

AGRO-MORPHOLOGICAL STUDY ON SEVERAL ACCESSIONS OF GARDEN CRESS (*LEPIDIUM SATIVUM* - BRASSICACEAE) IN IRAN

AFRA ROUGHANI¹, SEIED MEHDI MIRI^{2*}, MOHAMMAD REZA HASSANDOKHT³, PEJMAN MORADI⁴ AND VAHID ABDOSI¹

¹Department of Horticulture, Science and Research Branch, Islamic Azad University, Tehran, Iran

²Department of Horticulture, Karaj Branch, Islamic Azad University, Karaj, Iran

³Department of Horticulture, University of Tehran, Karaj, Iran

⁴Department of Horticulture, Saveh Branch, Islamic Azad University, Saveh, Iran

Abstract

In this research, the genetic variation of six Iranian garden cress (*Lepidium sativum*) accessions was studied by 21 agromorphological traits. A significant variation was observed in 6 out of 21 traits including shoot dry weight, petiole length, shoot height in flowering stage, petal length, and sepal length and width. The minimum shoot dry weight was in 'Ghayen', while, 'Isfahan II', 'Malayer' and 'Varamin' accessions showed higher weight than average. The shoot dry weight ranged between 0.5-4.3 g. Days to 75% flowering varied from 53 days (Isfahan I and Isfahan II) to 74 days (Ghayen), that the latter would be possible as a source for selection of late maturing types. The principal component analysis (PCA) and cluster analysis were used to identify the patterns of variation and detect the relationships among accessions. The first two PCAs could explain 92.9% of the total variance. Petal length and sepal length and width had the highest positive correlation in PCA1. The PCA2 included shoot dry weight, petiole length and shoot height in flowering stage. In cluster analysis, accessions classified in three major clusters. No relationships were found between genetic variation and geographical origin.

Key word: Cluster analysis, Genetic diversity, Germplasm, Morphological traits.

Introduction

Garden cress (*Lepidium sativum*) is an annual edible herb (Wadhwa *et al.*, 2012). This species belongs to Brassicaceae family which includes 338 genera and 3709 species (Warwick *et al.*, 2006). This family has economic importance and is native to Egypt and west Asia but also widely grows in temperate climates of all the world (Gokavi *et al.*, 2004; Rich, 1991).

The seedlings of garden cress with pungent taste are used in salads (Wadhwa *et al.*, 2012), that contains significant amount of iron, folic acid and vitamins A and C. Crude leaves have exceptionally high levels of protein (2.6 g/100g) as compared to common vegetables. This species is a good source of minerals and natural phenolic antioxidants; the seeds have a high amount of fat up to 16% (Sat *et al.*, 2013; Sharma & Agarwal, 2011; Malar *et al.*, 2014; Manohar *et al.*, 2012), and a high proportion of mucilage that is used for different therapeutic purposes (Gill *et al.*, 2012). Their seeds are exported as a drug from Iran to India, and Westward to Europe (Jahantab *et al.*, 2011; Parsa, 1959; Wadhwa *et al.*, 2012). Garden cress has been studied for its medicinal use as gastrointestinal stimulant, laxative, gastro-protective, tonic and digestive aid (Fleming, 1998; Nadkarni, 1986; Parsa, 1959), and also have several properties, such as antibacterial, diuretic, anti-rheumatic pain and antihypertensive (Duke *et al.*, 2002; Malar *et al.*, 2014; Nadkarni, 1986; Sharma & Agarwal, 2011; Tahseen & Mishra, 2013).

Garden cress is native to Iran, and is widely distributed as a cultivated plant eastward to Tibet (Parsa, 1959). Genetic diversity study is used for efficient utilization and for development of improved cultivar/varieties (Jan *et al.*, 2017). Genetic diversity can be studied using various methods such as agro-

morphological, biochemical and molecular markers. Morphological characteristic is important for understanding the native genetic potential that is critical for the establishment of long-term breeding programs. Wild accessions have a good starting material for breeding purposes and also possess a number of useful agronomic traits including resistance to disease, insect, nematode and tolerance to abiotic stresses, which could be used in breeding programs (Warwick *et al.*, 2009). The purpose of current research is study of agromorphological diversity of garden cress in Iran.

Material and Methods

Six accessions of garden cress (*Lepidium sativum*) were provided by Research Institute of Forests and Rangelands, Tehran, Iran and local farmers (Table 1). The field study was performed at Research field of Islamic Azad University, Karaj Branch, Karaj, Iran (35°73'N, 50°73'E, 1313 m). The location has a semi-arid climate and the soil is clay loam. The seeds of each accession were sown at 6 lines with 150×20 cm spacing. The seedlings were fertigated once using 50 ml of liquid fertilizer (Grow More; 20-20-20 NPK plus micronutrients), three weeks after planting.

Table 1. Plant materials used in the study.

Accessions	Population code	Altitude (m)
Arak	RIFR-12647	1708
Isfahan I	RIFR-23873	1600
Ghayen	RIFR-37528	1655
Isfahan II	-	1570
Malayer	-	1725
Varamin	-	918

The field layout was a randomized complete block design with six replications. Twenty one agro-morphological traits including leaf number, shoot height, shoot fresh and dry weight, leaf length, width and thickness, petiole length and thickness, root length, root fresh and dry weight, shoot height in flowering stage, flower length, petal length and width, sepal length and width, stamen height, pistil length and width were collected from three random plants in each replication. The vegetative features were evaluated five weeks after planting and floral traits were recorded on 75% flowering of each accession.

The quantitative data was tested for homogeneity using Minitab statistical package (ver. 16.0). Pearson correlation coefficients, analysis of variance and Ward's cluster analysis were conducted by SPSS software (ver. 21). Mean comparison was carried out using Duncan's multiple range test at 5% probability level.

Results and Discussion

Agro-morphological variation is important to screen best genotypes in field experiment. The diverse agro-morphological based genotypes are useful for further biochemical and molecular evaluation (Jan *et al.*, 2017). The summary of results is presented in Table 2. A significant variation was observed in 6 out of 21 traits including shoot dry weight, petiole length, shoot height in flowering stage, petal length, and sepal length and width.

The minimum shoot dry weight was in 'Ghayen', while, 'Isfahan II', 'Malayer' and 'Varamin' (3.0, 2.9 and 3 g, respectively) showed higher weight than average (2.4 g). Shoot dry weight ranged from 0.5 to 4.3 g. The range of petiole length was 3.8-10.7 cm and the accessions of 'Varamin' (9.4 cm) and 'Malayer' (8.4 cm) had longer petiole. This is comparable to the range of petiole length in Turkish accessions which was reported to be between 1 to 13.5 cm (Bona, 2014).

The shoot height in flowering stage ranged between 32.1 to 67.0 cm in 'Arak' and 'Isfahan I', respectively with average value of 50.2 cm. These results were similar to previous reports such as Adam *et al.* (2011), Mohammed (2012), and Bedassa *et al.* (2013), who reported average range of 50, 59.3 and 61 cm, respectively, while Zhan *et al.* (2009), Shehzad *et al.* (2011), and Sharma *et al.* (2015) found higher averages (80-113.1 cm) (Adam *et al.*, 2011; Bedassa *et al.*, 2013; Mohammed, 2012; Sharma *et al.*, 2015; Shehzad *et al.*, 2011; Zhan *et al.*, 2009).

Days to 75% flowering varied from 53 days ('Isfahan I' and 'Isfahan II') to 74 days ('Ghayen'), that makes it possible to select for early to late maturity types. Late maturity genotypes have low sensitivity to warm temperature thus these have a longer planting period and are resistant to bolting. Average days to flowering in Ethiopian populations were recorded from 65.3 to 86.7 in SNNPR and Tigray, respectively (Mohammed, 2012) and 15 local genotypes of Udaipur had similar average of 86.7 days (Sharma *et al.*, 2015). Flowers of all accessions have 4 petals and sepals except of 'Ghayen' that have 5 in some samples. Sepals were white margined, purplish on middle-upper part with scattered hairs that makes them look liked some wild Turkish garden cress (Bona, 2014). Stamen

numbers were 6, except of 'Ghayen' and 'Arak' which had 5 stamens. Reduction in floral organ number is common within the genus of *Lepidium*, and over half of the species have only two or four stamens, rather than the usual six. Similar to earlier reports on garden cress, inflorescence in all accessions were racemes (Bona, 2014; Brotonegoro & Wiharti, 2001; Zhan *et al.*, 2009). Petals were four, thin and delicate with color of white, white-pink or pink and some of them had red dots (Arak and Isfahan I). The petals in about one quarter of the *Lepidium* species are absent, and they are not developed in many others (Al-Shehbaz, 1986; Bowman & Smyth, 1998; Endress, 1992).

There was a significant correlation between fresh and dry weight of root with shoot height, leaf length and width. The results also showed positive correlation between leaf thickness and leaf number, but negative association was observed between petiole length and leaf thickness (Table 3). Since leaf blade is the edible part of garden cress, and on the other hand, nitrate accumulated in petioles (Chen *et al.*, 2004; Chiu *et al.*, 2004; Kawthar *et al.*, 2014; Wang & Li, 2004; Yosoff *et al.*, 2015), there up on long petiole is not a desirable characteristic. The maximum petiole length was recorded in commercial accessions of 'Malayer' and 'Varamin', whereas the minimum was observed in race genotypes of 'Ghayen' and 'Arak', so we recommend using these accessions in future breeding programs.

The principal component and cluster analysis based on 6 significant agro-morphological traits were used to identify the patterns of variation and to detect relationships among genotypes within a subset of 6 accession representing 5 geographical regions. Application of both the methods is recommended to extract the maximum amount of information from the matrix data. The first principal component analysis (PCA1) explained 63.0% of the total variation present in the accessions dataset. Petal length, sepal length and width had the highest positive correlation in PCA1. The second principal component (PCA2) accounted for 29.9% of the total variation. The PCA2 had a positive correlation with shoot dry weight and petiole length and negative correlation with shoot height in flowering stage (Table 4).

The cluster analysis classified the accessions in 3 major clusters (Fig. 1). The first cluster composed of 'Malayer', 'Varamin' and 'Isfahan II' accessions. The second included 'Isfahan I' and 'Ghayen' and the third cluster consisted of 'Arak'. Analysis of variance of the groups showed significant differences ($p \leq 0.05$) among clusters in six quantitative traits (Table 5). Accessions with the largest shoot dry weight and petiole length were grouped into cluster 1. Although petal length, sepal length and width were greater in cluster 1 and 3 compared to cluster 2. In this study, no relationship was found between genetic variation and geographical origin, and accessions of different regions were arranged in the same group. Similarly, Sabaghnia *et al.* (2015) indicated that the garden cress accessions did not cluster separately from their origin and those collected from different geographic areas tended to be grouped in the same group. This might be migration of the garden cress materials from one region to another sites through farmer to farmer exchange of seeds (Sabaghnia *et al.*, 2015).

Table 2. Mean value for 21 agro-morphological traits of six garden cress accessions.

Accessions	Leaf number	Shoot height (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Leaf length (cm)	Leaf width (cm)	Leaf thickness (cm)	Petiole length (cm)	Petiole thick (mm)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Shoot height in flowering stage (cm)	Flower length (mm)	Petal length (mm)	Sepal length (mm)	Sepal width (mm)	Stamen height (mm)	Pistil length (mm)	Pistil width (mm)	
Arak	35.3a	16.2a	10.7 a	1.9ab	7.7ab	3.4b	0.5a	4.6d	2.1b	11.7a	1.3a	0.2a	62.5a	33.3a	27.2a	8.8a	1.8a	21.3a	22.8ab	13.5a	
Isfahan I	27.5ab	18.0 a	21.6a	2.3ab	8.7ab	3.6ab	0.4ab	5.9cd	2.4ab	14.6a	1.4a	0.2a	43.5b	29.0b	23.2bc	8.1a	1.5c	19.0 a	19.8b	12.1a	
Ghayen	22.3b	13.1a	10.7a	1.2b	6.4b	3.4b	0.4ab	5.1d	2.1b	14.7a	0.7a	0.1b	47.3 b	29.5ab	21.8c	13.3a	1.6bc	18.1a	21.2ab	13.1a	
Isfahan II	25.7ab	18.4 a	51.7a	3.0a	9.5a	4.0ab	0.4b	6.9bc	2.4ab	15.8a	1.3a	0.2a	47.9 b	31.5ab	26.0ab	9.1a	1.8ab	20.0 a	21.6ab	12.7a	
Malayer	24.7ab	18.1a	22.0a	2.9a	9.8a	4.6a	0.4ab	8.4ab	2.7a	13.8a	1.4a	0.2a	51.1b	31.7ab	26.8a	9.2a	1.8a	19.9a	24.4a	13.2a	
Varamin	27.0ab	19.9a	23.2 a	3.0a	9.1a	4.2ab	0.4b	9.4a	2.5ab	13.4a	1.4a	0.2a	51.5b	32.5ab	26.7ab	9.7a	1.7ab	20.3 a	22.6ab	13.4a	
Mean	26.9	17.4	25.6	2.4	8.6	3.9	0.4	6.8	2.4	14.1	1.3	0.2	50.2	31.2	25.3	9.6	1.7	19.7	22.0	12.9	
Range	14-38	9.5-22.6	3.5-139.4	0.5-4.3	4.7-12.9	2.6-5.4	0.3-0.5	3.8-10.7	1.6-3.0	10.5-19.5	0.3-2.4	0.08-0.3	32.1-67.0	26.0-35.1	19.4-29.9	6.3-26.0	1.4-1.9	0.6-0.9	16.8-23.6	17.7-27.0	10.4-15.3
S.E	6.4	3.6	26.7	0.8	1.6	0.6	0.05	1.8	0.04	2.5	0.3	0.06	7.7	2.3	2.7	3.9	1.2	1.8	2.9	1.2	
F. value	1.4 ^{ns}	1.1 ^{ns}	0.9 ^{ns}	4.1*	2.5 ^{ns}	2.7 ^{ns}	2.2 ^{ns}	13.0*	2.07 ^{ns}	0.9 ^{ns}	1.4 ^{ns}	2.7 ^{ns}	3.2*	2.1 ^{ns}	4.0*	0.5 ^{ns}	6.4*	1.0 ^{ns}	2.2 ^{ns}	0.8 ^{ns}	

*p<0.05; ns = Not significant; SE= Standard error

Table 3. Correlation coefficient for 21 agro-morphological traits studied on *L. sativum* populations.

Traits	Shoot height	Leaf numbers	Shoot fresh weight	Shoot dry weight	Shoot dry weight	Leaf length	Leaf width	Leaf thick	Petiole length	Petiole thick	Root length	Root fresh weight	Root dry weight	Shoot height in flowering stage	Flower length	Petal length	Sepal length	Sepal width	Stamen height	Pistil length
Leaf Numbers	0.364																			
Shoot fresh weight	0.122	-0.263																		
Shoot dry weight	0.769**	0.330	0.126																	
Leaf length	0.753**	0.259	0.219	0.912**																
Leaf width	0.558**	0.027	0.281	0.686**	0.773**															
Leaf thick	-0.247	0.480*	-0.136	-0.227	-0.259	-0.316														
Petiole length	0.516*	-0.182	0.265	0.634**	0.616**	0.771**	-0.467*													
Petiole thick	0.619**	0.114	0.092	0.732**	0.732**	0.763**	-0.299	0.661**												
Root length	0.347	-0.134	0.220	0.243	0.246	0.187	-0.356	0.163	0.209											
Root fresh weight	0.778**	0.417	0.098	0.847**	0.867**	0.636**	-0.008	0.467*	0.659**	0.350										
Root dry weight	0.528*	0.360	0.349	0.721**	0.693**	0.552**	0.200	0.441*	0.455*	0.233	0.848**									
Shoot height in flowering stage	0.055	0.275	0.079	-0.093	-0.140	-0.081	0.305	-0.112	-0.311	-0.345	-0.033	0.099								
Flower length	0.087	0.065	0.297	0.202	0.102	0.113	0.217	0.240	-0.069	-0.233	0.185	0.390	0.760**							
Petal length	0.157	0.184	0.004	0.386	0.249	0.133	0.197	0.324	0.119	-0.254	0.274	0.416	0.433*	0.706**						
Petal width	0.054	0.075	-0.086	-0.005	0.033	0.061	0.124	0.011	0.051	-0.149	-0.088	-0.268	0.191	0.085						
Sepal length	-0.014	0.075	0.146	0.197	0.176	0.216	0.007	0.299	0.041	-0.172	0.089	0.262	0.322	0.520*	0.609**	-0.022				
Sepal width	0.168	0.199	-0.040	0.360	0.304	0.312	-0.061	0.269	0.120	-0.184	0.283	0.338	0.417	0.543*	0.736**	-0.045	0.832**			
Stamen height	0.152	0.078	0.472*	0.100	0.124	0.064	0.272	0.118	-0.228	0.000	0.174	0.400	0.525*	0.520*	0.480*	-0.059	0.414	0.393		
Pistil length	-0.205	-0.339	0.244	-0.024	-0.038	0.033	-0.049	0.253	-0.148	-0.101	-0.063	0.200	0.328	0.527*	0.433*	-0.147	0.674**	0.479*	0.524*	
Pistil width	-0.314	-0.0399	0.107	-0.207	-0.229	-0.152	-0.021	0.068	-0.379	-0.166	-0.177	0.048	0.239	0.453*	0.366	-0.117	0.475*	0.372	0.340	0.825**

*, **: Correlation is significant at the 0.05 and 0.01 level respectively (2-tailed)

Table 4. Principle component analysis for six accessions based on 6 significant agro-morphological traits.

Traits	Component	
	1	2
Shoot dry weight	0.313	<u>0.563</u>
Petiole length	0.240	<u>0.612</u>
Shoot height in flowering stage	0.354	<u>-0.498</u>
Petal length	<u>0.504</u>	0.037
Sepal length	<u>0.465</u>	-0.187
Sepal width	<u>0.499</u>	-0.156
% of Variance	63.0	29.9
Cumulative %	63.0	92.9

Lower genetic distances indicate a close genetic relationship, whereas high genetic distances indicate a large distant genetic relationship. Hybridization between distantly related individuals are expected to give better offspring than those between closely related genotypes. Therefore, prior knowledge of the genetic distance between genotypes is important in designing a breeding program (Mohammed & Tesfaye, 2015). On the basis of cluster grouping, crossing between 'Arak' and 'Malayer' could be recommended to get high heterozigosity. Also, 'Isfahan II', shows very similar traits to the commercial accessions of

'Varamin' and 'Malayer' and the lack of information at genetic level, makes it difficult to evaluate the authenticity of these ecotypes. However further analysis is required to verify the origin of this ecotype.

In PCA, first and second components revealed 92.9 % of total variance and they were projected in score plot graphic (Fig. 2). The score plot presents four groups of accessions. All the commercial accessions consisting 'Isfahan II', 'Varamin' and 'Malayer' were in the same group. The score plot not fully fits with that obtained by the cluster analysis, but in both analysis methods, accessions of 'Malayer', 'Varamin' and 'Isfahan II' were in the same group and 'Arak' was in a separate one.

Agro-morphological analysis revealed a significant variation among garden cress accessions. Similar to our findings, relatively high genetic variation was also reported in garden cress germplasm by morphological traits (Mohammed, 2012; Sabaghnia *et al.*, 2015) and molecular markers (Bansal *et al.*, 2012; Kumar *et al.*, 2012; Kaur *et al.*, 2015; Mohammed, 2012). It could be due to the fact that *L. sativum* is a moderately cross-pollinated species with a sporophytic self-incompatibility system (Dayal & Singh, 1985). Also, market exchange could increase genetic recombination which could result to high variation within accessions (Mohammed & Tesfaye, 2015).

Table 5. Mean value for 6 agro-morphological traits of three groups of garden cress accessions.

Cluster	Shoot dry weight (g)	Petiole length (cm)	Shoot height in flowering stage	Petal length (mm)	Sepal length (mm)	Sepal width (mm)
1	2.99a	8.17a	50.09b	26.54a	18.05a	8.15a
2	1.87b	5.61b	45.19b	22.60b	16.32b	6.94b
3	1.91b	4.69b	62.55a	27.23a	18.39a	8.57a
F. value	7.22*	15.39*	9.93*	10.04*	8.35*	23.37*

* $p \leq 0.05$

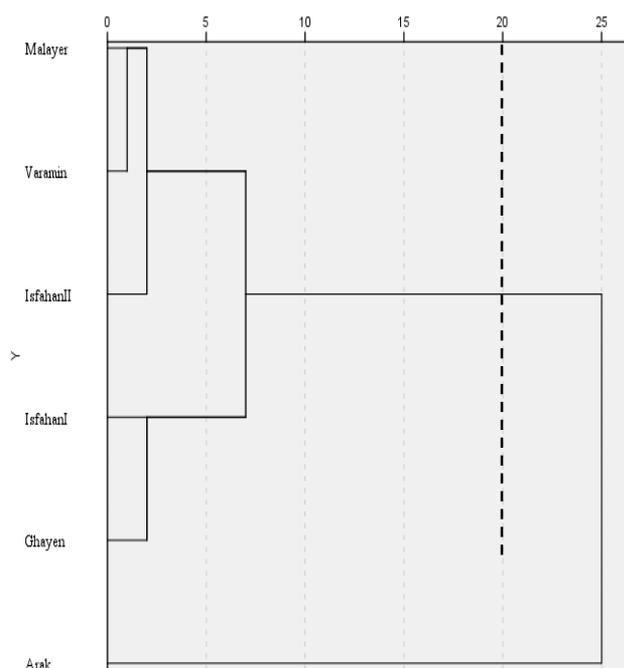


Fig. 1. Dendrogram showing the clustering patterns of the 6 accessions based on agro-morphological traits with Ward's method.

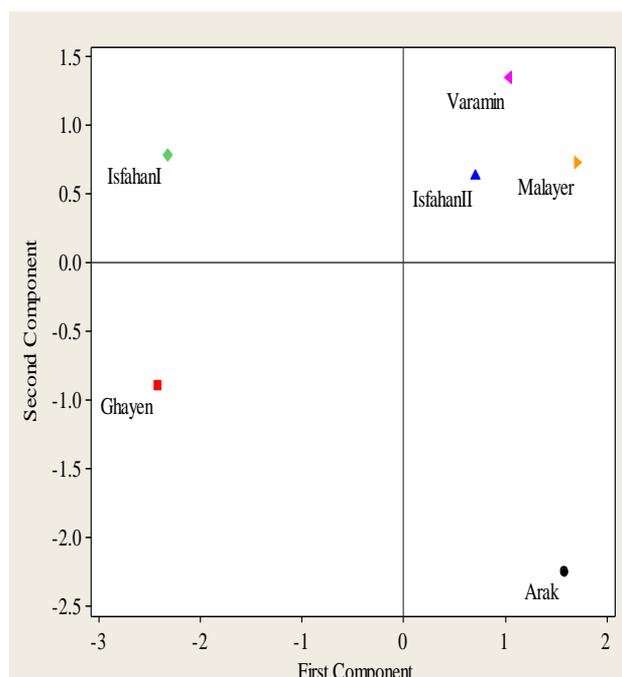


Fig. 2. Score plot diagram resulting from principal components analysis based on the first two principal components.

Conclusion

In the present study, agro-morphological research was found to be useful in genetic diversity evaluation of *Lepidium sativum*. The evaluation of garden cress accessions showed that 'Ghayen', which exceeded in days to 75% flowering and had a short petiole length, could be appropriate as promising material to develop *new varieties* by cross-breeding techniques. This study is a first step for identifying promising accessions for subsequent analysis such as study of genetic linkage between accessions and nutritional value, genotyping and chemical analysis.

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