

# Selection of Promising Early Ripening Progenies and Assessment of Earliness Heritability in the Breeding Program of Apple

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## Summary

The experiment was conducted on 560 apple progenies, 6 and 7-year-old, in a pre-ultimate selection of breeding program for early and mid-early cultivars in 2014-2015. The progenies were assessed for crop bearing precocity, pomologic, organoleptic characteristics and ripening time as well as bloom beginning, flowering period, yield per tree and tree vigor. The initial screening was performed based on ripening time, yield per tree and tree vigor. The selected 63 progenies with the highest performance were then classified in 9 distinct ripening groups by use of a modified ripening phenology scale of apple descriptor (D.U.S). Overall, 53 hybrids were individuated as very early, early, mid-early and mid-ripening fruits with high fruit acceptability. The selected hybrids benefitted both lofty bloom densities and low to moderate tree vigor. The progeny classification was achieved also by cluster analysis for which were formed 3 distinct groups and more sub-clusters. Nine early-ripening, high-yielding with moderate to weak vigor were native half-sib progeny of newly released 'Sharbati', 'Sheikh Ahmed' and red big fruit sized 'Heidarzadeh', together with introduced 'Jonathan', 'Yellow Spur' and 'Glockenapfel' parents. 'Sheikh Ahmed' progenies had the highest frequency. Some superior types as the full-sib 'Heidarzadeh'×'Early Red One' were also individuated.

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## Key words

breeding, early ripening, selection, tree vigor, pomology

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## Introduction

Binomial apple (*Malus×domestica* Borkh.) has become the standard designation as a complex inter-specific hybrid (Korban and Skirvin, 1984). The overall objectives of modern apple breeding programs besides earliness, precociousness, and high-yielding are to increase the fruit marketability (Hancock et al., 2008), emphasizing high fruit quality, tree growth habit and regular bearing (Laurens, 1999). To assess fruit quality, sensory analysis helps making the final selections, whereas accurate fruit descriptions are elaborated (Deslauriers et al., 1995). Considering that most of the early Iranian and introduced apples produce small fruits with low storability, there was a need of early cultivars with standard size and firm flesh, so the present breeding program was started to replenish such void. At the final phase of a breeding program, 9 selections were released with various ripening time ranged from late to very late ripening with good fruit size, color and taste (Mihai et al., 2011). High yielding apple sub-lines obtained by Alspach and Oraguzie, (2010) initially consisted of 119 half-sib and 50 full-sib families. Variance components of Julian harvest date of the sub-lines were estimated for fruit weight and sensory. Pirlak et al., (2010) collected 25010 wild genotypes in order to produce early apples tolerant to apple scab. Two early apples released by Raseira et al., (1992), 'BR-1' selected for productivity, size and earliness showed modest quality and 'BR-2' with good quality suffered from inconsistent yield.

## Parent selection and screening

In order to make proper parent selection, 108 commercial apple cultivars grown in the National Cultivars Collection established in Horticulture Research Station of Kamal Shahr located in Karaj, Alborz province, 45 km far from Kamalshahr, were assessed for phenological, pomological traits, yield-related parameters and tree vigor, in 2003-2005 (Hajnajari, 2010). In 2005, the performed 44 cross combinations led to 100,000 hybrid seeds. The 7,000 grown seedlings were roughly screened by using morphological markers for which 720 2-year-old hybrids were selected and established in the hybrid orchard during 2007-2008 (Chashnidel and Hajnajari, 2012).

## Materials and Methods

In this pre-final phase of breeding program, the single bearing trees of 560 half-sib and full-sib progenies, obtained from various cross combinations between native Iranian and introduced Cvs of apple consisting of Very Early ripening Cvs Golab-e Sahne, Yellow Transparent; Early Cvs Golab-e Kohanz', Sharbati; Mid Cvs Red Delicious, Golden Smoothie, Stayman, Ardabil2, Jonathan; Late Cvs Top Red Delicious, Ganny Beauty; Very Late Cvs Akhlemad-e Mashhad and Yellow Spur were observed. The hybrid orchard was established in Meshkin Abad Horticulture Research Station, located in Karaj-Iran. The single hybrid trees were assessed for certain phenological, pomological, growth traits and yield per tree. The assessments were carried out in two growing seasons, 2014 - 2015, on the large subset of 8 and 9-year-old genotypes according to a modified Guideline of apple D.U.S (Distinction, Uniformity and Stability) by expanding bloom beginning in to 9 different classes (Hajnajari et al., 2008). The appraised phenological traits included flowering period, deduced

by the temporal interval between the bloom beginning to the end, and assessment of fruit ripening time. The productive traits as flowering intensity, fruit fall and yield per tree were estimated, as well. Other botanical traits including flower size, reciprocal pistil-anthers posture, petals arrangement (free, closed and partially overlapped) were registered daily, besides tree vigor and growth habit as mandatory data to distinct systematically the hybrids from each other for future release. The pomologies as fruit shape, weight, size, length, diameter, length/diameter ratio, stalk length and thickness, width-depth values both for eye and stalk holes, sepal length, background color and over-color, russeting area, lenticels number, skin wax thickness and flesh firmness were registered. Biochemical attributes such as pH, Total Soluble Solids (TSS) and Titrable Acidity (TA), and further sensory features as the aroma, sweetness/sourness, juice content, crispness and general acceptance were scored. Fruit tests for pomological traits were performed in a completely randomized design in 10 replicates (fruit), and for supplementary chemical examinations in 3 replicates. Grouping was achieved based on the number of classes for each of the phenological and morphological traits described in the apple Guidelines for Distinctness, Uniformity and Stability (D.U.S) (Hajnajari et al., 2008), as well as simple cluster analysis. Each hybrid was considered as a treatment (replicate). For all of the traits in exam, the ranking was also achieved, taking into account the min and max levels of gene's expression of each trait registered during cultivar evaluation program in the National Cultivars Collection as the main gene pool. After the test of uniformity of variances and the normality, the data were analyzed by SAS 9.1. statistical package (SAS Institute, Cary, NC). Mean comparisons were performed using Duncan test ( $P \leq 0.05$ ). The screening was carried out based on early ripening and yield-class on all the progeny and cluster analysis was used in order to group high yield progenies. The trials of hybrid comparisons in terms of tree performance, growth habit and tree vigor and panel test for selection of promising hybrid cultivars were provided.

## Results and Discussion

Based on the results, 63 promising genotypes were selected among the 560 genotypes. Overall, 53 hybrids were individuated as very early, early, mid-early and mid-ripening fruits and high fruit quality. The analysis of variance for the quantitative traits of fruits harvested from the 31 superior apple genotypes showed a significant difference at 1% of probability (Table 1).

### Phenological traits

#### *Bloom beginning*

In 2015, ranking the data of flowering phenology of the 560 full-sib and half-sib hybrids based on a modified D.U.S classification, instead 5 classes, demonstrated a detailed distribution of the progenies (HYs) in 9 Coded groups: Code 1 - Very early flowering: 20 (HYs), Code 2- Very early to early: 16 HYs, Code3- Early: 84 HYs, Code 4- Early to mid: 157 HYs, Code 5- Mid: 55 HYs, Code6 - Mid to late: 38 HYs, Code7 - Late: 19 HYs, Code 8- Late to very late: 27 HYs, Code 9 - Extremely late: 11 HYs. Studying the distribution rhythm, it became clear that 332 hybrids from different families took part as early flowering in a crescent gradient, as a normal distribution of the quantitative trait.

**Table 1.** Analysis of variance for the quantitative traits of fruits harvested from superior apple genotypes

Hybrid genotype	Fruit weight (g)	Fruit length (mm)	Fruit diameter (mm)	Length: diameter ratio	Pedicle length (mm)	Pedicle thickness (mm)	Peduncle width (mm)
265	98.50 <sup>a</sup>	56.41 <sup>ab</sup>	60.88	0.92 <sup>bcdef</sup>	19.48 <sup>bc</sup>	1.91 <sup>cdef</sup>	18.62 <sup>b</sup>
337	93.38 <sup>ab</sup>	56.86 <sup>ab</sup>	60.54 <sup>aba</sup>	0.90 <sup>bcdefg</sup>	14.81 <sup>defg</sup>	2.12 <sup>bcdef</sup>	14.06 <sup>fgh</sup>
430	91.40 <sup>abc</sup>	59.66 <sup>a</sup>	57.15 <sup>cde</sup>	1.00 <sup>ab</sup>	13.00 <sup>fghi</sup>	1.92 <sup>cdef</sup>	14.08 <sup>fgh</sup>
247	90.18 <sup>abc</sup>	55.20 <sup>abc</sup>	58.46 <sup>bcd</sup>	0.90 <sup>bcdefg</sup>	13.90 <sup>efgh</sup>	2.06 <sup>bcdef</sup>	14.70 <sup>def</sup>
216	90.17 <sup>abc</sup>	59.71 <sup>a</sup>	57.43 <sup>cde</sup>	0.98 <sup>abc</sup>	15.21 <sup>def</sup>	2.00 <sup>bcdef</sup>	10.27 <sup>Lmn</sup>
479	84.06 <sup>bcd</sup>	51.00 <sup>cd</sup>	59.30 <sup>abc</sup>	0.81 <sup>defghi</sup>	24.23 <sup>a</sup>	1.97 <sup>bcdef</sup>	16.58 <sup>c</sup>
269	84.05 <sup>cd</sup>	55.41 <sup>abc</sup>	57.98 <sup>cd</sup>	0.90 <sup>bcdefg</sup>	9.88 <sup>kLm</sup>	2.42 <sup>b</sup>	12.42 <sup>ij</sup>
482	80.23 <sup>cde</sup>	48.00 <sup>defgh</sup>	57.35 <sup>cde</sup>	0.78 <sup>fghi</sup>	22.70 <sup>a</sup>	1.84 <sup>def</sup>	15.54 <sup>cde</sup>
480	75.60 <sup>def</sup>	47.49 <sup>defgh</sup>	56.41 <sup>de</sup>	0.77 <sup>ghi</sup>	21.66 <sup>ab</sup>	1.85 <sup>def</sup>	14.33 <sup>efg</sup>
81	74.14 <sup>defg</sup>	45.30 <sup>efghi</sup>	56.64 <sup>de</sup>	0.73 <sup>i</sup>	12.55 <sup>fghijk</sup>	1.90 <sup>cdef</sup>	20.76 <sup>a</sup>
529	72.45 <sup>defgh</sup>	47.58 <sup>defgh</sup>	53.76 <sup>eg</sup>	0.82 <sup>defghi</sup>	10.17 <sup>hijklm</sup>	1.90 <sup>cdef</sup>	15.96 <sup>cd</sup>
613	69.56 <sup>efghi</sup>	48.71 <sup>defg</sup>	57.36 <sup>cde</sup>	0.84 <sup>defghi</sup>	13.74 <sup>fgh</sup>	2.26 <sup>bcd</sup>	14.38 <sup>efg</sup>
412	68.34 <sup>efghi</sup>	50.12 <sup>de</sup>	54.24 <sup>eg</sup>	0.90 <sup>bcdefg</sup>	7.71 <sup>mno</sup>	2.14 <sup>bcd</sup>	8.97 <sup>no</sup>
258	66.93 <sup>fghij</sup>	49.76 <sup>def</sup>	52.74 <sup>ghi</sup>	0.88 <sup>bcdefgh</sup>	12.65 <sup>ghijk</sup>	2.06 <sup>bcdef</sup>	13.03 <sup>ghi</sup>
557	62.00 <sup>ghijk</sup>	45.81 <sup>efgh</sup>	53.76 <sup>eg</sup>	0.80 <sup>efghi</sup>	8.94 <sup>Lmn</sup>	2.28 <sup>bcd</sup>	12.30 <sup>ij</sup>
425	61.44 <sup>ghijk</sup>	57.09 <sup>ab</sup>	51.08 <sup>hij</sup>	1.06 <sup>a</sup>	17.12 <sup>cd</sup>	1.64 <sup>efg</sup>	14.15 <sup>fgh</sup>
610	60.96 <sup>hijk</sup>	55.44 <sup>abc</sup>	51.17 <sup>hij</sup>	0.93 <sup>abcde</sup>	12.90 <sup>fghij</sup>	2.10 <sup>bcdef</sup>	6.23 <sup>p</sup>
518	59.97 <sup>hijk</sup>	50.11 <sup>de</sup>	49.72 <sup>j</sup>	0.94 <sup>abcd</sup>	5.37 <sup>op</sup>	2.11 <sup>bcdef</sup>	10.37 <sup>Lm</sup>
60	58.64 <sup>ijk</sup>	43.94 <sup>ghij</sup>	55.39 <sup>ef</sup>	0.74 <sup>i</sup>	11.02 <sup>ghijk</sup>	1.91 <sup>cdef</sup>	11.97 <sup>ij</sup>
401	58.42 <sup>ijk</sup>	52.26 <sup>bcd</sup>	49.36 <sup>jk</sup>	1.00 <sup>ab</sup>	8.10 <sup>mno</sup>	1.83 <sup>def</sup>	11.80 <sup>ijk</sup>
651	57.04 <sup>ijk</sup>	45.51 <sup>efghi</sup>	50.27 <sup>ij</sup>	0.89 <sup>bcdefgh</sup>	7.38 <sup>mnop</sup>	2.39 <sup>bc</sup>	11.32 <sup>jkL</sup>
221	55.13 <sup>jkl</sup>	44.20 <sup>ghij</sup>	50.40 <sup>j</sup>	0.81 <sup>defghi</sup>	22.20 <sup>a</sup>	1.36 <sup>gh</sup>	11.81 <sup>ijk</sup>
546	54.86 <sup>jkl</sup>	49.38 <sup>def</sup>	53.07 <sup>gh</sup>	0.86 <sup>cdefghi</sup>	14.52 <sup>defg</sup>	2.13 <sup>bcd</sup>	14.60 <sup>ef</sup>
527	54.20 <sup>jkl</sup>	44.01 <sup>ghij</sup>	49.31 <sup>jk</sup>	0.84 <sup>defghi</sup>	16.60 <sup>de</sup>	1.62 <sup>fg</sup>	12.83 <sup>hi</sup>
555	53.78 <sup>kl</sup>	47.87 <sup>defgh</sup>	51.23 <sup>hij</sup>	0.90 <sup>bcdefg</sup>	10.02 <sup>ijklm</sup>	1.67 <sup>efg</sup>	10.58 <sup>klm</sup>
381	43.78 <sup>lm</sup>	44.46 <sup>ghij</sup>	47.46 <sup>k</sup>	0.89 <sup>bcdefgh</sup>	7.41 <sup>mnop</sup>	1.85 <sup>def</sup>	10.17 <sup>Lmn</sup>
399	36.70 <sup>m</sup>	43.80 <sup>ghij</sup>	44.47 <sup>l</sup>	0.92 <sup>bcdef</sup>	4.62 <sup>p</sup>	3.25 <sup>a</sup>	8.42 <sup>o</sup>
23	35.93 <sup>m</sup>	45.14 <sup>fghij</sup>	42.48 <sup>lm</sup>	1.00 <sup>ab</sup>	6.12 <sup>nop</sup>	1.98 <sup>bcdef</sup>	9.45 <sup>mno</sup>
24	31.94 <sup>m</sup>	40.91 <sup>ij</sup>	42.95 <sup>lm</sup>	0.90 <sup>bcdefg</sup>	7.90 <sup>mno</sup>	2.07 <sup>bcdef</sup>	11.72 <sup>ijk</sup>
396	30.98 <sup>m</sup>	40.30 <sup>j</sup>	42.17 <sup>m</sup>	0.90 <sup>bcdefg</sup>	7.38 <sup>mnop</sup>	1.95 <sup>bcdef</sup>	10.33 <sup>Lm</sup>
285	11.47 <sup>n</sup>	22.60 <sup>k</sup>	28.04 <sup>n</sup>	0.80 <sup>efghi</sup>	12.65 <sup>fghijk</sup>	1.11 <sup>h</sup>	6.87 <sup>p</sup>

Numbers with same letters in each row have no significant difference at the 5% level based on Tukey test.

Cod 4 as Early to mid class contained 157 HYs with the highest frequency. Furthermore, the result of cluster analyses of the progenies using the registered data of all the traits including fruit ripening time, precociousness, flowering beginning, flowering end, flowering period, flowering density, petal arrangements, stigma/anther posture, the percentage of fruit fall at the pre-harvest stage, tree growth vigor and yield per tree clustered them in three subgroups (Fig. 1). Similar dual grouping was applied on flowering phenology of 10 chance seedling as promising apple genotypes whereby were classified into 8 subgroups in absence of any genotype in Code 9 (Zand Fani and Hajnajari, 2012). Meier et al., (1994) presented the BBCH (Biologische Bundesanstalt, Bundessortenamt, Chemische Industrie) phenological descriptor with a more fragmented stage of flower phenology that may serve for more specific studies. Opportune selections were applied after the severe spring frost in Karaj-Iran in 2002, by which only 20 of 108 cultivars could keep the crop till harvest time, among them 'Bel du Puntuaz', 'Bel du Boskoop' and 'IRI4' due to the short flowering period, or late flowering in case of 'Prim Gold', 'Starking' and 'Yellow spur' (Hajnajari and Eccher, 2006). Nevertheless, high expression levels of certain traits may have controversial aspect, e.g. increasing the span of the flowering period provides profuse amounts of pollen and a potential pollinator, but it also increases a risk of disposal to spring frost. Based on the results of Chashnidel et al. (2009), the flowering period is correlated strongly with the time of fruit ripening.

#### *Time of fruit ripening*

According to D.U.S Guidelines, the collected data regarding the time of fruit ripening of inter and intrafamily progenies were ranked into 9 different classes of ripening time:

**Code 1** - Very early ripening: 6 HYs; **Code 2** - Very early to early; 83 HYs, **Code 3** - Early: 114 HYs; **Code 4** - Early to mid: 68 HYs; **Code 5** - Mid to late: 2 HYs; **Code 6** - Mid to late: 24 HYs; **Code 7** - Late: 18 HYs; **Code 8** - Very late: 24 HYs; **Code 9** - Very late: 20 HYs.

It was concluded that 271 hybrids were inserted in the four earliest classes, within them 53 superior progenies carrying highest yield and middle fruit size traits. Here below, an extended calendar of ripening time delivered in 9 classes based on D.U.S guideline is presented: Very early ripening (18/May- 27/June), Early ripening (28/June-17 July), Early- mid-ripening (18 July- 27 July) and Mid ripening (28 July- 6 August). Among them, a superior hybrid as the earliest progeny ripened as early as 18 May, registered by L8G502 (Aidard × Heidarzadeh) and the summer apple L8G538 a half-sib progeny of 'Sheikh Ahmad' as the max expression value regarding the latest phenology in the Mid ripening class was harvested on 6 August. These results confirm the high efficiency of screening applied on the young offspring by morphological markers during the first stages of the breeding program (Chashnidel and Hajnajari, 2012).

#### *Flowering period*

The results of ranked data related to the 'Flowering period' would resolve many aspects of the selected hybrids in future orchard management, such as the use of proper pollinators. The need of late flowering cultivars where spring frost occurs

frequently would be resolved by the promising hybrids ranked as 'Short flowering' as well as late flowering classes. The results of the grouping of genotypes based on 'Short flowering' consisted of five different classes. Code 1- Very short flowering period (4-6 days): 31 genotypes; Code 2- Short flowering period (7-8 days): 45 genotypes; Code 3 - Moderate flowering period (9-10 days): 288 genotypes; Code 4 - Long flowering period (11-12 days): 57 genotypes, Code 5 -Very long flowering period ( $\geq 13$  dd.): 8 genotypes. The flowering period showed to be ranged from a minimum of 5 days in the hybrid L8G523 with maternal 'Sheikh Ahmed' and a maximum of 12 dd in the hybrid L1G61 with half-sib 'Soltani Shabestar'. The mean span of the flowering period was recorded as 10 dd., in 2015. Although the flowering sequence is controlled genetically, temperature fluctuations may easily postpone or hasten significantly bloom calendar; notwithstanding our observations confirmed that the genetic order of flowering among genotypes is maintained. At cellular level, the change in the developmental fate of primordial meristem is controlled by environmental and endogenous signals (McDaniel et al., 1992). The genetic interactions that control the floral transition were described in Arabidopsis. The essential feature was that the time at which flowering occurs is determined by antagonistic action of the parallel pathways that promote and monitor developmental age, environment and the repressive action of floral inhibitors (Yaron and Dean., 1998).

#### *Yield performance*

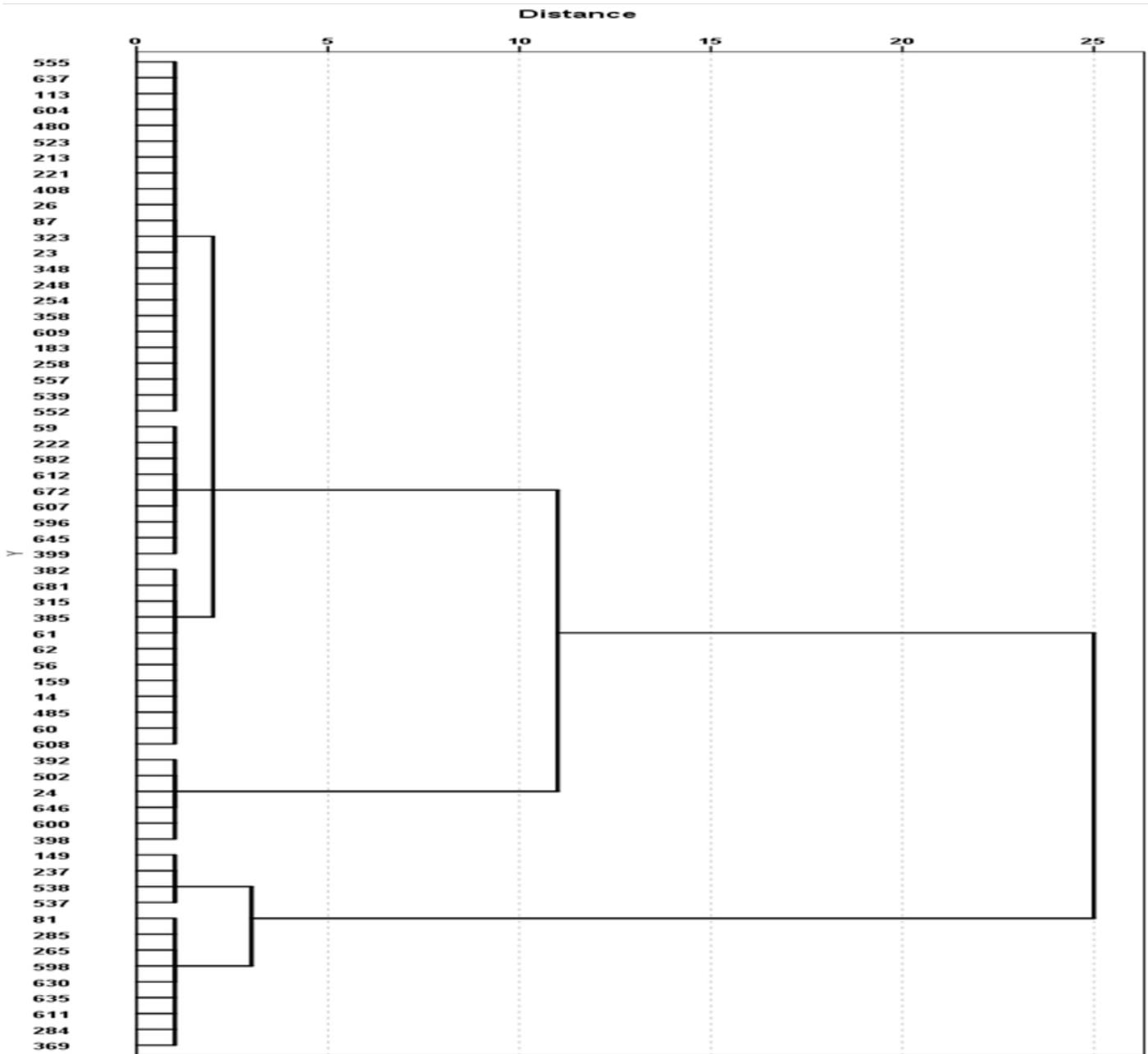
The hybrid genotypes were classified according to their yield performance into seven groups: Very weak (87 hybrids), Weak (83 hybrids), Medium (82 hybrids), Good (66 hybrids), Very good (55 hybrids), Excellent (36 hybrids), and Extremely excellent (27 hybrids).

#### *Bloom density and Flower structure*

The recorded flowering densities of the genotypes were generally high, 80 to 100%. The petal arrangements ranged from dominant type figured in the half-overlapping, to the free type petals. Investigating the trait of the stigma position in relation to the anthers showed that the stigma was longer than the anthers in most hybrid genotypes. These traits will serve for cultivar registration (D.U.S).

#### *Fruit fall*

The results regarding percentage (%) of fruit fall at pre-harvest stage showed a min of 1% in the coded progenies as 113, 149, 237, 285, 358, 399, 480, 523, 537, 538, 555, 557, 604, 607, 609, 611, 612, 630, 635 and 637, which all belonged to native early 'Sheikh Ahmad', an early parent characterized with cylindrical fruit, heavy cropping, weeping growth habit and very low tree vigor. These results strengthen the results of the multiannual artificial inbreeding field trials where 'Sheikh Ahmad' was reported as a self-compatible by Moradi and Hajnajari (2014), even though the max fruit drop of 80 %, was noted in two hybrids, the first '392' and the second '502', the offspring of 'Sheikh Ahmad' and 'Heidarzadeh', relatively. A total of 15 progenies of 'Sheikh Ahmad' were selected in this study, including 358, 399, 523, 537, 538, 555, 557, 604, 607, 609, 611, 612, 630, 635 and 637 with excellent yield and least fruit



**Figure 1.** Dendrogram of the traits studied in 63 hybrid apple genotypes. The studied traits include fruit ripening time, precociousness, flowering beginning, flowering end, flowering period, flowering density, petal arrangements, stigma/anther posture, the percentage of fruit fall at the pre-harvest stage, tree growth vigor and yield per tree.

fall at 1%, while hybrid 392 had the highest fruit drop within the same family. However, the general tendency among the progenies confirms the privilege of the selected maternal parent.

*Tree vigor*

The two growth components of tree vigor consisting of tree height and canopy shading area were examined in the 8-year-old trees. Grouping the data evidenced that the tree height varied from 2.5 to 3.5 m. and the canopy shading area of genotypes was ranked between a min value of 1.59 sqm (square meters) in the half-sib 113 derived from ‘Yellow spur’ and a max of 10.82 sqm in the full-

sib 81 (‘Ardabil2×Heidarzadeh’). The overall mean value of whole genotypes resulted in 4.7 sqm. The moderate tree vigor preserves the capacity of higher flower differentiation and an increased number of trees per unit of area. The new cultivars with columnar growth habits, such as the ‘Wjckic McIntosh’, allow the on row tree cultivation distance to be reduced up to 25 cm (Blazek and Kelinova, 2011). The upright growth assures reduced shading area and so is considered as the closest tree habit to the columnar type. The 20-year-old trees of newly released Iranian apple ‘Sharbati’ with upright growth and a span of 4.5 sqm shading area is adapted for intensive orchard establishment.

### Cluster analysis of phenological traits

The dendrogram (Fig. 1) obtained from cluster analysis of data, at 9 Euclidean distance, the genotypes were categorized into three groups. Nine high-yielding hybrids with weak to moderate tree vigor were known as the progeny of 'Sheikh Ahmad', 'Heidarzadeh', 'Sharbati', 'Jonathan', 'Yellow spur' and 'Glockenapfel', respectively. The highest frequency of early progenies was attributed to the indigenous parents, especially 'Sheikh Ahmed'. Based on the results, among 9 ripening classes the highest number of progenies was placed in the first class of ripening as Very Early, within them 44 marked by mean tree vigor, very low percentage of fruit fall and half-overlapping petals. Within the half-sib progenies of 'Sheikh Ahmed' 20 superior hybrids were selected, meanwhile the progenies of 'Haidarzadeh', 'SoltaniShabestar' and 'GolBahar' were found in decreasing order of frequency in the group (Fig. 1). A number of 6 hybrids were clustered in the second group with the common attribute of excellent performance accompanied with a high percentage of fruit fall in the pre-harvest stage. Once again, four open pollinated progenies of 'Sheikh Ahmad', a progeny of 'Glockenapfel' and the full-sib 'Hydrzadeh×Aidard' were inserted in this group. Thirteen identified genotypes in the third group had in common some traits like late ripening, least fruit drop, lack of color change in ripened fruit and almost identical tree height of 3 m. (Fig. 1). In the third clustered group were listed a pair of 'Sheikh Ahmad' half-sib progenies, two cross-bred of 'Heidarzadeh×Ardebil' followed by other pair hybrids of 'StarkanRoge', an open pollinated offspring of 'Granny Smith' and the crossbred 'Early Red One×Winsap'.

### Pomological traits

#### Quantitative attributes

Based on the results of analysis of variance, the studied traits of fruit such as weight, length, diameter, length/diameter ratio, stalk length, stalk thickness, depth and width of stalk hole were significantly different ( $P \leq 0.01$ ) in the progenies (Table 1). According to the mean comparisons, the highest fruit weight was registered in the following hybrids 337, 430, 216, 265, 247 in decreasing order as 93.3, 91.4, 90.1, 98.5, and 90.1 g. respectively, and in a similar order for fruit length the longest fruits, (56.8, 59.6, 59.7, 56.4, and 55.2 mm, respectively). The biggest fruit diameter (60.8 and 60.1 mm) was observed in genotypes 265 and 337. The highest ratio of length to diameter was recorded in genotypes 430, 425, 401 and 23. However, the quality of the samples was partially damaged during storage due to the cold room failure, so the present values can be improved. Genotypes 479, 482, 480 and 221 showed the longest stalk length (24.2, 22.7, 21.6, and 22.2 mm, respectively). The thickest stalks (3.2 and 2.4 mm) were observed in genotypes 399 and 269. Genotypes 81 and 265 had the widest stalk hole (20.7 and 18.6 mm) (Table 1).

#### Qualitative traits

In order to study the qualitative traits of fruit size, shape, size of the eye, sepal length, skin wax, background color and over-color, flesh color, number of lenticels and russetting area were observed. Based on the results of this study, fruit quality traits for fruit size in the genotypes of medium and big size were dominant with the highest mean values and the maximum fruit yield among

genotypes. In terms of the fruit shape, most of the genotypes overall had conical or spherical shape. Most of the progenies had middle eye size and sepal length, and also medium skin wax was recorded in most of the progenies. In terms of fruit color, the lowest number of genotypes had a greenish-white background color, but the largest number was yellowish-green. None or very small russeted area was observed. A large number of genotypes had a limited number of lenticels and white flesh color was assigned as the dominant color in the genotypes.

#### Data cluster analysis based on quantitative and qualitative traits of fruit

The dendrogram (Fig. 2) derived from cluster analysis was based on quantitative and qualitative fruit traits data. These were placed within 10 Euclidean distances and the genotypes were clustered into three groups. Based on the results, the highest number of 14 genotypes placed in the third group carrying high fruit weight (69 to 98 g), moderate skin wax, green-yellowish background color and lack of russet. In the second group, 11 identified progeny had the fruit weight ranged between 53 to 61 g and the fruit diameter from 49 to 53 cm. The lowest number of six genotypes was placed in the first subgroup characterized by fruit weight ranged between 11-43 g, and a lower fruit length/diameter ratio. Other similar breeding program persisted for 40 years, beginning from 100,000 seedlings and 66 plus hybrids were selected. Full-sib (Starking×Jonathan) and (Golden Delicious×Jonathan) were released while other 69 promising hybrids were left to be studied in the Fruit Research Institute at Čačak-Serbia (Tešović et al., 1994).

### Sensory assessment

Based on the results of the sensory evaluation (Table 2), the highest fruit aroma score ( $\geq 70\%$ ) was obtained in genotypes 265, 399, 529, 555, 610 and 14-2, though hybrid 555 could surpass others for a good flavor ( $\geq 72\%$ ). Ideal sweetness was voted for the progeny 221, 247, 399, 412, 555 and 142, ( $\geq 70\%$ ). Two genotypes, 216 and 481, had low sweetness and extreme sourness according to the panel test results. Biosynthesis of phenolic compounds depends on the time of ripening and the cultivar. Phenolic compounds play a decisive role in nutritional quality and sensory characteristics such as taste and aroma, color, odor, bitterness and astringency of apples (Hampson et al., 2000; Way et al., 1990).

Most of the genotypes except 14, 544 and 481 had substantial amounts of fruit juice and high flesh firmness. High variability was found in the peel thickness from thin to slightly thick. The F1 progeny 258, 412, 555 and 610 were marked by excellent fruit color. Finally, according to all of the features considered in the panel test, genotypes 216, 265, 399, 421, 425, 520, 529, 610 and 14-2 were judged as the highest grade of general acceptability ( $\geq 72\%$ ). Studying 48 phenotypic traits among 56 Early flowering almond accessions, 'Sattarbai' was characterized by crescent dry fruit, thin paper shell, and high kernel/dry fruit weight ratio ( $>0.65$ ) (Giordani et al., 2017). Labuschang et al., (2004), in a 3-year qualitative sensory evaluation of the progeny of different parents, concluded that the progeny of 'Golden Delicious' was superior to 'Anna'. Sensory tests were evaluated in 3-year-old trees on M26 rootstocks in Karaj. 'Gala' was ranked as the first in terms of flesh firmness and sensory evaluations, while 'Galashniga'

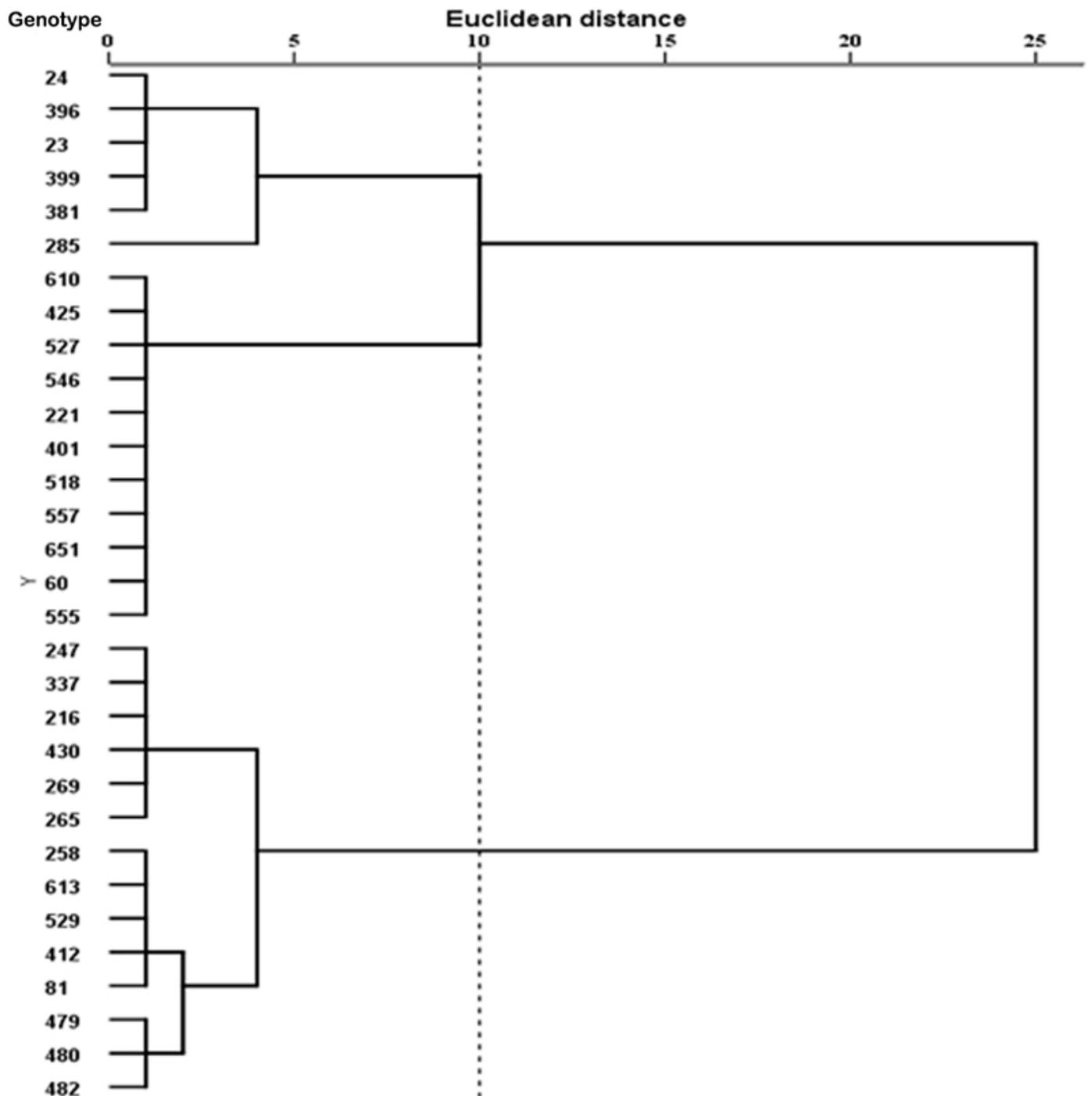


Figure 2. Dendrogram obtained from quantitative and qualitative traits examined in fruits of 31 hybrid apple genotypes

was more desirable in terms of flavor, sweetness, juiciness, fruit firmness, peel thickness and flesh color (Mizani et al., 2013). The effect of genetic diversity of early apples on storability was charged through monthly sensory tests. It was shown that not necessarily the early apples suffered a low storage potential. The native cultivars Sheikh Ahmad, Assali and Mashhad-e Nouri besides the introduced ‘Ozark Gold’ could preserve general acceptability even after 90 days in cold storage (Hajnajari and Eshghi, 2013).

### Correlation between phenological traits

The results of correlation studies confirmed that the performance of progeny had no significant relationship with flowering time, flowering density, fruit ripening time and tree height. Tree yield had a direct and significant relationship with the percentage of fruit fall. An inverse relation was found between lateness and a decrease in fruit fall. Fruit ripening time had no significant relationship with tree growth, flowering time, flowering density and tree height (Table 3). Different researches exposed useful correlations between late leafing and late flowering (Janicks

**Table 2.** Mean comparison of fruit sensory assessments regarding superior progenies of apple.

Genotype	Aroma	Taste	Sweetness	Sourness	Juice	Firmness	Peel thickness	Color	overall acceptability
14	42	22	46	2	38	28	32	32	38
81	46	68	62	18	64	68	50	60	66
216	52	50	24	67	68	44	42	66	70
221	64	58	72	34	72	40	50	62	68
246	38	36	46	24	54	44	36	52	56
247	64	68	70	22	70	44	50	58	60
258	58	44	38	34	72	78	54	82	64
265	72	62	64	6	72	60	36	68	70
399	76	68	82	18	74	36	40	48	70
412	60	68	78	4	78	10	20	92	74
425	52	58	54	10	76	86	58	54	72
481	38	30	20	50	42	40	38	38	50
482	56	61	47	19	60	59	50	51	62
520	64	58	62	26	68	60	56	60	70
529	70	68	60	18	82	74	38	58	70
544	38	24	42	16	36	20	40	36	52
555	74	72	82	20	76	38	36	88	82
557	40	52	46	46	52	48	48	42	50
610	70	62	60	28	64	50	46	80	72
2-14	70	68	82	18	76	44	42	70	76

Scores from 0 (Very Low) to 100% (Very High).

et al., 1996). This study confirmed a negative correlation between tree vigor and productivity, because high yielding hybrids were clustered as weak to moderate tree vigor.

### Heritability of traits

In order to perform the genetic analysis of traits, components of genetic variance, environmental and phenotypic variances, and broad sense heritability ( $h^2$ .s) of 5 important traits were calculated based on the equation:  $100 = VG / VP \times h^2$ .s: (1-4) (Table 4).

According to Farshadfar (1988), the  $h^2$ .s of traits is ranked into three ranges as High ( $h^2 \geq 50\%$ ), Medium ( $h^2$ : 20% - 50%) and Low ( $h^2 \leq 20\%$ ). The results of the present research showed that the highest (64.10%), medium (41.15%) and low (5.75%)  $h^2$ .s values pertained relatively to tree yield, ripening time and length of the flowering period. It seems that the proper parent selection in the present research as high yielding and early

parents had a definitive role in the progeny segregation. It is in agreement with the opinion of Breen et al. (2016) as  $h^2$ .s of flowering period resulted moderate due to the notable influence of temperature influence on the phenology of flowering. The variation of flowering phenology caused by climatic oscillations is remunerated by the dominant genetic basis of the plant material. Brown (1960) reported the progeny mean for a season of ripening is close to the mid-parent value except in crosses between late-ripening varieties, in which the progeny mean is earlier than the mid-parent. Our results are in agreement with Brown report as late ripening 'Golden Delicious' postponed the mean ripening time of the progeny. Again, the reason of moderate  $h^2$ .s assessed for other studied traits in the present research, firstly for fruit fall, may be attributed to acceptable orchard management. Factors like annual bearing, drip irrigation and tree age, 6 to 7-year-old hybrid trees, as near to economical age of productivity, may be affected notably in a well maintained orchard. Secondly, moderate  $h^2$ .s obtained for tree vigor trait, though affected partly by

**Table 3.** Pearson's correlation coefficients among phenological traits

Trait	Yield	Tree vigor	Fruit color change	June drop	Ripening time	Flowering period	Flowering density	Tree height
Yield	1							
Growth vigor	0.03 ns	1						
Fruit color change	0.22 ns	0.15 ns	1					
June drop	0.24*	0.13 ns	0.29*	1				
Fruit ripening time	0.01 ns	-0.001 ns	-0.50**	-0.31*	1			
Flowering period	0.04- ns	0.003 ns	0.15 ns	0.07 ns	-0.05 ns	1		
Flowering density	0.05 ns	-0.11 ns	0.05 ns	0.08 ns	0.13 ns	0.01 ns	1	
Tree height	-0.01 ns	0.65**	0.009 ns	-0.02 ns	-0.09 ns	0.02 ns	-0.04 ns	1

\*\*,\* Significant at 1% and 5% level, correspondingly. ns: no significant difference.

**Table 4.** Heritability values of some measured traits

Trait	Environmental variance (VE)	Genetic variance (VG)	Phenotypic variance (VP)	Heritability (%)
Flowering period	0.9	0.055	0.955	5.75
Yield	0.92	1.64	2.56	64.10
Growth vigor	0.2	0.073	0.273	26.82
June drop	94.4	50.23	144.63	34.73
Fruit ripening time	112.8	78.88	191.68	41.15

maternal and/or paternal vigor, may presume that factors like soil characteristics, cultivation distance and pruning type plus environmental factors can also influence the phenotypic traits. Clearly, the rate of success during selection depends on the level of  $h^2$ , whereas a higher level of heritability will accelerate the selection procedure.  $h^2$  rates of estimates would enable breeders to predict genetic improvement of the plant material through various selection methods marked by different pressures. Others found the kernel weight could be used as a selection criterion for almond breeding (Imani and Shamili, 2018). The inheritance of time of flowering is polygenically controlled. A fair estimate of the mean flowering date of the progeny can be obtained from the mid-flowering time of the parents (Janick et al., 1996).

## Conclusion

As the last biennial part of this breeding program, 63 hybrids were selected with excellent to very good performance among 560 full-sib and half-sib hybrids. Also, 19 early high-yielding progenies with weak to medium tree vigor were identified. The highest frequency of early genotypes comprised the half-sib progeny of native parents, especially 'Sheikh Ahmad'. Bloom phenology data makes available the valuable outputs such as determination of pollinators groups and best pollinators. However, it should also take into consideration the S-allele constitution besides the time of flowering.

It also provides insights into individuation of tolerant progeny to spring frost as the tolerant 'IRI4' with a concise flowering period of 4 days withstood against extreme late frost in Karaj, during 2003 and 2004 (Hajnajari and Eccher, 2006). The studied cross-bred hybrids of apple evidenced a normal gradual interfamily distribution, all in concordance with the principle of segregation of quantitative traits. The overall degree of polymorphism was assessed studying 21 traits of 18 collected apple accessions according to economically valuable traits; cluster analysis distinguished three groups in which the accessions showed a clear separation based on yield and fruit weight (Mratinić and Fotirić, 2012). However, alongside the cross-hybridization as the classic breeding method, modern transgenic tools are often used to enhance crop production. Targeted genome editing technology is discussed for its potential use to improve the traits of horticultural crops through genome engineering (Subburaj et al., 2016). The present breeding program in Iran has lasted 15 years, 72 promising genotypes were selected (Fig. 3a - i), within them also the half-sib progenies of Jonathan (Fig. 3a, b, h, and j). Full-sib (Fig. 3c) and half-sib (Fig. 3d, e, f, and k) progenies of native 'Sheikh Ahmad' had high acceptability and some introduced parents gave place to excellent full-sib progenies ('Stayman' × 'Early Red One') (Fig. 3i).



**Figure 3 (a).** L3G203 (Jonathan♀)-Ripening time: 21 June, Shape: Spherical, Diameter 50 mm, Ground color: green, Over color: Sharp red, Lenticels intensity: numerous evident Stalk: short.



**Figure 3 (b).** L3G216 (Jonathan♀)-Ripening time: 9 July, Shape: Sphere- conical, Diameter 60 mm, Ground color: lemon colored, Over color: light purple, Stalk: short.



**Figure 3 (c).** L10G694 (Sheikh Ahmad♀×HeidarZadeh♂)-Ripening time: 10 July, Shape: oval, Diameter 60 mm, Ground color: lemon colored, Over color: semi-inclusive red tenuous, Stalk: short.



**Figure 3 (d).** L9G651 (Sheikh Ahmad♀)-Ripening time: 10 July, Shape: oval, Diameter 60 mm, Ground color: green yellow, Over color: red, Lenticels: white, Stalk: short.



**Figure 3 (e).** L6G413 (Sheikh Ahmad♀)-Ripening time: 14 July, Shape: conical, Diameter 60 mm, Ground color: lemon colored, Over color: violet striated, Lenticels intensity: limited, Stalk: short.



**Figure 3 (f).** L9G623 (Sheikh Ahmad♀)-Ripening time: 18 July, Shape: Spherical, Diameter 70 mm, Ground color: white yellow, Over color: glossy red, Stalk: short.



**Figure 3 (g).** L5G352 (Sheikh Ahmad♀)-Ripening time: 20 July, Shape: elliptical, Diameter 70 mm, Ground color: pale green, Over color: Dark violet highly striated, Stalk: short.



**Figure 3 (h).** L3G192 (Jonathan♀)-Ripening time: 22 July, Shape: Spherical, Diameter 80 mm, Ground color: green pale yellow, Over color: Dark red, Lenticels intensity: numerous evident, Stalk: medium.



**Figure 3 (i).** L1G55 (Stayman♀×Early Red one♂)-Ripening time: 24 July, Shape: plate, Diameter 70 mm, Length 40 mm, Ground color: lemon colored, Over color: violet semi-inclusive striated, Lenticels density: medium, Stalk: short.



**Figure 3 (j).** L5G308 (Jonathan♀)-Ripening time: 24 July, Shape: Spherical, Diameter 80 mm, Ground color: pale yellow, Over color: merry red, Lenticels: large evident, Stalk: short.



**Figure 3 (k).** L6G404 (Sheikh Ahmad♀)-Ripening time: 25 July, Diameter 70 mm, Shape: Spherical, Ground color: pale yellow, Over color: Dark red glossy, Stalk: short.



**Figure 3 (l).** L8G550 (Sheikh Ahmad♀)-Ripening time: 20 July, Diameter 70 mm, Shape: Spherical, Ground color: green, Over color: Tenuous red, Stalk: short.

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