Evaluation of Iranian Native Apple (*Malus* x *domestica* Borkh) Germplasm using Biochemical and Morphological Characteristics

Javad FARROKHI [1]
Reza DARVISHZADEH [2]
Hamid HATAMI MALEKI [3(✉)]
Lotfali NASERI [4]

Summary

In this study, fifty six native apple genotypes from different geographical regions of Iran were evaluated based on 16 different biochemical and morphological characters using augment design. Analysis of variance showed significant difference between studied genotypes. Considering descriptive statistics, there was high level of genetic variation in this plant material. Regarding simple correlation between studied characters, fruit weight as one of the most important item, was positively and significantly correlated with fruit volume, leaf size and canopy axile. Principle component analysis revealed that the first seven principle components (PCs) were accounted 74.7% of the total variation. Cluster analysis using Ward method classified the 56 genotypes into four groups. As regards to clustering pattern, distribution of the cultivars was independent from their geographical distribution. The present study shows that Iranian apple genotypes possess high level of genetic variation that is useful for breeding.

Key words

apple, genetic variability, cluster analysis, principal component analysis

[1] Department of Horticulture, Urmia University, Urmia, Iran
[2] Department of Agronomy and Plant Breeding, Urmia University, Urmia, Iran
[3] Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Maragheh, 55181-83111, Maragheh, Iran
✉ e-mail: hatamimaleki@maragheh.ac.ir
[4] Department of Horticulture, Urmia University, Urmia, Iran

Received: July 29, 2013 | Accepted: October 15, 2013

ACKNOWLEDGEMENTS
The authors would like to thank Kahriz Agricultural Stations (KAS) Personnel for their coordination to provide plant materials and Institute of Biotechnology, Urmia University, Iran, for financial support of this work. The authors thank Professor Larisa Gustavsson from Sveriges lantbruksuniversitet, Skåne, Sweden for critical reading and correcting the manuscript.

Introduction

Apple (*Malus domestica* Borkh.) is the fourth most important fruit crop worldwide, after citrus, *Vitis* and banana (FAOSTAT, 2008). It is the most ubiquitous and well-adapted of the temperate fruit crop species that is grown in areas ranging from high latitude regions of the world where temperature may reach –40°C, to high elevations in the tropics where two crops may be grown in a single year (Janick et al., 1996). The primary centers of *Malus* germplasm domestication were in Asia Minor, the Caucasus, Central Asia, Himalayan India and Pakistan, and Western China. The "Old Silk Road" from the Black Sea to western China had an important role in the evolution of cultivated apple (Juniper et al., 1999). According to historical and genetic documents, Iran has important role in introducing apple from East Asia to the ancient Rome and Greece (Gharghani et al., 2009). Iran is the fourth apple producer country in the world after China, USA and Poland. Cultivated apple is supposed to be the result of inter-specific hybridization. The denomination *Malus* × *domestica* has been generally accepted as appropriate scientific name for cultivated apple (Korban and Skirvin, 1984).

Estimating genetic diversity and determining the relationships among germplasm collections enhances efficiency of its management and genetic improvement (Geleta et al., 2005). Future of breeding programs depends on the availability of genetic variability to increase productivity. Morphological characterization of trees and fruits is the first and the most important step for the description, classification and characterization of germplasm collections. Trees are described by focusing on vegetative growth vigor, tree height, canopy width, crown diameter, leaf size, internodes length, and leaf chlorophyll content (Badenes et al., 2000). Apple fruits are characterized using maturity indices including firmness, sugar, starch, acid content, and ethylene concentration (Crisosto, 1994) as well as marketability indices (flesh and background color and fruit shape) used by consumers for differentiate cultivars (Arnon, 1949; Smith, 1971; Cripps et al., 1993).

Multivariate statistical techniques, which simultaneously analyze multiple measurements on each individual under investigation, are widely used in analysis of genetic diversity irrespective of whether it is morphological, biochemical, or molecular marker-based. Among the multivariate techniques, cluster analysis and Principal Component Analysis (PCA) are most commonly employed (Mohammadi and Prasanna, 2003). Multivariate analysis has been used frequently for genetic diversity analysis in many crops such as barley, *Hordeum vulgare* L. (Cross, 1992); Sorghum, *Sorgum bicolor* L. Moench, (Ayana and Bekele, 1999); wheat, *Triticum* spp (Hailu et al., 2006); Peanut, *Arachis hypogaea* L. (Upadhyaya et al., 2009); vineyard peach, *Prunus persica* L. Batsch, (Nikolic et al., 2010) and Apple, *Malus* sp. (Mratinić and Fotirić-Akšić, 2011). Considering the importance of apple in Iran, study of genetic diversity and information on relationships among native old varieties and new types would be desirable in order to allow for better management and preservation of genetic resources and their utilization within plant breeding programs. Until now, there were some reports about genetic diversity of Iranian apple germplasm based on DNA markers (Gharghani et al., 2009; Farrokhi et al., 2011; Naseri et al., 2011), but there was not any report about morphological classification of Iranian apple genotypes. Therefore, this study was conducted for evaluation of Iranian apple germplasm and classification of them using morphological characters.

Materials and methods

Plant material

Fifty six apple genotypes from all over of Iran were grafted on the seedling rootstocks, and planted at augmented design (Federer, 1956) in Kahriz Agricultural Station, northwest of Iran (44.58N, 37.4E). In the first and second blocks 18 genotypes and in the third one 17 genotypes had been planted. In each block three varieties including 'Golden Delicious', 'Red Delicious' and 'Gala' had been planted as control varieties. The trees were ten-years old with five meters distances in a rectangle planting design. Cultivars were managed in according to standard apple orchard management. The trees training system was central leader.

Characters measurements

Ten fruits per genotype were hand harvested randomly at commercial maturity stage. After harvesting, the samples were immediately transferred to the cold room. Fifteen mature leaves

Table 1. Characteristics of apple genotypes, abbreviated names, scale and method used for their measurement

Character	Abbr. name	Scale	Tool or method
Fruit weight	FW	gr	Digital balance model
Fruit volume	FV	cm^3	Submerging fruit in a scaled cylinder
Fruit length	FL	mm	Digital caliper
Fruit diameters	FD	mm	Digital caliper
Fruit firmness	FF	kg cm^{-2}	Skin was removed and firmness measured by using a Magness-Taylor pressure tester equipped with an 8 mm probe for small fruits and 11 mm for big ones
Number of Days from Full Bloom to Ripening	DAFBR	day	
Organic acid content	OA	g·100 ml^{-1}	Method described by Saini et al. (2001)
Total soluble solids	TSS	°Brix	Refractometer
Vitamin C	Vit-C	mg 100 ml^{-1}	Method described by Saini et al. (2001)
pH	pH	-	pH meter
Chlorophyll index	CI	-	Chlorophyll meter
Leaf size	LS	-	Leaf area index meter
Canopy axils	CA	-	1= close, 2= semi open, 3=open
Crown diameter	CD	mm	Digital caliper
Tree height	TH	mm	Tape measure
Internodes length	IL	mm	Tape measure

were picked from the mid part of the shoots in June and late July. Overall sixteen biochemical and morphological characters were measured as described in Table 1.

Statistical analysis

Descriptive statistics (mean, standard error, standard deviation, and coefficient of variation) for each of 16 studied characters were calculated. Relationships between all characters were calculated by Pearson correlation coefficients. In order to identify the patterns of morphological variations and also importance of characters in each component, Principal Component Analysis (PCA) was conducted through correlation matrix. Clustering of genotypes into similarity groups was performed using Ward Method based on squared Euclidean distances. Prior to squared Euclidean distance calculation, the data were standardized. These analyses were processed using SPSS 13.0 statistical software.

Results and discussion

Descriptive analysis

Univariate analysis of variance depicted significant difference between studied genotypes (data not shown). Some descriptive statistics such as minimum, maximum, mean, standard deviation and coefficient of variation for each of the 16 studied characters are shown in Table 2. Among the studied characters, the highest coefficients of variation were corresponded to fruit weight, crown diameter and organic acid content of fruit with values of 87.5, 41.97 and 37.5%, respectively (Table 2). Descriptive statistics analysis revealed high genetic variability among studied apple genotypes for the studied characters. This broad genetic variability is the basis for applied crop breeding that allows for selection of superior genotypes. Descriptive statistics analysis was also used for studying genetic variability in some other horticultural crops such as Iranian almond genotypes, *Prunus spp.* (Nikoumanesh et al., 2011) and apple, *Malus* × *domestica* Borkh. (Costa et al., 2011). The color of fresh fruit of all studied genotypes was white except for 'Meshkie Germez' and 'Shahrood 6' genotypes that were in red color under the skin and flesh similar to some foreign cultivars such as 'Boskoop' and 'Cripps Red'. In this sense, 'Shahrood 6' was more discrete than other 'Shahrood' series (Figure 1). Naseri et al. (2011) using SSR fingerprinting also proved that 'Shahrood 6' grouped together with 'Malling2' apart from other 'Shahrood' series.

Figure 1. Perfect fruit and fruit in longitude section of 'Shahrood 6' genotype

Relationships between characters

Most important characters of the majority plants are quantitative characters that are largely influenced by the environment and hence have a low heritability. Therefore, the response to direct selection for these characters may be unpredictable unless there is good control of environmental variation (Hatami Maleki et al., 2011). Commonly, plant breeders prefer to select for related characters that indirectly increase target character. Hence, simple correlations among 16 biochemical and morphological characters were calculated and presented in Table 3. Considering correlation results, fruit weight was positively and significantly correlated with fruit volume (r=0.47), leaf size (r= 0.41) and canopy axile (r= 0.32). In open canopy trees light could penetrate well into the canopy causing increase of photosynthesis rate and transfer of carbohydrates from the leaves to the fruits. Of course, leaf characteristics play an important role in efficiency of influenced light. Avery (1969) also showed the importance of transferred carbohydrates to fruits. In directed studies with $^{14}CO_2$ in apple it was confirmed that the fruits are

Table 2. Biochemical and morphological characteristics of the 56 apple genotypes

Character	Mean	Std. Deviation	CV%	Maximum	Minimum
FW	0.08	0.07	87.5	0.95	0.01
FV	71.93	14.53	20.2	221.00	19.00
FL	49.55	4.82	9.86	85.00	30.00
FD	52.31	6.08	11.6	95.00	4.00
FF	8.35	1.4	16.86	17.10	3.20
DAFB	133.55	3.5	2.66	179.00	127.00
OA	0.20	0.074	37.05	0.98	0.02
TSS	12.20	0.741	6.07	18.00	9.00
Vit-c	9.84	1.93	19.58	27.70	1.40
$_{p}$H	4.34	0.15	3.41	5.21	3.43
CI	39.18	7.26	18.53	94.60	11.10
LS	4589.50	969.15	21.11	13044.00	1023.00
IL	25.93	4.82	18.58	47.50	2.10
CA	1.87	0.44	23.78	3.00	1.00
CD	1560.80	654.9	41.97	5225.00	125.00
TH	2631.10	407.7	15.5	5843.00	1380.00

For character names see Table 1.

Table 3. Correlation matrix of studied characteristics in 56 apple genotypes

Character	FW	FV	FL	FD	FF	DAFB	OA	TSS	Vit-C	pH	CI	LS	IL	CA	CD
FV	0.47*														
FL	0.17	0.67*													
FD	0.21	0.56*	0.66*												
FF	0.09	-0.06	-0.07	-0.04											
DAFB	-0.09	-0.17	0.10	-0.11	0.20										
OA	0.23	-0.15	-0.17	-0.12	0.13	0.22									
TSS	-0.22	-0.07	0.04	-0.03	0.54*	0.28	-0.10								
Vit-C	-0.08	0.00	-0.09	-0.03	0.33*	-0.42*	-0.06	0.27*							
pH	-0.31*	0.04	0.01	0.01	0.05	-0.09	-0.21	0.29*	0.38*						
CI	-0.11	-0.05	0.10	0.03	-0.12	0.21	-0.17	0.00	-0.21	0.24					
LS	0.41*	0.57*	0.19	0.30*	0.19	-0.06	0.13	0.01	0.04	-0.02	-0.13				
IL	0.05	0.26	0.26	0.26*	0.04	-0.04	-0.10	-0.23	-0.09	-0.19	0.09	0.14			
CA	0.32*	0.12	0.08	-0.04	0.02	0.13	0.30*	-0.15	-0.19	-0.26	0.07	0.24	0.02		
CD	-0.02	-0.02	0.18	0.09	0.25	0.01	-0.09	0.26	-0.10	0.01	0.11	-0.19	0.13	0.25	
TH	-0.13	0.07	0.01	0.15	-0.19	-0.13	0.01	-0.42*	-0.07	-0.07	-0.07	0.16	0.41*	-0.07	-0.18

For character names see Table 1.

as strong sink in assimilation of photosynthetic products from leaves (Hansen, 1980). In this study fruit volume was positively correlated with fruit length (r=0.67) and fruit diameter (r=0.56). Plant height was correlated positively with internodes length (r=0.41). Number of days from blooming to ripening was correlated with total soluble solids (r=0.54) and vitamin C content (r= 0.33). Similarly, Agric (2004) reported that apple harvest date was significantly correlated with fruit vitamin C levels and this correlation remained consistent across different years. In peach, significant correlation was also found between ripening time and soluble solids content (Nikolic et al., 2010). Total soluble solids are an index for photosynthetic products such as soluble sugars. It is an important biochemical factor that its level is increased simultaneously with fruit development. The proportion of total soluble solids/organic acid could determine the final taste of fruits. Results revealed the existence of negative relation between fruit weight and acidity (r=-0.32).

Principal Component Analysis (PCA)

PCA revealed the first seven principle components (PCs) with eigen values greater than value 1, accounted for 74.7% of the total variation (Table 4). The first component (PC1), explaining 17.7% of the total variation, was negatively associated with fruit weight, fruit volume, fruit length, fruit diameter, organic acid content, leaf size, internodes length, and tree height. Hence, genotypes with high values of PC1 have lower fruit quality and appearance as well as smaller tree size (Table 4). Generally, PC1 corresponds to variables correlated with fruit marketability and tree growth vigor. The second component (PC2) accounting for 12.8% of the total variation was negatively correlated with fruit length, fruit

Table 4. Eigenvalues, proportion of total variability, eigenvector and correlation between the original variables and the first seven principal components (PCs) for 56 apple genotypes

Item	PC axis						
	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalue	2.8253	2.0412	1.807	1.634	1.4459	1.1942	1.0044
Proportion	0.177	0.128	0.113	0.102	0.09	0.075	0.063
Cumulative	0.177	0.304	0.417	0.519	0.61	0.684	0.747
	Eigenvector						
Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
FW	-0.342*	-0.175	-0.215*	-0.274*	-0.215	-0.162	-0.057
FV	-0.439*	-0.145	0.063	-0.107	0.206	-0.1	0.252
FL	-0.387*	-0.225*	0.294*	0.252*	-0.066	0.032	0.232
FD	-0.429*	-0.136	0.249*	0.125	0.082	0.064	0.103
FF	0.127	-0.464*	-0.105	-0.117	0.303*	0.271*	-0.087
DAFB	0.182	-0.37*	-0.082	0.359*	-0.005	-0.211	-0.085
OA	-0.237*	-0.182	0.078	0.007	-0.444*	0.218	-0.399*
TSS	0.228*	-0.436*	0.264*	-0.102	0.250*	0.197	0.066
Vit-C	-0.152	0.276*	0.319*	-0.423*	-0.091	0.16	-0.031
pH	0.075	0.003	0.489*	-0.109	0.136	0.226	-0.449*
CI	0.058	-0.034	0.232*	0.482*	-0.21	-0.036	-0.223
LS	-0.189*	-0.234*	-0.35*	-0.268*	0.222*	-0.051	-0.37
IL	-0.30*	0.064	-0.156	0.331*	0.296*	-0.033	-0.117*
CA	-0.027	-0.155	-0.335*	0.049	-0.462*	0.473*	0.039
CD	-0.04	0.199*	-0.174	0.178	0.203	0.663*	0.275
TH	-0.199*	0.323*	-0.152	0.208*	0.293*	0.096	-0.458*

For character names see Table 1.

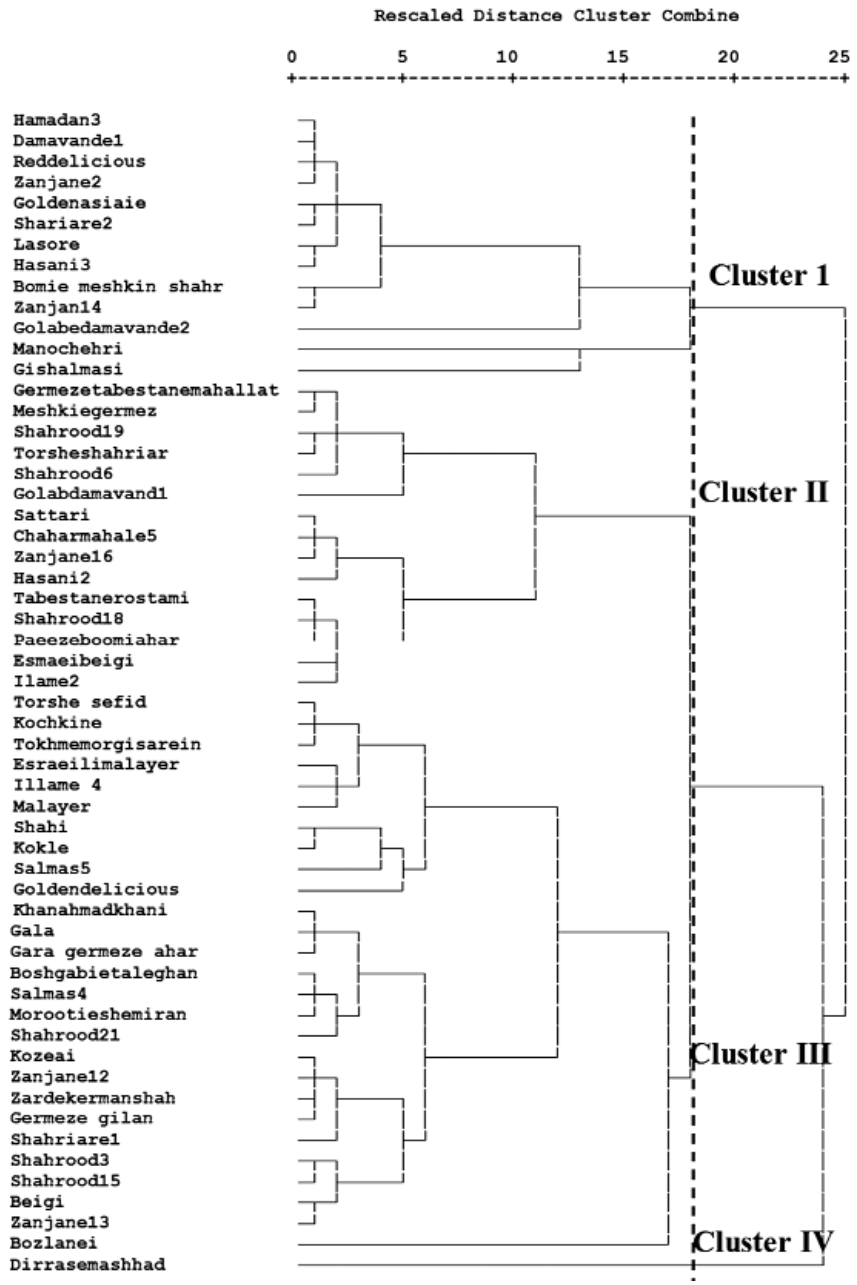

Figure 2. Dendogram for the 56 apple genotypes collected from different regions of Iran produced by Ward's clusters analysis; based on 16 morphological characters (scale. squared Euclidean distance)

firmness, total soluble solids, number of days from full blooming to ripening and leaf size (Table 4). It had also positive correlation with tree height (Table 4). According to PC2, the majority of variables associated with fruit ripening were in opposition to vegetative variables of tree. It supports the existence of negative relationships between vegetative and reproductive growth in fruit crops (Forsley, 1986; Barden et al., 1989). Moreover, PC2 manifested that early ripening cultivars were characterized with small leaf and small fruit size with low acceptable marketing factors. Third component (PC3) was correlated with fruit quality characters such as vitamin C, and total soluble solids (Table 4). Moreover, PC3 had significant relation with pH, fruit length, and fruit diameter. The third component suggested that fruit shape and fruit biochemical characters could be located in one index. Similarly, in apricot, Mratinic et al. (2011) found that the fruit shape and yield as well as vitamin C and total soluble solids content could be positioned in the same index. The fourth up to seventh component explained 10.2%, 9%, 7.5%, and 6.3% of the total variation, respectively (Table 4). In the study on Serbian apple cultivars (Mratinić and Fotirić-Akšić, 2011), it was shown that the first three components explained more than 65% of the total trait variation. Mratinić and Fotirić-Akšić (2011) reported that the first component was negatively and strongly associated with ripening time and period from blooming time until the harvest time, but positively and strongly associated with flowering phases and mineral content. Also, Mratinić and Fotirić-Akšić (2011) reported that the second component was positively and strongly associated with flowering phases, but negatively with yield, meanwhile, the third component explained chemical properties such as titratable acidity, total sugars content, and soluble solids content. In their studuies (Mratinić and Fotirić-Akšić, 2011) character such as fruit weight, an important yield component, was not useful in distinguishing accessions. Generally, this research supported that principal component analysis (PCA) is a useful tool for identification of the most significant variables in the biochemical and morphological data set of apple. PCA previously has been used for germplasm evaluation in several different fruit species such as pomegranate (Mars and Marrakchi, 1999), loquat (Badenes et al., 2000), olive (Rotondi et al., 2003; Cantini et al., 1999), almond (Nikoumanesh et al., 2011), and apple (Mratinić and Fotirić-Akšić, 2011).

Cluster analysis

Hierarchical cluster analysis could be properly utilized in assessing the similarity or dissimilarity among individuals and for clarification of relationships among them (Costa et al., 2011). According to dendrogram generated by Ward methods based on squared Euclidean distance, the 56 studied apple genotypes were classified into four separate groups (Figure 2). The large number of genotypes were placed in cluster III (27 genotypes) followed by cluster II (15 genotypes), cluster I (13 genotypes) and cluster IV (1 genotype). This showed high level of morpho-

logical variation among Iranian apple genotypes. The existence of genetic diversity among Iranian apple germplasm has supported by several reports (Gharghani et al., 2009; Farrokhi et al., 2011; Naseri et al., 2011).

In the study of Serbian native apple cultivars (Mratinić and Fotirić-Akšić, 2012), the analysis of variance revealed significant differences among the accessions and among years for some characters as well. Mratinić and Fotirić-Akšić (2012) classified studied Serbian *Malus* sp. accessions into three distinct groups using cluster analysis. In this study, there was not any relationship between clustering pattern and geographical distribution. One of its reason can be related to synonymes, homonymes and misnymes; For example in cluster I both genotypes ('Red Delicious' and 'Damavande1') mainly are the same, but during the time the orchrders in central part of Iran have changed its name to a new one ('Damavande1'). In previous study, it have been showed that two genotypes ('Dirrasemashhad') and ('Salmas4') have the highest genetic similarity with each other and were classified within the same group (Farrokhi et al., 2011), but herein 'Dirrasemashhad' is located in group IV far away from genotype 'Salmas4'.

Conclusions

To sum up, apple genotypes from Iran possess a considerable diversity based on biochemical and morphological characters that is useful for germplasm management and for utilization in breeding programs. Considering simple correlations, most of apple characters had not any significant relationships with each other. Principal component analysis (PCA) is a useful tool for identification of the most significant variables among several characters and both biochemical and morphological characters could be combined in the same indices. By means of heirarchial cluster analysis, studied germplasm was classified in four distinguished groups. The information of this research could be very useful for determination of the most different genotypes to be used as parents for mapping populations as well as in hybrid breeding programs.

References

Agric J. (2004). Relation between harvest date and Vitamin-C. Food Chem 52: 8031-8038.

Arnon D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1-15.

Avery D. J. (1969). Comparisons of fruiting and deblossomed maiden apple trees, and of non-fruiting trees on a dwarfing and an invigorating rootstock. New Phytol 68: 323-336.

Ayana A., Bekele E. (1999). Multivariate analysis of morphological variation in sorghum (*Sorghum bicolor* L. Moench) germplasm from Ethiopia and Eritrea. Genet Resour Crop Ev 46: 273-284.

Badenes M. L., Martinez-Calvo J., Llacer G. (2000). Analysis of a germplasm collection of loquat (*Eriobotrya japonica* Lindl). Euphytica 114: 187-194.

Barden J. A., Delvalle T. B. G., Myers S. C. (1989). Growth and fruiting of delicious apple trees as affected by severity and season of pruning. J Am Soc Hort Sci 114: 184-186.

Cantini C., Cimato A., Sani G. (1999). Morphological evaluation of olive germplasm present in Tuscany region. Euphytica 109: 173-181.

Costa F., Apellina L., Longhia S., Guerrac W., Magnagoa P., Porrob D., Soukoulisa C., Salvia S., Velascoa R., Biasiolia F., Gasperia F. (2011). Assessment of apple (*Malus* × *domestica* Borkh.) fruit texture by a combined acoustic-mechanical profiling strategy. Postharvest Biol Tec 61: 21-28.

Cripps J. E. L., Richard L. A., Mairata A. M. (1993). 'Pink Lady' Apple. HortScience 28: 1057-1060.

Crisosto C. H. (1994). Stone fruit maturity indices: a descriptive review. Postharvest News Inf 5: 65-68.

Cross R. J. (1992). A proposed revision of the IBPGR barley descriptor list. Theor Appl Genet 84: 501-507.

FAOSTAT (2008). Food and Agriculture Organization of the United Nations. Available at: http://faostat. Fao.Org/336.default.Asp. (Accessed 20 May 2012)

Farrokhi J., Darvishzadeh R., Naseri L., Azar M. M., Hatami Maleki H. (2011). Evaluation of genetic diversity among Iranian apple (*Malus* × *domestica Borkh*.) cultivars and landraces using simple sequence repeat markers. Aust J Crop Sci 5: 815-821.

Federer W. T. (1956). Augmented (or hoonuiaku) designs. Hawaiian Planters 2:191-208.

Forsley C. G. (1986). Training and pruning apple trees. Available at. http.//eap.mcgill.ca/CPTFP_7.htm. (Accessed 27 July 2012)

Geleta L. F., Labuschagne M. T., Viljoen C. D. (2005). Genetic variability in pepper (*Capsicum annuum* L.) estimated by morphological data and amplified fragment length polymorphism markers. Biodivers Conserv 14: 2361-2375.

Gharghani A., Zamani Z., Talaie A., Oraguzie N. C., Fatahi R., Hajnajari H., Wiedow C., Gardiner S. E. (2009). Genetic identity and relationships of Iranian apple (*Malus* × *domestica* Borkh.) cultivars and landraces, wild *Malus* species and representative old apple cultivars based on simple sequence repeats (SSR) marker analysis. Genet Resour Crop Ev 56: 829-842.

Hailu F., Merker A., Singh H., Belay G., Johansson E. (2006). Multivariate analysis of diversity of tetraploid wheat germplasm from Ethiopia. Genet Resour Crop Ev 54: 83-97.

Hansen P. (1980). Yield components and fruit development in 'Golden Delicious' apples as affected by the timing of nitrogen supply. Scientia Horticulturae 12: 243-257.

Hatami Maleki H., Karimzadeh G., Darvishzadeh R., Sarrafi A. (2011). Correlation and sequential path analysis of some agronomic traits in tobacco (*Nicotiana tabaccum* L.) to improve dry leaf yield. Aust J Crop Sci 5: 1644-1648.

Janick J., Cummins J. N., Brown S. K., Hemmat M. (1996). Apples. In: Janick J, and Moore JN (eds) Fruit Breeding, vol 1: Tree and Tropical Fruits. John Wiley & Sons, New York, USA.

Juniper B. E., Watkins R., Harris S. A. (1999). The origin of the apple. Acta Horticulturae 484: 27–33.

Korban S. S., Skrivin R. M. (1984). Nomenclature of the cultivated apple. Hortic Sci 19: 177-180.

Mars M., Marrakchi M. (1999). Diversity of pomegranate (*Punica granatum* L.) germplasm in Tunisia. Genet Resour Crop Ev 46: 461–467.

Mohammadi S. A., Prassana B. M. (2003). Analysis of genetic diversity in crop plants-salient statistical tools and considerations. Crop Sci 43: 1235-1248.

Mratinić E., Fotirić-Akšić M. (2011). Evaluation of phenotypic diversity of apple (*Malus* sp.) germplasm through the principle component analysis. Genetika 43: 331-340.

Mratinić E., Fotirić-Akšić M. (2012). Phenotypic diversity of apple (*Malus* sp.) germplasm in south Serbia. Braz Arch Biol Technol 55: 349-358.

Mratinic E., Popovski B., Milosevic T., Popovski M. (2011). Evaluation of Apricot Fruit Quality and Correlations Between Physical and Chemical Attributes. Czech J Food Sci 2: 161–170.

Naseri L., Darvishzadeh R., Azar M. M., Alizadeh A. (2011). Molecular characterization and similarity relationships among some Iranian native and commercial apple (*Malus* × *domestica* Borkh.) cultivars using simple sequence repeat markers. J Hortic Sci Biotechnol 86: 527-533.

Nikolic D., Rakonjac V., Milatovic V., Fotiric M. (2010). Multivariate analysis of vineyard peach [*Prunus persica* (L.) Batsch.] germplasm collection. Euphytica 171: 227-234.

Nikoumanesh K., Ebadi A., Zeinalabedini M., Gogorcena Y. (2011). Morphological and molecular variability in some Iranian almond genotypes and related *Prunus* species and their potentials for rootstock breeding. Scientia Horticulturae 129: 108-118.

Rotondi A., Magli M., Ricciolini C., Baldoni L. (2003). Morphological and molecular analyses for the characterization of a group of Italian olive cultivars. Euphytica 132: 129-137.

Saini R. S. (2005). Laboratory Manual of Analytical Techniques in Horticulture. Agrobios, Jodhpur. India.

Smith M. W. G. (1971). National Apple Register of the United Kingdom. Ministry of Agriculture, Fisheries and Food, London, UK.

Upadhyaya H. D., Reddy L. J., Dwivedi S. L., Gowda C. L. L., Singh S. (2009). Phenotypic diversity in cold-tolerant peanut (*Arachis hypogaea* L.) germplasm. Euphytica 165: 279-291.

---

acs78_54