Agro-Morphological Variation in Some Iranian Melon (Cucumis melo L.) Genotypes Revealed by Multivariate Analysis

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ABSTRACT
Diversity of 9 local Iranian genotypes of melon was studied based on 28 quantitative and qualitative agro-morphological traits, using multivariate statistical methods. Analysis of variance showed significant differences between genotypes for all quantitative traits. However, the studied melon genotypes were previously clustered in two main groups of inodorous and cantalupensis, cluster analysis classified them in four different groups based on all traits, in particular outlaying Shadegani genotype from Inodorous group. Clustering was also supported by principal components analysis (PCA). The first two PCs contributed 60% of the total variability and demonstrated variations mainly on the basis of yield related traits including fruit number, day to flower, day to harvest, fruit weight, fruit length and other distinctive traits such as rind pattern, peduncle attachment strength, fruit firmness and aroma. Accordingly, agro-morphological traits could be used for variation studies in melons and results might be useful for improving breeding programs.

Key words: Agro-morphological characterization, Cluster analysis, Diversity evaluation, Genetic improvement, Principal components analysis, Ward’s method.

INTRODUCTION
Melon (Cucumis melo), an important tropical and subtropical vegetable crop of family cucurbitaceae, is highly polymorphic in fruit morphology and yield related traits. Iran as third leading country in melon production (FAO 2014), is considered to be one of the primary diversity centers for melon (Raghami et al. 2014); But unfortunately the most known traditional landraces of Iranian melon are exposed to genetic erosion due to introduction and cultivation of improved non-native commercial varieties. Extensive collection and diversity evaluation of local melon accessions is not only vital for conservation purposes, but also for genetic improvement throughout selecting valuable genetic resources in breeding programs (Sarkar and Kundagrami, 2015). Availability of genetic variation among local genotypes of melon is a determining factor in success of breeding programs. Higher genetic distance between parents leads to better selection of desirable parental combinations and achieving higher heterosis in their progeny (Arya et al., 2017).

Various techniques are available for evaluating diversity in different collections of plant genotypes using pedigree records, morphological, biochemical and molecular markers (Esghii and Akhundova, 2010). Morphological characterization of genotypes is the first step in description and classification of germplasm and has been proven as an effective, inexpensive and easy method to measure with straightforward detection in comparison with other approaches (Darvishzdeh and Hatami 2012). Multivariate analysis techniques such as principal component analysis (PCA) and cluster analysis are essential tools for measuring and classifying the variability in plant genetic resources based on agro-morphologic and physiological traits (Singh et al., 2017).

Genetic diversity in different genotypes of melon has been analyzed worldwide, based on various techniques such as assessment of morphological traits (Laghetti et al. 2008; Escríbano and Lazaro 2009), molecular markers such as isozymes (McCreight et al. 2004), amplified fragment length polymorphism (AFLP) (Yashiro et al. 2005), random amplified polymorphic DNA (RAPD) (Sensoy et al. 2007; Yi et al. 2009) and simple sequence repeat (SSR) (Hanane et al. 2015). There are also a few studies on the genetic structure of Iranian melon (Abdollahi et al. 2015; Danesh et al. 2015; Kohpayegani and Behbahani 2008; Nasrabadi et al. 2012; Raghani et al. 2014); But there are still many accessions, landraces and improved cultivars remaining to be studied.

In present study, agro-morphological diversity among some Iranian Cucumis melo genotypes belonging to inodorous and cantalupensis groups were studied in order to increase understanding of the genetic variability of Iranian genotypes.

MATERIALS AND METHODS
Seeds of 10 melon genotypes were used in this study, including nine Iranian accessions belonging to inodorous
and cantaloupensis groups and one exotic commercial cultivar belonging to inodorous group (Table 1). All seeds were obtained from Horticultural Science Research Institute of Iran.

The experiment was conducted in the research field of Tabriz University, Tabriz, Iran, as a randomized complete block design with three replicates and 10 plants per plot. Seeds were directly sown in double rows (2 m apart) with 60 cm spacing. Irrigation (furrow system), fertilization, hand weeding and other management practices were performed when required throughout the growing period. Data were collected on five randomly selected plants from the middle row of each plot. 28 agro-morphological characteristics were scored, including 15 qualitative fruit related traits according to UPOV (The International Union of Protection of New Varieties of Plants) descriptors (UPOV 2014) and 13 additional quantitative traits (Table 2).

Analysis of variance (one-way ANOVA) and Duncan test for mean comparison were conducted on quantitative parameters to determine the differences between varieties. Cluster analysis (UPMGA, based on Squared Euclidean distance measure using WARD method) was performed on all variables, to estimate the multivariate relationships among genotypes. Principal component analysis (PCA) was also done to check the results of UPGMA-based clustering. All statistical analysis was carried out using IBM SPSS Statistics 23 software.

**RESULTS AND DISCUSSION**

**Quantitative and Qualitative variability among genotypes**

Fruit appearances of 10 melon genotypes are shown in Fig 1 and their Morphological performance data is presented in Table 3. Fruit morphology traits including shape, rind coloring pattern, flesh color and aroma were extremely divergent among studied genotypes. Inodorous fruits varied in shape from elongated (Dargazi, Shadegani and Minoo), to Ovate (Jalallii and Charjoo) and round (Honeydew). But cantaloupensis group were all Broad elliptic types. Inodorous fruits except Shadegani attached hardly to stem at maturity, but cantaloupensis genotypes absicised when mature. Fruit odor in cantaloupensis group was medium to very strong and in inodorous group very weak to medium, except in Shadegani fruits with strong odor. Rind color, grooves and patches showed highly diverse pattern among genotypes. According to Munger and Robinson (1991), melons of cantaloupensis group are defined as medium-large size fruits, smooth, scaly or netted rind of variable color, aromatic with sweet, juicy flesh and abscission layer at maturity and inodorous group as large-sized non-aromatic, non-climacteric and long-storing fruits, with smooth or wrinkled, but not netted rind. However, there are still other types with characteristics not included in this classification. This is especially a challenge for inodorous type Iranian accessions which differ from other melons belonging to the var. inodorus, in their netted skin surface and rugby ball-shaped fruits and it is suggested to consider them in other group called Iranisans (Lotfi and Kashi, 1999).

Mean comparison of quantitative characters in studied melon genotypes revealed significant variation for all 13 traits (Table 4). Lateral branch number and fruit number values in cantaloupensis group were significantly higher than inodorous group. But high number of lateral branches cannot be a differentiating characteristic for melons of cantaloupensis group according to previous studies which used lower lateral branching Cantaloupe genotypes as parental lines (Luan et al., 2010). Lateral branching is typically associated with producing bisexual and female flowers and in this study, multi-lateral branching genotypes (cantaloupensis group and Shadegani genotype from inodorous group) were significantly early flowering and early harvesting plants, bearing more fruits. Fruit weight was negatively correlated with number of fruits (data not shown); hence less fruiting genotypes of each group had higher fruit weight. Fruits formed near the crown and after more internodes in inodorous group have more source leaves and less competing sinks, resulting in significantly higher fruit weight and yield, compared with cantaloupensis group (Feyzian et al., 2009). Despite almost equal diameters between two major groups, fruit length was higher in inodorous group, resulting in long shaped fruits (ie: high length/diameter ratio), except Honeydew variety which is a local Iranian melon. Cavity size was negatively correlated with length and length/width ratio (Table 4).

**Table 1:** List of C. melo genotypes assessed for genetic variation analysis. Genotypes names, Bioclimatic Zone and their assignment to varietal groups.

| Genotype name | Type of genotype | Source | Cultivar group |
|---------------|-----------------|--------|----------------|
| Charjoo       | Traditional cultigen | Iran-Golestan-Gonbad | Inodorous |
| Dargazi       | Traditional cultigen | Iran-Khorasan-Dargaz  | Inodorous |
| Garmak        | Traditional cultigen | Iran-Isfahan | Cantaloupensis |
| Honey Dew     | Commercial cultivar | Unknown | Inodorous |
| Jalallii      | Traditional cultigen | Iran-Semnan-Eyvanakey | Inodorous |
| Magasii       | Traditional cultigen | Iran-Khorasan-Neyshaboo | Cantaloupensis |
| Minoo095      | Traditional cultigen | Iran-Khorasan | Inodorous |
| Samsouri      | Traditional cultigen | Iran-Varamin | Cantaloupensis |
| Saveii        | Traditional cultigen | Iran-Varamin | Cantaloupensis |
| Shadegani     | Traditional cultigen | Iran-Golpayegan | Inodorous |
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PGMA and PCA based cluster analysis

The unweighted pair group method arithmetic (UPGMA) using Squared Euclidean distances matrix based on the agro-morphological markers, grouped the analyzed genotypes into four clusters according to melon types of cantaloupensis and inodorous (Fig 2). As seen from the dendrogram (Fig 2), inodorous group except Shadegani genotype are classified in one major group and all cantaloupensis genotypes are clustered in one major group beside Shadegani. Among inodorous genotypes, Minoo was the most divergent and was clustered in a different subgroup, which is in agreement with distinct characteristics in this genotype compared to other inodorous types (Tables 3 and 4). Three genotypes of Garmak, Samsouri and Saveii were very similar according to most traits and were merged closely in one cluster. Shadegani and Magasii genotypes with relatively more similarity (Tables 3 and 4) were clustered in one subgroup in the main cluster of cantaloupes near the inodorous. Shadegani was different than inodorous group mainly based on aroma and abscission of peduncle and Magasii was different than cantaloupensis group based on rind netting and soluble solids content.

In previous studies, Shadegani was clustered as an *inodorous* local cultivar (Abdollahi *et al*., 2015). The discrepancies between Shadegani and the *inodorous* types might be due to the differences in cultivation history and adaptation of populations to local conditions and also the high rate of out crossing in melon landraces and cultivars, which causes gene flow between populations and lose of characteristic traits among them.

### Table 2: Analyzed traits for genetic variation analysis of Iranian melon genotypes.

| Trait                                      | Unit, Interval or class                   |
|--------------------------------------------|-------------------------------------------|
| **Qualitative Traits**                     |                                           |
| Fruit: shape in longitudinal section       | 1 Round, 3 Broad elliptic, 5 Medium elliptic, 7 Ovate, 9 Elongated |
| Fruit: change of skin color from young fruit to maturity | 3 early in fruit development, 5 late in fruit development, 7 very late or no change |
| Fruit: ground color of skin                | 3 White, 5 Yellow, 7 Green, 9 Grey       |
| Fruit: intensity of ground color of skin   | 3 Light, 5 Medium, 7 Dark                |
| Fruit: hue of ground color of skin         | 1 absent/very weak, 3 Orange, 5 Ochre, 7 Greenish, 9 Greyish |
| Fruit: rate of change of skin color from maturity to over maturity | 1 Absent/very slow, 3 Slow, 5 Medium, 7 Fast |
| Fruit: size of dots                        | 1 Absent, 3 Small, 5 Medium, 7 Large     |
| Fruit: color of dots                       | 1 Absent, 3 White, 5 Yellow, 7 Green     |
| Fruit: size of patches                     | 1 Absent, 3 Small, 5 Medium, 7 Large     |
| Strength of attachment of peduncle at maturity | 1 Very weak, 3 Weak, 5 Medium, 7 Strong, 9 Very strong |
| Fruit: width of grooves                    | 1 Absent, 3 Narrow, 5 Medium, 7 Broad    |
| Fruit: depth of grooves                    | 1 Absent/very shallow, 3 Shallow, 5 Medium, 7 Deep, 9 Very deep |
| Fruit: color of grooves                    | 1 Absent, 3 White, 5 Yellow, 7 Green    |
| Fruit: main color of flesh                 | 1 Yellowish white, 3 Greenish white, 5 Green, 7 Orange |
| Fruit: Aroma                               | 1 Very weak, 3 Weak, 5 Medium, 7 Strong, 9 Very strong |
| **Quantitative Traits**                    |                                           |
| Lateral branch number                      | To include all branches of more than 12.5 cm on the main stem |
| Day to first female flower opening         |                                            |
| Day to harvest                             |                                            |
| Number of fruits per plant                 | Fruits of at least 7.5 cm in diameter     |
| Yield                                      | (g.plant⁻¹)                              |
| Fruit weight                               | (g)                                      |
| Fruit length                               | (cm)                                     |
| Fruit diameter                             | At position of maximum fruit diameter (cm) |
| Flesh pulp thickness                       | At position of maximum fruit diameter (cm) |
| Cavity thickness                           | At position of maximum fruit diameter (cm) |
| Ratio length/diameter                      | (N) measured with a penetrometer         |
| Fruit pulp firmness                        | (°Brix) measured with a digital refractometer |

diameter ratio (data not shown), resulting in relatively bigger cavity versus thinner pulp in round genotypes. Total soluble solids value did not show a regular pattern among genotypes; After Honydew as a commercially improved cultivar, Charjoo (*inodorous* group) and Magasii (*cantaloupensis* group) contained the highest amount of total soluble solids.

In previous studies, Shadegani was clustered as an *inodorous* local cultivar (Abdollahi *et al*., 2015). The discrepancies between Shadegani and the *inodorous* types might be due to the differences in cultivation history and adaptation of populations to local conditions and also the high rate of out crossing in melon landraces and cultivars, which causes gene flow between populations and lose of characteristic traits among them.
The result of the PCA showed that five Principal Component axes (PC) had Eigenvalues greater than 1 and all together accounted for 92.7% of the total variability. The first component, which explained 43.6% of the total variance, was strongly and positively correlated with yield related traits including day to flower, day to harvest, yield, fruit weight, fruit length, fruit length/diameter ratio and other distinctive traits such as peduncle attachment strength and fruit firmness; and was negatively correlated with lateral branch number, fruit number, aroma and main color of flesh. The second component with 16.42% of total variance was positively associated with traits fruit diameter, pulp thickness and rate of color change at maturity; and negatively with traits width, depth and color of grooves.

In general, the results obtained from PCA were in agreement with UPGMA clustering (Fig 3). Attending to the first and second principal components, the two dimensional PCA plot graphic showed a classification of the melon genotypes in four groups: a first group formed by multi-lateral branching genotypes with high number of early flowering and early harvesting small round aromatic fruits with patches and grooves on rind, corresponding to the genotypes Garmak, Samsouri and Saveii. The second group consisted of two genotypes Magasii and Shadegani, which were separated from other groups based on intermediate values for traits multi-lateral branching, day to flowering and harvesting, Total soluble solids and aroma and also for extreme values of color change rate at maturity and over-

### Table 3: Expression of qualitative fruit related traits in local Iranian melon genotypes.

| Genotype  | Shape in longitudinal section | Change of skin color | Ground color of skin | Intensity of ground color of skin | Hue of ground color of skin |
|-----------|------------------------------|----------------------|----------------------|----------------------------------|----------------------------|
| Charjoo   | Ovate                        | Late                 | Green                | Medium                           | Greyish                    |
| Dargazii  | Elongated                    | Late                 | Grey                 | Medium                           | Greenish                   |
| Garmak    | Broad elliptic               | Late                 | Green                | Light                            | Ochre                      |
| HoneyDew  | Round                        | Late                 | White                | Light                            | Greenish                   |
| Jalalii   | Ovate                        | Late                 | Yellow               | Dark                             | Absent                     |
| Magasii   | Broad elliptic               | Early                | Green                | Medium                           | Greyish                    |
| Minoo     | Elongated                    | Early                | Yellow               | Medium                           | Greenish                   |
| Samsouri  | Broad elliptic               | Late                 | Yellow               | Light                            | Ochre                      |
| Saveii    | Broad elliptic               | Late                 | Yellow               | Light                            | Ochre                      |
| Shadegani | Medium elliptic              | Early                | Yellow               | Medium                           | Orange                     |

| Genotype  | Color change From maturity to over maturity | Size of dots | Color of dots | Density of patches | Strength of attachment of peduncle at maturity |
|-----------|---------------------------------------------|--------------|---------------|--------------------|-----------------------------------------------|
| Charjoo   | Medium                                      | Large        | Green         | Dense              | Medium                                        |
| Dargazii  | Absent                                     | Medium       | Green         | Medium             | Very strong                                   |
| Garmak    | Absent                                     | Small        | Yellow        | Very dense         | Very weak                                     |
| HoneyDew  | Absent                                     | Absent       | Absent        | Absent             | Very strong                                   |
| Jalalii   | Absent                                     | Absent       | Absent        | Dense              | Very strong                                   |
| Magasii   | Fast                                        | Large        | Green         | Absent             | Very weak                                     |
| Minoo     | Absent                                     | Absent       | Absent        | Absent             | Very strong                                   |
| Samsouri  | Slow                                        | Absent       | Absent        | Very dense         | Very weak                                     |
| Saveii    | Slow                                        | Medium       | Green         | Very dense         | Very weak                                     |
| Shadegani | Fast                                        | Absent       | Medium        | Medium             | Very weak                                     |

| Genotype  | Width of grooves | Depth of grooves | Color of grooves | Main color of flesh | Aroma |
|-----------|------------------|------------------|-------------------|--------------------|-------|
| Charjoo   | Absent           | Absent           | Absent            | Green              | Medium|
| Dargazii  | Absent           | Absent           | Absent            | Greenish white     | Very weak|
| Garmak    | Narrow           | Medium           | Green             | Green              | Medium|
| HoneyDew  | Absent           | Absent           | Absent            | Greenish white     | Medium|
| Jalalii   | Absent           | Absent           | Absent            | Yellowish white    | Very weak|
| Magasii   | Broad            | Shallow          | Green             | Green              | Very strong|
| Minoo     | Broad            | Medium           | Green             | Yellowish white    | Weak |
| Samsouri  | Narrow           | Medium           | Green             | Greenish white     | Strong|
| Saveii    | Narrow           | Medium           | Green             | Orange             | Strong|
| Shadegani | Broad            | Medium           | Green             | Orange             | Strong|
### Table 4: Mean comparison of quantitative traits in local Iranian melon genotypes.

Means within rows followed by the same letter are not significantly different from each other (Duncan's post hoc analysis of subsets, P = 0.05).

| Genotype   | Lateral branch number | Day to first flower (day) | Day to harvest (day) | Yield (g/plant⁻¹) | Number of fruits per plant | Fruit weight (g) | Fruit length (cm) |
|------------|-----------------------|----------------------------|----------------------|-------------------|---------------------------|----------------|-----------------|
| Charjoo    | 5.4±0.22de            | 41.6±1.04b                 | 84.6±0.65cd          | 3419.80±411.16abc | 1.7±0.21c                 | 1948.7±143.03cd | 22.0±0.89c       |
| Dargazii   | 4.6±0.31fg            | 43.1±0.34ab                | 85.4±0.92bc          | 4050.30±162.45ab  | 1.8±0.29bc                | 2230.70±56.58b  | 25.0±0.46b       |
| Garmak     | 13.2±0.32a            | 33.1±0.64d                 | 69.9±1.26f           | 3468.20±314.36abc | 3.3±0.36a                 | 1051.50±71.47c  | 11.4±0.52g       |
| HoneyDew   | 4±0.00g               | 44±0.57a                   | 89.3±0.92a           | 4318.40±275.16ab  | 2.1±0.31bc                | 2053.6±45.54bc  | 19.4±0.48d       |
| Jalalii    | 4±0.00g               | 42.9±0.57ab                | 88.1±0.74ab          | 3539.90±369.85abc | 1.7±0.21c                 | 2079.0±68.43bc  | 23.2±0.84c       |
| Magasii    | 6±0.00d               | 34.9±0.74d                 | 76.5±1.34e           | 2838.10±381.36c   | 2.6±0.26abc               | 1090.40±51.77e  | 13.1±0.54g       |
| Minoo      | 5.1±0.3ef             | 44.6±0.48a                 | 86.4±1.23abc         | 4405.00±331.18a   | 1.8±0.25bc                | 2446.3±49.99a   | 30.5±0.53a       |
| Samsouri   | 7±0.33c               | 32.9±0.99d                 | 74.7±0.78e           | 2681.60±398.08bc  | 2.7±0.37ab                | 1024.6±5.63e    | 12.7±0.39g       |
| Savei      | 9±0.33b               | 33.5±0.79d                 | 75.9±1.15e           | 2927.50±203.81c   | 3±0.3a                    | 1004.0±10.35e   | 13.9±0.38f       |
| Shadegani  | 6±0.00d               | 37.4±0.73c                 | 81.7±1.31d           | 3313.80±353.23bc  | 1.9±0.23bc                | 1804.20±44.14d  | 17.7±0.67e       |
| C.V.       | 41.99                 | 13.27                      | 8.67                 | 32.83             | 41.36                     | 34.45           | 33.22           |

| Genotype   | Fruit diameter (cm) | Ratio length/diameter | Flesh pulp thickness (cm) | Cavity thickness (cm) | Fruit pulp firmness (N) | Soluble solids (%Brix) |
|------------|---------------------|-----------------------|----------------------------|-----------------------|------------------------|------------------------|
| Charjoo    | 14.40±0.38ab        | 1.52±0.05d            | 3.30±0.08ab               | 7.65±0.32ab           | 7.63±0.42b             | 10.87±0.41a            |
| Dargazii   | 10.25±0.42e         | 2.48±0.1b             | 2.90±0.12bc              | 4.65±0.32d            | 7.40±0.49b             | 7.26±0.15d             |
| Garmak     | 12.35±0.49c         | 0.92±0.02g            | 2.70±0.23b               | 7.60±0.29b            | 5.32±0.22cd            | 7.58±0.37cd            |
| HoneyDew   | 14.70±0.39a         | 1.32±0.03e            | 3.30±0.17ab              | 7.85±0.29ab           | 9.29±0.28a             | 11.47±0.34a            |
| Jalalii    | 13.25±0.42abc       | 1.76±0.05c            | 3.85±0.18a               | 5.75±0.31c            | 8.16±0.52b             | 7.10±0.38d            |
| Magasii    | 12.00±0.66cd        | 1.09±0.02f            | 2.45±0.23c               | 7.10±0.24b            | 3.06±0.19e             | 9.58±0.47b            |
| Minoo      | 10.95±0.26de        | 2.80±0.06a            | 2.90±0.14bc              | 5.15±0.22cd           | 7.93±0.35b             | 8.14±0.18bcd           |
| Samsouri   | 14.00±0.34ab        | 0.91±0.02g            | 2.95±0.26bc              | 8.10±0.34a            | 4.53±0.13d             | 7.33±0.46d            |
| Savei      | 14.15±0.57ab        | 0.99±0.03f            | 3.25±0.27ab              | 7.65±0.26ab           | 5.73±0.27c             | 8.11±0.9cd            |
| Shadegani  | 13.15±0.58bc        | 1.36±0.07e            | 2.80±0.2bc               | 7.45±0.4ab            | 5.17±0.14cd            | 9.12±0.42bc           |
| C.V.       | 15.54                | 42.38                 | 23.22                    | 21.56                 | 31.40                   | 22.99                  |

**Fig 1:** photographs of fruits of *Cucumis melo* genotypes used in present study.

**Fig 2:** UPGMA cluster analysis of local Iranian melon genotypes by Ward’s grouping method and using Euclidean distances. Dendrogram is based on qualitative and quantitative agro-morphological traits.
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maturity and fruit firmness. Charjoo, Dargazi, Honeydew and Jalali were clustered in the third group based on yield related traits, despite variations in different shape related characteristics. Minoo genotype was an outlier due to extreme values of yield related traits and rind pattern characteristics.

CONCLUSION

In this study, different multivariate approaches including Mean comparison, PCA analysis and two-way cluster analysis were applied to describe and understand the extent of genetic variability in melon genotypes. Present classification using agro-morphological markers revealed a broad diversity in 9 local Iranian melon genotypes. We believe distinct sub-clusters among two major groups of cantalopensis and inodorous type, due to transition of dominant traits from cantalopensis and other groups and fixation by farmer selections. Since melon is a cross pollinated plant with broad variation among different varieties, different genotypes could be introduced as suitable parents in melon breeding programs. Results of this study could be utilized in germplasm management activities and improvement of genetically diverse melon accessions.

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