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PRELIMINARY EVALUATION OF SOME ELICITED APPLE GENOTYPES CANDIDATE AS CULTIVARS USING MORPHOLOGICAL AND MOLECULAR MARKERS

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Abstract

Preliminary evaluation of 5 apple genotypes produced by seed selection in the germplasm of seed rootstocks in Sweida- Syria was carried out between 2005 and 2012, to study date of full flowering, maturity time, morphological characters of tree, shoots, leaves, flowers, and fruits, including chemical analysis. On the other hand, yield efficiency, fruit storability and the susceptibility to pests and physiological disorders were evaluated. Molecular characterization was achieved by using 8 pairs of SSR primers for the 5th genotypes in comparison with the two main commercial cultivars "Golden Delicious" and "Starking Delicious" to determine the genetic relationship between the studied genotypes and cultivars. The results divided the studied genotypes depending on the time of ripening into three groups: early, intermediate and late genotypes. All studied genotypes gave efficient and regular yield, and showed fixable fruit shape and size with variable storability due to maturity time. Molecular characterization reveled the ability of SSR technique to determine the genetic variation between the studied genotypes, the 8 primer pairs produced 22 alleles, 21 of them were polymorphic (95.45%). Cluster analysis divided the studied genotypes into 2 clusters, the first cluster included the two commercial cultivars Starking delicious and Golden delicious with one genotypes, while the second cluster contained the four remaining genotypes. Finally, the studied genotypes revealed high desired quantitative and qualitative traits, which assess to distribute these genotypes as new cultivars, and SSR technique was able to identify and revealed the genetic distance between the studied genotypes and commercial cultivars.
Key words: Apple, evaluation, Morphological characterization, SSR

Introduction

Apples are the second deciduous fruit crop in importance after grapes in Syria as in the world. The present production in the world and Syria are 76378738 and 334166 mt, respectively, which play an important role in Syrian commodities balance (FAO, 2012). Apple trees are widely distributed in Syria from the Southern to the coastal region. Many commercial cultivars were introduced, however farmers and consumers need more new cultivars which have desired commercial and agronomic traits (Janick et al., 1996). Producing high levels of yield under sustainable systems requires more achievements to be made through breeding programs (Wissuwa et al., 2009). Many breeding programs firstly depended on seeds planting and evaluation by fruit growers (Morgan and Richards, 1993), then followed by the hybridization between known parentages such as Stark rimson with Fujy (Guo and Zhang ,1993) or Golden delicious (Goffreda et al., 1995). Recently, local and selected apple cultivars which have useful traits have become the main part of breeding programs (Janick, 2000; El-Halabi et al., 2009). Usually, the identification of cultivars and hybrids depending on morphological characters, But numbers of these characters are limited, unstable and cannot always discriminate between closely related cultivars (Farrokhi et al.,2011). The emergence of PCR-based molecular markers such as simple sequence repeats (SSRs) give the opportunity of accurate genetic characterization of collections (Agarwal et al., 2008). However, SSR molecular technique widely used for characterization and genetic relationship among apple cultivars (Oliveira,2001; Muzher et al.,2007; Farrokhi et al., 2011; Király et al.,2012), and showed high competence to reveal the genetic variance between parents and their progenies (Coart et al., 2003), and to determine and document the parents of elicited cultivars (Kitahara et al., 2005).

According to the wide genetic base of seedling apple genotypes which produced by open pollination, and the necessity to produce new cultivars carry high desired qualitative and quantitative traits, the present investigation aimed to identify and evaluate these genotypes, and to study their genetic relationship with the main planted commercial cultivars in Syria Golden delicious and Starking delicious using SSR technique as a step to credence these new cultivars.

Material and Methods

The study was carried out between 2005 and 2012, at the Agricultural Scientific research center in Sweida province at the south of Syria, which is located at 1525 m altitude and the mean rainfall about 525 mm.

Plant Material

Five apple genotypes 15 years old produced by open pollination were selected through apple breeding program and named (G1, G2, G3, G4 and G5 genotypes), in addition to two commercial apple cultivars Golden Delicious and Starking Delicious for molecular characterization.

Morphological characterization and preliminary evaluation

All morphological and parametric traits were achieved according to IPGRI descriptor (IPGRI, 1997) with some modifications:

Date of full flowering and fruit set.

Morphological Traits of: tree, shoots, leaves, flowers and fruits.

Maturity time

Yield efficiency (K g/ tree)

Fruit storability: we used cold storage to evaluate the storability of studied genotypes.

Eating quality (dessert): A combined assessment of flavor, sweetness, juicy and acidity at optimum eating time

Fruit firmness: Recorded in K g c m^{-2}, on which is just ripe, using penetrometer with an 8 mm probe (IPGRI, 1997).

Chemical analysis for fruits which includes (Total soluble solids, total sugar content, pH and tetratable acidity)

Physiological disorders: evaluation of fruit susceptibility to bitter pit and water core in field.

Pests and diseases susceptibility: Based on a 1-9 scale of general field susceptibility (IPGRI, 1997). Pests: Wooly apple aphid, codling moth and rosy apple aphid. Fungi: Powdery mildew, apple scab and canker.

Molecular characterization

DNA extraction was achieved using CTAB protocol according to (Porebski et al., 1997) from the five genotypes and the two commercial cultivars Golden delicious (G.D) and Starking delicious (S.D). Estimation of the DNA concentration in all samples was done by using biophotometer plus (Eppendorf, Germany).

A set of 8 SSR primer-pairs (Table 1) derived from apple genome developed by (Silverberg-Dilworth et al., 2006) were used. 25 µ l of PCR reaction were added to 0.2 m l PCR tube as following: PCR amplification were performed in a 25 µ l vol containing 25 n g of genomic DNA, 1X PCR buffer, 2 m M of each dNTP, 1.5 m M $MgCl_2$, 2.5 µ l each of forward and reverse primers and 1 U of *Taq* polymerase. All SSR amplification will be performed in a Mastercycler gradient PCR, under the following conditions: an initial denaturation at 94ºC for 4 min, then 20 cycles at 94ºC for 1 min, annealing temperatures according to each primer for 1 min, 72ºC for 1 min, the annealing temperature will be reduced by 0.5ºC per cycle; the initial cycles will be followed by 20 cycles of 94ºC for 30s, annealing temperatures according to each primer for 1 min, 72ºC for 1 min and final 5 min 72ºC extension.

Table 1: SSR name, forward primer, reverse primer, allele size and No. Alleles

SSR name	Forward primer	Reverse primer	Allele size	No. Alleles
Hio4a05	GGCAGCAGGGATGTATTCTG	GTTTCATGTCAAATCCGATCATCAC	194-222	8
Hi04g05	CTGAAACAGGAAACCAATGC	GTTTCGTAGAAGCATCGTTGCAG	190-258	9
Hi07h02	CAAATTGGCAACTGGGTCTG	GTTTAGGTGGAGGTGAAGGGATG	246-276	10
Hi08c05	TCATATAGCCGACCCCACTTAG	GTTTCACACTCCAAGATTGCATACG	230-240	3
Hi08d09	AACGGCTTCTTGTCAACACC	GTTTACTGCATCCCTTACCACCAC	183-186	2
Hi08e06	GCAATGGCGTTCTAGGATTC	GTTTGGCTGCTTGGAGATGTG	134-138	4
Hi09a01	GAAGCAACCACCAGAAGAGC	GTTTCCCATTCGCTGGTACTTGAG	183-192	7
Hi12a02	GCAAGTCGTAGGGTGAAGCTC	GTTTAGTATGTTCCCTCGGTGACG	249-255	2

Data analysis

The experiment was designed in completely randomized design (CRD), data for parametric traits, fruit storability and chemical analysis (TS, TSS, TA, pH) were analyzed using analysis of variance (ANOVA) to compare genotypes means, mean comparison was achieved using LSD test ($p < 0.05$). Concerning genetic studies; genetic similarity (GS) was estimated according to Jaccard coefficient (Jaccard, 1908) and Cluster analysis using UPGMA method (unweighted pair-group method using arithmetic averages).

Results

Morphological characterization and Evaluation

Date of full flowering

The date of full flowering was the same for all studied genotypes at the end of April except Genotype G1 which was at the mid of April, while the fruit set was at the beginning of May.

Morphological traits

Tree: Growth habit ranged between upright to spreading (Table 2), shoots were vigor in all studied genotypes, genotype G5 revealed the lowest shoot length which was 46.8c m, while genotype G1 significantly revealed the highest shoot length and shoot diameter (75 c m and 7.4 m m, respectively) in comparison with all studied genotypes, followed by G3, G2, G4 and G5 for shoot length and G3,G4,G2, and G5 for shoot diameter. Shoot color was dark brown in genotype G3, while it was brown reddish for all other genotypes (Table 2).

Table 2: Tree habit, shoot length, shoot diameter, shoot color, leaves shape, leaves area, length and width of leaves.

genotype	Tree habit	Shoot length* (c m)	Shoot diameter (m m)	Shoot color	Leaves shape	Leaves margin	Leaves area	Leaves length (c m)	Leaves width (c m)
G1	Upright	75a	7.4a	brown reddish	Ovate	Dentate	Large	8.1b	5c
G2	Upright	67.8c	6.2c	brown reddish	Oval	Doubly dentate	Large	8.3a	5.5a
G3	Spreading	72b	7b	dark brown	Ovate	Serrate	Moderate	7.2d	5.2b
G4	Spreading	50.2d	6.4c	brown reddish	Ovate	serrate	large	8.1b	5c
G5	Upright	46.8e	5.6d	brown reddish	Oval	Doubly dentate	Moderate	7.5c	4.9c
L. S. D 5%		0.86	0.23					0.18	0.18

* 25 c m> weak, 25-45 c m intermediate, 45 c m< vigor.

Leaves: Genotype G2 significantly showed the highest leaf length and width (8.3 and 5.5 c m, respectively) of all other genotypes, while G3 revealed the lowest leaf length (7.2 c m) and G5 revealed the lowest leaf width (4.9 c m). On the other hand, the leaves shape was ovate in genotypes G1,G3 and G4, while it was oval in genotypes G2 and G5. Likewise, the leaf margin was serrate in genotypes G3 and G4, doubly dentate in genotypes G2 and G5, and dentate in G1 (Table 2).

Flowers: The color of petals was white with pinkish color of vines in all studied genotypes, the size of petals was intermediate except genotype G3 which was large.

Fruits: Data showed that the fruit shape was globose-conical in genotypes G2, G3 and G5, short – globose conical in genotype G1 and conical in G4 (Table 3). Fruit color was between light yellow in genotype G2 and dark red on green yellow ground in genotype G1. All genotypes revealed different fruit weight which was the highest (177.4 g) in genotype G3 and the lowest in genotype G2 (96.2 g) which was in significant with G3, G5 and G4. Genotype G3 and G4 were significantly higher in fruit length (7.2 and 7 c m, respectively) and diameter (7.3 and 7 m m, respectively) than G2 fruits length and diameter (5.7 and 6 c m, respectively) and in fruit length with G1 (6 c m). Fruit stalks were short in genotypes G1, G2, G3 and G4, and moderate in genotype G5 which was in significant with G2 (23 and 13.5 m m, respectively).

Table 3: Fruit shape, fruit color, fruit weight (g), fruit length and diameter, fruit stalk length, and maturity time.

genotype	Fruit shape	Fruit color	Fruit weight (g)	Length (c m)	Diameter (c m)	Fruit stalk length (m m)	Maturity time*
G1	Short-globose-conical	Dark red on green yellow ground	136.4ab	6b	7a	19.3ab	early
G2	Globose-conical	Light yellow	96.2a	5.7b	6b	13.5b	mid
G3	Oblong-conical	Mottled Pink on yellow ground	177.4a	7.2a	7.3a	21ab	mid
G4	Conical	Green-yellow slightly blushed	156.3a	7a	7a	21.7ab	late
G5	Globose-conical	Pink with striped dark pink on yellow ground	169.3a	6.4ab	7.2a	23a	late
L SD5%			50	0.9	0.9	8.2	

* maturity time: early genotype: mature in August, mid genotype: mature in the first half of September and late genotype: mature at The end of September and beginning of October

Maturity time there was a very clear difference in maturity time between genotypes (Table 3). They divided into three groups, early, mid and late. The genotype G1 matured early in mid August, and genotypes G2 and G3 matured at the first half of September, while genotypes G4 and G5 matured at the end of September and beginning of October.

- Yield efficiency: All studied genotypes showed high and regular yield except genotype G4 which characterized by alternate bearing and yield (60-70 K g/tree), while it was 70-80 K g/tree in genotypes G1, G3 and G5, and 90- 100 K g/tree in genotype G2.

Fruit storability: There were clear differences between genotypes for fruit storability, it was 4 months for genotype G1, 6 months for genotypes G2 and G3, while it was more than 8 months for genotypes G4 and G5. These results insured the relationship between maturity time and fruit storability.

Eating quality: All studied genotypes showed good flavor and juicy except G1 which was intermediate to good. The genotypes G4 and G5 revealed sweetness taste, while the other genotypes showed sour to sweet taste.

Fruit firmness: The genotype G2 revealed the highest firmness (4.6 K g . c m^{-2}) which considered as very firm according to (IPGRI,1997), followed by G4 (3.7 K g . c m^{-2}), while G1 revealed the least firmness (3.2 K g . c m^{-2}) and grouped with the other two genotypes in intermediate group (Table 4).

Table 4: Fruit firmness, Total soluble solids, Total sugar, Tetratable acidity, and pH

genotype	Fruit firmness K g. c m^{-2}	Total soluble solids (%)	Total sugar (g%)	Tetratable acidity (%)	pH
G1	3.2	15.6	12	0.5 a	3.4
G2	4.6	16	13.5	0.45a	3.65
G3	3.25	17.4	13	0.23b	3.5
G4	3.7	14.6	12.1	0.24b	4.7
G5	3.4	16	14.1	0.25b	4

L.S.D 5% for tetratable acidity: 0.18

Chemical analysis: (Table 4) showed that the highest percentage of total sugar was in genotype G5 (14.1%), while the genotypes G1 and G2 were significantly revealed higher percentage of tetratable acidity (0.5, 0.45% respectively) than the other genotypes. On the other hand, there were no significant difference between studied genotypes in total soluble solids and pH).

physiological disorders: All genotypes revealed low susceptibility for bitter pit due to the environmental and nutrient conditions. Concerning water core disorder, just genotype G2 showed low susceptibility, while the other genotypes were resistant.

Susceptibility for pest and diseases: The results showed that the genotype G3 revealed low susceptibility to wooly apple aphid (15%), while the other genotypes revealed very low susceptibility (3-5%). Concerning codling moth and rosy aphids, all genotypes revealed low susceptibility. Genotypes G2 and G5 showed low susceptibility (10%) to powdery mildew while G1, G3, and G4 revealed very low susceptibility. All genotypes didn't reveal any symptoms for apple scab and canker.

Molecular characterization

In the present study, the eight primer pairs were able to characterize the studied apple genotypes, which revealed 22 alleles, of which 21 were polymorphic (95.45%) among all genotypes. The number of alleles per primer-pairs ranged from 1 to 5 alleles (Figure. 1) with an average 2.75. Hi07h02 primer-pair produced 5 polymorphic alleles, while Hi12a02 primer-pair revealed one monomorphic allele. The size of all alleles ranged between 110 to 280 bp depending on primer pairs (Table 5).

Table 5. Total allelic forms, polymorphic alleles, %polymorphism and allele size

Primers	Total allelic forms	Polymorphic alleles	% polymorphism	Allele size/bp
Hi04a05	3	3	100	190-220
Hi04g05	3	3	100	220-240
Hi07h02	5	5	100	210-280
Hi08c05	3	3	100	220-230
Hi08d09	3	3	100	185-195
Hi08e06	2	2	100	110-130
Hi09a01	2	2	100	190-200
Hi12a02	1	0	0	230
Total	22	21	95.45	
Mean	2.75	2.63		

The genetic similarity based on Jaccard coefficient for studied apple genotypes and cultivars ranged from 0.5 between G3 with starking delicious(S.D) to 0.13 between G2 with G3 and G2 with G1 (Table 6). Golden delicious Cultivar (G.D) revealed the highest relationship with G2(0.33), while revealed the lowest genetic relationship with G1(0.21). Likewise, Starking delicious cultivar revealed the lowest genetic similarity with G1 (0.20).

Table 6. Genetic similarity matrices computed according to Jaccard coefficient from SSRs data.

	G. D	S. D	G1	G2	G3	G4	G5
G. D	1						
S. D	0.31	1					
G1	0.21	0.20	1				
G2	0.31	0.29	0.13	1			
G3	0.31	0.50	0.2	0.13	1		
G4	0.31	0.29	0.39	0.20	0.29	1	
G5	0.33	0.21	0.42	0.31	0.31	0.42	1

Cluster analysis grouped the 5 studied genotypes and the two commercial cultivars into 3 clusters, where S. D cultivar with G3 formed the first cluster, while the second cluster contains G. D cultivar with G5. The third cluster divided into two subclusters, the first one contains G1 and G5, while the second subcluster contains only one genotype G4 (Fig. 1)

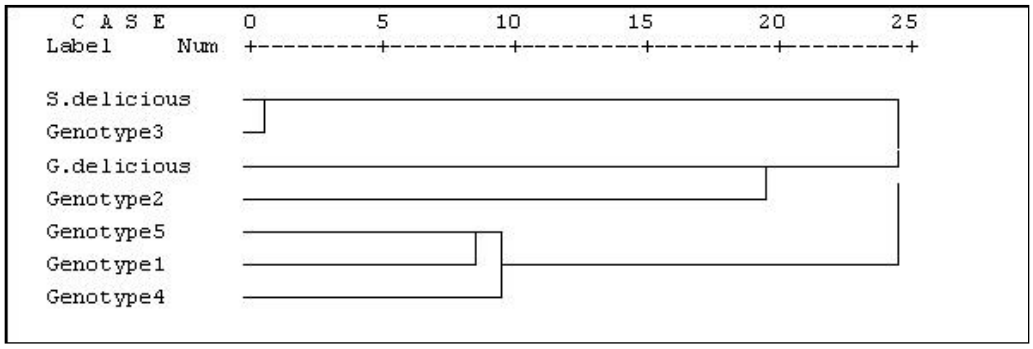

Figure 1: Cluster analysis as revealed by SSR data.

Discussion

Morphological characterization and evaluation

The genus Apple characterized by the highest genetic variation due to somatic mutations and genetic improvement through the long history of apple agriculture (Muzher et al., 2007). Morphological characters and agronomic traits play an important role to differentiate apple cultivars (Farrokhi et al., 2011). Our study showed that the studied genotypes revealed different behavior in phenological stages, and various characters of leaves and fruit which related to the genetic factors and environmental conditions especially the relative humidity, chilling period, growth temperature, location and rainfed system. Moreover, chemical analysis was able to differentiate between the studied genotypes, and some of them revealed high firmness according to (IPGRI, 1997). Fruit characters such as over color, ground color, fruit shape, fruit size, and maturity time were commonly used to evaluate apple germplasm (Grassi et al., 2002). On the other hand, Koc et al. (2009) used leaves chracters to identify two local apple cultivars and two apple rootstocks in Turky and they stated that the classification of the two rootstocks in the same group is too difficult depending on morphological parameters. While, Al-Halabi and Muzher (2015) studied the genetic diversity of some apple cultivars using morphological parameters for leaves and fruits which were able to discriminate between local and introduced apple cultivars. Researchers used chemicals analysis as a useful tool to differentiate between commercial apple cultivars and to evaluate apple germplasms (Misic et al., 1993; Blazek, 2002; Hudina, 2002; Wu et al., 2007; Muzher and Al-Halabi, 2012).

Susceptibility for pests and diseases: All studied genotypes revealed low and very low susceptibility to wooly apple aphid which gave the studied genotypes an important aspect. The low infection of codling moth and rosy aphids may due to the control strategy and protection program that used, while the absence of symptoms for apple scab and canker related to the environmental conditions (low relative humidity during flowering dates and fruit set. Wooly apple aphid is considered as the second pest after codling moth in Syria (Mansour, 2006). The

evaluation of Wooly apple aphid resistance is an important step to select apple cultivars and rootstocks (Ateyyata and Al-Antaryb,2009) and for apple rootstock breeding program (El-Halabi et al., 2013).

Molecular characterization

SSR considered as valuable marker in the genetic diversity and genetic relationship studies among apple species and cultivars (Hokanson et al. 2001). Our results stated that SSR markers revealed high polymorphic percentage and valuable number of alleles which reflected the high genetic variance among studied genotypes, and the size of alleles was in agreement with (Garkava- Gustavsson et al., 2008) except Hi12a02 which gave small size. Silverberg- Dilworth et al., (2006) stated that the number of observed alleles for each locus were 2 to 15 when they studied the genetic diversity and relationship among 68 apple genotypes using SSR marker. In some cases, the results showed disagreement between morphological characterization and molecular characterization because some characters aren't binding to SSR loci, and some traits like tree habit, shoot length and diameter may not detectable by molecular markers. On the other hand, it should be noticeable that some characters related to cytoplasmic inheritance which may cause the lack of argument between morphological and molecular markers (Gupta and Rustgi, 2004).

Conclusion

The studied genotypes revealed many agronomical and economical traits which lead to be creditable as new cultivars. On the other hand, SSR marker was very efficient tool to identify the genetic variability between the five studied genotypes and two cultivars.

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