

ASSESSMENT OF GENETIC DIVERSITY AMONG MELON ACCESSIONS USING GRAPHICAL PRINCIPAL COMPONENT AND CLUSTER ANALYSIS

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Assessment of genetic diversity among melon accessions using graphical principal component and cluster analysis

Abstract

Iran is a part of the secondary center of origin of melons, and therefore, there is a wealth of genetic variation of these species in this country. In this work, 14 morphological traits were studied in order to select the most discriminating traits. Significant difference among accessions was revealed for almost quantitative traits. KMO coefficient obtained was 0.723 that is explaining of appropriation of this test. The results of the principal component analysis (PCA) for the morphological traits indicated that the first three PCs explained 53.46% of the total variation. Principal component analysis results (PCA) for morphometric data indicated that samples of Semnan and Khorasan showed high degree of variation and were highly different from Sistan and this approved by cluster analysis. Cluster analysis grouped the 36 accessions into three clusters confirming the diversity of the collection that can be useful for selection and source of desired genes.

Key words: melon, selection, variability, germplasm, yemtic, cluster analysis.

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تحديد التباعد الوراثي للبطيخ باستعمال المكونات الجغرافية والتحليل العنقودي

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المستخلص

تحتل ايران المرتبة الثانية في مركز أصل البطيخ وعليه هي غنية بالتغايرات الوراثية. في هذه الدراسة تم التركيز على الصفات المورفولوجية لغرض الاعتماد عليها في الانتخاب. وجدت فروق معنوية بين التراكيب الوراثية في الصفات المظهرية. معامل (KMO) كانت 0.723 أستعملت في اختبار الصفات. نتائج تحليل المكونات الاساسية (PCA) للصفات المظهرية اوضحت بان ثلث PCA كانت 53.46% من مجموعة التغايرات الكلية اوضحت التحليل الاساس للصفات المظهرية لل PCA كانت لل سمنان Samnan وخرسان Khorasan حيث حتوت على تغايرات عالية اكبر سيستان Sistan ظهرت من التحليل العنقودي للصفات المظهرية التي شملت 36 مجموعة بوجود ثلاثة تباعد عنقودي الذي يمكن الانتخاب منها حسب ازواج الجينات المرغوبة.

كلمات مفتاحية: أنتخاب، تغايرات، صفات مظهرية، تحليل العنقودي

INTRODUCTION

Melon (*Cucumis melo* L.) not only is the most important fruit but also is a temperate and warm season crop in Iran from the genus *Cucumis* and family *Cucurbitaceae*. Different classifications by several intraspecific have been shown and it is the most diverse species (14). Assessment of genetic variation in different species is of interest and it is essential for the managing and protecting of plant genetic resources also show the way of practical applications such as broadening the genetic base of the species and exploitation of heterosis for breeders (15). Information about magnitude of genetic diversity will help breeders to select favorite parents in hybrid in production of suitable cultivar and also maintaining population diversity. Loss of genetic variation has resulted based on inbreeding, to overcome this problem diversity is a main concern in species (11). As stated by Escribano and Lázaro (5), morphological analyses is the main requirement in the initial assessment of genetic variation for classification and identification of genotypes and cultivars, morphological characterization has been done by several studies for local germplasms and indicate different genetic sources (2). Genetic variation of fruit species is not only to preserve the adequate genetic diversity for breeding programs but also it is essential for long-term survival for species (3). Morphological characterizations are suitable to be included in breeding program for the gaining genetic availability and use of genetic variation. To utilize genetic variation,

selection of suitable variables should be considered correctly and characterization regarding to morphological variability should be performed accurately in germplasm collections (4). One the important centers origin of melons is Iran, so high genetic variation is expectable for this species in this country (10). Several types of morphological, phenological and physiological have been utilized to identify the genetic diversity in melon (*Cucumis melo*). Morphological characters of some Iranian melon accessions have been used in order to classify them at the National Plant Gene Bank of Iran (8). For gaining of high yielding genotypes, it is necessary to utilize the best breeding approach also suitable information like heritability, variability and genetic gain, . The level of genetic variation in expression of a specific character for forecasting its breeding worth is defined by its heritability (9). The present study was designed to characterize some of the fruit and morphological traits of 36 selected melon accessions from the eastern warm and dry regions of Iran. These descriptive data will be useful for identifying melon accessions in this collection of germplasm, determining melon accessions which have the best quantities, to be used as parents in breeding programs.

MATERIALS AND METHODS

Thirty six melon accessions (Table 1) were obtained from National Plant Gene Bank of Iran that were collected from warm and dry zones and assessed under field conditions from Mar. to June 2016–2017.

Table 1. The name and code of 36 melon accessions

Number	Identity Code	Number	Identity Code	Number	Identity Code	Number	Identity Code
1	TN-345	10	KC-257236	19	KC- 357020	28	TN-278
2	KC-35700	11	TN-629	20	TN621	29	TN-272
3	TN-271	12	TN-92-334	21	TN-92-306	30	KC-357104
4	KC-357250	13	TN-628	22	TN92-302	31	KC-357079
5	KC-357238	14	TN-92-319	23	KC- 357044	32	357063
6	TN-441	15	TN-623	24	KC-357154	33	KC-357047
7	TN-92401	16	TN92317	25	KC-357009	34	KC357062
8	TN-377	17	TN92312	26	KC-357067	35	Sefidak
9	TN-277	18	TN622	27	KC-357100	36	Suski

This study was carried out at the Agriculture and Natural Resource Research Station of Zahak, in southeast of Iran, under normal condition cultivation. All accessions were sown in two replication as incomplete block design (Square lattice), the texture of soil was sandy-loam. Fertilizers with 80 kg.ha⁻¹ of

P2O5 and 100 kg.ha⁻¹ of N from urea utilized before planting. Three seeds were sown in each hole at a distance of 0.5 × 0.5 m and six plants for each genotype assigned in each incomplete block. The fourteen traits were evaluated at the ripening of the fruits.

1.1- Evaluated Traits and analyses

Evaluated traits included, fruit length, fruit weight, number of fruit, total Soluble Solids (TSS) that measured by hand refractometer, Single plant yield, flesh diameter, cavity diameter, plant length and physiological traits like Chlorophyll (CHL), that machine SPAD-502 portable chlorophyll meter was used to measure leaf chlorophyll content, Canopy temperature, this trait measured by infrared thermometer, held 0.5-1 m from the edge of the plot and approximately 50 cm above the plant, relative water content (RWC), Days to maturity and finally the trait root length measuring were done on the main root of the plant, when roots pulled out from the soil and measured by meter when the plant life is finished. Principal Component Analysis (PCA) and K-means cluster analysis were performed to assess the genetic diversity and distribution of accessions, statistical analyses were performed by software R (15).

RESULTS AND DISCUSSION

2.1- Principal component analysis

Based on ANOVA a significant difference between accessions studied for quantitative traits was observed indicating that the traits studied could be useful and important for accession differentiation. 14 quantitative morphological characteristics of melon accessions were used as identification roles for classification in multivariate analysis. Scree plot showing the number of components for the estimated traits of melon accessions that explained by percentage of variance for each component (Fig. 1). KMO coefficient approximately more than 0.6 is suitable, it indicates that the PCA method will be useful for the data (6). In this study the KMO

coefficient obtained was 0.723 that is explaining of appropriation of this test at good. In this analysis the characteristics with an eigenvalue of one were included and others discarded. Through PCA of 14 morphological variables of the 36 melon accessions, 7 principal components were determined with 84.72% cumulative contribution. The values obtained by PCA called eigen values are often used to settle on how many factors to retain. The results in table 2 focused on first three components and graphically represented in figure 1 and table 2 estimated melon based on their effective factors with eigenvalues more than one which all together explained 53.46 of total variability. The two major principal components covered 14 characteristics and explained 40% of the results. Through the PC 1 and PC 2 from PCA, each accession was plotted on the scatter plot and 36 of melon accessions were categorized into three groups. The first component (PC1) was highly associated with, fruit weight, fruit length and fruit width. Meanwhile, the PC2 was highly correlated with the number of fruit. Szamosi et al. (14) in morphological evaluation and comparison of 58 Hungarian and Turkish melon (*Cucumis melo* L.) germplasm reported the first PC explained 29%, the second 25% and the third 10% of the morphological variation making a total of 64% and PC1 and PC2 were mostly constructed from diameter of fruit, diameter of seed cavity, fruit weight, thickness of flesh. Trimech *et al.* (16) reported the PCA plot according to the two first components, accounting to 49.68 % of the total variation and second component was correlated to the fruit length.

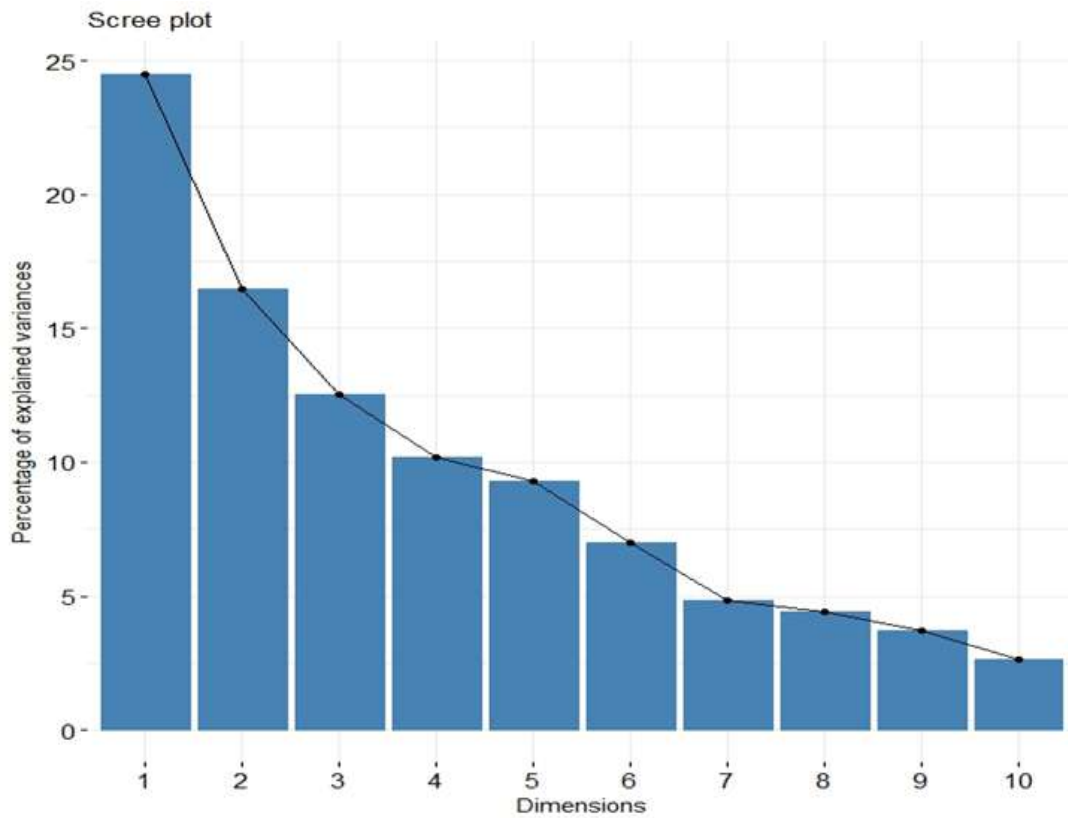


Figure 1. Scree plot and respective eigenvalues

Table 2. Eigenvalues, proportion and cumulative of PCA for the estimated traits of melon

Traits	PC1	PC2	PC3
Eigenvalues	3.43	2.30	1.75
Proportion	24.50	16.45	12.51
Cumulative	24.50	40.95	53.46

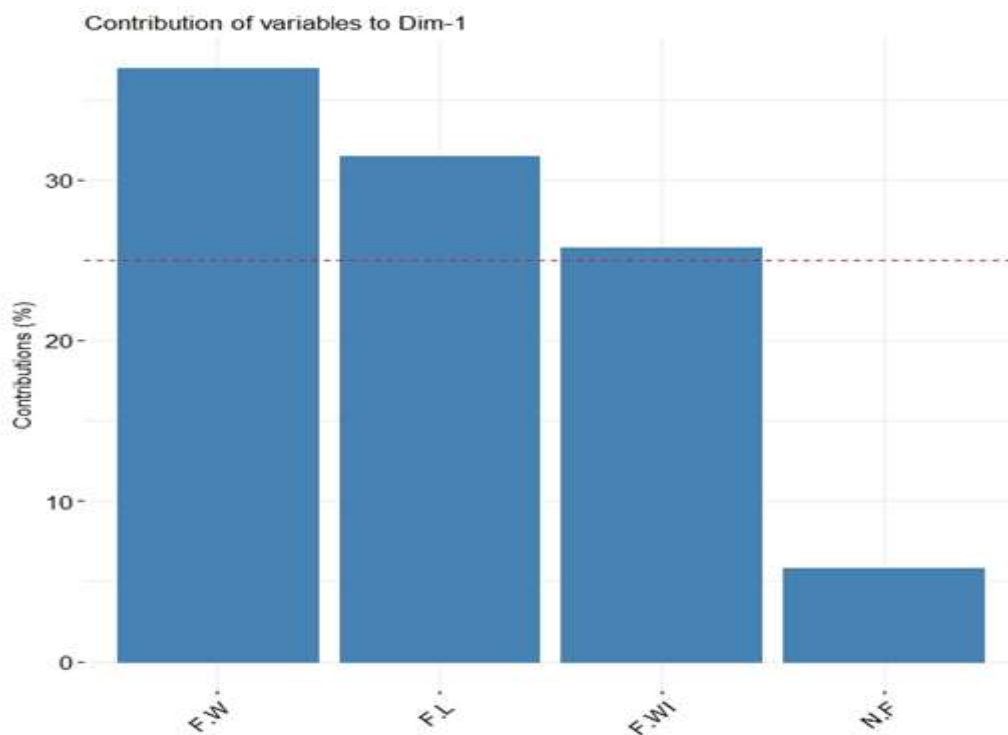


Figure 2. Contribution of variables to PC1

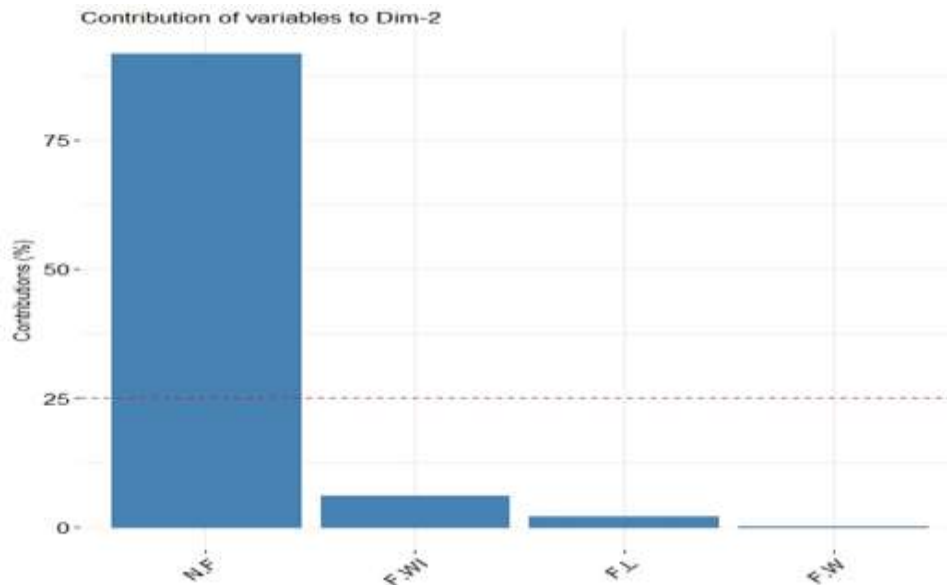


Figure 3. Contribution of variables to PC2

Data in Table 2, figure 1, 2 and 3 shows that PC1 accounted for about 25% of total variation, PC2 contribute 17%, PC3 contribute 13%, while PC4 and PC5 explained 12% and 9% respectively. In the PC biplot (Figure 4, 5) the contribution of accessions on the observed variation is illustrated as distance between the traits with respect to PC1 and PC2. The biplot showed that fruit length, fruit width, fruit weight, cavity diameter and number of days to maturity contributed the most in variability among the accessions. In a PC scatter plot, accessions located near each other are considered to have a similar contribution with respect to the different variables studied. By

contrast, genotypes placed far from each other are more diverse. Based on the PCA, biplot, and scatter plot, accessions number 6, 16 and 36 had the maximum variability for fruit length, width and weight and can be used in breeding programs successfully (Figure 4). Ali-Shtayeh *et al.* (1) reported morphological traits showed a large variation for the Palestinian melon cultivars also results of the PCA based on qualitative and quantitative traits showed that 67 % of the total variability and the fruit traits were of high discriminating level and were consistent in their contribution in the first two components and therefore could be used for melon characterization.

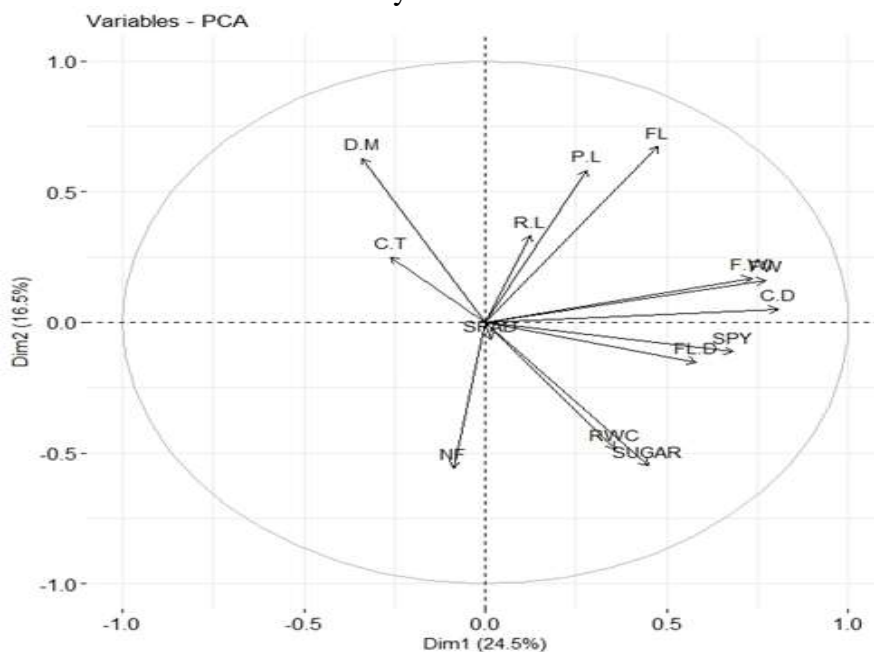


Figure 4. Biplot between PC-1 and 2 showing contribution of various traits in variability among different accessions

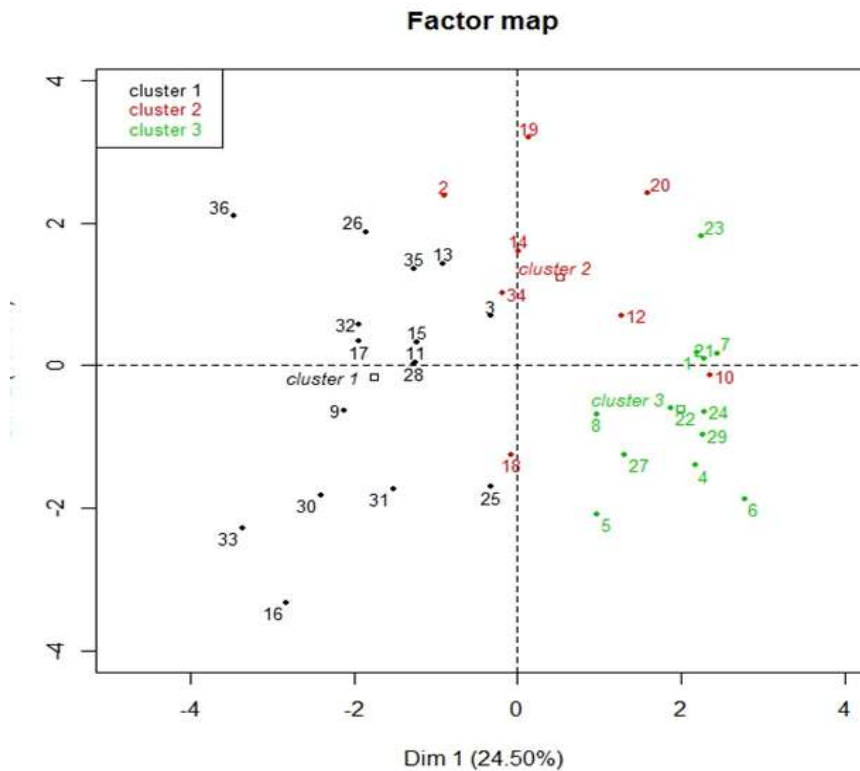


Figure 5. Biplot graph for 36 accessions

3.1- Cluster Analysis

Fundamental problem in cluster analysis is to determine the best estimate of the number of clusters, which has a deterministic role on the clustering results. Choosing a useful clustering method is another critical step in clustering. K-

means clustering is one of the most popular clustering techniques. The cluster analysis grouped the 36 melon accessions into three clusters based on different traits and their level of variability.

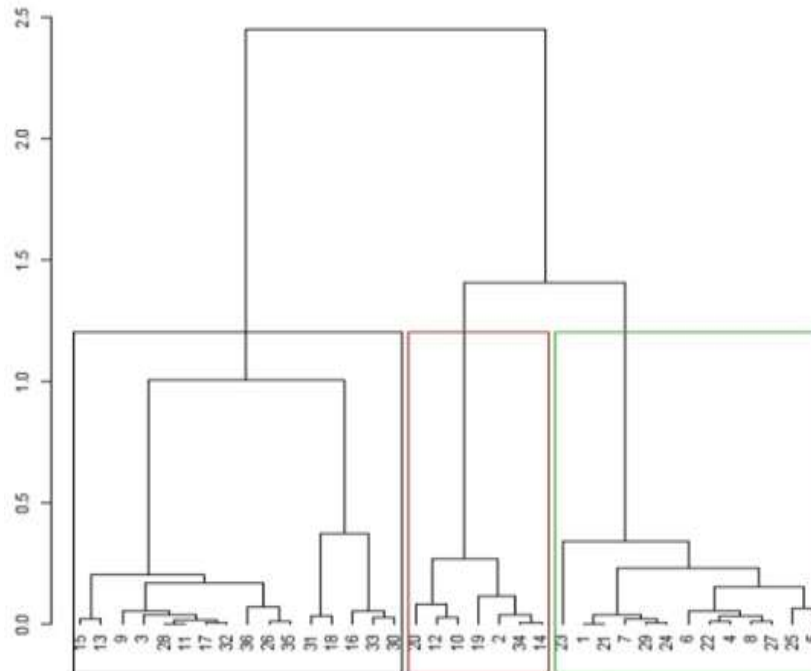


Figure 6. Dendrogram by cluster analysis of the investigated melon accessions

Table.3 Estimation of trait means for all clusters depending on variability

Trait	Cluster 1	Cluster 2	Cluster 3
Number of fruit	1.72	2	1.81
Fruit weight	2880.45	1872.5	1300.62
Fruit length	21.27	26.75	16.87
Fruit width	14.18	12.93	11.718
Total solid soluble	7	4.37	4.09
Single plant yield	3989.09	3727.25	2318.93
Flesh diameter	8.5	6.01	7
Cavity diameter	3.95	3.41	3.01
Plant length	152	203.12	139.68
Chlorophyll	48.90	46.75	48.68
Canopy temperature	28.45	27.37	29.25
Relative water content	0.76	0.62	0.65
Days number to maturity	85.81	90.37	88.68
Root length	37.09	39.375	34.43

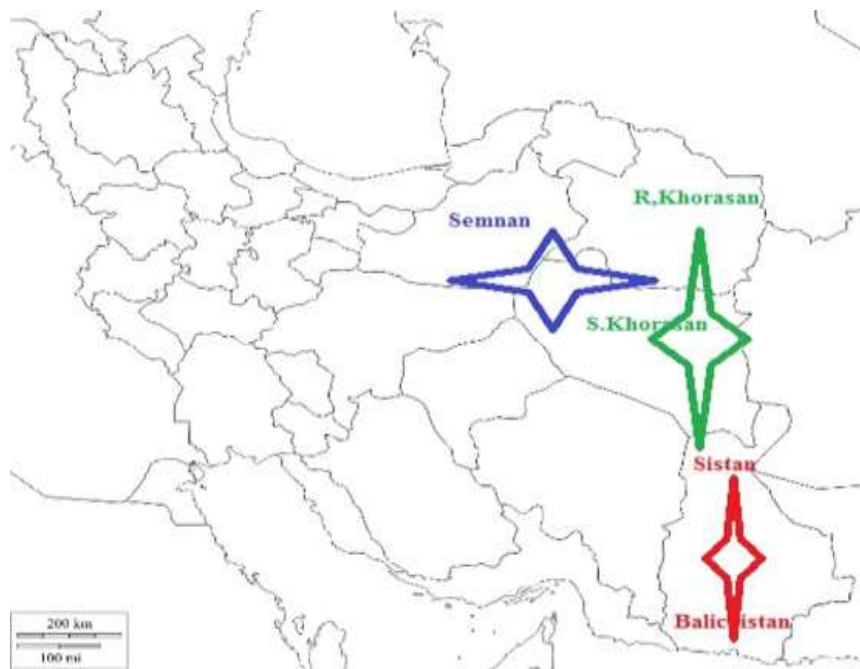


Figure 7. Distribution of evaluated melon accessions in Iran

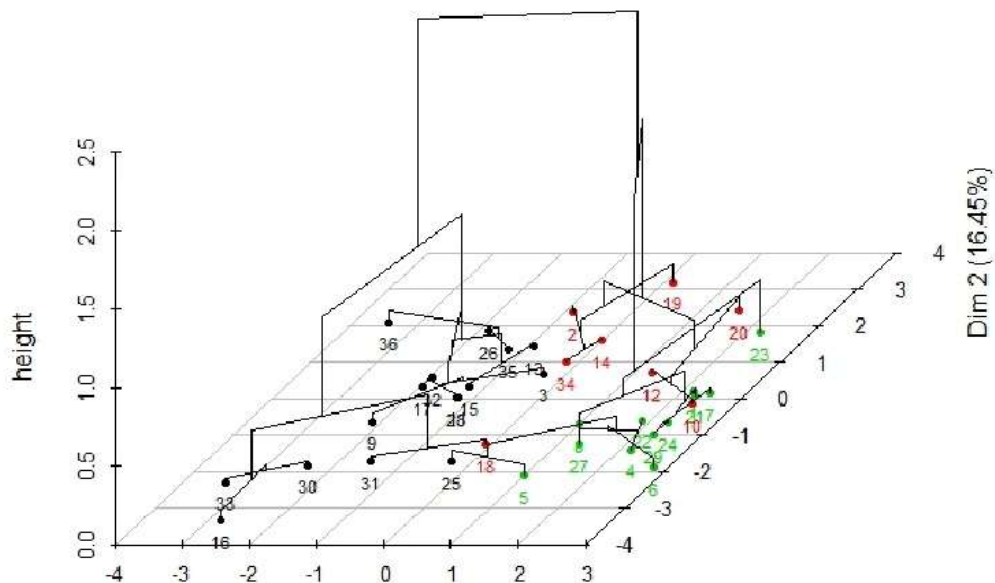


Figure 8. Hybrid cluster and principal components

Clusters 1-3 comprised 13, 7 and 16 accessions, respectively. Accessions number 11 and 28; 1 and 21; 14 and 34 were the most genetically related genotypes. The accessions in cluster 1 had higher values compared to all other clusters for all traits investigated in this study except for number of fruit, fruit length, plant length, canopy temperature, days number to maturity and root length, indicate that this cluster comprised accessions with almost early maturity by high plant yield and this cluster could be used for gaining high value in breeding program to gain better yield. Cluster 2 contained the highest mean values for number of fruit per plant, fruit length, plant length, days number to maturity and root length, in this cluster were found accessions by late maturity. In cluster 3 we can find accessions with high value for canopy temperature. (1) reported in the dendrogram generated by morphological characters, similar accessions in terms of fruit characteristics have positioned in similar clusters and the generated UPGMA dendrogram has discriminated the collected accessions into three main clusters, also similar results for fruit size reported by Naroui Rad *et al.* (7) under drought stress condition. The variation in warm and dry zone melon populations reflects the heterogeneous and heterozygous nature of accessions. Obvious differences were observed in fruit shape and dimensions and fruits color and shape. The same results reported by researchers especially for fruit size (5, 12, 13).

Conclusions

Findings of the study provided a useful tool for determining the genetic diversity among melon warm and dry zone of Iran. In particular, the selected characteristics such as fruit weight, fruit length and width using PCA could increase the efficiency in management melon accessions and also provide the proper maintenance level to maintain variation based on genetic relationships. The results of the investigations were also very useful to choose the precious accessions especially for fruit size, like fruit width, fruit length and fruit weight for further breeding programs.

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REFERENCES

1. Ali-Shtayeh, M.S., R.M., Jamous, M.J., Shtaya, O.B., Mallah, I. S., Eid, and S.Y.A., Zaitoun, 2017. Morphological characterization of snake melon (*Cucumis melo* var. *flexuosus*) populations from Palestine. *Genetic Resources and Crop Evolution* 64, 7-22
2. Decker-Walters, D.S., J. E., Straub, S.M., Chung, E., Nakata, and H. D., Quemada, 2002. Diversity in free-living populations of *Cucurbita pepo* (*Cucurbitaceae*) as assessed by random amplified polymorphic DNA. *Syst Bot* 27, 19-28
3. Esquinas Alcazar, J., 2005. Protecting crop genetic diversity for food security Political, ethical and technical challenges. *Nat. Rev. Genet* 6, 519-526
4. Giraldo, E., Lopez., M. Corrales, and J. I., Hormaza, 2010. Selection of the most discriminating morphological qualitative variables for characterization of fig germplasm. *J. Am. Soc. Hortic. Sci* 135, 240-249
5. Jagosz, B., 2011. The relationship between heterosis and genetic distances Based on RAPD and AFLP Markers in Carrot. *Plant Breed* 130, 574-579
6. Kaiser, H.F., 1974. An index of factorial simplicity. *Psychometrika* 39, 31-36
- Mingjin, Y., 2005. Methods of determining the number of clusters in a data set and a new clustering criterion, Polytechnic Institute. Virginia
7. Naroui Rad, M., M. Mohammad Ghasemi, , and J., A. Koohpayegani, 2017. Evaluation of melon (*Cucumis melo*. L) genotypes aiming effective selection of parents for breeding directed at high yield under drought stress condition, *Journal of Horticultural Research*, p. 125
8. Naroui Rad, M.R., M., Allahdoo, and H.R., Fanaei, 2010. Study of some yield traits relationship in melon (*Cucumis melo* L.) germplasm gene bank of Iran by correlation and factor analysis. *Trakia J. Sci* 8, 27-32
9. Naroui Rad, M. R., H. R., Fanaei, and A., Ghalandarzahi, 2017. Integrated Selection Criteria in Melon Breeding. *International Journal of Vegetable Science* 23, 125-134
10. Raghani, M., Z., Zamani, A.I., Lopez-Sese, M.R. Hasandokht, and M. R., Fattahi

- Moghadam, 2014. Genetic diversity among melon accessions from Iran and their relationships with melon germplasm of diverse origins using microsatellite markers. *Plant. syst. Evol* 300, 139-151
- 11.Ramanatha, R.V.,and T., Hodgkin, 2002. Genetic diversity and conservation and utilization of plant genetic resources. *Plant Cell Tissue and Organ Cult* 68, 1-9
- 12.Sensoy, S., S., Büyükalaca, and K., Abak, 2007. Evaluation of genetic diversity in Turkish melons (*Cucumis melo* L.) based on phenotypic characters and RAPD markers. *Genetic Resources and Crop Evolution* 54, 1351-1365
- 13.Staub, J. E., A. I., López-Sesé, and N., Fanourakis, 2004. Diversity among melon landraces (*Cucumis melo* L.) from Greece and their genetic relationships with other melon germplasm of diverse origins. *Euphytica* 136, 151-166
- 14.Szamosi, C., I., Solmaz, N.,Sari, and C., Bársony, 2010. Morphological evaluation and comparison of Hungarian and Turkish melon (*Cucumis melo* L.) germplasm. *Scientia Horticulturae* 124, 170-182
- 15.Team, R.C., 2015. R a Language and Environment for Statistical Computing. Available at: <http://www.r-project.org>
- 16.Trimech, R., Y., Zaouali, A., Boulila, L., Chabchoub, I., Ghezal, and M., Boussaid, 2013. Genetic variation in Tunisian melon (*Cucumis melo* L.) germplasm as assessed by morphological traits. *Genetic Resources and Crop Evolution* 60, 1621-1628.