eJBio

Electronic Journal of Biology, 2016, Vol.12(3): 302-308

Genetic Diversity and Classification of Wild *Arum* (Araceae) Species Using Morphological Characters in Iran

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Citation: Joudi L, Mehregan I, Assadi M, et al. Genetic diversity and classification of wild *Arum* (Araceae) Species Using Morphological Characters in Iran. Electronic J Biol, 12:4

Received: May 24, 2016; **Accepted:** June 24, 2016; **Published:** July 01, 2016

Research Article

Abstract

Assessment of genetic diversity is primarily useful to classification of plant species. In this study, a multivariate statistical analysis was performed on morphological characters of *Arum* L. species in Iran. Totally, 29 qualitative and quantitative morphological characters of species were evaluated. Cluster analyses by ward method classified the genotypes based on morphological traits in three groups. Cluster 1 had 1 genotype. Cluster 2 had 2 genotypes and cluster 3 had 2 groups. Dendrogram of Genetic Relationships among *Arum* species which was constructed by the UPGMA method demonstrated that 4 main groups existed in the collection at the highest level of hierarchy (0.34). Using the results from morphological studies, differential characteristics were obtained in the principal component analysis. The result of principal component analyses introduced two principal components with eigenvalue more than one which contributed 99.075% of the total variability. Classification of the cluster analysis was confirmed by principal component analysis and good variety of different traits to species based on similarities and differences have separated. In conclusion, morphological traits can identify and classify species of this genus as a systematic application.

Keywords: *Arum*; Cluster analysis; Principal component analysis.

Introduction

The family Araceae with 3790 species in 117 genera, have a worldwide distribution and are found in a wide range of environments [1,2]. Most of the aroids are tropical and contain members from terrestrial, aquatic, and epiphytic habitats although there are many aroids indigenous to temperate climates. The family Araceae is most easily diagnosed by the inflorescence which a spadix unbranched spike is bearing small bractless flowers subtended by a modified leaf called a spathe. The plants have adapted in response to climatic, ecological and biotic conditions (i.e., selective pressures) according to the various habitats occupied in the different regions of Europe and the Middle East [3]. The genus *Arum* with 28 species have distributed in Europe, North Africa, the Middle East and Central Asia [4,5]. In Iran, *Arum* species grow naturally in mountains, red and alluvial soils, near water canal, rocky places and forests. Amorphophallus Decne. is especially noteworthy because of the enormous tubers produced by certain species (*Arum giganteum*). The largest species: *Amorphophallus titanum* Becc. can produce tubers weighing approximately 70 kg. Genetic variability is more likely to be found between species from completely different environmental conditions [6]. Evidence from morphology, as an important feature in the diagnosis and taxonomy of plants and as a basic language used to describe and identify, classify and study the relationship between evolutionary and phylogenetic relationships of plants, through morphological characteristics has always been popular [7]. The aim of this study was to evaluate several quantitative and qualitative characteristics of the species, with emphasis on the traits of spath and spadix which could be used as important characteristics to distinguish the species of this genus for taxonomic identification.

Materials and Method

Plant material and morphological evaluation

In order to conduct morphometric researches, all species of *Arum* were collected from different locations of Iran reported in the flora of Iran that *Arum* species were introduced before [8]. The number of collected plants in each location was 5 plants. The collected plants had an uneven distribution in different parts of Iran: *A. maculatum* from 11 locations. (North, North West), *A. kotschyi* from 12 locations (North, North West, North East, Tehran), *A. korolkowii* from 11

locations (North, North West, North East, Tehran), *A. virescens* from 9 locations (North, North West, East), *A. conophalloides* from 8 locations (East), *A. giganteum* from 3 locations (East and Center). Table 1 represents only the samples which already have a herbarium code. The selected plants were growing under ambient environmental conditions like e.g. light, temperature, soil condition, etc. The minimum and maximum of each parameter were recorded. Due to the small differences in between species, the values would overlap if the average has taken into account. The samples were sent to the Forests and Rangelands Institute (TARI) and were identified based on Flora of Iran and the world scientific resources [8]. Lab analyses were carried out at the Herbarium of Research Institute of Forests and Rangelands (TARI) in 2014 and 2015. The following quantitative morphological variable were petiole length (cm), leaf blade length and wide (cm), peduncle (cm), spathe length and wide (cm), spathe tube length (cm), pistillate zone, staminode zone and staminate zone (mm). Some of morphological characters were not measurable quantitatively and qualitative characters were used for analysis. Qualitative characters used in the experiment were as follows: Tuber shape, arrangement of leaves and inflorescence, leaf blade shape, leaf blade apex and inner and outer section of spath. In order to conduct cluster analysis, the minimum and maximum yield was used, while qualitative characteristics were coded as attributes of two or more cases.

Statistical analysis

Collected data in 2014 and 2015 years were

Table 1. Species characteristic and species herbarium code.

Species	Origin, voucher
Arum ***maculatum***	Iran, Prov. N. Gorgan, Ramsar 200-500 m, (69216 TARI).
A. kotschyi	Iran, Prov. N. Mazandaran, Siahbishe 2200 m, (27355 TARI).
A. korolkowii	Iran, Prov. N. Mazandaran, veisar 1600 m, (50656TARI).
A. virescens	Iran, Prov. N. Mazandaran, Polsefid 670 m, (36826TARI).
A. conophalloides	Iran, Prov. W. Hamedan, Nahavand 2600 m, (22222TARI).
A. giganteum	Iran, Prov. W. Khorramabad, Rimele 1800 m, (2558TARI).

Table 2. Mean and standard deviation of each variable.

Variables	N	Mean	Std. Deviation
Petiole length (min)	6	17.8333	9.82683
Petiole length (max)	6	40.6667	8.43010
Leaf Blade Length (min)	6	11.8333	8.93122
Leaf Blade Length (max)	6	20.0000	5.32917
Leaf Blade Wide (min)		6.0000	4.64758
Leaf Blade Wide (max)	6	11.6667	2.50333
Peduncle Length (min)	6	20.5000	12.40564
Peduncle Length (max)	6	44.6667	15.97081
Spathe Length (min)	6	17.1667	12.96791
Spathe Length (max)	6	32.0000	20.27807
Spathe Wide (min)	6	3.6667	2.42212
Spathe Wide (max)	6	6.7500	4. 60163
Spathe Tube Length (min)	6	15.0000	12.74363
Spathe Tube Length (max)	6	25.8333	20.49797
Spathe Tube Limb (min)	6	11.1667	12.09408
Spathe Tube Limb (max)	6	20.9167	20.50467
Pistillode Zone (min)	6	13.5000	8.64292
Pistillode Zone (max)	6	21.6667	11.25463
Staminate Zone (min)	6	6.3333	4.45720
Staminate Zone (max)	6	8.5000	4.23084
Staminode Zone (min)	6	6.1667	4.91596
Staminode Zone (max)	6	9.5000	4.84768
Tuber Shape	6	1.1667	0.40825
Leaf O Flower	6	1.8333	0.40825
Leaf Blade Shape	6	1.5000	0.83666
Leaf Blade Apex	6	1.1667	0.40825
Spathe Tube Color	6	1.5000	0.83666
Inner Section of Spathe	6	2.1667	0.75277
Outer Section of Spathe	6	1.8333	0.40825
Valid N (list wise)	6		

combined and analyzed. Statistical analysis and draw dendrograms were done by SAS 9.1. The mean and standard deviation was prepared and shown in Table 2. The correlation analyses were performed following the methods of and Snedecor and [9,10]. In order to describe the classification of studied species cluster analysis using the ward and principal component analysis (Factor Analysis) and also ordination attributes (Principal Component Analysis) were performed on traits. The Euclidean distance coefficients were used in the assessment of the similarity of morphological characters in the cluster analysis [11].

Results

Correlation

The correlations among all pairs of variables are shown in Table 4. Petiole length (min) was significantly correlated with leaf blade length (r=0.891*), leaf

Table 3. Morphological quantitative variable in species characteristic.

Species	Petiole length (cm)	Leaf Blade Length (cm)	Leaf Blade Wide (cm)	Peduncle Length (cm)	Spathe Length (cm)	Spathe Wide (cm)	Spathe Tube Length (cm)	Spathe Tube Limb (cm)	Pistillode Zone (mm)	Staminate Zone (mm)	Staminode Zone (mm)
Arum ***maculatum***	10-74	7-22	2-10	4-18	11-19	4.5-7.5	6-9	3-6.5	6-10	2-3	3-4
Arum ***kotschyi***	9 -37	8-17	3-13	13-51	7.5-17	1.5-3	6-13	4-10	7-15	3-5	3-7
Arum ***korolkowi***	12-35	8-16	5-13	16-64	14-20	1.5-2	11-17	5-6	12-15	6-8	5-7
Arum ***virescens***	21-55	9-17	5-8	35-55	13-27	3.5-6	12-21	8.5-18	13-20	5-10	5-8
Arum ***conophalloides***	20-35	9-18	6-11	20-40	15-39	3-7	15-30	11.5-25	13-40	6-10	5-15
Arum ***giganteum***	15-35	10-30	10-15	35-40	43-70	8-15	40-65	35-60	20-30	10-15	6-16

Table 4: Correlation coefficients between studied traits expressed as averages combining two years. (*p<0.05, ** p<0.01).

	PL min	P L max	L B L min	LBL max	LBW min	LBW max	PEL min	PEL max	SL min	SL max	SW min	SW max	STL min	STL max
PLmin														
PL max	-0.117													
LBL min	0.891*	-0.325												
LBL max	0.733	-0.187	0.895*											
LBW min	0.942**	-0.378	0.968**	0.791										
LBW max	0.306	-0.859*	0.632	0.495	0.602									
PEL min	0.846*	0.147	0.631	0.333	0.725	0.045								
PEL max	-0.022	-0.15	-0.099	-0.512	0.043	0.142	0.394							
SL min	0.914*	-0.325	0.981**	0.886*	0.979**	0.587	0.631	-0.111						
SL max	.960**	-.335	.940**	0.829*	0.968**	0.496	.681	-0.149	0.957**					
SW min	0.821*	0.023	0.866*	0.961**	0.791	0.275	0.486	-0.500	0.878*	0.849*				
SW max	0.861*	-0.070	0.877*	0.946**	0.823*	0.304	0.511	-0.498	0.886*	0.906*	0.982**			
STL min	0.947**	-0.346	0.977**	0.836*	0.996**	0.577	0.700	-0.041	0.990**	0.979**	0.839*	0.868*		
STL max	0.956**	-0.375	0.959**	0.802	0.991**	0.564	0.715	-0.034	0.967**	0.991**	0.808	0.859*	0.992**	

	STM min	STM max	PZ min	PZ max	STZ min	STZ max	SDZ min	SDZ max	TS	LOF	LBS	LBA	STC	ISS	OSS
PL min															
PL max															
LBL min															
LBL max															
LBW min															
LBW max															
PEL min															
PEL max															
SL min															
SL max															
SW min															
SW max															
STL min															
STL max															

	PL min	PL max	LBL min	LBL max	LBW min	LBW max	PEL min	PEL max	SL min	SL max	SW min	SW max	STL min	STL max
STM min	0.950**	-0.325	0.982**	0.855*	0.987**	0.560	0.697	-0.090	0.980**	0.986**	0.857*	0.894*	0.995**	0.994**
STM max	0.960**	-0.293	0.956**	0.833*	0.965**	0.498	0.715	-0.125	0.948**	0.990**	0.850*	0.904*	0.975**	0.988**
PZ min	0.955**	-0.310	0.957**	0.773	0.996**	0.545	0.768	0.072	0.975**	0.957**	0.793	0.816*	0.991**	0.982**
PZ max	0.663	-0.393	0.421	0.250	0.574	0.166	0.516	-0.002	0.47	0.688	0.318	0.473	0.565	0.652
STZ min	0.919**	-0.390	0.966**	0.808	0.994**	0.621	0.680	0.024	0.988**	0.954**	0.800	0.819*	0.993**	0.977**
STZ max	0.945**	-0.241	0.802	0.523	0.915*	0.359	0.894*	0.275	0.837*	0.886*	0.605	0.660	0.894*	0.912*
SDZ min	0.920**	-0.327	0.989**	0.855*	0.989**	0.607	0.680	-0.027	0.994**	0.951**	0.845*	0.855*	0.993**	0.973**
SDZ max	0.833*	-0.494	0.704	0.503	0.817*	0.429	0.634	0.028	0.730	0.879*	0.528	0.657	0.806	0.868*
TS	-0.391	0.368	-0.265	0.184	-0.422	-0.326	-0.652	-0.818*	-0.233	-0.314	0.169	0.080	-0.346	-0.402
LOF	0.391	-0.368	0.265	-0.184	0.422	0.326	0.652	0.818*	0.233	0.314	-0.169	-0.080	0.346	0.402
LBS	-0.499	-0.425	-0.308	-0.493	-0.257	0.382	-0.318	0.674	-0.304	-0.460	-0.642	-0.688	-0.319	-0.356
LBA	0.108	-0.329	-0.155	-0.184	0.000	-.0130	-0.020	-0.143	-0.082	0.169	-0.135	0.027	0.000	0.100
STC	-0.304	0.766	-0.335	0.045	-0.463	-0.668	-0.356	-0.644	-0.082	-0.365	0.148	0.039	-0.394	-0.449
ISS	0.734	-0.557	0.600	0.299	0.743	0.460	0.653	0.288	0.611	0.760	0.311	0.447	0.709	0.780
OSS	0.391	-0.368	0.265	-0.184	0.422	0.326	0.652	0.818*	0.233	0.314	-0.169	-0.080	0.346	0.402

	STM min	STM max	PZ min	PZ max	STZ min	STZ max	SDZ min	SDZ max	TS	LOF	LBS	LBA	STC	ISS	OSS
STM min															
STM max	0.991**														
PZ min	0.979**	0.954**													
PZ max	0.578	0.646	0.555												
STZ min	0.976**	0.942**	0.992**	0.525											
STZ max	0.878*	0.878*	0.933**	0.714	0.891*										
SDZ min	0.985**	0.954**	0.986**	0.464	0.992**	0.861*									
SDZ max	0.815*	0.855*	0.795	0.935**	0.778	0.863*	0.734								
TS	-0.331	-0.344	-0.425	-0.508	-0.366	-0.637	-0.316	-0.556							
LOF	0.331	0.344	0.425	0.508	0.366	0.637	0.316	0.556	1.000**						
LBS	-0.385	-0.475	-0.263	-0.425	-0.215	-0.254	-0.267	-0.370	-0.293						
LBA	0.014	0.098	-0.028	0.798	-0.037	0.174	-0.116	0.556	-0.200	-0.293					
STC	-0.376	-0.370	-0.429	-0.531	-0.429	-0.537	-0.365	-0.616	0.878*	-0.429	-0.293				
ISS	0.710	0.746	0.722	0.905*	0.695	0.848*	0.640	0.959**	-0.759	-0.159	0.542	-0.794			
OSS	0.331	0.344	0.425	0.508	0.366	0.637	0.316	0.556	-1.000**	1.000**	0.293	0.200	-0.878*	0.759	

*p<0.05, ** p<0.01, Petiole Length=PL, Leaf Blade Length=LBL, Leaf Blade Wide=LBW, Peduncle Length=PL, Spath Length=SL, Spath Wide=SW, Spath Tube Length=STL, Spath Tube Limb=STM, Pistillode Zone=PZ, Staminate Zone=STZ, Staminode Zone=SDZ, Tuber Shape=TS, Leaf O Flower=LOF, Leaf Blade Shape=LBS, Spath Tube Color=STC, Leaf Blade Apex=LBA, Inner Section of Spath=ISS and Outer Section of Spath=OSS

blade wide (r=0.942**) and spath length (r=0.960**). On the other hand spath length could be effected on spath tube length (r=0.990**), spath tube limb (r=0.986**), pistillode zone (r=0.957**), staminate zone (r=0.954**) and staminode zone (r=0.951**). Staminate zone was correlated on pistillode zone(r=0.992**), Staminode zone effected on pistillode zone(r=0.986**) and staminate zone (0.992**). In qualitative traits, arrangement of leaves and flowers was effective on tuber shape (1.000**). The other characters expressed a non-significant correlation. Therefore, the function of the each of the traits is assessed in its performance on petiole length and inflorescence components. Positive and significant correlation between petiole length and the structure of florescence could be originated from genotypes ability in competition of light absorption in order to promote the photosynthesis process to reach to the reproductive stage.

Cluster analyses

Data analyses using Ward and UPGMA methods are shown in Figures 1 and 2. Cluster analyses by ward method based on all morphological characters from each genotype shown in Figure 2. Cluster analysis categorized genotypes into three groups. Cluster 1 had 1 genotype. Cluster 2 had 2 genotypes and cluster 3 had 2 groups. Cluster 3 could be further divided into 2 subgroups. The first cluster is allocated only *Arum giganteum* that is endemic in west of Iran and has its own unique morphological characteristics. Cluster 2 contained 2 species including *Arum conophalloides* and *Arum viresences*. Cluster 3 was divided into 2 groupings, including *Arum maculatum* and sub groups with *Arum kotschyi* and *Arum korolkowii*.

Dendrogram of genetic relationships among *Arum* species constructed by the UPGMA method demonstrated that 4 main groups existed at the highest level of hierarchy (0.34). According to the results of dendrogram *A. giganteum* and *A. maculatum* replaced in independent groups. Group 3 divided into 2 subgroups consist of *A. conophalloides* and subgroup 2. The second subgroup included *A. virescense*, *A.kotschyi* and *A. korolkowii.*

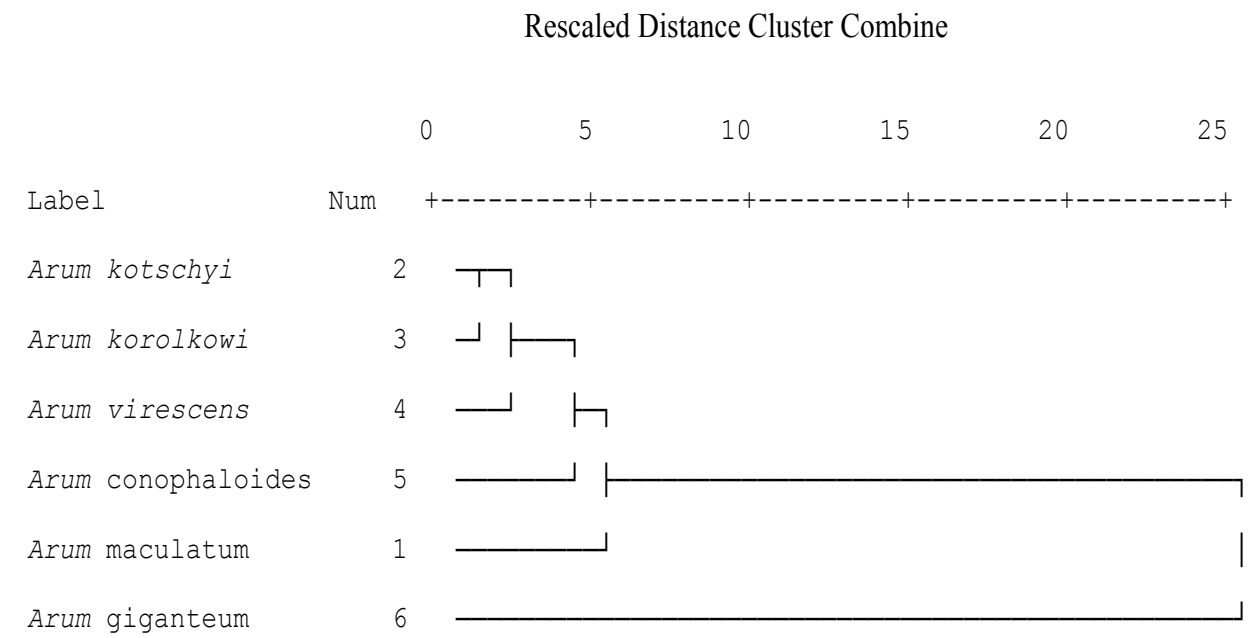

Figure 1. Cluster analysis of genotypes based morphological traits (UPGMA).

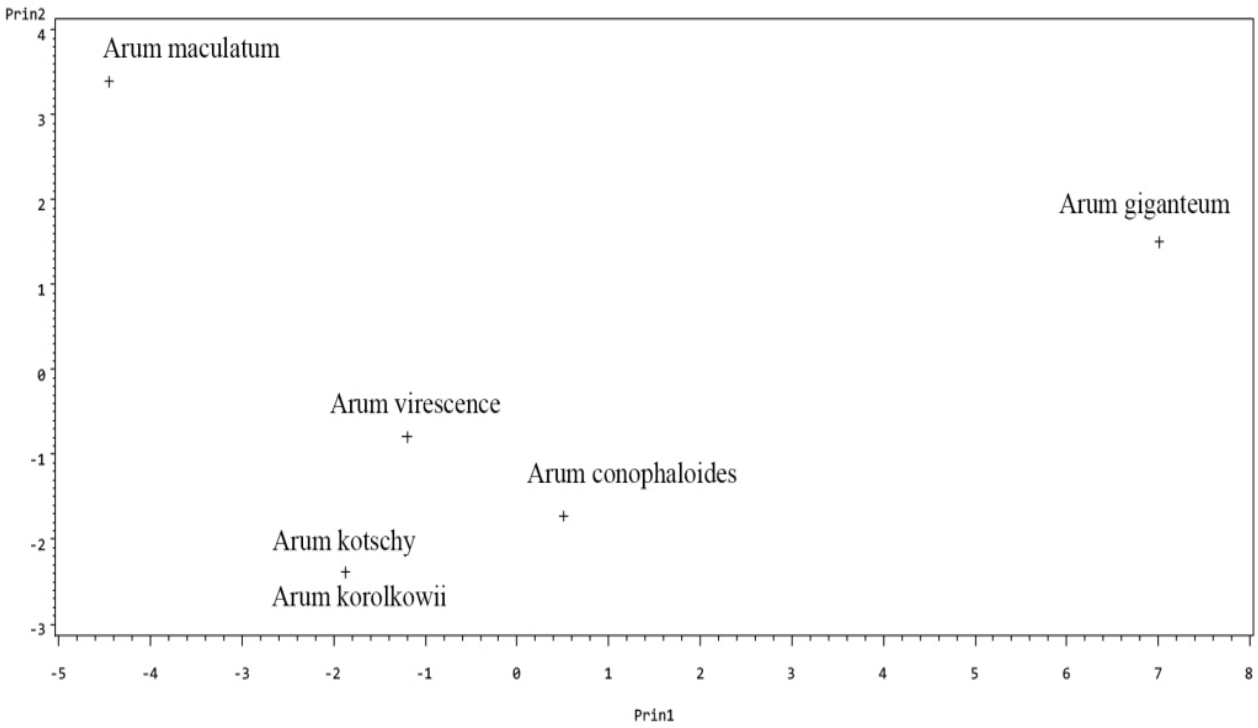

Figure 3. Scatter plot PC1 and Pc2 in *Arum* genotypes.

Principal components analysis (PCA)

The results of principal component analysis on *Arum* species data, including the Eigen values and cumulative variances of correlation matrix of *Arum* species traits are presented in Tables 5, Table 6 and Figure 3. It is clear that the first principal component of *Arum* species data accounts 62.256% of total variability in the data, while the second principal component accounts for 20.680% of the total variability. Finally, together the first and second components accounts for 82.936% of the total variability.

The first four principal components are orthogonal with each other and extract maximum of total variability (about 99.075%). Based on the correlation between features and components are given in Table 5: In first principal component petiole long, leaf blade length and wide, spath long, spath tube long, staminode, staminate and pistillode zone have positive correlation. The second principal component; spath wide and spath tube color had positive correlation.

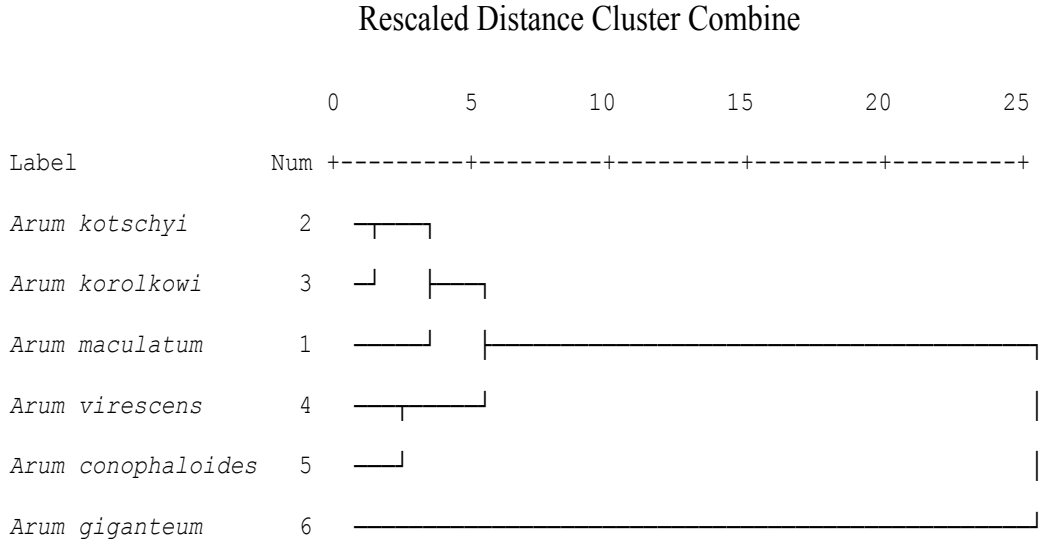

Figure 2. Cluster analysis of genotypes based morphological traits by using Ward's method.

Table 5: Eigen value, proportion and cumulative explained by two PC Principal Component Eigen value proportion cumulative.

Principal component	Total	% of Variance	Cumulative %
PC1	18.054	62.256	62.256
PC2	5.997	20.680	82.936
PC3	2.572	8.868	91.804
PC4	2.109	7.271	99.075

Table 6: Vector loadings explained by the first two PC.

Traits	PC1	PC 2	Traits	PC1	PC 2
PL(min)	0.959*	0.110	STM max	0.982*	0.145
PL max	-0.379	0.426	PZ min	0.982*	0.040
LBL min	0.944*	0.205	PZ max	0.676	-0.271
LBL max	0.760	0.598*	STZ min	0.971*	0.072
LBW min	0.989*	0.035	STZ max	0.934*	-0.203
LBW max	0.555	-0.213	SDZ min	0.963*	0.148
PEL min	0.747	-0.204	SDZ max	0.885	-0.209
PEL max	0.025	-0.874	TS	-0.462	0.854
SL min	0.952*	0.220	LOF	0.462	-0.854
SL max	0.984*	0.151	LBS	-0.334	-0.616
SW min	0.772	0.631*	LBA	0.120	-0.274
SW max	0.829	0.548*	STC	-0.492	0.815*
STL min	0.985*	0.119	ISS	0.813	-0.473
STL max	0.998*	0.062	OSS	0.462	-0.854
STM min	0.986*	0.149			

Discussion

There are six species of *Arum* in Iran that most of them have been adapted in north and west. In the current research, *Arum* species from different places were analyzed using morphological characters using Ward, UPGMA and PCA methods. Classical taxon definition and circumscription in the genus *Arum* only partially match our morphometric traits [12]. As shown in Figures 1 and 2, identity the sections and subsections and species are extremely challenged and it seems obvious that there are some varieties in classification of plants in Iran [5]. In the research conducted by Peter Boyce in 1993, *Arum rupicola* and *Arum conophalloides* reported as synonym plants. In Turkey and Iran, Lance Chilton discovered the *A. rupicola* on the Aegean island of Lesbos which usually displays a massive conic-clyindric spadix-appendix borne on a short, rather stout stipe; this form is the plant known as *A. conophalloides*. Toward the Turkish border with Syria, the spadix appendix is often much more slender with a rather poorly defined stipe, this is the plant described as *A.rupicola*. *A. rupicola* in other classification of Boyce and has been situated in Tenufila subsection with *A. jacquemonti* and *A. korolkowii*. Based on morphological characteristics and using the Ward and UPGMA analyses *A. korolkowii* is completely separated from *A. conophalloides* and *A. giganteum* and were introduced as independent plants.

Engler's taxon contains 8 species that *A. italicum* and *A. maculatum* differ markedly because of their horizontal-rhizomatous tubers. In this study, *A. maculatum* replaced in separated group. *A. conophalloides* is a closer match to the *A. protologue* than *A. jacquemontii*. The only change to be made is the adoption of Boissier's earlier name, *A. rupicola*, for the species.

In another study the color of floral chamber wall was used as a feature to help the classification. The floral chamber wall can be seen bicoloured: dark purple in its upper part and pale green (translucent) in its lower part. This kind of floral chamber wall is observed in *A. orientale, A. rupicola, A. purpureospathum*, and *A. elongatum*, but not in *A. maculatum*, *A. italicum, A.*

concinnatum or *A. cylindraceum*. This character also can separate *A. maculatum* and *A. kotschyi* and *A. korolkowii* that can be placed with *A. rupicola* in the same groups. Also regarded to Boyce, ecologically, differences between two types of cryptic and flag inflorescence can separated *A. maculatum* and *A. korolkowii* whereas cryptic inflorescence species like *A. maculatum* grow in wooded or scubby area, but flag species (*A. korolkowii* and *A. rupicola*) usually inhabit in open or rocky areas.

The results of PCA showed peduncle length aligned with the characteristics of inflorescences. Field studies suggest that peduncle length can be an important factor in pollination biology and investigation by has shown that spadix appendix odour is of considerable importance in determining the predominant pollinator plants [13]. Bedalov and Grayum worked on pollen of some and noted the similarity in the pollen of different species [14,15]. It will be interesting to see which separates the morphological characteristics of some species and can also be used to separate the species Iran by the correlation characteristic between species. Makhadmeh et al. showed that *Arum* populations of the same species or having a common genome were grouped in the same cluster, regardless of the collection site [16,17]. The wide range of genetic distance was represented by the high level of DNA polymorphism occurring among *Arum* species.

Conclusion

The current study assessed the levels of genetic variation of 6 *Arum* species in Iran, to provide a baseline for further studies and conservation strategies. Molecular markers significantly need for separation between *Arum* species. Also based on this study, a chemo diversity study of these species is recommended, as well as the establishment of in situ and ex situ field gene banks to protect this plant and the development of legal measures to conserve this species and consider this plant as one of those that must be protected.

Acknowledgement

The authors would like to express their thanks to Islamic Azad University, Tehran, Science and Research Branch for providing the facilities necessary to carry out the work.

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