



GENETIC DIVERSITY AND TAXONOMIC STUDIES OF *ALLIUM AKAKA*

AND *A. ELBURZENSE* NATIVE TO IRAN USING MORPHOLOGICAL CHARACTERS

Sajad JAFARI¹, Mohammad Reza HASSANDOKHT*¹, Mahdi TAHERI², Abdolkarim KASHI¹

¹ Department of Horticultural Sciences, College of Agriculture and Natural Resources,
University of Tehran, Karaj, Iran

² Soil and Water Research Department, Zanzan Agriculture and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Zanzan, Iran

Received: April 2017; Accepted: June 2017

ABSTRACT

Two *Allium* species (*A. akaka* S.G. Gmelin and *A. elburzense* W.) native to Iran are used locally as the fresh vegetables and in medical therapy. They are not cultivated, but are collected from the wild, thus, will soon be threatened with extinction. In this study, the diversity of 15 wild accessions (4 accessions of *A. elburzense* endemic of Iran and 11 accessions of *A. akaka*) collected from the north-western part of Iran were evaluated with the use of 16 qualitative and 16 quantitative characteristics. The morphological characters with high heritability included leaf length, flower number in umbel, inflorescence diameter, leaf dry weight, bulb fresh weight, weight of 100 seeds, seed length and seed length/width. Results of the principal component analysis indicated that 92.52% of the observed variability was explained by the first six components. The first two components explained about 64.74% of the total observed variability. The first and third hierarchical cluster analysis included all accessions of *A. akaka*. The accessions of *A. elburzense*, except one, were placed in a separate cluster. These morphological descriptors can successfully apply for evaluating morphological diversity of *Allium* wild accessions and can help in horticultural usage.

Keywords: *A. akaka* S.G. Gmelin, *A. elburzense* W., broad-sense heritability, cluster analysis, morphological diversity

INTRODUCTION

Genus *Allium* (Amaryllidaceae, subfamily Alliioideae Herb., tribe Allieae) comprises more than 900 species worldwide (World Checklist of Selected Plant Families 2014) classified into 15 subgenera and 57 sections (Fritsch & Maroofi 2010; Fritsch & Abbasi 2013). Only subgenus *Amerallium* Traub has a second center of diversity in North America (Friesen et al. 2006; Gurushidze et al. 2010). According to Fritsch and Maroofi (2010), *Allium* is represented in Iran by 121 species. Thereafter several new species and subspecies from *Allium* were described and newly recorded for the Iranian territory (Razyfard et al. 2011; Fritsch & Abbasi 2013; Fritsch & Amini Rad 2013, Akhavan et al. 2015; Aryakia et al. 2016). *Allium* subg. *Melanocrommyum* (Webb &

Berthel.) Rouy is one of the largest subgenera in the genus and comprises 170 species and subspecies, and is diverse in Southwest Asia, particularly in Iran and Turkey. Among the Iranian species of *Allium*, 76 species and subspecies are assigned to subgenus *Melanocrommyum* (Fritsch & Abbasi 2013). According to the latest report (Fritsch & Abbasi 2013), 24 taxa (23 species) have been placed in this subgenus. Many species of this section are endemic to Iran, although a few species occur in the neighboring countries as well. This botanically diverse genus of the Alliaceae family is a horticulturally important crop with a potential for different purposes, such as spices, vegetables, medicinal or ornamental usage (Fritsch & Abbasi 2013). Two species (*A. akaka* S.G. Gmelin and *A. elburzense* W.) are known as ‘Valak’ in Iran and are used as fresh vegetables and

*Corresponding author:
e-mail: mrhassan@ut.ac.ir, Tel./fax: 00982632248721

medical agents. These plants are not cultivated, but are collected from the wild. Their populations have declined drastically during recent years, and will soon be threatened with extinction (Akhavan et al. 2015). To prevent the extinction of wild plant genotypes, *ex situ* conservation of germplasm resources was pioneered by Vavilov (1926) and nowadays, germplasm collections hold over 6 million crop plant accessions world-wide (Smykal et al. 2008). The study of genetic diversity for both germplasm management and breeding has received much attention. Collection, conservation and subsequent characterization and assessment of *Allium* germplasm could produce beneficial knowledge about the extent and pattern of genetic diversity, developing an important database for the support of several objectives, such as agricultural management, phylogenetic and taxonomic classification, evolutionary and ecological studies or breeding programs (Aryakia et al. 2016). Morphological descriptors are widely used in germplasm groups and remain the only legitimate marker type accepted by the international union for the protection of new cultivars of plants (UPOV). Morphological traits represent the action of numerous genes and thus contain high information value (Smykal et al. 2008). For characterization and evaluating morphological diversity of *Alliums*, it is important to use descriptors with high heritability and stability. Given the constraints of descriptors and according to the required performance (Podgornik et al. 2010), it might be necessary to use discriminative descriptors (Aryakia et al. 2016).

The main objective of the present study was to prepare a collection of germplasms and to evaluate the morphological variation in 15 accessions of two Iranian edible *Allium* species, collected from origin sites to prevent their extinction.

MATERIAL AND METHODS

Plant material and field site description

Intact bulbs of two wild *Allium* species were collected from 15 natural regions of Iran (Table 1; Fig. 1). These species included *A. akaka* S.G. Gmel (11 accessions A1 – A11) and *A. elburzense* W. (4 accessions E1-E4) belonging to the *Melanocrommyum* subgenus and sections of *Acanthoprason* and *Asteroprason*, respectively. A total of 15 bulbs of

each *Allium* accession were cultured in each plot (in three replicates) with distance of 0.2×0.3 m for each plant. The climate parameters of the region were obtained from the Iran meteorological organization (Table 1). Soil physical and chemical properties of the origin sites and field are presented in Table 2.

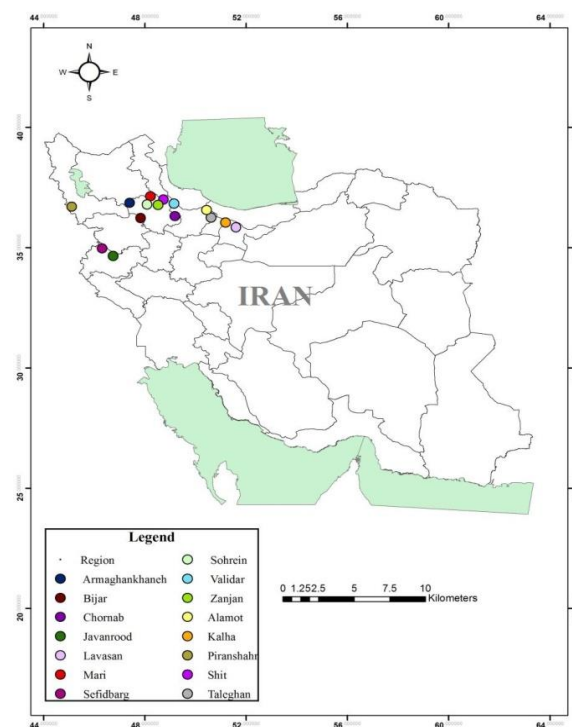


Fig. 1. Collecting sites of *Allium akaka* and *A. elburzense* accessions in Iran

Morphological parameters studied

A total of 16 qualitative and 16 quantitative morphological traits were investigated in their natural conditions in two phenological stages; at anthesis, for flowering and vegetative characters, and at the end of the season, for seed traits. Quantitative traits, based on IPGRI (2001) were analyzed including leaf length (cm), leaf width (mm), flower number in umbel, inflorescence diameter (cm), leaf dry weight (%), bulb fresh weight (g), bulb dry weight ($g \cdot 100 g^{-1}$ FW), leaf TSS (Total Soluble Solids) (%), bulb TSS (%), bulb height (mm), bulb width (mm), 100-seed weight (g), seed length (mm), seed width (mm), seed length/width ratio and seed thickness (mm) (some traits are additional information based on our experiences). 16 qualitative characteristics on inflorescence, scape, leaf and seed were assessed according to the list of descriptors given by the IPGRI (2001).

Heritability of quantitative traits

The ANOVA of quantitative traits was analyzed and the genotype and phenotype variance, general heritability and genetic and phenotypic coefficient variability (CV) were calculated based on the mathematical expectation of treatment and error mean squares using the following equations (Falconer 1989):

$$\begin{aligned}\sigma_g^2 &= MS_g - MS_e/r \\ \sigma_p^2 &= \sigma_g^2 + \sigma_e^2/r \\ \sigma_E^2 &= \sigma_p^2 - \sigma_g^2 \\ GCV &= \sqrt{\sigma_g^2}/X \\ PCV &= \sqrt{\sigma_p^2}/X \\ h^2 &= (\sigma_g^2/\sigma_p^2) \times 100\end{aligned}$$

where: σ_g^2 = genotype variance, MS_g = mean squares of genotype, MS_e = mean squares of error, r = replication, σ_p^2 = phenotype variance, σ_e^2 = error variance, σ_E^2 = Environment variance, GCV = Genetic CV, PCV = Phenotype CV, X = mean and h^2 = heritability.

Data analysis

Mean, minimum, maximum, variance, standard error (SE) and CV% of quantitative traits and frequency occurrence (%) of qualitative variables for each species were determined using SPSS v.21. For quantitative traits Pearson correlation was determined. In order to evaluate the information contained in the collected morphological data, principal component analysis (PCA) was carried out. Mean values per species were used to create a correlation matrix from which the standardized PCA scores were extracted and Scatter plot on the first two PCA was performed. PCA was used to identify the most important traits in the data set (Mousavizadeh et al. 2015). Cluster analysis was performed using the Ward's methods and Euclidean distance and a dendrogram was calculated.

RESULTS AND DISCUSSION

Characteristics of accessions

The wild accessions of two *Allium* species studied here were collected from different elevations (1853 to 2454 asl) and different climate (cold and semi-arid, semi-arid and ultra-cold and semi-wet and cold) (Table 1). They grow in all slopes that modify shading and humidity conditions. They tolerate well any type of soil: the sandy-loamy to loamy-sandy

with good drainage, low salinity and neuter pH. These species of *Allium* sprouted in Iran from late March to early May. Seeds matured from about early June to early July. In natural conditions, due to the indiscriminate harvesting of bulbs and aerial parts before seed formation, the frequency of these species is from intermediate to low with the concomitant risk of disappearance. Therefore they should be conserved in gene banks or botanical gardens. It concerns especially *A. elburzense*, which is a rare endangered species that only grows in Alborz Mountain.

The results of qualitative morphological measurements and descriptions and its frequency (%) are given in Tables 3 and 4. Results showed that some of the qualitative morphological characteristics, as bulb skin color, seed coat color, leaf number and foliage attitudes were similar across the studied genotypes. However, a high level of morphological diversity concerning other traits was observed at intra- and inter species level (Table 3 & 4). In agro-ecological conditions of field experiment, time of flowering was from late April to early June, and within this period was classified as early (A1, A2, A3, A4, A10 and A11), mediate (A5, A6, A7, A8, A9, E1 and E2) and late (E3 and E4). According to Fritsch and Abbasi (2013), the flowering time depends on environment conditions, such as temperature and precipitation at flowering (Aryakia et al. 2016). Morphological diversity of this trait, which was observed at intra- and interspecific levels, is also used in the evaluation of taxonomic positioning of different plants, which was important in evolutionary process responsible for reproductive diversification (Friedman & Harder 2005). Inflorescence fragrance was light in accessions of *A. akaka* [(except A3 that was light (75.1%) and medium (24.8%)] and medium in accessions of *A. elburzense* [(except E1 that was medium (66.7%) and light (33.3%)] (Table 3). No strong fragrance was observed among these 15 accessions of two *Allium* species. In previous studies (Nogueira et al. 2001; Jurgens 2004; Aryakia et al. 2016), inflorescence fragrance was used for taxonomic arrangement or pollination biology. Flower colors in *A. akaka* were white (37.4%) and pink (62.6%) in A1, white (17%) and pink (83%) in A2 and pink in other accessions of the species.

Table 1. Wild accessions of two *Allium* species collected from Iran and the climate parameters of their stands

Species	Symbol	Province-Region	Altitude	Longitude	Latitude	AminT	AmaxT	AT	TP	DMT	Climate
<i>A. ataka</i> S.G. Gmel.	A1	Kermanshah-Javanrood	1997	46°44'	34°29'	5.5	18.6	13.3	610	17.18	cold mediterranean
<i>A. ataka</i> S.G. Gmel.	A2	Kermanshah-Sefidbarg	1906	46°33'	34°21'	5.8	19.4	14	606	16	cold mediterranean
<i>A. ataka</i> S.G. Gmel.	A3	West Azerbaijan-Piranshahr	2185	46°68'	36°17'	5.1	16.3	13.1	677.1	24.29	cold and semi-arid
<i>A. ataka</i> S.G. Gmel.	A4	Kurdistan-Bijar	2022	47°36'	35°52'	4.5	17.4	12.5	347.7	26.9	cold and semi-arid
<i>A. ataka</i> S.G. Gmel.	A5	Zanjan-Armaghankhaneh	2255	47°33'	36°59'	4.3	17.8	11	316.5	23.3	semi-arid and ultracold
<i>A. ataka</i> S.G. Gmel.	A6	Zanjan-Sohrein	2280	47°31'	36°58'	4.2	17.6	10.5	328	22.75	semi-arid and ultracold
<i>A. ataka</i> S.G. Gmel.	A7	Zanjan-Mari	2360	47°29'	36°55'	4.1	17.4	10	326.7	24	semi-arid and ultracold
<i>A. ataka</i> S.G. Gmel.	A8	Zanjan-Chornab	2140	47°37'	37°21'	4.3	17.7	10.9	322	21.6	semi-arid and ultracold
<i>A. ataka</i> S.G. Gmel.	A9	Zanjan-Zanjan	1970	47°57'	37°08'	4.4	18.1	11.1	313.1	19.5	semi-arid and ultracold
<i>A. ataka</i> S.G. Gmel.	A10	Zanjan-Shit	1862	48°45'	37°11'	5.6	18.6	15.5	451	13.89	moderate mountain
<i>A. ataka</i> S.G. Gmel.	A11	Zanjan-Validar	1853	48°38'	36°95'	5.8	18.5	15.9	462.3	11.5	moderate mountain
<i>A. elburzense</i> W.	E1	Qazvin-Alamot	2330	50°09'	36°21'	4.2	16.5	10.5	483	17	semi-wet and cold
<i>A. elburzense</i> W.	E2	Alborz-Taleghan	2175	50°49'	36°12'	4.7	17.4	11.4	471	16.29	cold mediterranean
<i>A. elburzense</i> W.	E3	Alborz-Kalha	2454	51°17'	36°44'	4.3	15.7	11.8	411.2	37.87	semi-wet and cold
<i>A. elburzense</i> W.	E4	Tehran-Lavasan	2182	52°05'	35°59'	4.5	15.3	11.4	286.5	14	moderate mountain
Field site	-	Alborz-Karaj	1348	51°10'	35°41'	9.1	23.4	15.5	252.3	10.7	semi-arid with cold winter

AminT – average of minimum temperature (°C); AmaxT – average of maximum temperature (°C); AT – average of temperature (°C); TP – total precipitation (mm); DMT – days with maximum temperature equal 0 and below.

Table 2. Some physical and chemical properties of soil from the origin sites and field

Sites	Symbol	Depth (cm)	SSP (%)	EC (dS/m)	pH	TNV (%)	OC (%)	OM (%)	P (ava) (mg/kg)	K (ava) (mg/kg)	N (%)	Sand (%)	Silt (%)	Clay (%)	Texture
Javanrood	A1	0-30	21.5	0.592	7.01	2.6	0.73	1.3	15.2	94	0.075	71	24	5	sandy-loam
Sefidbarg	A2	0-30	24	0.553	7.27	2.5	0.7	1.2	13.6	102	0.07	68	26	6	sandy-loam
Piranshahr	A3	0-30	23	0.675	7.18	2.4	0.91	0.83	10.7	110	0.09	74	19	7	sandy-loam
Bijar	A4	0-30	22	0.535	7.88	2.5	0.28	0.49	2	189	0.025	86	8	6	loamy- sand
Armaghankhaneh	A5	0-30	21.5	0.518	7.12	2.4	0.92	1.24	12.3	194	0.09	63	29	8	sandy-loam
Sohrein	A6	0-30	23	0.561	7.2	2.6	0.81	1.13	14.7	116	0.084	65	21	14	sandy-loam
Mari	A7	0-30	27	0.512	7.62	2.9	0.62	1.06	13.6	238	0.062	58	32	10	sandy-loam
Chomab	A8	0-30	24	0.732	7.36	2.8	1.3	1.38	16	211	0.122	60	27	13	sandy-loam
Zanjan	A9	0-30	28	0.760	7.3	3.1	0.99	1.5	19.4	125	0.1	54	30	16	sandy-loam
Validar	A10	0-30	32	0.938	7.35	5.2	1.29	1.42	18.7	153	0.133	61	25	14	sandy-loam
Shit	A11	0-30	27	1.08	7.48	4.9	0.99	1.5	22	181	0.1	66	24	10	sandy-loam
Alamot	E1	0-30	26	0.817	7.52	2.9	1.03	0.95	19.5	115	0.105	69	22	9	sandy-clay-loam
Taleghan	E2	0-30	24	0.931	7.03	2.6	0.94	0.8	23.6	144	0.09	60	30	10	sandy-loam
Kalha	E3	0-30	31	1.376	7.09	2.3	1.64	2.83	11.8	116	0.182	70	20	10	sandy-loam
Lavasan	E4	0-30	23	0.668	7.69	4.1	0.87	1.5	6.8	89	0.087	86	10	4	loamy- sand
Field	-	0-30	42	2.318	7.65	9.4	0.83	1.83	28.4	376	0.101	34	40	2.6	loamy

SSP – Soil Saturation Percentage, EC – Electrical Conductivity, TNV – Total Neutralizing Value, OC – Organic Carbon, OM – Organic Matter, ava – available

Table 3. Frequency (%) of qualitative characteristics studied in 11 wild accessions of *A. atkaka* S.G. Grmel collected in Iran

Traits	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
TF	early (100)	early (100)	early (100)	early (100)	mediate (100)	mediate (100)	mediate (100)	mediate (100)	mediate (100)	early (100)	early (100)
FC	white (37.4), pink (62.6)	white (17), pink (83)	pink (100)	pink (100)	pink (100)	pink (100)	pink (100)	pink (100)	pink (100)	pink (100)	pink (100)
IF	light (100)	light (100)	light (75.1), medium (24.8)	light (100)	light (100)	light (100)	light (100)	light (100)	light (100)	light (100)	light (100)
AC	yellow (100)	yellow (100)	yellow (100)	yellow (100)	yellow (100)	yellow (100)	yellow (100)	yellow (100)	yellow (100)	yellow (100)	yellow (100)
SD	erect (100)	erect (100)	erect (100)	erect (100)	erect (100)	erect (100)	erect (100)	erect (100)	erect (100)	erect (100)	erect (100)
SDJA	medium (100)	medium (100)	medium (50), narrow (50)	medium (30), narrow (70)	narrow (100)	narrow (100)	narrow (100)	narrow (100)	narrow (100)	narrow (100)	narrow (100)
SL	intermediate (100)	intermediate (100)	intermediate (100)	intermediate (100)	intermediate (100)	intermediate (100)	intermediate (100)	intermediate (100)	intermediate (100)	intermediate (100)	intermediate (100)
FOLC	grey-green (100)	grey-green (100)	grey-green (100)	grey-green (100)	dark green (100)	dark green (100)	dark green (100)	dark green (100)	dark green (100)	dark green (100)	dark green (100)
DLW	medium (100)	medium (100)	weak (100)	weak (100)	weak (100)	weak (100)	weak (100)	weak (100)	weak (100)	weak (100)	weak (100)
FCR	medium (100)	medium (100)	medium (100)	medium (100)	medium (100)	medium (100)	medium (100)	medium (100)	medium (100)	medium (100)	medium (100)
SDB	globe (100)	globe (100)	globe (100)	globe (100)	globe (100)	globe (100)	globe (100)	globe (100)	globe (100)	globe (100)	globe (100)
PBSU	highly variable (100)	highly variable (100)	variable (100)	variable (100)	variable (100)	variable (100)	variable (100)	variable (100)	variable (100)	variable (100)	variable (100)

TF – time of 50% flowering, FC – flower color, IF – inflorescence fragrance, AC – anther color, SD – scape direction, SDIA – scape diameter, SL – scape length, FOLC – foliage color, DLW – degree of leaf waxiness, FCR – foliage cracking, SDB – shape of mature dry bulbs, PBSU – population bulb shape uniformity

Table 4. Frequency (%) of qualitative characteristics studied in four wild accessions from *A. elburzense* W. collected in Iran

Trait	E1	E2	E3	E4
Time of flowering (50 % flowering)	Mediate (100)	Mediate (100)	Late (100)	Late (100)
Flower color	Pink (100)	Pink (100)	Pink (100)	Purple (22), Pink (78)
Inflorescence fragrance	Medium (66.66), Light (33.33)	Medium (100)	Medium (100)	Medium (100)
Anther color	Purple (100)	Purple (100)	Purple (100)	Purple (100)
Scape direction	Erect (52.7), Indirect (47.3)	Erect (100)	Erect (66.7), Indirect (33.3)	Erect (58.4), Indirect (41.6)
Scape diameter	Medium (100)	Medium (82), Narrow (18)	Medium (93.4), Narrow (6.6)	Medium (30), Narrow (70)
Scape length	Intermediate (100)	Intermediate (100)	Intermediate (100)	Intermediate (100)
Foliage color	Grey- green (100)	Grey- green (100)	Dark green (100)	Dark green (100)
Degree of leaf waxiness	Weak (100)	Weak (100)	Weak (100)	Weak (100)
Foliage cracking	Medium (36.6), Strong (63.4)	Medium (55), Strong (45)	Medium (50), Strong (50)	Medium (50), Strong (50)
Shape of mature dry bulbs	Globe (100)	Globe (100)	Globe (100)	Globe (100)
Population uniformity of bulb shape	Variable (100)	Variable (100)	Variable (100)	Variable (100)

Table 5. The range of variability in *Allium* species quantitative traits, symbol, mean, min, max, standard error (SE) and standard deviation (SD)

Traits	Symbol	Unit	Mean	Min	Max	SE	SD
Leaf length	LL	cm	19.94	16.58	24.6	0.6	4.325
Leaf width	LW	mm	22.226	18.08	27.84	0.69	4.676
Flower number in umbel	FNU	-	78.26	53.66	111.88	2.66	20.325
Inflorescence diameter	IS	cm	7.922	5.6	7.58	0.121	0.99
Leaf dry weight	IDW	(%)	9.82	9.53	12.44	0.111	1.431
Bulb fresh weight	BFW	g	21.69	17.06	29.17	0.97	5.758
Bulb dry weight	BDW	(g/100 g FW)	19.16	17.1	20.81	0.3	3.164
Leaf TSS	LTSS	(%)	3.35	2.97	3.77	0.055	1.214
Bulb TSS	BTSS	(%)	18.51	16.46	20.67	0.337	4.308
Bulb height	BH	mm	25.36	21.47	31.46	0.764	4.961
Bulb width	BW	mm	20.89	18.14	26.12	0.599	4.32
100-seed weight	SW100	g	0.234	0.209	0.280	0.003	0.034
Seed length	SL	mm	2.31	1.83	3.08	0.12	3.19
Seed width	SW	mm	1.93	1.51	2.37	0.27	4.41
Seed length/width ratio	Seed L/W	-	1.19	1.22	1.4	0.08	2.37
Seed thickness	STh	mm	1.51	1.29	1.80	0.3	3.05

Table 6. ANOVA, variance components, coefficient of variance and broad-sense heritability of studied quantitative traits of *Allium* species

Traits	MS		Variance components				CV (%)		h ² (%)
	genotype	error	pheno- type	genetic	environ- ment	environ- ment	genetic	pheno- type	
LL***	16.22**	2.88	6.37	4.45	1.92	9.63	22.30	31.93	69.84
LW	21.48**	7.719	9.73	4.59	5.14	23.15	20.64	43.79	47.12
FNU	1173.3**	169.3	21.85	14.29	7.56	9.66	18.26	27.92	74.78
IS	2.11**	0.36	0.82	0.58	0.24	3.03	7.36	10.39	70.85
LDW	6.359**	0.918	2.43	1.81	0.61	6.23	18.47	24.70	74.76
BFW	42.37**	6.9	16.42	11.82	4.6	21.21	54.51	75.72	71.99
BDW	5.07*	2.29	2.45	0.93	1.52	7.97	4.84	12.80	37.77
LTSS	5.13**	1.41	2.18	1.24	0.94	5.08	6.70	11.78	56.88
BTSS	0.138**	0.054	0.06	0.03	0.03	1.07	0.84	1.91	43.75
BH	26.3**	5.88	10.73	6.81	3.92	15.46	26.84	42.30	63.45
BW	16.15**	5.03	7.06	3.71	3.35	16.05	17.74	33.80	52.50
SW100	0.008**	0.002	0.00	0.00	0.001	0.57	0.85	1.42	60
SL	1.735**	0.38	0.71	0.45	0.25	10.97	19.55	30.52	64.06
SW	2.14*	0.92	1.02	0.41	0.61	31.78	21.07	52.85	39.86
Seed L/W	0.005**	0.001	0.00	0.00	0.0006	0.06	0.11	0.17	66.66
STh	3.011**	0.755	1.26	0.75	0.50	33.33	49.80	83.13	59.90

* P<0.05, ** P<0.01; *** Symbols as in Table 5

In the *A. elburzense*, the flower color was pink in the accessions of E1, E2 and E3 and purple (22% and pink (78%) in E4. Variation in flower color is a common feature between and within *Allium* species (Fritsch and Abbasi 2013), and in other bulbous plants (Torskangerpoll et al. 2005). Anther color was recorded as yellow in *A. akaka* accessions and purple in *A. elburzense* accessions. It was reported that it is much less variable, but may present usable taxonomic characteristics in some cases (Fritsch & Abbasi 2013; Aryakia et al. 2016), but in our study, variable features of anther color were noted at inter and intraspecific level, which can be used for taxonomic classification of *Allium* species. Scape direction predominantly was erect in *A. akaka*, but in accessions of *A. elburzense* was erect in E1 and E2, erect and indirect in E3 and E4. Many *Allium* species proved to be erect in flowering stage, but in some cases, the scape remains permanently curved near the apex (Choi & Cota-Sanchez 2010). Scape diameter in *A. akaka* was medium (A1 and A2), narrow (A5, A6, A7, A8, A9, A10 and A11), medium (50%) and narrow (50%) and in A3 and medium (30%) narrow (70%) in A4. In *A. elburzense*, the

scape diameter was medium in E1, medium (82%) and narrow (18%) in E2, medium (93.4%) and narrow (6.6%) in E3, and medium (30%) and narrow (70%) in E4. Scape length was intermediate in all accessions of both species. Generally, morphological diversity in inflorescence and scape characteristics might be due to a variation in chromosome number (Gurushidze et al. 2012; Chae et al. 2014, Aryakia et al. 2016), natural interspecific hybrids (Gurushidze et al. 2010), inbreeding depression and transgressive segregation (Porta et al. 2014). In *A. akaka*, the foliage color was grey-green and dark-green. The foliage color in *A. elburzense* was grey-green in the accessions of E1 and E2 and dark-green in E3 and E4. The degree of leaf waxiness was weak and medium in *A. akaka*, and weak in all the accessions of *A. elburzense*. The foliage cracking in accessions of *A. akaka* was medium, and in *A. elburzense* was variable in each accession (medium and strong). The shape of mature dry bulbs in all the accessions was globe and population uniformity of bulb shape was highly variable. The pictures of different parts and growth phases of *A. akaka* and *A. elburzense* are presented in Fig. 2.



Fig. 2. Different parts and growth phases of two species of *Allium*: a = *A. akaka* S.G. Gmelin, Javanrood accession (a1 = inflorescence, a2 = anther, a3 = parts of a flower, a4 = ovary maturing, a5 = seeds, a6 = bulbs, a7 = Leaves), e = *A. elburzense* W., Kalha accession (e1 and e3 = inflorescence, e2 = parts of a flower, e4 = anther, e5 and e6 = ovary maturing, e7 = seeds maturing, e8 = bulbs)

Table 7. Means comparison of the different quantitative characters of 15 wild accessions from two *Allium* species (A = *A. akaka*, E = *A. elburzense*)

Accession	LL* (cm)	LW (mm)	FNU	IS (cm)	LDW (%)	BFW (g)	BDW (g/100g FW)	LTSS (%)	BTSS (%)	BW (mm)	BH (mm)	SW100 (g)	SL (mm)	SW (mm)	Seed L/W	STh (mm)
A1	21.58 ^{bc}	27.84 ^a	107.55 ^{ab}	7.45 ^a	10.15 ^{de}	29.12 ^a	18.93 ^{ab}	3.14 ^{b-d}	16.98 ^{de}	30.43 ^a	26.12 ^a	0.269 ^{ab}	2.92 ^{ab}	2.19 ^{ab}	1.33 ^a	1.80 ^a
A2	23.66 ^{ab}	26.7 ^{ab}	111.88 ^a	7.58 ^a	9.53 ^{de}	27.59 ^a	18.74 ^{ab}	3.33 ^{cd}	17.42 ^{c-e}	30.2 ^a	24.62 ^{ab}	0.280 ^a	3.08 ^a	2.37 ^a	1.30 ^a	1.74 ^a
A3	19.4 ^{c-e}	20.83 ^{c-e}	77.55 ^{cd}	6.52 ^{bc}	12.44 ^a	29.17 ^a	20.18 ^a	3.38 ^{cd}	18.75 ^{cd}	31.46 ^a	24.16 ^{ab}	0.266 ^{ab}	2.78 ^{bc}	2.21 ^{ab}	1.26 ^a	1.66 ^{ab}
A4	24.6 ^a	25.09 ^{ac}	81.66 ^{cd}	6.16 ^{b-e}	10.21 ^{de}	18.1 ^b	20.14 ^a	3.43 ^{ac}	17.55 ^{c-e}	22.26 ^b	19.68 ^c	0.259 ^{bc}	2.73 ^{b-d}	2.11 ^b	1.29 ^a	1.71 ^a
A5	19.83 ^{c-e}	19.41 ^{d-e}	84 ^{cd}	6.07 ^{c-e}	11.23 ^{bc}	20.6 ^b	19.32 ^{ab}	3.44 ^{ac}	16.46 ^e	25.39 ^b	18.78 ^c	0.216 ^f	1.99 ^{ef}	1.76 ^{ef}	1.13 ^{bc}	1.38 ^{de}
A6	20.75 ^{b-d}	22.65 ^{b-e}	66.88 ^{df}	5.67 ^e	9.73 ^{de}	20.56 ^b	18.41 ^{ab}	3.33 ^{ac}	19.13 ^{cd}	25.21 ^b	20.72 ^{bc}	0.209 ^f	1.83 ^h	1.58 ^{ef}	1.16 ^{bc}	1.29 ^e
A7	20.6 ^{b-d}	18.08 ^e	91.66 ^{bc}	6.82 ^b	10.29 ^{c-e}	20.61 ^b	17.16 ^b	3.46 ^{ac}	20.67 ^a	25.02 ^b	21.44 ^{bc}	0.215 ^f	1.87 ^{gh}	1.66 ^{df}	1.13 ^{bc}	1.42 ^{b-d}
A8	20.93 ^{b-d}	21.86 ^{b-e}	79.55 ^{cd}	6.4 ^{b-d}	10.29 ^{c-e}	21.16 ^b	17.1 ^b	3.58 ^{ab}	20.24 ^{ab}	24.53 ^b	20.92 ^{bc}	0.217 ^f	1.97 ^{gh}	1.6 ^{df}	1.23 ^{ab}	1.33 ^e
A9	17.74 ^{de}	23.82 ^{a-d}	77.66 ^{cd}	6.11 ^{b-e}	10.55 ^{cd}	20.15 ^b	18.32 ^{ab}	3.32 ^{ab}	19.37 ^{ac}	23.47 ^b	19.6 ^c	0.213 ^f	1.83 ^h	1.63 ^{df}	1.12 ^{bc}	1.29 ^e
A10	19.46 ^{c-e}	22.04 ^{b-e}	79 ^{cd}	6.48 ^{bc}	10.21 ^{de}	19.74 ^b	20.51 ^a	2.97 ^{b-d}	19.48 ^{ac}	24.51 ^b	20.52 ^{bc}	0.219 ^f	2.01 ^{fh}	1.77 ^{cf}	1.14 ^{bc}	1.38 ^{de}
A11	20.63 ^{b-d}	21.75 ^{b-e}	74.66 ^{c-e}	6.52 ^{bc}	10.28 ^{c-e}	20.61 ^b	19.96 ^{ab}	3.02 ^d	18.53 ^{de}	24.49 ^b	20.55 ^b	0.224 ^{ef}	2.27 ^g	1.84 ^{b-d}	1.23 ^{ab}	1.52 ^{bc}
E1	16.84 ^e	19.94 ^{c-e}	78.77 ^{cd}	6.67 ^{bc}	10.63 ^{cd}	20.78 ^b	18.19 ^{ab}	3.55 ^{od}	16.46 ^{ab}	24.47 ^b	19.28 ^c	0.236 ^{de}	2.44 ^{ef}	2.21 ^{ab}	1.10 ^c	1.55 ^{bc}
E2	16.58 ^e	21.81 ^{b-e}	57.33 ^{ef}	5.6 ^e	11.52 ^b	19.83 ^b	20.81 ^a	3.77 ^{b-d}	19.16 ^{cd}	23.67 ^b	19.86 ^c	0.235 ^{de}	2.5 ^{de}	2.29 ^a	1.09 ^c	1.62 ^{ab}
E3	19.5 ^{c-e}	21.28 ^{c-e}	53.66 ^e	6.24 ^{b-e}	10.31 ^{c-e}	20.3 ^b	19.53 ^{ab}	3.18 ^{b-d}	19.36 ^{ac}	23.88 ^b	18.89 ^c	0.247 ^{od}	2.59 ^{de}	2.25 ^{ab}	1.15 ^{bc}	1.63 ^{ab}
E4	17.02 ^e	20.23 ^{c-e}	54.66 ^e	5.72 ^{de}	10.04 ^{de}	17.06 ^b	20.13 ^a	3.26 ^{b-d}	18.1 ^{b-e}	21.47 ^b	18.14 ^c	0.215 ^f	1.92 ^{gh}	1.51 ^f	1.27 ^a	1.4 ^{de}

Means followed by the same letter in column are not significantly different at 5% (based on Duncan multiple range test). * Symbols as in Table 5

Table 8. Bivariate correlations between qualitative traits in two *Allium* species

	LL	LW	FNU	IS	LDW	BFW	BDW	LTSS	BTSS	BW	BH	SW100	SL	SW	Seed L/W	STh
LL***																
LW	0.556*															
FNU	0.615*	0.519*														
IS	0.490	0.456	0.868**													
LDW	-0.424	-0.387	-0.234	-0.248												
BFW	0.311	0.472	0.650**	0.723**	0.226											
BDW	-0.150	0.086	-0.389	-0.323	0.347	-0.091										
LTSS	-0.211	-0.251	-0.110	-0.281	0.387	-0.109	-0.260									
BTSS	-0.109	-0.296	-0.319	-0.246	-0.020	-0.234	-0.234	0.017								
BH	0.330	0.394	0.659**	0.702**	0.237	0.985**	-0.083	-0.121	-0.227							
BW	0.472	0.590**	0.743**	0.772**	0.009	0.930**	-0.138	-0.169	-0.096	0.914**						
SW100	0.475	0.618*	0.480	0.621*	0.101	0.718**	0.243	-0.034	-0.429	0.665**	0.673**					
SL	0.408	0.588*	0.401	0.569*	0.121	0.658**	0.312	-0.005	-0.467	0.600*	0.602	0.981**				
SW	0.167	0.389	0.233	0.429	0.264	0.525*	0.334	0.149	-0.413	0.479	0.415	0.864**	0.924**			
Seed L/W	0.630*	0.617*	0.439	0.469	-0.248	0.498	0.114	-0.316	-0.288	0.445	0.594*	0.614*	0.544*	0.184		
STh	0.378	0.525*	0.359	0.537*	0.119	0.575*	0.351	-0.013	-0.448	0.512	0.556*	0.939**	0.969**	0.896**	0.532*	

* P<0.05, ** P<0.01; ***Symbols as in Table

Quantitative traits

We studied the quantitative morphological traits in the natural conditions and in field cultivation, calculating the mean, maximum, minimum, standard error and standard deviation (Table 5). Analysis of variance exhibited highly significant differences ($P < 0.01$, $P < 0.05$) for all the 16 traits among the 15 accessions of two species *Allium*, indicating the presence of high degree of morphological variation among the accessions studied (Table 6). The percent values of variance components (phenotype, genetic and environment), environmental CV (%), phenotype CV (%), genetic CV (%) and broad sense heritability (%) for various traits are presented in Table 8. The characters that showed the greatest variation considering the phenotype CV% were leaf width, bulb height, bulb fresh weight, seed width and seed thickness, and characters with low phenotype CV(%) included inflorescence diameter, bulb dry weight, bulb TSS, leaf TSS, weight 100 seeds and seed length/width. The characters with high heritability percent included leaf length, flower number in umbel, inflorescence diameter, leaf dry weight, bulb fresh weight, leaf TSS, bulb height, bulb width, weight 100 seeds, seed length, seed length/width and seed thickness. So, these traits could be introduced as reliable ones in selection, segregation and classification of wild *Allium* accessions. Morphological classification provides useful guidelines to evaluate species relationships and to develop further insight for plant breeders and gene bank management (Khadivi-Khub et al. 2012). There were significant differences among accessions for all the evaluated quantitative morphological traits (Table 7). Accessions A1 and A2 of *A. akaka* produced large leaves, bulbs and seeds (leaf length and width, bulb height and width, bulb fresh and dry weight, weight 100 seed, seed L/W, seed length, width and thickness) and had the largest flower number in umbel, inflorescence diameter and leaf TSS. The inflorescence diameter is a fascinating context involving in all levels of research with respect to phylogeny and taxonomy (Mytnik-Ejsmont et al. 2015), ornamental applications (De Souza et al. 2012) and biological aspects (Fabbro & Korner 2004; Lambrecht & Dawson 2007). Accession A3 had high leaf dry weight, bulb fresh and dry

weight, bulb width and height, weight 100 seed, seed width, seed L/W and seed thickness. The accession A4 produced large leaves and had high bulb dry weight, leaf TSS, seed L/W and seed thickness. The accessions of A7 and A8 had the highest leaf and bulb TSS among all accessions. Accessions of A9, A10, A11, E1, E2, E3 and E4 had high bulb dry weight and bulb TSS. The comparison of means showed that the accessions of *A. elburzense* produced smaller plants in terms of leaf length and width, bulb height and width, flower number and inflorescence diameter than *A. akaka*.

Correlations between traits

The bivariate correlations between 16 plant characters are shown in Table 8. In the correlation matrix, most of the traits showed high correlation with each other, which indicate that some traits could be selected for breeding programs to save time and labors (Wang et al. 2014). The leaf length was positively correlated with leaf width ($r = 0.556$), flower number in umbel ($r = 0.615$) and seed length/width ($r = 0.630$). The flower number in umbel was correlated with inflorescence diameter ($r = 0.868$), bulb fresh weight ($r = 0.650$), bulb height ($r = 0.659$) and bulb width ($r = 0.743$). The seed thickness had positive correlation with leaf width ($r = 0.525$), inflorescence diameter ($r = 0.537$), bulb fresh weight ($r = 0.575$), bulb height ($r = 0.556$), weight 100 seeds ($r = 0.939$), seed length ($r = 0.969$), seed width ($r = 0.896$) and seed length/width ($r = 0.532$). Pavlovic et al. (2007) investigated the phenotypic correlations of some onion (*Allium cepa* L.) bulb traits and reported a high correlation between dry matter with bulb TSS ($r = 0.93$) and total mineral content ($r = 0.74$). Wang et al. (2014) investigated the Pearson correlation of some qualitative traits of 375 garlic accessions and reported that bulb yield was found to be strongly positively correlated ($r = 0.99$) with bulb weight and bulb diameter ($r = 0.71$).

Principal component analysis

In order to assess the patterns of variation, principal component analysis (PCA) was conducted by simultaneously considering all the 16 variables. The first six principal components (PCs) with eigenvalue above 0.5 explained 92.52% of the variability amongst the 15 accessions under study (Table 9).

Table 9. Eigenvalues and cumulative variance for six major factors obtained from principal component analysis (PCA) for two *Allium* species

Traits	PCA1	PCA2	PCA3	PCA4	PCA5	PCA6
LL*	0.188	0.162	0.798	-0.298	-0.024	-0.027
LW	0.210	0.390	0.668	-0.144	-0.194	0.106
FNU	0.657	0.110	0.341	-0.518	-0.005	0.265
IS	0.666	0.356	0.180	-0.523	-0.225	0.116
LDW	0.302	0.114	-0.454	0.666	0.405	0.012
BFW	0.912	0.338	0.156	0.021	-0.007	0.049
BDW	-0.240	0.376	0.022	0.766	-0.331	0.136
LTSS	-0.118	0.089	-0.173	-0.032	0.958	-0.004
BTSS	-0.065	-0.297	-0.100	-0.028	0.010	-0.941
BH	0.930	0.276	0.123	0.027	-0.015	0.060
BW	0.857	0.282	0.350	-0.097	-0.064	-0.084
SW100	0.417	0.806	0.359	0.053	0.018	0.134
SL	0.332	0.876	0.290	0.072	0.015	0.162
SW	0.214	0.958	-0.040	0.050	0.092	0.120
Seed L/W	0.335	0.165	0.820	0.128	-0.154	0.143
STh	0.263	0.882	0.274	0.085	-0.005	0.151
Eigenvalue	7.69	2.65	1.81	1.14	0.828	0.657
Variance (%)	48.12	16.62	11.35	7.14	5.17	4.10
Cumulative (%)	48.12	64.74	76.1	83.24	88.41	92.52

*Symbols as in Table 5

PC1 accounted for 48.12% of the total morphological variation for the traits. PC1 includes flower number in umbel, inflorescence diameter, bulb fresh weight, bulb height and width as the traits with the largest coefficients, all with positive sign. The traits of bulb and leaf TSS had low values for PC1, while the traits related to seed morphology exhibited moderate to high positive weight on PC1. PC2 accounted for 16.62% of the variance and was more related to the seed morphological traits, which contributed with high coefficients except for seed length/width ratio. The characters with greatest positive weight on PC3 (accounted 11.35%) were leaf length, leaf width and seed length/width ratio. PC3 seems to be more related to yield contributing traits. PC4 accounted for 7.14% of the variance and had positive amount for dry weight leaf and bulb. All the traits related to leaf size, flower and bulb TSS and leaf TSS had

negative values for PC4. Wang et al. (2014) evaluated the morphological diversity of 212 accessions of *A. sativum* L. in China and reported that the principal component analysis of 21 quantitative traits revealed eight principal components with eigenvalue above 1 and explained 71.35 % of total morphological variation. The aim of PCA is to determine the number of main factors that could be extracted to reduce the number of effective parameters to discriminate genotypes. In addition, associations between traits emphasized by this method may correspond to genetic linkage between loci controlling traits or to a pleiotropic effect (Iezzoni & Pritts 1991; Rakonjac et al. 2010). Morphological characterization is the first step for the description and classification of germplasm and statistical methods like PCA are useful tools for screening the accessions in a collection (Mousavizadeh et al. 2015).

Cluster analysis

The relationship among the 15 accessions of *Allium* based on genetic distance values was determined by cluster analysis using Ward’s methods (Table 10, Fig. 3). According to the dendrogram, the 15 accessions could be classified into three clusters. Cluster A with 10 accessions, 9 of *A. akaka* and 1 of *A. elburzense* are characterized by LDW, LTSS, BTSS and seed length/width ratio higher than cluster C, in which are also accessions of *A. akaka*. In the cluster B, there are three accessions of *A. elburzense* that are characterized by lower means of most traits with the exceptions for LDW, BDW, and BTSS. The two accessions of *A. akaka* are placed in cluster C, which grouped plants with highest means of most traits except LDW, BDW, LTSS and (Table 10). Compared with the other clusters, the C cluster possesses the accessions with the largest leaves and bulbs that constitute potential to select high yield candidate plants. As observed, the accessions of *A. elburzense* of the section *Asteroprason*, were placed, except E1, in the same cluster, which confirms separation the species belong to different sections. So, the two studied species of *Allium* are well separated by morphological quantitative traits based on clustering. Based on proximity matrix, the two studied here *Allium* species are placed at Euclidean distance of 34.5 (Table 11).

Table 10. Cluster and total means for quantitative traits of *Allium* accessions

Traits	Cluster mean			Total mean
	A	B	C	
LL*	20.07	17.02	22.62	19.94
LW	21.54	20.23	27.27	22.226
FNU	79.13	54.66	109.71	78.26
IS	6.34	5.72	7.51	7.922
LDW	10.58	10.04	9.84	9.82
BFW	21.14	17.06	28.35	21.69
BDW	18.92	20.13	18.83	19.16
LTSS	3.34	3.26	3.23	3.35
BTSS	18.66	18.1	17.2	18.51
BH	25.08	21.47	30.31	25.36
BW	20.56	18.14	25.37	20.89
SW100	0.227	0.215	0.274	0.234
SL	2.17	1.92	3	2.31
SW	1.83	1.51	2.28	1.93
Seed L/W	1.17	1.27	1.31	1.19
STh	1.45	1.4	1.77	1.51

*Symbols as in Table 5

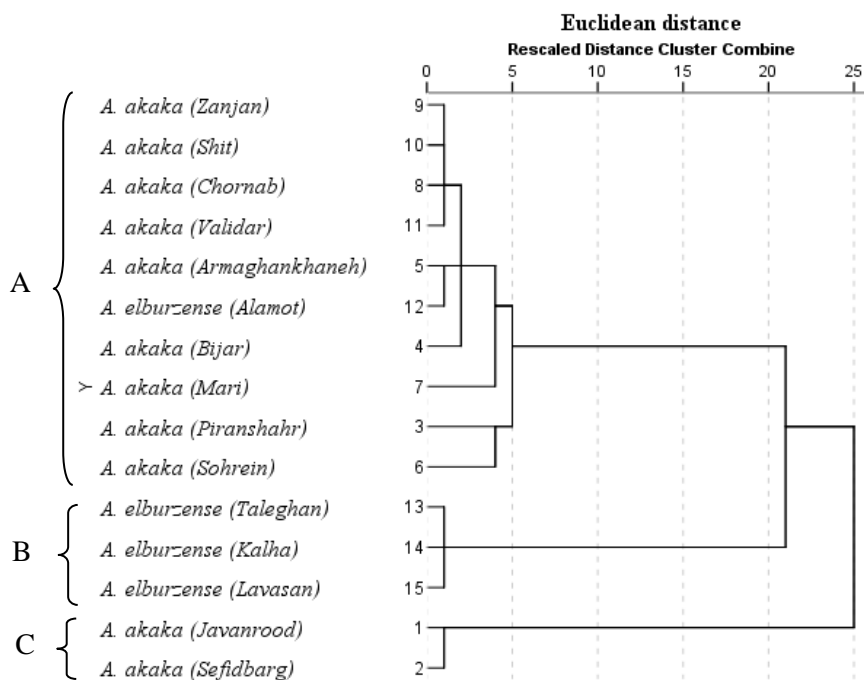


Fig. 3. Dendrogram of grouping for 15 accessions of two *Allium* species

Table 11. Proximity matrix for *Allium* species

Case	Euclidean distance	<i>Allium elburzense</i> W.
	<i>Allium akaka</i> S.G. Gme	
<i>Allium akaka</i> S.G. Gme	0	
<i>Allium elburzense</i> W.	34.5	0

Scatter plot

The 15 accessions of two *Allium* species were clustered into five groups using the PCA1 and PCA2 scores (Fig. 4). It did not support the results of the cluster analysis; since accessions were mostly distributed in value of 0 to 1 of PC1, except the accession A9. The A group included five accessions of *A. akaka* (A3, A4, A6, A10 and A11) with fewer flower number in umbel and larger bulbs than total mean. Three accessions of *A. elburzense* (E1, E2 and E3) are accounted for B group, which includes accessions with smaller leaves, flower number in umbel, bulb fresh weight, bulb width and bulb height, and with seed width greater than mean of all accessions.

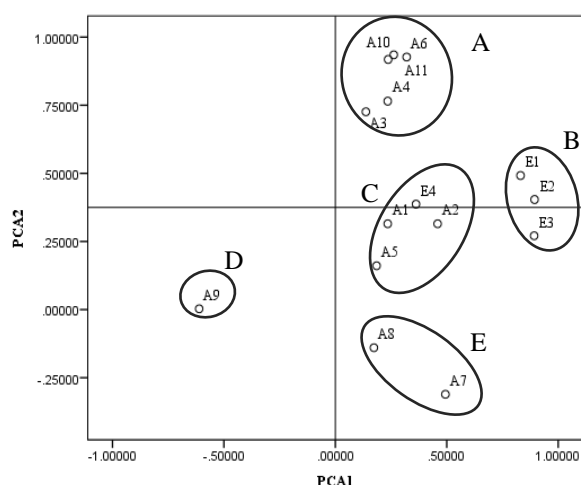


Fig. 4. Factor scores for the first two principal component analysis (PCA) for 15 accessions of two *Allium* species

In the C group, there were three accessions of *A. akaka* (A1, A2 and A5) and one accession of *A. elburzense* (E4) with larger leaves, flower number

in umbel, bulb fresh weight, bulb width and traits related to the seed size. In the D group, there was accession of *A. akaka* (A9) with smaller leaf length, bulb dry and fresh weight, bulb size and seed size. There were two accessions of *A. elburzense* (A7 and A8) in the E group with larger leaf length, flower number in umbel, bulb and leaf TSS, bulb height, and with smaller leaf width, bulb fresh and dry weight, bulb width and seed size than the mean of all accessions. The accessions collected in near provinces did not gather into the same group, revealing that the morphological traits of accessions are variable among different locations within the close provinces.

The diversity of accessions within the geographical region might be due to the heterogeneity and genetic architecture of accessions and developmental traits (Singh 1991) that has been reported in different crop species (Alemayehu & Becker 2002; Singh et al. 2004, Bhargava et al. 2007). The accessions of a particular cluster having desirable genes for a specific trait can be hybridized with the other accessions of different clusters, which may facilitate to accumulate favorable genes in hybrids. The hybrids thus obtained may be fixed by selecting transgressive segregants, followed by recurrent selections in advanced generations, which may lead to the development of high yielding varieties with desirable components.

CONCLUSION

In this study, the accessions of *A. akaka* and *A. elburzense* were evaluated. The results of evaluation of morphological diversity revealed that an enormous amount of variability exist in the studied *Allium* accessions growing naturally in Iran. Cluster analysis grouped the accessions of greater similarity but the clusters did not include only accessions from the same species, indicating heterogeneity of accessions within a given geographical region. Multivariate analysis of 16 traits showed that most of the variations were accounted by the first six PCs. The main traits that accounted for more variability in both PC1 and PC2 include flower number in umbel, inflorescence diameter, bulb fresh weight, bulb height, bulb width, seed length, seed width and seed

thickness. Thus, these traits are important in distinguishing the material under study. A wide range of diversity at the intra- and interspecific level of wild accessions of *A. akaka* and *A. elburzense* is important for the study of phylogeny and taxonomic arrangement, evolution and ecology and breeding programs.

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