Journal of Plant Molecular Breeding (JPMB)
Vol. 3/ Issue 1/ June 2015/ 35-49

Study of genetic diversities and relatedness of Iranian citrus genotypes using morphological and molecular markers

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Received: March 2015 Accepted: June 2015

Abstract
Having knowledge about genetic relationships among accessions is necessary for developing breeding strategies to produce improved cultivars. In present study, genetic diversity and inter-relationship among 29 genotypes of citrus were comparatively analyzed using morphological and RAPD markers. Significant variability was observed among citrus genotypes for 61 quantitative and qualitative morphological characters of leaves, fruits and seeds. Furthermore, the RAPD markers revealed a high polymorphism rate (91.82 %). A pair-wise similarity value between genotypes ranged from 0.14 to 0.97 with average of 0.62. Both morphological and molecular analysis indicated a high degree of variation among studied genotypes. In current research, genotypes "pummelo" and "mandarin" were confirmed as true species of citrus in distinct cluster. Results of present study proved that both of morphological and molecular markers are potential tools for determining genetic diversities and genetic relationships of citrus genotypes and can be used in citrus breeding programs.
Keywords: Citrus, Cluster analysis, Genetic variability, Molecular markers.

Introduction

Citrus is one of the most economically important fruit crops in worldwide (20). *Citrus* and its closed relatives are represented by 28 genera from tribe Citreae of subfamily Aurantioideae in family Rutaceae (32). Citrus taxonomy and phylogeny are very complicated, controversial and confusing, mainly due to sexual compatibility between *Citrus* and its related genera, the high frequency of bud mutations and the long history of cultivation and wide distribution (24).

Elucidating relationships, taxonomy, and diversity are important for developing breeding strategies, conserving biodiversity, and improving breeding efficiency. Understanding the genetic diversity in citrus is also critical for characterizing germplasm, controlling genetic erosion and registration of new cultivars (12, 4).

Morphological characterization in combination with molecular markers would be more rewarding in terms of accurate identification and characterization of most closely related cultivars at intra-specific level. Molecular marker techniques are routinely used for proper characterization, management and conservation of germplasm collections of horticultural species (16).

Among molecular markers, random amplified polymorphic DNA (RAPD) markers have been employed most widely for characterization of plant species. RAPD markers are simple, fast, and sensitive. They require no prior knowledge about DNA sequence and can amplify a large number of DNA fragments for reaction (35).

RAPD markers are routinely used for proper characterization, management and conservation of germplasm collections of *Citrus* species. For example, RAPD has been used to generate linkage map for citrus (6). Federici *et al.* (11) examined the phylogenetic relations of 88 accessions representing 45 *Citrus* species and six related genera by utilizing RFLP and RAPD markers. Overall, these previous studies demonstrated that molecular markers are powerful tools for elucidating genetic diversity, determining parentage, and revealing phylogenetic relationships among various citrus species. Nicolosi *et al.* (24) used RAPD, SCAR, and cpDNA markers to elucidate phylogenetic relationships and genetic origins of hybrids in 36 accessions of *Citrus* and one accession from each of four related genera and indicated that *Fortunella* is phylogenetically close to *Citrus* while the other three related genera are distant from *Citrus* and from each other. Dehestani *et al.* (9) evaluated the genetic diversity in 52 genotypes of Navel orange in Mazandaran province (Iran) using RAPD marker and reported high polymorphism (70.13%). Malik *et al.* (20) investigated genetic diversity and inter-relationship among 22 cultivars of *C. sinensis* based on morphological and RAPD markers. In their study, RAPD markers proved to be useful for germplasm characterization and diversity analysis in *C. sinensis* cultivars. Pal *et al.* (27) studied genetic variability and relationships of mandarins using morphological and molecular markers. Their study revealed that both morphological and molecular markers can be successfully utilized for inferring genetic diversity and genetic relationship of mandarin group. Tripolitsiotis *et al.* (33) evaluated genetic similarity among 36 accessions of the Greek *Citrus* germplasm using RAPD and ISSR markers and indicated that both techniques were proven to be equally analytical with an average discrimination power above 0.9. The RAPD and ISSR markers were highly correlated and clustering based on their results are highly correspondence. *Citrus* accessions formed separate clusters according to their species, even though sweet orange and mandarin cultivars revealed high affinity, while lemons were more divergent.

Little is known about the genetic variability of the Iranian citrus accessions. The objective of the present

study was to assess genetic diversity and relationship of some important *Citrus* genotypes using morphological and RAPD markers.

Materials and methods

Plant material and sample collection

A total of 29 genotypes of Citrus were collected from Iran Citrus Research Institute, located at Tonekabon, Iran**.** These genotypes were used for morphological and molecular studies (Table 1). Flower, leaf and fruit samples of each genotypes were collected for confirmation of taxonomic identity, characterization and DNA extraction.

Table 1. Plant materials utilized for morphological and RAPD analysis.

Plant code	Common name	Scientificname	Plant code	common name	Scientific name
G1	Sour orange	*Citrus aurantium*	G61	Unknown natural type	*Citrus* sp.
G2	Marssorange	*Citrus sinensis*	G63	Unknown natural type	*Citrus* sp.
G3	Thomson navelorange	*Citrus sinensis*	G65	Unknown natural type	*Citrus* sp.
G4	Local orange (Siavaraz#1)	*Citrus sinensis*	G67	Unknown natural type	*Citrus* sp.
G5	Local orange (Siavaraz#2)	*Citrus sinensis*	G70	Unknown natural type	*Citrus* sp.
G6	Local orange (Siavaraz#3)	*Citrus sinensis*	G71	Unknown natural type	*Citrus* sp.
G7	Local orange (Siavaraz#4)	*Citrus sinensis*	G72	Unknown natural type	*Citrus* sp.
G8	Moallemkoh (Natural type)	*Citrus* sp.	G73	Unknown natural type	*Citrus* sp.
G9	Shelmohalleh(Natural type)	*Citrus* sp.	G74	Unknown natural type	*Citrus* sp.
G10	Atabakimandarin	*Citrus reticulata*	G76	Unknown natural type	*Citrus* sp.
G11	Unshiumandarin	*Citrus unshiu*	G78	Unknown natural type	*Citrus* sp.
G12	Dancymandarin	*Citrus reticulata*	G79	Unknown natural type	*Citrus* sp.
G13	Bamimandarin	*Citrus reticulata*	G80	Unknown natural type	*Citrus* sp.
G14	Local mandarin	*Citrus reticulata*			
G15	Clementinemandarin	*Citrus clementina*			
G16	Pummelo	*Citrus grandis*			

Morphological characters

For achieving uniformity in current study only genotypes from Iran Citrus Research Institute were used. 15 leaves, 10 flowers and 10 fruits were randomly collected from each plant with three replications. 61 morphological characters (qualitative and quantitative) of flower, leaf, fruit and seed were determined according to the International Plant Genetic Resources Institute (IPGRI) protocols (13).

Samplings were done by randomly collection of 15 leaves, 10 flowers and 10 fruits from each plant in three replications. According to the criteria provided by protocols of International Plant Genetic Resources Institute (IPGRI) 61 morphological characters (qualitative or quantitative) of flowers, leaves, fruits and seeds were determined (13). All of the 61 morphological characters were converted to bi- and multi-state code. A pair-wise similarity matrix was generated based on simple matching coefficient method using software NTSYS ver. 2.10e (29). A cluster analysis was performed using

the unweighted pair group method with arithmetic average (UPGMA) based on simple matching coefficient using XLSTAT software version 2012.3.01 (2). Principal coordinate analysis (PCo) was also carried out for studying correlations among the variables and establishing relationships among genotypes using the Genalex ver 6.5 software (28). The two-way Mantel test (21) for goodness of fit for the UPGMA cluster was also performed using the NTSYS ver. 2.10e software.

Table 2. Statistical analysis and results of genetic diversity of 29 citrus genotypes.

Row	Primer Name	Primer Sequence 5' ⟶ 3'	Annealing temperature	Total number of Bands	Number of Polymorphic Bands	% polymorphism	PIC
1	OPB-12	CCTTGACGCA	37	15	13	86/66	0.216
2	OPE-09	CTTCACCCGA	37	16	16	100	0.120
3	OPA-04	AATCGGGCTG	37	15	14	93/33	0.263
4	OPA-07	GAAACGGGTG	37	19	19	100	0.235
5	OPA-08	GTGACGTAGG	37	10	9	90	0.282
6	OPA-19	CAAACGTCGG	37	12	11	91/66	0.267
7	OPG-05	CTGAGACGGA	37	11	9	81/81	0.247
8	OPG-06	GTGCCTAACC	37	16	14	87/5	0.226
9	OPB-08	GTCCACACGG	35	14	12	85/71	0.215
10	OPA-12	TCGGCGATAG	35	13	12	92/30	0.249
11	OPA-05	AGGGGTCTTG	37	9	8	88/88	0.224
12	OPA-18	AGGTGACCGT	37	19	18	94/73	0.197
13	OPM-11	GTCCACTGTG	37	17	15	88/23	0.265
14	OPM-14	AGGGTCGTTC	35	12	12	100	0.309
15	OPM-18	CACCATCCGT	37	11	10	90/90	0.189
16	OPG-04	AGCGTGTCTG	37	16	15	93/75	0.267
17	OPC-07	GTCCCGACGA	37	16	15	93/75	0.223
18	OPA-10	GTGATCGCAG	37	16	15	93/75	0.213
19	OPA-09	GGGTAACGCC	37	12	11	91/66	0.266
Mean	-	-		14/15	13/05	91/82	0.230

DNA isolation

From each genotyepe, five young leaves were taken and total genomic DNA was isolated from leaves using the CTAB (hexadecyltrimethylammonium-bromide) method (22). The DNA concentration was determined spectrophotometrically (Nano Drop 1000) at 260 nm and its quality was checked by electrophoresis on 0.8 % agarose gel. The extracted DNA was diluted to 20ng/µl and stored at -20°C for PCR amplification.

PCR amplification

Thirty RAPD primers were initially screened and finally 19 primers that produced scorable polymorphic bands were selected for further analysis (Table 2).

DNA amplification was carried out in 25 µL reactions containing 20 ng of template DNA, 0.2 mM dNTPs, 10µM primer, 2.5 µL of 10× PCR Buffer (CinnaGen, Iran), 3 mM of magnesium chloride, 17.2 µL ddH_2O and 1.5 unit of Taq polymerase (CinnaGen, Iran). PCR amplification was carried out in a PTC-10096V Thermocycler (MJ Research,

Inc, USA). The thermal cycler conditions for PCR reactions were an initial denaturation of 1 min at 94°C followed by 40 cycles comprising 1 min at 94°C, 1 min at 35-37°C (annealing temperature was optimized for each primer) (Table 2), and 1 min and 30 s at 72°C. An additional step of 7 min at 72°C was used for final extension. Amplification products were separated by electrophoresis (8.3 $V.cm^{-1}$) in 1.5% agarose gel and stained by ethidium bromide (10µg.ml^{-1}). A photographic record was taken under UV illumination.

Table 3. Cophenetic coefficients obtained from algorithms with similarity coefficient.

	UPGMA algorithm	Simple connection algorithm	Complete connection algorithm
Dice similarity coefficient	0.972	0.979	0.986
Jaccard similarity coefficient	0.975	0.984	0.989*
Simple matching similarity coefficient	0.979	0.977	0.986

Data analysis

Only clear and repeatable amplification products were scored as 1 for present bands and 0 for absent ones. Data were analyzed with the NTSYS-pc software package version 2.10 (29). A cluster analysis was performed using the unweighted pair group method with arithmetic average (UPGMA) based on simple matching coefficient using XLSTAT software version 2012.3.01 (2). The representativeness of dendrograms was evaluated by estimating cophenetic correlation for the dendrogram and comparing it with the similarity matrix, using Mantel's matrix correspondence test (21). The result of this test is a cophenetic correlation coefficient, r, indicating how well the dendrogram represents similarity data. The percent of polymorphism was calculated using the formula (number of polymorphic bands/ total bands). polymorphism information content (PIC) was calculated for dominant markers that the allelic relationship between their bands was unclear with the formula PIC=Σ [2fi (1-fi)]. The principal coordinate analysis (PCo) of the original binary data matrix was also performed using Genalex ver 6.5 software (28).

Results and Discussion

Morphological analysis

The average genetic similarity among citrus genotypes was 0.47, with values ranging from 0.22 to 0.70. Genotypes G61 and G65 showed the highest degree of similarity (0.70), indicating that these pairs are closely related genotypes. On the other hand, the pummelo (G16) and G74 genotypes indicated the lowest similarity values (0.22).

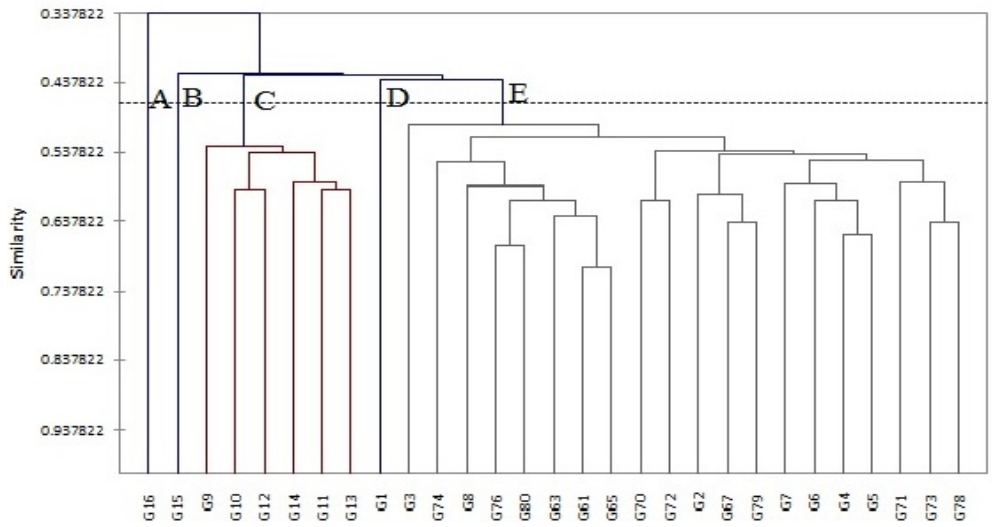

Figure 1. Dendrogram generated using UPGMA, showing relationships between 29 citrus genotypes based on morphological data.

The dendrogram obtained with 29 genotypes based on 61 quantitative and qualitative morphologic characteristics, separated citrus genotypes into five clusters (A, B, C, D and E), which diverged at a similarity index of 0.47 (Figure 1). Cluster A comprised pummelo (G16). Cluster B comprised Clementine mandarin (G15). Cluster C was divided into two sub-clusters, so that, sub-cluster C1 consisted of Shelmohalleh (G9) and sub-cluster C2 contained Dancy (G12), Local (G14), Bami (G13), Unshiu (G11) and Atabaki (G10) mandarins. In current study, Clementine (G15) and Unshiu (G11) were classified into two distinct clusters. In view of morphological characteristics of mandarins, similar results have been reported in previous studies (18, 7). Cluster D, contained sour orange (G01). Cluster E, the largest group, consisting genetically unknown local genotypes (G8, G61, G63, G65, G67, G70, G71, G72, G73, G74, G76, G78, G79 and G80), Siavaraz (G4, G5, G6 and G7), Thomson Navel (G3) and Marss (G2) oranges. Within this cluster, the genetically unknown local genotypes G61 and G65 showed 0.70 genetic similarities. The Siavaraz oranges (G4, G5, G6 and G7) were very similar to Thomson navel (G3) (Figure 1). Rouhi Ghorabaie *et al.* (30) reported high similarity among oranges using morphological traits which is in agreement with the present study.

The cophenetic analysis comparing the UPGMA cluster analysis and the simple matching similarity matrix demonstrated a correlation r= 0.86, indicating that data in matrix was well represented by the dendrogram.

Principal coordinate analysis:

Principal Coordinate Analysis was drawn with two dimensional graph using 61 quantitative and qualitative morphologic characteristics (Figure 2).

A two-dimensional plot generated from PCA showed four groups which supporting the clustering pattern of the UPGMA dendrogram, except for genotype sour orange (G01) which was included in oranges group, while it was present in cluster D in UPGMA clustering.

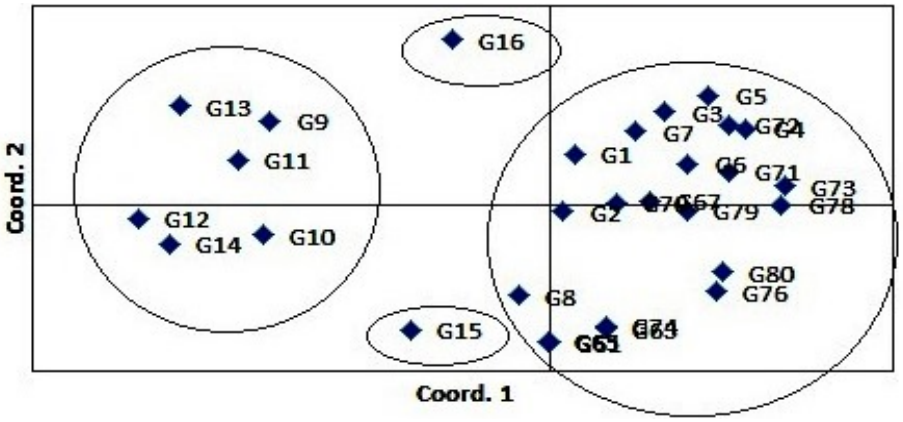

Figure 2. Principle Coordinate Analysis (PCo) ordination based on morphological data.

The analysis oriented the first three principal components, which contributed 62.27 % of the total variability of collected genotypes. Maximum variability was contributed by the first coordinate (27.07 %) followed by the second coordinate (20.47 %), and the third coordinate (14.72 %).

RAPD analysis

From total of 19 screened primers, 269 bands with high intensity were scored. The number of bands scored per primer combination ranged from 9 (OPA-05) to 19 (OPA-07 and OPA-18), with a mean of 14. Overall, the polymorphic band number varied between 8 (OPA-05) and 19 (OPA-07), with a mean of 13. The PIC values for the 19 primers ranged from 0.120 to 0.309, with an average of 0.230 (Table 2).

In order to classify genotypes based on RAPD data, Dice, Jaccard and the Simple matching similarity coefficient were calculated. Based on comparison of the correlations in matrices of similarity, each matrix of similarity was used to draw clusters using UPGMA algorithms, simple and complete connection. Cophenetic coefficient was calculated for every cluster. This coefficient shows the severity of similarity between matrix and cluster. Therefore, greater number resulted from the comparison between coefficient matrix and cophenetic matrix, indicating goodness of fit of the cluster analysis to the similarity matrix (23). Accordingly, Jaccard similarity coefficient and UPGMA algorithms were chosen as the most compatible similarity coefficient and clustering algorithm (Table 3).

A similarity matrix was calculated using RAPD data according to Jaccard coefficient (14). dendrogram was constructed using the UPGMA method (Figure 3). Cophenetic correlation between ultrametric similarities of tree

and similarity matrix was found to be high (r= 0.98, P < 0.01), suggesting that the cluster analysis strongly represents the similarity matrix. The genotypes studied had similarity values ranging from 0.14 to 0.97.

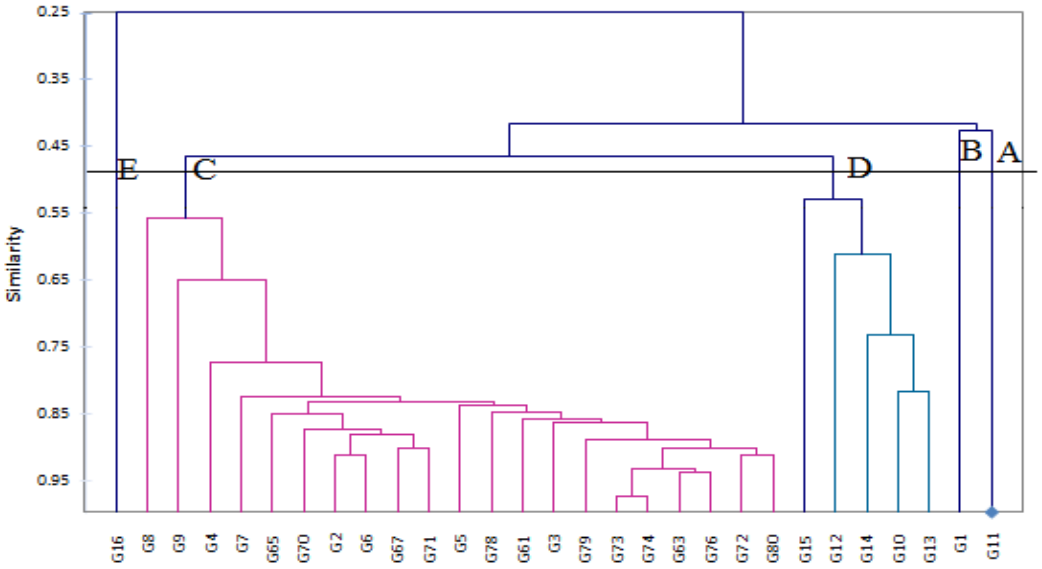

Figure 3. Dendrogram generated using UPGMA, showing relationships between 29 citrus genotypes, using RAPD data.

Results of similarity matrix showed that the highest genetic similarity (0.97) was existed between genotypes G74 and G73 and the lowest genetic similarity (0.14) was observed between genotypes of pummelo and Local mandarin. UPGMA dendrogram was generated by RAPD data and average similarity (0.49) for all genotype pairs was used as a cut off value for defining the clusters (Figure 3). From this dendrogram, 29 genotypes could be classified into five classes (A, B, C, D and E).

Considering the dendrogram (Figure 3), cluster A, included sour orange (G1). Sour orange showed similarity values of 0.26 and 0.42 with pummelo and mandarin, respectively. According to previous works, have suggested that sour orange is a natural hybrid between mandarin and pummelo (5, 4, 1) which was consistent with this study. Unshiu mandarin (G11) is placed into cluster B. The cluster C, the largest group, consist of unknown local genotypes (G61, G63, G65, G67, G70, G71, G72, G73, G74, G76, G78, G79 and G80), Siavaraz (G4, G5, G6 and G7), Thomson navel (G3) and Marss (G2) oranges. Within this cluster, the genotypes G74 and G73 showed 0.97 genetic similarity. Sweet orange showed low level of genetic diversity according to lots of previous studies (19, 25, 26, 34, 20). It is notified that, most of sweet oranges were mutations of unique ancestor tree. However, despite differences in morphological characters, genetic variation of sweet orange was low (10). According to a recent study using a

large number of oranges, there is a high level of genetic similarity among oranges (34). Furthermore, accessions arising from spontaneous mutations are also often difficult to be distinguished (4). According to our data, Siavaraz genotypes were high similar to Thomson navel orange (G3), indicating that probably originated from bud mutation, and the idea was supported by SSR analysis (17, 15, 30).

Shelmohalleh (G9) had high similarity (0.68) to Siavaraz 3, whereas based on morphological data it was clustered in mandarin group. Also, Moallemkoh (G8) showed high similarity to Siavaraz 2 and Thomson navel. The present findings were consistent with the results of Jannati *et al.* (15). Using SSR markers, they reported that Moallemkoh (G8) had similarity to Thomson and Siavaraz and they concluded that this genotype probably obtained through the hybridization between them or a bud mutation.

Cluster D comprised with two subclusters, D1 including Clementine mandarin (G15) and D2 consisting of Dancy (G12), Local (G14), Bami (G13) and Atabaki (G10) mandarins. Within subcluster D2, high genetic similarity (0.82) was observed between the genotypes of Bami (G13) and Atabaki (G10) in spite of morphological differences. In both RAPD and morphological analysis, Clementine (G15) and Unshiu (G11) were classified into two distinct clusters. Coletta Filho *et al.* (8) reported that they belong to two different groups. A high level of polymorphism (86%) was also reported by Campos *et al.* (7) in mandarins based on morphological and AFLP markers. Although Coletta Filho *et al.* (8) reported very narrow genetic base of mandarin group using RAPD marker and proposed mandarin group as a single species mandarin. Mandarins are one out of three citrus types that Barrett and Rhodes (5) proposed as true species and a number of researches (24, 4, 34) supported this idea.

Using both of morphological and RAPD markers, Pummelo (G16) was placed into group E separately and showed a little similarity in comparison with other genotypes. Pummelo was reported as one of the three true citrus species by Barrett and Rhodes (5) and most of subsequent studies were followed by this statement (11, 24, 4, 34). Thus, Pummelo has played an important role as the parent of many citrus fruits, such as lemons, oranges and grapefruits.

Principal coordinate analysis:

The principal Coordinate analysis was performed for better visualization of relationship among studied accessions. The classical principal Coordinate analysis (PCo) is likely an example of dimensionality reduction. The results of PCo are demonstrated in Figure 4.

A two-dimensional plot generated from PCo showed seven groups which was found to be almost similar to the clustering pattern of UPGMA dendrogram. The reason behind observing low differences is that two to three of the first components cannot represent the diversity of primary variables (total number of bands). In a 2D plot analysis, genotypes Shelmohalleh (G9) and Moallemkoh

(G8) were stayed together at the same group, whereas, in UPGMA clustering they were presented at two different clusters. Clemantine mandarin (G15) also formed a separate group in 2D plot but in dendrogram, it was placed at cluster D.

The analysis demonstrated that the first three principal components have contributed 77.74 % of total variability of collected genotypes. Maximum variability was created by the first component (46.17 %) followed by the second (20.44%), and the third components (11.13 %). In molecular set of data, two or three of the first components can defined about 10-20 percent of changes, in which, are not statistically suitable for graphic display, but represents genetically desirable sample of total genome (31). In present study, 77.74% of total changes were determined mainly because of measuring only a few numbers of components. The applied primers have covered only a little chromosomal regions and have poor dispersion in various parts of genome. Hence, further investigation using more primers is necessary for covering whole plant genome.

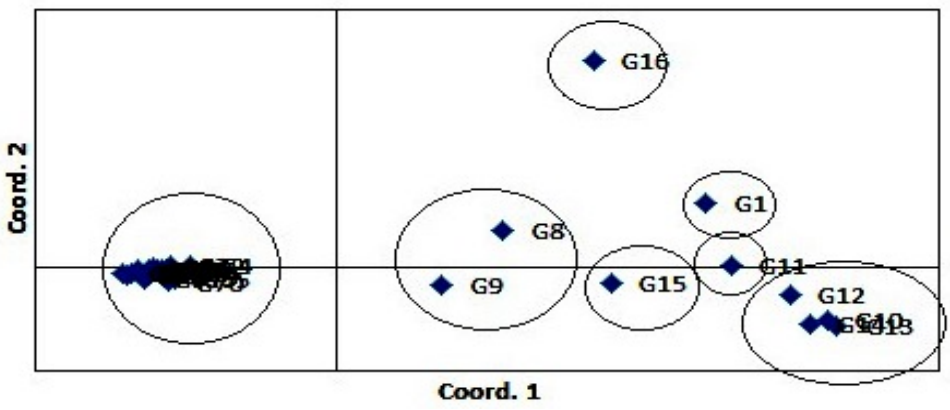

Figure 4. Principle Coordinate Analysis (PCo) ordination based on RAPD data

Comparison between RAPD and morphological data

Comparing matrices of RAPD and morphological data showed a weak correlation between dendrograms (r= 0.47, P=0.99) following 500 random permutations with the Mxcomp procedure from NTSYS program. Despite the weak correlation between morphological and molecular analysis, similar groups were placed at respective dendrograms. Formation of five clusters was consistently found in both analyses, however, some discrepancies could be found between two dendrograms. For example, Clementine mandarin (G15) was clearly separated in morphological analysis, however, it was grouped in subgroup D1 based on RAPD analysis. Another discrepancy concerning the Shelmohalleh (G9) that clustered into mandarin genotypes within subgroup C1 in morphological dendrogram, but it was clustered closely to orange genotype within cluster C based on molecular analysis. Similar results were

found in mandarins by Koehler-Santos *et al.* (18), who detected differences between dendrograms generated from morphological and SSR data, and suggested that morphological and molecular differences were apparently independent, due to different selection and evolutionary factors. The reasons for the non-correlation or weak correlation between morphological and molecular markers can be fallowing by these acts: 1- Low number of primers that are probably not cover as well genomic level and resulting in a weak correlation between the genes those controllers the molecular and morphological traits. 2- Morphological traits are affected by the environment or the effects of genotype and environment interaction or maybe have involved the effects of dominance, epistatic and pleiotropic or different allelic combinations may be lead to similar phenotypes and the resulting morphological differences that is not consistent with genetic differences. 3- Morphological characteristics are compared by RAPD sequences that may be have various levels of changes in evolutionary, as a nucleotide change that can alter RAPD phenotype but morphological trait may be preserve due to compatibility despite random mutations contingency. 4- Most of the genome of eukaryotic organisms are comprises non-coding regions that during evolution were exposed to mutation. RAPD sequences existed in the coding and non-coding sequences. Therefore many of the molecular markers are created in non-coding regions that have not linkage with coding genes (3).

Conclusion

Comparison of morphological and molecular characterization data is of immense importance to conclude the extent of genetic diversity present in the set of cultivars (20). Generally, the applied primers in present study showed high PIC that indicating polymorphism and high efficiency. Furthermore, the morphological data showed highly variation among the selected citrus genotypes. However, since morphological variation influences by environmental conditions, more accuracy will be achieved by application of molecular markers for grouping the genotypes. In both RAPD and morphological analysis, 29 genotypes were classified into five groups. however, some difference could be found between two dendrograms. Although the correlation between morphological and RAPD data is low, both techniques can be used complementarily in citus genetic diversity. This study represented the first attempt to use morphological trait with RAPD markers to study genetic diversity of Iranian citrus genotypes. Present study revealed that morphological and molecular markers could be successfully utilized for inferring genetic diversity and genetic relationship of citrus. Results derived from present study are useful for citrus breeding programs and enhancing citrus industry.

Acknowledgments

The help and cooperation received from Citrus Research Institute, I.R. Iran is fully acknowledged.

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مطالعه تنوع ژنتیکی و روابط بین ژنوتیپ‌های مرکبات ایرانی با استفاده از نشانگرهای مورفولوژیک و مولکولی

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چکیده:

داشتن اطلاعات در مورد روابط ژنتیکی در میان ارقام برای توسعه استراتژی‌های اصلاحی به منظور تولید ارقام اصلاح شده ضروری است. در این پژوهش، تنوع ژنتیکی و روابط خویشاوندی بین ۲۹ ژنوتیپ مرکبات با استفاده از نشانگر مورفولوژیکی و مولکولی RAPD مورد بررسی قرار گرفت. تنوع قابل ملاحظه‌ای در بین ژنوتیپ‌های مرکبات با استفاده از ۶۱ صفت کمی و کیفی بدست آمده از برگ، میوه و بذر مشاهد شد. همچنین نشانگر RAPD چند شکلی بالایی (۹۱/۸۲ درصد) را نشان داد. تشابه بین ژنوتیپ‌ها در محدوده بین ۰/۱۴ تا ۰/۹۷ با میانگین ۰/۶۲ بود. هم نشانگر مورفولوژیکی و هم نشانگر مولکولی تنوع بالایی را در بین ژنوتیپ‌های مورد مطالعه نشان دادند. در پژوهش حاضر، ژنوتیپ‌های نارنگی و پوملو به عنوان گونه‌های حقیقی مرکبات در خوشه‌های مجزا قرار گرفتند. نتایج پژوهش حاضر بیانگر آن است که هر دو نشانگر دارای پتانسیل بالقوه‌ای در تعیین تنوع و روابط ژنتیکی ژنوتیپ‌های مرکبات می‌باشند و می توانند در برنامه‌های اصلاحی مرکبات استفاده گردند.

کلمات کلیدی: مرکبات، تجزیه خوشه‌ای، تنوع ژنتیکی، نشانگر مولکولی.