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PRODUCTION OF A DIVERSE RANGE OF EARLY RIPENING APPLE PROGENIES THROUGH SCALAR SCREENING FOR PHENO-MORPHO AND YIELD TRAITS

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SUMMARY

Seeking to promote the earliness traits in apples (*Malus* × *domestica* Borkh.), this study evaluated 246 half-sib descendants of their mature trees for pheno-morphology, pomological, and sensory attributes. From a productivity criterion, the gene pool performance ranked in 12 resistance and susceptibility classes to spring frost. The two selected groups comprised five and 23 descendants, assigned by high and moderate tolerance in 2019. In the following season, assessing 246 progenies for bloom intensity led to the selection of 80 outstanding hybrids. The earliness traits showed a high promotion of the appearance of new classes of earliness. The ripening time distribution of the bearing progenies consisted of six categories, namely, four extra-early (June 5 to 17), 18 far-early (June 22 to 30), 22 very early (July 2 to 9), 21 very early-early (July 11), nine early plus to control (July 19), and six progenies (July 25). Subsequent pomological and sensory analyses led to the choice of 18 progenies for high fruit set, well-formed fruits, different colors, and superior organoleptic qualities. The results authenticated the efficiency of breeding methods, supported by heritability and morphological markers to predict the earliness traits.

Keywords: Apple (*Malus* × *domestica* Borkh.), breeding, heritability, earliness traits, spring cold tolerance, morphological markers, phenological stages, fruit setting

Key findings: Promoting earliness traits were associated with large fruit size, red color, and apple acceptability. Five promising apple progenies with spring cold tolerance, late bloom-early, and dual-purpose continued for release.

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INTRODUCTION

Considering climate change, emerging pests, and economic challenges in the fruit industry worldwide, breeders have prioritized the release of resistant and high-yielding apple cultivars (CVs) (Hjalmarsson and Tomic, 2012; Laurens *et al.*, 2018). Since 2002, Iran has undertaken significant projects focused on producing early-ripening and high-yielding apple CVs with enhanced quality. Similar breeding programs for improvement in apple production have also ensued in Belgium (Røen *et al.*, 2000) and China (Gao *et al.,* 2011).

Researchers have shown the extraordinary competence of morphological markers as screening methods (Gasi *et al*., 2010; Hajnajari, 2010). An extended flowering period facilitates reliable pollination; however, it further prolongs the vulnerability of flowers to chilling stress. Therefore, a shorter flowering period has become an escape defense mechanism (Eccher and Hajnajari, 2006). The breeding programs for early apple cultivar development proceeded in two distinct stages. At the first stage, the breeding, centered on improving early-mid CVs, commenced in 2002, producing 7,200 full-sib and half-sib progenies. The 720 pre-selected apple progenies based on morphological markers underwent transplanting into the first selection plots (Chashnidel and Hajnajari, 2012).

In this study, the entire 246 hybrids earlier screened through marker-assisted selection bore investigation for cold hardiness, bloom and ripening phenology, and morpho-pomological characteristics. Setting apart 80 out of 246 promising progenies as the most promising helped determine the apple tree's productivity. Past studies also demonstrated extensive research about cold tolerance in apples (*Malus domestica* Borkh.) (Wisniewski *et al.,* 2015; Liang *et al.,* 2020). In the studied apple progenies, the spring cold damage varies, leading to classifying progenies into 12 classes of resistance, where the top four resistance classes contained 15 hybrids with the highest performance.

The 17 early-ripening but late-blooming progenies attained the desirable fruit size, peel color, organoleptic characteristics, and high consumer acceptance. In 2020, the ripening phenology revealed a significant overlap with the breeding target for earliness, expanding it to six different classes with extra early, far early, very early, very early-early, early, and early-mid ripening. The 18 early and high-performance selected progenies passed to the adaptation trials in eight provinces countrywide. In most apple breeding programs, few commercial parental genotypes have been utilized (Sestras and Sestras, 2023). Therefore, the presented study tried to accentuate the role of endemic CVs for earliness traits with the desired results, taking advantage of the germplasm formed on the Silk Road (Gharaghani *et al*., 2009).

MATERIALS AND METHODS

Plant materials

In the second stage of apple cultivar (CV) breeding, the target focused on estimating heritability values for promoting earliness and productivity traits of the 520 progenies in the first selection plot. Based on their previous study, the six apple CVs, i.e., Sheikh Ahmad, HeidarZadeh, Soltani Shabestar, Ardebil1, Gala, and Golden Spur, became the choice maternal parents. Precisely, 330 half-sib progenies were choices for screening the 5,000 half-sib offsprings, aged 1-2 years, using morphological markers for earliness at the juvenile stage. The rooted seedlings, transplanted in the second selection plot, had no replicates, with the number of progenies reduced to 246 due to adverse conditions up to 2010.

The earliness traits, i.e., blooms beginning, full bloom, bloom end, ripening time, and bloom intensity, reached appraising (Table 1). Additionally, fruit yield per tree, 34 pomological attributes, and sensory analyses continued based on the Apple Descriptors in

Table 1. Pheno-morphological abbreviation of apple fruit attributes, biochemical, and sensory traits progenies.

Trait	Abbrev.	Trait	Abbrev.	Trait	Abbrev.
Bloom beginning	BB	Stalk hole depth	ST.hd	Color intensity	Cl.int
Bloom end	BE	Stalk hole width	ST.hw	Color type	Cl.Ty
Full bloom	FB	Eye depth	EY.D	Flush	Flu.
BB end	BBe	Eye width	EY.W.	Strip width	ST.Wd
Bloom intensity	B.Int	Flesh firmness	Fl.Fir	Stalk Hole Russet	ST.H Ru
Days after bloom end	DAFB	Total soluble solids	TSS	Cheek Russet	CH.Ru
Flowering dens. % at FB	FS1	Titratable acidity	TA	Lenticel umber	LE.N
Fruit set % 10 DAFB	FS2	Acidity	pH	Lenticel size	LE.Si
Fruit set % at June drop	FS3	Fruit size	Si	Flesh color	FLE.CL
Fruit set % at RT	FS4	Fruit shape	Sh	Carpels space	CA.SP
Ripening time	RT	Eye ribs	ER	Fragrance	Fra.
Fruit length	F. LE	Eye size	ESi	Flavor	Fla.
Fruit diameter	F. DI	Peel grass	Pegr	Sweetness	Swe.
F. Length/F. Diameter	FLE.F.DI	Peel wax	Pewx	Sourness	Sou.
Fruit weight	F.WE	Peel thickness	Peth.	Juiciness	Jui.
Stalk thickness	ST.thi	Background color	Bg.cl	Flesh hardiness	FlEH.
Stalk length	ST.Le	Over color ratio	OCLR	Acceptability	Accep.

2019–2020 (Watkins and Smith, 1982). The second selection plot contained 246 half-sib apple progenies of 10 to 11-year-old trees, with drip irrigation, spaced 3 m × 3 m, then grown in spindle-training form. The orchard sits at the Meshkin Dasht Station, Temperate Cold Fruit Research Center, Karaj, Iran (54° 50' N, 55° 35' E, and 1312 m alt.).

First season evaluation

With the unpredictable climatic conditions, vast gene pool, and no replications, the mentioned apple experiments ran annually. In mid-February 2018, an unusual temperature increase broke tree dormancy, resulting in a precocious bloom. Subsequently, the temperature dropped to -3 °C in a cloudless sky. On March 08, 2019, the severe cold stress damaged the progenies at various flowering stages. A day later, another unexpected temperature drop to -5 °C occurred. In such circumstances, the resistance level measurement was according to the crop unit's maintenance at each biological growth stage up to ripening. During the pre- and post-stress phases, the data recording emerged on the variables, i.e., bloom beginning, full bloom, bloom end, bloom intensity, flowering period, fruits, and fruit number. A few tolerant apple progenies underwent analysis of 30 pomological traits, including the pH, titratable acidity, total soluble solids measurements, and panel tests.

Second season evaluation

Phenology survey

In 2020, a comprehensive phenological appraisal through various earliness parameters (bloom beginning, full bloom, bloom end, bloom intensity, flowering period, and ripening) progressed on all apple progenies belonging to the six diverse progeny families. All the collected data served to classify the apple progenies based on their bloom beginning, ranging from very early to very late flowering. Reciprocal pollinizer determination helped rank the germplasm through a modified classification for ripening. Comparative analysis of the data related to bloom beginning and ripening time led to identifying the dual-purpose apple progenies: late bloom-early ripening.

Plant productivity

Assessing the bloom intensity at full bloom, attained by the ratio of canopy size/bloom intensity ranked into ten varying decimal classes, determined the productivity potential

Table 2. The progenies used for multivariate analyses.

Number	Name	Number	Name	Number	Name
1	L1G10	18	L5G12	35	L8G31
2	L1G12	19	L5G28	36	L8G32
3	L1G16	20	L5G31	37	L9G2
4	L1G19	21	L6G21	38	L9G6
5	L1G20	22	L6G23	39	L9G10
6	L1G28	23	L6G24	40	L9G19
7	L2G10	24	L6G28	41	L9G21
8	L2G12	25	L6G29	42	L9G30
9	L2G15	26	L7G4	43	L9G31
10	L2G16	27	L7G19	44	L10G11
11	L2G22	28	L7G23	45	L10G18
12	L2G23	29	L8G4	46	L10G21
13	L2G30	30	L8G8	47	L10G25
14	L3G15	31	L8G9	48	L10G29
15	L4G21	32	L8G11	49	L10G30
16	L4G24	33	L8G16		
17	L4G25	34	L8G21		

of the progenies. The top two classes (90%–100% and 70%–89%) encompassed 80 of the 246 progenies. The 80 outstanding apple hybrids gained further evaluation for fruit set percentage (FSP). Six flowering shoots per tree, totaling 480, became choices, with flower buds counted as the first stage of the fruit set (FS1). Ten days after bloom end (DAFB) recorded fruitlet numbers at FS2. Fruit number per shoot was visible after the biological stages of the June drop (FS3) and at ripening time (FS4).

Pomological traits

The 34 morpho-physical traits, including fruit weight, flesh firmness, shape, color (background and over color, type, and hue), length and diameter, peel thickness, wax, stalk, eye characteristics, and relative hole details, were measured on 10 samples per apple progeny. Biochemical trait measurements occurred on total soluble solids, titratable acidity, and pH. The final selection of fruit acceptability, succeeded by sensory analysis through aroma, taste (sweet, sour, and sour-sweet), juiciness, and flesh hardness, as assayed by expert panelists. The principal component analysis (PCA) and cluster analyses progressed on 49 superior hybrids for productivity, fruit diameter, and acceptability to assess the similarities among the apple hybrids based on their quantitative and qualitative traits (Table 2).

Statistical analysis

The experimental layout had a factorial arrangement using a completely randomized design. The analysis of variance (ANOVA) and means comparison engaged Duncan's Multiple Range Test (DMR) using the SPSS software (Duncan, 1955). The other statistical analysis performed used the R-software.

RESULTS AND DISCUSSION

Cold resistance in apple progenies

The unusual increase in temperature in mid-February 2018, followed by a severe frost in March 2019, resulted in significant crop losses of the mature hybrid progenies. However, the damage varied among the genotypes, leading to the classification of progenies into 12 classes of resistance (Table 3). The top four resistance classes contained 15 hybrids with the highest performance. Progenies L8G16Y and L2G18 were remarkable, with 100% fruit yield per tree (Y/tr), and L5G10Y, L5G18Y, L5G11Y, and L8G14Y, with 95% Y/tr attained "Very High Resistant" and "High Resistant" classification in the 1st and 2nd classes, respectively.

Table 3. The apple progenies distributed in 12 different resistance classes to spring frost in 2019.

Level of Tolerance	Cropping %	Number of Progeny	The Resistance/Sensitivity Levels
Maximum resistance	100	2	L8G16Y, L2G18Y
High resistance	95	4	L5G10Y, L5G18Y L5G11Y, L8G14Y
Good resistance	90	8	L2G21Y, L3G20Y, L3G24Y, L3G28Y, L3G29Y, L10G18Y, L9G21Y, L6G22
Normal resistance	85	1	L7G24Y
High tolerance	80	22	L1G13Y, L1G15Y, L2G9Y, L2G19Y, L2G20Y, L3G6Y, L3G14Y, L3G22Y, L4G20Y, L4G26Y, L4G27Y, L5G12Y, L5G15Y, L6G19Y, L6G23Y, L6G26Y, L7G16Y, L8G23Y, L9G1Y, L9G19Y, L9G31Y, L10G18Y
Normal tolerance	70	21	L1G21Y, L1G28Y, L2G12Y, L3G21Y, L3G25Y, L3G30Y L5G5Y, L5G6Y, L5G19Y, L5G26Y, L5G20Y, L6G15Y, L6G28Y, L6G30Y, L8G11Y, L8G18Y, L9G4Y, L9G9Y, L10G14Y, L10G19Y, L10G24Y
Semi tolerance	60	26	L1G3Y, L2G14, L2G28Y, L3G9Y, L4G24Y, L4G21Y, L4G12Y, L5G13Y, L5G22Y, L6G8Y, L6G13Y, L7G6Y, L7G10Y, L7G14Y, L7G19Y, L7G25Y, L7G30Y, L8G9Y, L8G22Y, L8G31Y, L9G3Y, L9G16Y, L9G24Y, L9G27Y, L10G26Y, L10G32Y
Semi-sensitive	50	19	L1G12Y, L2G15Y, L2G24Y, L3G11Y, L4G22Y, L4G18Y, L4G5Y, L6G5Y, L6G11Y, L6G12Y, L6G14Y, L7G9Y, L8G19Y, L8G25Y, L8G28Y, L9G2Y, L9G10Y, L10G23Y, L10G27Y
Sensitive	40	21	L1G19Y, L2G17Y, L3G19Y, L4G29Y, L5G2Y, L5G21Y, L5G24Y, L5G28Y, L6G27Y, L7G18Y, L7G20Y, L7G29Y, L8G4Y, L8G26Y, L8G27Y, L9G12Y, L9G26Y, L9G28Y, L10G31Y, L10G11Y, L10G7Y
Very sensitive	30	23	L2G25Y, L2G29Y, L1G18Y, L3G15Y, L4G16Y, L5G4Y, L5G9Y, L5G16Y, L5G27Y, L5G25Y, L5G17Y, L6G17Y, L6G21Y, L7G4Y, L7G27Y, L7G28Y, L8G15Y, L8G17Y, L9G22Y, L9G13Y, L9G17Y, L9G20Y, L9G6Y
High sensitive	20	25	L1G29Y, L2G30Y, L3G10Y, L3G13Y, L3G16Y, L3G23Y, L4G14Y, L4G17Y, L5G29Y, L6G3Y, L6G4Y, L6G16Y, L6G20Y, L6G24Y, L6G29Y, L7G22Y, L7G26Y, L7G29Y, L7G31Y, L8G8Y, L8G30Y, L9G15Y, L9G18Y, L9G25Y, L10G29Y
Maximum sensitive	5-10	33	L1G9Y, L1G14Y, L1G17Y, L2G13Y, L2G16Y, L2G22Y, L2G26Y, L3G7Y, L3G17Y, L3G26Y, L3G27Y, L4G9Y, L5G7Y, L6G9Y, L6G18Y, L7G1Y, L7G2Y, L7G11Y, L7G12Y, L7G21Y, L7G23Y, L8G7Y, L8G20Y, L8G21Y, L9G14Y, L9G29Y, L9G30Y, L10G8Y, L10G13Y, L10G20Y, L10G21Y, L10G22Y, L10G30Y

Other eight showed "Good Resistance" in the 3rd class, with progeny L7G24Y recorded with 85% Y/tr, occupying the 4th position as "Normal Resistance." The 5th class comprised 22 apple hybrids as highly tolerant, exhibiting 80% Y/tr. (Table 3, Figure 1). Other genetic attributes, such as short-flowering and secondary-flowering periods, were visible in 20 of 108 apple CVs in Karaj, Iran, mostly considered different escape mechanism types (Eccher and Hajnajari, 2006). Pfleiderer *et al.* (2019) reported that climatic events could enhance the risk of frost damage to apple orchards by up to 10% when the temperature rises by 2 °C.

Progenies productivity

The relationship between the apple progenies and their respective maternal parents was considerable. Heritability estimates facilitate whether an individual's phenotype can predict parental performance (Currie, 2000). The latest study assessed inter- and intra-relationships between the apple parental genotypes and their progenies and evaluated cold resistance based on Yield/tree (percentage). Out of the 12 half-sib progenies of the maternal Soltani Shabestar, they had a good bearing of tasteful fruits, i.e., four, five, and three descendants exhibited low,

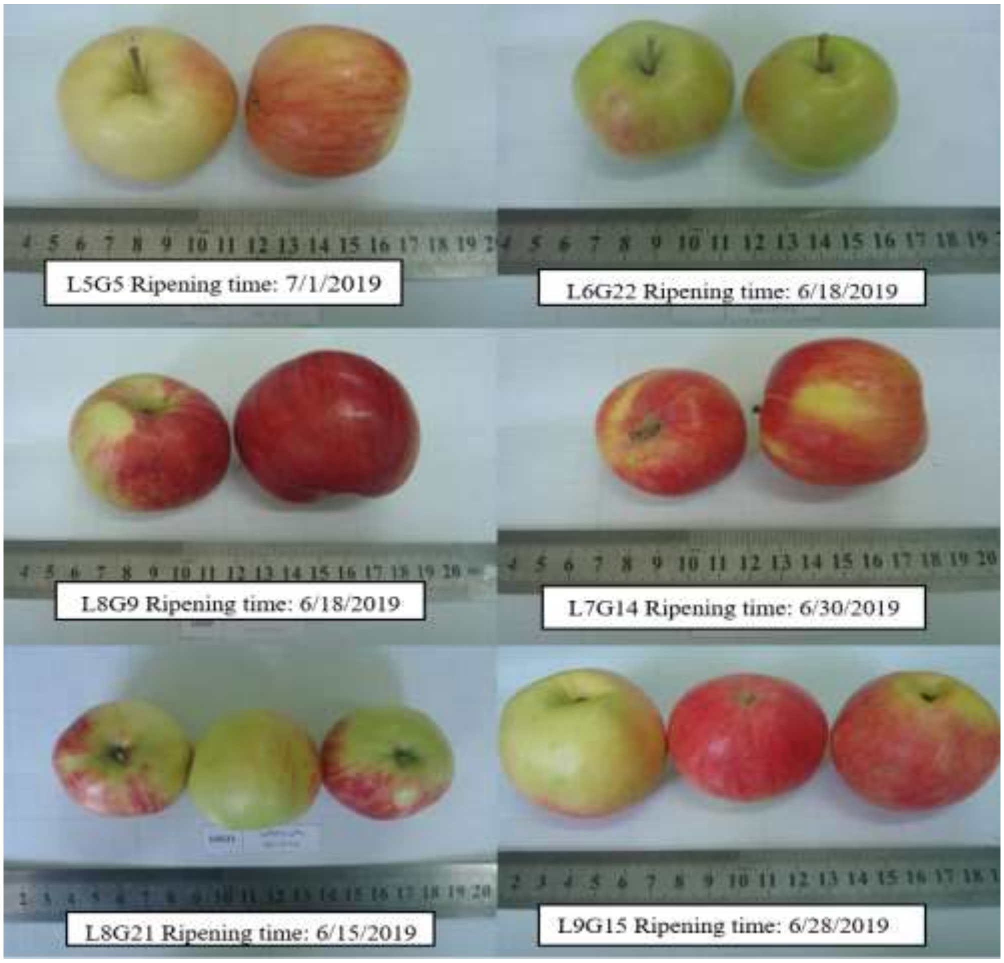

Figure 1. Appearance of some of the selected apple progenies with different levels of cold tolerance in 2019.

moderate, and high productivity, respectively (Figure 2). Among the 25 progenies of the heavy cropping genotype, Sheikh Ahmad emerged with cylindrical fruits, with the 11 and two apple progenies ranked with moderate and high productivity. Similarly, 30 descendants of the moderately cropping CV Heidarzadeh with large red fruits yielded 11 and 19 progenies with moderate and low-productive genotypes.

Remarkably, the total absence of high-performance genotypes confirmed the significant influence of the maternal parents in the productivity potential. The exceptional fruit quality of the CV Heidarzadeh makes it an ideal candidate for hybridization with high-yield apple CVs (Figure 2). Nevertheless, the influence of foreign pollens on the productivity behavior of half-sib progenies may have negligible retention. Seven and four high-cropping progenies (Code 3) belonged to the apple genotypes Soltani Shabestar and Sheikh Ahmad (Figure 2). The existing 38 weak cropping (Code 1) explains the irregular productivity distributions (Figure 2). Past studies reported similar correlation and co-heritability of tree performance among 13 apple genotypes for fruit number per tree (Srivastava *et al.*, 2012). The transmission of traits from the maternal parent to the progenies can refer to cytoplasmic and genetic-cytoplasmic heritability (Lorenzetti *et al.,* 1980).

In breeding programs, using half-sib progenies is time-saving and cost-effective, provided that appropriate parent selection transpired (Kumar *et al.,* 2020; Motyleva *et*

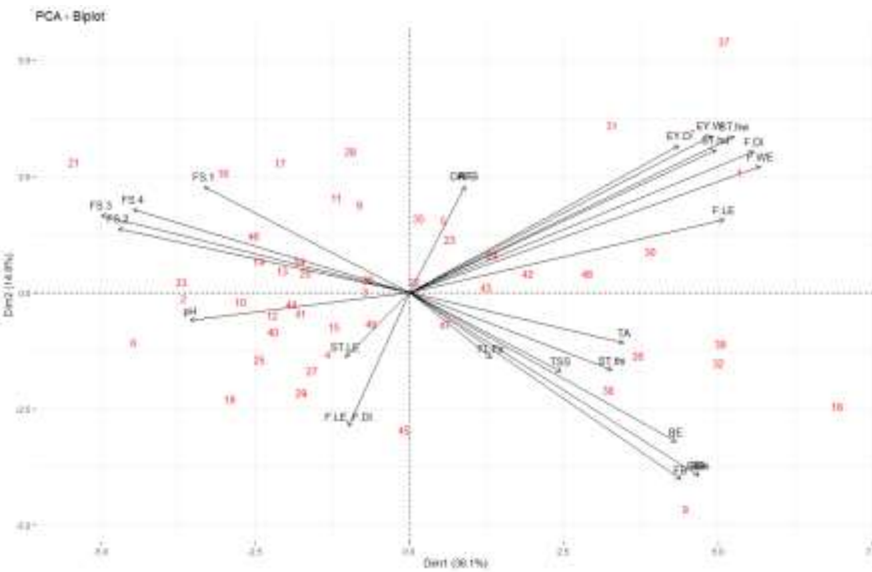

Figure 2. Biplot of the principal component analysis (PCA) based on quantitative variables evaluated in 49 apple progenies. The listed names of traits and progenies appear in Tables 1 and 2.

al., 2021). However, genetic factors, temperature fluctuations, and site interactions influenced the apple traits, bloom beginning, bloom period, and ripening time (Legave *et al.*, 2015). The genetic components exhibited a variation range of 26 days for bloom beginning - from March 21 to April 16, with a rare subgroup of 17 late bloom-early progenies. However, it also showed the genetic contrast between late-flowering and high-yield performance. The aggregation of 132 hybrids in the early ripening time class would have resulted from narrow-sense heritability and additive variance (Lorenzetti *et al.,* 1980). The limited number of Apple maternal parents led to high selection pressure. Despite the influence of year and site on bloom beginning, the sequence and order of the phenomenon remained constant within the half-sib gene pool, being CV-dependent.

In five full-sib apple families, exploring the genetic determinism of flowering behavior used Markovian and linear models. Meanwhile, regularity was apparent in continuous and predictable flowering, with the occasional irregularity attributed to high annual shoot synchronism (Durand *et al.,* 2017; Upadyshev, 2022). The selection of promising apple hybrids attained collation by considering available attributes, such as flowering phenology, earliness, tree productivity, and fruit quality data from the 80 half-sib progenies. The classification facilitates the identification of apple's single hybrids that possess the desired traits. The results identified 15 apple progenies with very early flowering, 38 early, 10 mid, and four with late flowering (Table 4).

Flowering period

Within the early-flowering apple group, a subgroup with 34 hybrids passed from bloom beginning to bloom end over five days (21 to 25 March), where 15 progenies (44.1%) demonstrated the highest FSP at 10 DAFB. A maximum of 38 progenies initiated bloom beginning on 29 March. Within the 132 progenies, 38 (28.8%) exhibited the utmost FSP.

Comparison of progenies to control for earliness

The evaluation of ripening time phenology revealed a significant overlap with the breeding target for earliness traits in 2020. A modified classification of ripening time was distinct

Table 4. Classification of 246 apple progenies based on ripening time in 2020.

Earliness class	Ripening time	Progeny	Number of hybrids	Fruit set % at harvest	Fruit diameter (mm)	Acceptability
Extra early	June 4	L3G24	1	6.667	45.5	-
	June 11	L2G27	1	7	50.981	-
	June 16	L1G28, L9G19	2	29.5, 8.667	44.996, 44.274	85, 81.67
Far early	June 22	L2G30	1	18.167	48.366	36.67
	June 24	L2G9, L2G16, L2G17, L4G21, L8G31, L9G21	6	5.167, 9.333, -, 12.5, 13.333, 15.5	-, 45.293,-, 47.752, 54.215, 48.451	-, 50, -, 75, -, 41.67
	June 26	L2G23	1	13	47.004	73.33
	June 27	L1G12, L3G15, L8G28	3	27.5, 11.333, 9.5	45.969, 51.778, 43.8	63.33, 61.67, -
	June 28	L2G15	1	9.833	53.036	68.33
	June 30	L4G24, L4G25, L5G28, L6G21, L8G16, L10G11,	6	17.167, 22.5, 13.167, 33.333, 27.833, 1.5	48.406, 50.386, 40.332, 48.772, 43.52, 45.38	63.33, 46.67, 71.67, 75, 78.33, 56.67
Very early	July 2	L6G23, L8G4, L10G32	3	11.833, 3.667, 5.33	49.876, 41.329, -	80, 48.33,-
	July 4	L1G10, L7G28, L7G29, L8G8, L8G9, L8G11, L8G21, L8G32, L9G10, L10G21, L10G25	11	6.5, 5.167, 4, 9.833, 12.5, 3.833, 11.167, 7.667, 1.167, 17, 3.167	62.119, -, 52.538, 56.609, 61.757, 53.598, 48.28, 48.862, 58.395, 44.031, 51.939	83.33, - و-, 73.33, 80, 75, 43.33, 41.67, 65, 70, 43.23
	July 6	L5G2, L5G31, L7G23, L9G31, L10G27, L10G29	6	7.5, 8.167, 11.667, 2, 1.167, 3.42	54.452, 47.605, 54.879, 54.964, -, 57.551	78.33, 68.33, 53.33, 61.67, -, 55
	July 7	L6G30	1	11.967	39.089	-
	July 9	L6G27	1	11.667	43.726	-
Very early-early	July 11	L10G18, L10G19, L10G30, L1G16, L1G3, L2G10, L2G12, L2G22, L5G10, L5G5, L6G18, L6G24, L6G28, L6G29, L7G19, L8G14, L9G23, L9G29, L9G30, L9G4, L9G6	21	7.833, 7.167, 5, 11.333, 15.5, 8.333, 9.423, 17.5, 15.684, 2.667, -,4, 14.333, 9.5, 13.333, 9.432, -,-,8.333,- ,4.833	45.646, -, 48.459, 47.848, -, 60.922, 53.105, 49.615, 56.621, 49.689, 48.224, 55.977, 55.088, 46.232, 42.589, 49.343,-, 54.468, 54.455, 50.718, 53.538	51.67, -, 55, 48.33,-, 78.33, 50, 70, 60, 63.33, -, 51.67, 45, 46.67, 53.33, 53.33, -, -, 68.33, -, 56.67
Early	July 19	L10G31, L1G19, L2G24, L4G17, L5G15, L5G19, L5G21, L7G8, Control 'Golab Kohanz'	9	1, 7.833, 3,- , 7.64,- ,-, 4.322,-	57.944, 45.603, 59.863, 50.217, 55.905, -,-, 69.487, -	-, 50, -, -,50, -, -, 51.67, -
Mid-early	July 25	L1G20, L5G12, L5G13, L7G4, L9G12, L9G13	6	15.5 ,3.667,- ,5.167,- ,-	49.233, 60.849, -, 54.954, -, 59.915	48.33, -, -, 53.33, -, -

Underlined (-): high-performing hybrids.

compared with the Apple Descriptor (UPOV, 2005), expanding the earliness ranking to six classes (extra early, far early, very early, very early-early, early, and early-mid ripening) (Table 4). All 79 high-performance apple progenies fell into these six earliness subgroups with the complete exclusion of late-ripening descendants. For instance, the extra early apple hybrids, L3G24, L2G27, L1G28, and L9G19, reached harvest on June 4, 11, 16, and 21, respectively, with the last two progenies displaying acceptability high ratings of 85 and 82 scores. The distribution analysis of ripening time ranked the 12 hybrids in the very early class, maturing from June 22 to 28, while 20 hybrids in the early class ripened from June 30 to July 4.

The components of the early-mid and mid-ripening classes, comprising 19 and 14 apple hybrids, ripened between July 6–11 and July 19–25, respectively (Table 4). Importantly, none of the hybrids was notable in the mid-late, late, late-very late, and very late classes, indicating the success of the breeding strategies (Table 4). Comparing the mean ripening time of July 15 with the early native apple Golab Kohanz under the same environmental conditions at 1300 masl, 65 half-sib hybrids exhibited a privileged position in terms of earliness. The selected hybrids in the classes, i.e., extra early, very early, early, and early-mid, preceded the control by 40, 24, 16, and nine days, respectively, significantly promoting earliness traits. The remaining 14 mid-ripening apple progenies ripened two to 25 days later than the control. These results confirmed the effectiveness of the apple parents' selection based on heritability estimates and offspring screening through morphological markers (Koohneshin *et al.,* 2018). Tancred *et al.* (1995) also reported similar findings. They released the earliest apple CVs, i.e., Earlidel, Summerdel, and GB-63-43 Jonathan, emphasizing the high heritability of ripening time and the crucial role of selected parents in producing early apples. Therefore, in selection plots, the time-consuming assessments of progenies primarily aim to identify those with the highest performance under the existing environmental conditions, subsequently proceeding to regional adaptation trials (Table 3).

Dual-purpose hybrids

Efforts to develop spring cold-resistant apple CVs have proven unsuccessful (Rodrigo, 2000). Late-bloom CVs still became the safest solution against stress in various fruit species. Among the 79 evaluated apple progenies, the max and min bloom beginning dates were evident on March 21 in L3G24 and L2G27 and April 10 in L5G12, with a significant recorded interval of 21 days. Excluding 40 Julian progenies, 18 early bloom-beginning hybrids exhibited an ascending order of ripening time, from June 4 to June 30, 2020 (Table 5). For instance, the progenies, L7G28, L6G21, L4G23, and L1G28, exhibited bloom beginning on March 28, and ripened in June 16 and 24 and July 2 and 4, respectively. Progenies L1G10 and L2G9 exhibited bloom beginning on March 29, reaching harvest on July 4. The progenies L5G28, L2G9, and L10G25 exhibited bloom beginning on March 31 and ripening on June 24, 30, and July 14, respectively. Apple progenies L8G8 and L9G10 had bloom beginning–ripening time starting on April 2 and July 4 (Table 5).

A gradient of dual-purpose combinations of late bloom-early ripening time materialized, overrunning the control 'Golab Kohanz,' with a ripening time of July 30. Notably, late-bloom hybrids L9G12, L2G12, L7G8, and L5G12, from April 11 to 13, corresponded with Julian ripening times of 25, 11, 19, and 25, respectively (Table 5). Seventeen late-bloom beginning progenies had desirable fruit size, peel color, organoleptic characteristics, and high acceptance. Employing remote sensing systems can mitigate the risk stress associated with sensitive CVs to avoid insurance costs and crop losses (Zhu *et al.,* 2021). A proposed sequential phenological model could indicate an average flowering advancement of -1.6 ± 0.9 days every 10 days, leading to an earlier bloom onset in early April (Unterberger *et al.,* 2018). This study confirmed that utilizing collected data on tree growth habit, vigor, pheno-morpho-pomologies, and FSP of the bred progenies appears appropriate through classic breeding.

Fruit productivity potential

The FSP assessment, being a primary objective of breeding, is an accepted indicator of the tree-productivity potential. Based on the DMR test, the mean FSP evaluation for 80 apple progenies had the highest bloom intensity presented, i.e., the progeny L6G21 exhibited the utmost FSP (33.3%), followed by L1G12, L8G16, and L1G28 with 27.5% to 29.5% values. The apple progenies L2G30 and L4G25 had FSP values of 20.2% to 22.5%, with the lower values found in L4G24 (17.2%) and L4G17 (17.5%). However, the minimum FSP (8.33%) emerged in the progeny L5G31.

Distribution of progenies based on ripening time

Progeny distribution based on ripening time showed that fruit over the color of four extra-early hybrids started to appear from June 4 to June 16, while 18 far-early apple hybrids gained harvest between June 22 and June 30. The two classes of very-early and very-early-early consisted of 22 and 21 progenies, respectively, with the highest frequency. The other eight early hybrids matured on July 19, similar to the control, with the ripening time ranging from July 2 to July 9 and July 11, 2020. Only six apple progenies attained the early-mid ripening time classification four days after the control. Overall, 73 of 79 apple progenies were into the category of six classes of earliness occurring between June 4 and July 19, based on the modified ripening time classification (Table 4). The development of 21 extra and far-early progenies was evident in June, with a red peel, good size, and high organoleptic quality, representing an innovative achievement, besides several Julian hybrids.

Variability in over color

According to free pollination, the resulting half-sib progenies may receive various pollen sources in the collection. Meanwhile, the maternal part plays an influential role, enhancing the biological diversity, leading to apple progenies with increased variability in fruit color, over-color, size, and organoleptic characteristics. A smaller group had the 17 late cold-tolerant apples manifested with three full red and 10 semi-colored apples. The highest variability in over-color occurred among the 80 high-performance genotypes, with 11 red, 35 semi-red, 24 yellow, and one green. The influence of the apple CV Soltani Shabestar registered with a striped pink color in some of the progenies resembling the maternal part, with the yellow over-color and conical fruit shape of the genotype Sheikh Ahmad in more of its descendants.

Analysis of variance of pomological traits

Analysis of variance helped evaluate the flowering and ripening phenology traits (FSP, fruit diameter, length, and weight) and biochemical traits (total soluble solids, titratable acidity, and pH) for 71 of 80 apple progenies. Significant ($p \leq 0.01$) differences were distinct among the apple progenies for all studied traits, indicating a high level of genetic variability within the population. Despite the meaningful promotion of earliness traits, the analysis revealed differences in flowering phenology traits, FSP, June drop, and ripening time. The 68 half-sib apple progenies exhibited substantial genetic variations in precocity, late-flowering, fruit traits (diameter, length, shape, background color, and over-color), flesh firmness, biochemical characteristics, and tree vigor. Each apple fruit attribute, including color (Wang *et al.,* 2020), flesh firmness (Bejaei *et al.,* 2021), diameter and length (Li *et al.,* 2015), and total soluble solids (Kumar *et al.,* 2018), considerably determined the fruit quality.

Reflecting the reliable environmental conditions for all apple progenies for physical and biochemical fruit parameters, the observed significant differences can refer solely to the apple genotypes' genetic makeup. However, rootstock type, planting distance, and training systems can also influence these characteristics (Rotondi *et al.,* 2003). Cice *et* *al.* (2023) studied the morphological and physicochemical traits of 31 apple CVs. They reported the highest inter-varietal diversity, concluding a significant proportion of the variation in fruit weight and physicochemical characteristics. Previous research has also highlighted the importance of maternal selection in obtaining desired traits, such as red-flesh apple hybrids (Neumüller and Dittrich, 2017).

Multivariate analysis

The principal component analysis (PCA) proceeded for 25 quantitative traits of the apple. The generated biplot of 49 apple progenies on the first two principal components (PC) used the quantitative traits (Table 2, Figure 2). The biplot allows the visual identification of the most significant features based on their vector length and recognizes the apple progenies with the highest values for them. Additionally, the graphical representation of the angles between the vectors enunciates the correlation between the attributes, where acute and obtuse angles indicate positive and negative correlations, respectively. As shown in Figure 2, the correlation between traits depicted in the figure aligns with the results obtained from Pearson's correlation coefficient.

The PC1 provided a maximum variability of 36.1% of the total variation among the apple progenies. The variables fruit diameter, length, weight, and stalk-hole width made the optimal contributions to this component. The investigated apple progenies showed significant differences in terms of these attributes. The renaming of three promising progenies, L1G10 (1), L2G10 (7), and L8G9 (31), to Baharan, Sorkhin, and Nobaran, respectively, reached formal release as CVs in 2022, together with L9G2 (37), which displayed the maximum values for fruit weight, fruit diameter, and stalk-hole width. All gained a position in the upper right part of the biplot, away from the plot origin. The PC2 explained 14.8% of the total variability; the most crucial variables referred to the flowering phenology (full bloom and bloom beginning). Progenies located in the lower right portion and farther from the center exhibited those with later-bloom beginning.

The PCA results using qualitative traits showed that PC1 accounted for 22.5% of total variability. The key features contributed to this component were those related to sensory tests, including acceptability, flavor, juiciness, and flesh hardness. The PC2 explained 17.1% of trait variations in the studied apple progenies, i.e., flavor, color intensity, and over-color ratio were the most significant variables. Progenies L1G28, L6G23, L5G28, and L8G16 had better quality from others for sensory test attributes, including acceptability, flavor, flesh hardness, and juice attributes (Figure 3).

A cluster analysis ran on 49 apple progenies to examine their similarity, using qualitative traits, resulting in a dendrogram that classified the progenies into two distinct groups. The progenies' grouping depended on their degree of similarity in the investigated traits. As a result, classifying the progenies bore two main groups. The first cluster comprised 11 progenies characterized by late bloom and big fruit size, i.e., L7G4 (26), L9G9 (38), L2G12 (8), L5G12 (18), L9G2 (37), L8G11 (32), L8G9 (31), L1G10 (1), L2G10 (7), L8G8 (30), and L9G10 (39) (Figure 4). The second cluster contained two subgroups. The apple progenies in the first subgroup exhibited intermediate values for bloom phenology and fruit size. However, dispersion occurred for most apple progenies in the second subcluster. Distinction between the two main clusters appeared with differences in bloom phenology and fruit attributes, such as size, depth, length, and weight.

The first cluster comprised the progenies characterized by favorable taste, juicier and firmer flesh, and higher acceptability, including the apple progenies L1G10, L8G8, L2G10, L8G9, L8G11, L1G28, L5G28, L6G21, L10G21, L8G16, L4G21, L2G15, L9G2, L6G23, and L9G10. Additionally, these hybrids displayed fruit over-color and high-color intensity. The second cluster comprised two subclusters, with the first consisting of 21 hybrids and the lowest scores for over-color and color intensity.

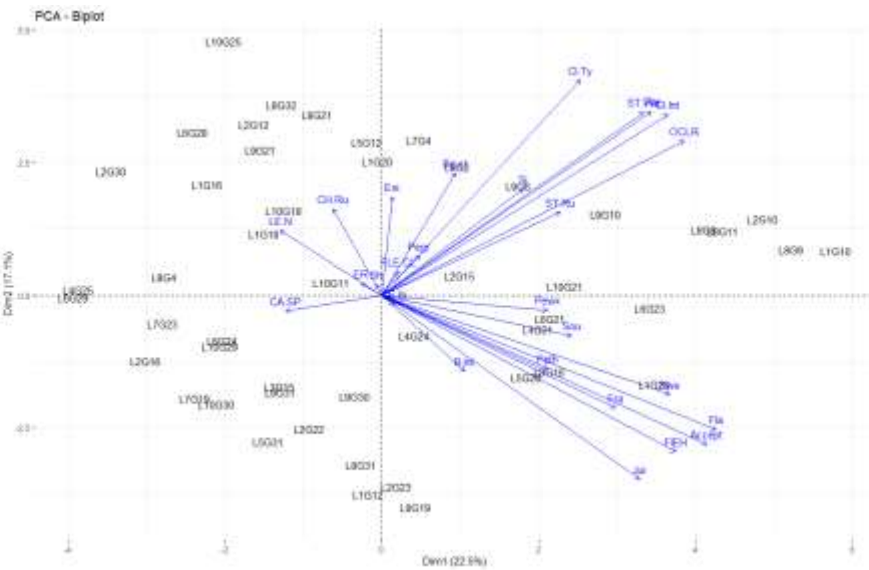

Figure 3. Biplot of the principal component analysis (PCA) based on qualitative variables evaluated in 49 apple progenies, with list of trait names in Table 1.

Selection of promising apple hybrids

The apple progenies' final selection depended on the chain of screening and selection using the collected field and laboratory data. The apple progenies sustained spring coldness at the top of the biological activity, such as bud swelling, flowering stages, and fruitlet formation. Nevertheless, the unexpected stress became an integral part of the breeding program as the resistance and susceptibility levels in 2019. The final selection focused on identifying the apple progenies in six advanced classes of earliness as highly demanded by the market. Four extra early hybrids, with a mean fruit diameter ranging from 44.27 to 50.98 mm and FSP values between 7 and 29.5, exhibited exceptional acceptability and outperformed by earliness.

Several exceptional apple hybrids were prominent within, i.e., extra-early— L1G28 (85) and L9G19 (81.67), far-early—L5G28 (71.67), L6G21 (75), and L8G16 (78.33), and very-early—L1G10 (83.33), L8G9 (80.75), L5G2 (78.33), L5G31 (68.33), L9G31 (61.67), and L6G23 (80). These apple hybrids exhibited high acceptability, satisfying FSP, exceptional organoleptic quality, and high yields (Table 4). More red and semi-red hybrids surpassed the control. Further selection continued in choosing the apple progenies L1G12, L2G15, L2G23, L4G21, L3G15, L4G24, L10G21, L6G23, L8G8, L5G31, L9G30, and L2G22 based on fruit shape and flesh firmness (Table 4). The same breeding objectives through 13 bi-parental hybridizations involving early, mid, and late apple CVs resulted in 36 families, transplanted onto their roots with no replicate, focusing solely on flesh texture, background, flesh color, and flavor and taste (Tancred *et al.,* 1995). Supply of firstlings is a psychologically sensitive endeavor, where a few days of earliness can be economically meaningful. Advanced earliness is given in a gradual crescent order of earliness from early (eight) to very-early-early (21), very-early (22), far-early (18), and extra-early (four) progenies. Therefore, supplying the crop could be as soon as eight, 17, 39, and 43 days earlier than the control.

CONCLUSIONS

The results concluded the selection of 18 promising apple genotypes comprising extra-early, far-early, and very-early, with spring cold tolerance. Five CVs out of 18 selected apple hybrids attained registration and release. The selection process relied on earliness, productivity, fruit size, flesh firmness, and overall acceptability through sensory analysis comprising aroma, flesh hardness, juiciness, taste, and flavor. Early apple CVs have limited water requirements, high demand in the market, reduced storage, higher nutritional value, and do not require pesticides against codling moth.

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