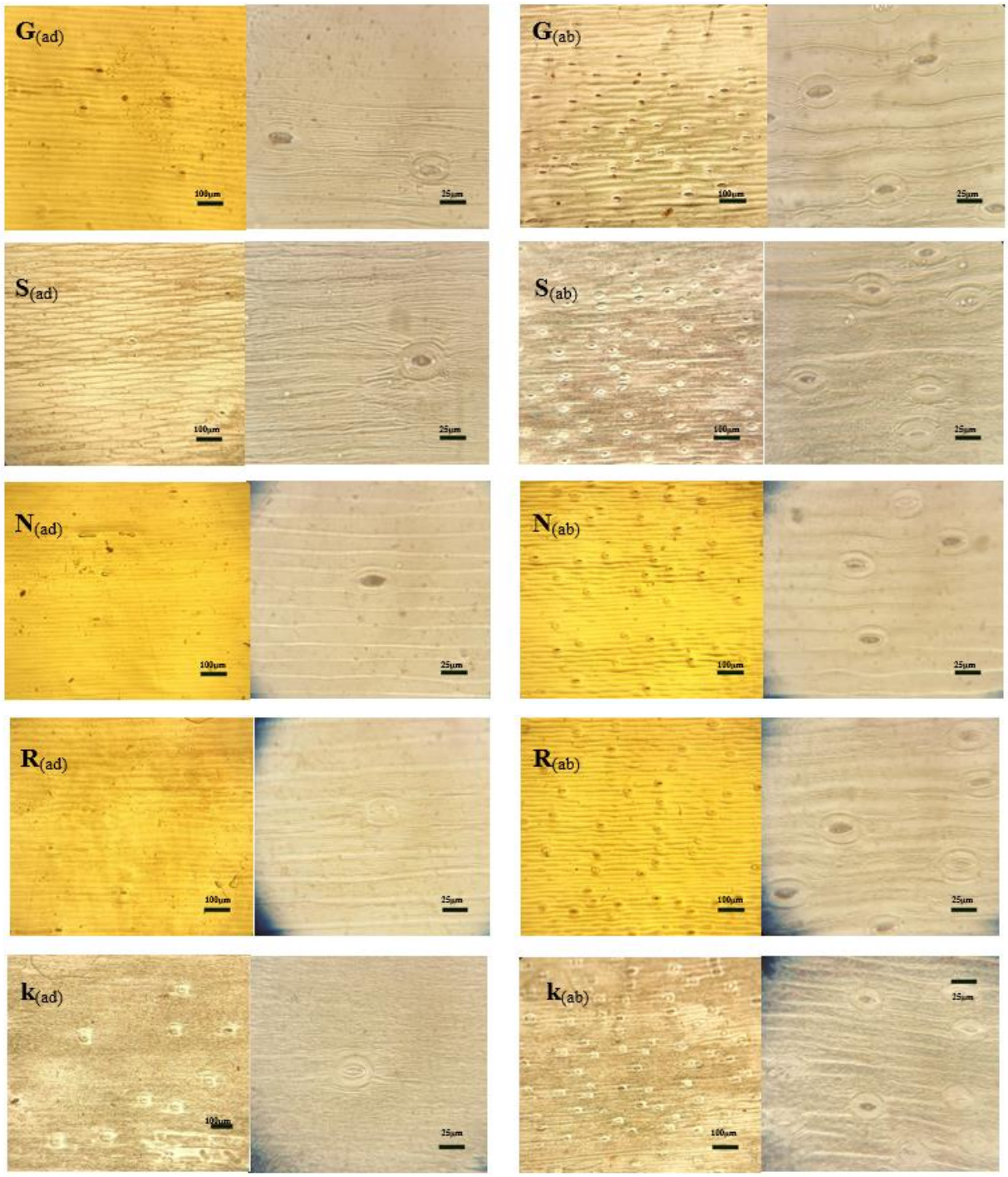

Fig. 6. Stomatal details of *Galanthus transcaucasicus* populations on the upper (ad) surface and lower (ab) surface of leaves

Also, there was a great correlation between the width of the outer and width of the inner perianth segments, the length of the leaf and length of the stem, the length of the outer and length of the inner perianth, and the width and length of the inner perianth segments in Bulgarian *Galanthus elwesii* (Sidjimova, 2006). No other comparable morphometric studies on *G. transcaucasicus* existed in the available literature for a comparison with this study.

The accessions were very similar according to the cluster analysis dendrogram based on

morphological features, perhaps because the habitats of all accessions from these populations had similar conditions, i.e. deciduous, wet, rain forests with humus-rich soils and humid air. Of course, the samples were separated into two main groups by geographical conditions. *G. transcaucasicus* from the western regions of the Caspian Sea, including accessions from Rasht and Khalkhal, were grouped in one category, whereas *G. transcaucasicus* of the central part of Caspian shores was placed in another group. Meanwhile, *G. transcaucasicus* from the eastern regions of the Caspian Sea, including accessions from Gorgan, were identified in both the first and second groups.

According to anatomical observations, the cells on the lower (abaxial) epidermis were spherical and short, but on the upper (adaxial) epidermis were oblong and strongly elongated (Fig. 4). Mesophyll was usually comprised of 3-5 layers of rounded parenchymatous cells, commonly separated into two halves by air cavities, bundle sheaths, and vascular bundles. The guard cells in the *G. transcaucasicus* accessions were bean-like in shape. Guard cells in Amaryllidaceae are usually bean-like in shape (Paliwal, 1969). The leaf anatomy of *Transcaucasicus* species was very similar to *Caucasus* species. In a study on the leaf anatomy of the *Galanthus* L. genus, the leaf anatomy of the *Caucasus* species was described (Davis and Barnett, 1997).

The number of stomata on the upper surface was less (ranging from 0.16 to 0.6 per mm^2) than their count on the lower surface (ranging from 3.77 to 9.51 per mm^2) (Table 6). In amphistomatous leaves, the density of stomata is usually higher on the lower surface (abaxial) than on the upper (adaxial) surface of the leaf, and although there are many environmental factors (e.g. light quality, light intensity, and air humidity) that play an essential role in stomatal density, the genetic construct of species can play a major role in stomatal density (Camargo and Marenco, 2011).

In a study on *Pancratium maritimum* L. or sea daffodil (Amaryllidaceae), the thickness of the leaves, as well as the number and size of the stomata varied significantly, which correlated consistently with general climate parameters (Perrone et al., 2015). Currently, the available literature lacks information on the anatomical features of *G. transcaucasicus*, and, thus, a comprehensive comparison cannot be made with the current research.

Conclusion

The results of this study revealed a low range of variability among morphological features of *G. transcaucasicus* accessions, likely representing a clone-population structure of one species.

Anatomically, there is a significant difference between populations from different regions. Therefore, in general, it seems that the genetic characteristics of the species, and the range of environmental conditions in which the species are distributed, play a role in the formation of the type and characteristics of the stomata and guard cells. The results of this study provide information for breeding programs, conservation and genetic storage of Iranian *G. transcaucasicus*. It is necessary to do more research on the characterization and evaluation of more accessions from other regions, especially to find genetic diversity at the molecular level.

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Conflict of interest

The authors indicate no conflict of interest for this work.

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