International Journal of Horticultural Science and Technology
Journal homepage: http://ijhst.ut.ac.ir

Influence of Improved Seedling Rootstocks on Pomological and Organoleptic Traits of Four Apple Cultivars

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ARTICLE INFO

Article history:

Received: 20 May 2021,
Received in revised form: 21 August 2021,
Accepted: 8 November 2021

Document type:

Research paper

Keywords:

Crab apple,
Fruit quality,
Genetic improvement,
Pomology,
Seed rootstock

ABSTRACT

The influence of five open-pollinated seedling rootstocks and two marketed seed lots (as the controls) were assessed on pomological, biochemical, and organoleptic traits of four apple cultivars (*Malus×domestica* Borkh.), grown in Meshkin Abad Horticultural Research Station (Karaj, Iran). The maternal parents, as the three crabs 'Zinati', 'Morabbaei', and 'Azayesh' along with the standard trees 'Northern Spy' and 'Golden Karaj' were selected as the seed sources through a breeding program in 2003. The present study was achieved on the 4- and 5-year-old trees, spindle formed, planted in 3.5×4 m, and drop irrigated in 28 combinations (four cultivars onto 5+2 rootstocks, distributed in four distinct parcels). The investigation was carried out on pomological traits based on UPOV scales, as well as biochemical and organoleptic traits. Higher fruit length, diameter, and weight were recorded for the cultivars grafted onto 'Northern Spy', followed by 'Zinati' and 'Morabbaei' seed sources. 'Zinati' F1 crab seedling induced higher flesh firmness combined with the four scions. The crab seedling rootstocks 'Zinati' followed by 'Azayesh' caused a higher organoleptic scent, flavor, and sweetness attributed by the panel members. Moreover, pH, TA, and TSS were influenced by scion, while rootstock effect was negligible. These results indicated that F1 half-sib seedling rootstocks positively influenced the pomological and organoleptic traits and improved the fruit's quality.

Introduction

Despite the widespread use of vegetative rootstocks globally, seed rootstocks are used under specific environmental and edaphic conditions. The superficial root system, lack of good anchorage, and incompatibility with heavy calcareous soil, along with unsuitable and uneven topography, as well as fragmented orchards, are the main restrictive factors of using vegetative rootstocks in Iran. Consequently, the growers have no choice other than the use of seed rootstocks (Saghafian Larijani et al., 2021).

Rootstocks have been used for propagating temperate fruit trees for more than 2000 years. In the mid-19th century, practically all rootstocks were raised from seeds obtained from indigenous wild populations of pome-fruits and stone-fruits. Nowadays, apple seeds used by nurseries are mainly from a mixture of native/local and imported commercial cultivars (mostly vigorous). In a genetic improvement

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DOI: 10.22059/IJHST.2021.324031.469

program for the production of genetically pure seeds, the maternal parents are selected based on a series of growth and productive traits. In this way, the orchards' lack of tree canopy uniformity will be reduced (Hajnajari, 2018).

Apple breeding is a long-term endeavour, and breeders have been eager to exploit the species' broad genetic and phenotypic diversity to fulfil consumer expectations for new cultivars (Hancock et al., 2008). Rootstocks selection is one of the most critical aspects of fruit quality. It has an essential role in orchard performance by influencing potential tree density, precocity, cropping efficiency (Daugaard and Callesen, 2002; Al-Hinai and Roper, 2004), ripening, storability, mineral composition, size, and firmness (Marini et al., 2002). Moreover, rootstocks improve fruit tree tolerance to environmental stress and control tree size (Webster, 2001). Rootstock can influence scion leaf and fruit mineral concentrations and indirectly affect fruit quality and yield (Fallahi et al., 2018). Other than rootstocks and varieties (genetic factors), environmental and agronomic factors are strongly influenced the final apple quality (Vanoli and Buccheri 2012; Serra et al., 2016; Musacchi and Serra 2018).

Fruit size, shape, and productivity are under polygenic control, implying that there will be a broad and continuous range of expression of all these characters in the seedlings when two cultivars are inter-crossed. The range of variation is related to the expression of the characters in the parents (Janick et al., 1996; Naschitz and Naor 2005; Iglesias et al., 2012). The control treatments, composed of open-pollinated seeds, belong to several cultivars, are recycled from processing industries, and are genetically impure. Open-pollinated seeds characterized by heterosis provoke tree vigour as an undesirable factor in modern fruit growing. The high genetic variability of impure seeds induces as many hormonal packages as the number of used seedlings, leading to the least possible uniformity of the propagated trees. The awful economic consequences will reflect in yield components.

Fruit quality is assessed by its appearance (taste, aroma, crunchiness, firmness, color, size, shape, and absence of defects) and then by its eating quality (Abbott et al., 2004; Talluto et al., 2008; Musacchi and Serra, 2018). These attributes are evaluated by utilizing a trained sensory panel or instrumentally measured (Brookfield et al., 2011). However, some apple characteristics assessed via trained sensory analysts can vary significantly from instrumental predictions (Hoehn et al., 2003; Harker et al., 2006). Therefore, the latest developments in the field of sensory evaluation and instrumental analysis lay further emphasis on the interaction between instrumental analysis and sensory attributes, such as hardness, crispness, and crunchiness (Barreiro et al., 1998; Harker et al., 2002; Mehinagic et al., 2004; Ioannnides et al., 2007; Ross, 2009). Generally, researchers have attempted to measure flesh firmness, soluble solid content (SSC), and titratable acidity (TA) to define fruit quality (Hoehn et al., 2003).

Several studies have revolved around the quality of many fruits through instrumental and sensory analyses (Allegra et al., 2015; Sortino et al., 2015; Gentile et al., 2016), and particularly on apples (Karlsen et al., 1999; Donati et al., 2006; Skendrović Babojelić et al., 2007), as apple orchard profitability relies upon producing high yields of marketable fruit. Since fruit size and yield are essential, rootstock researchers regularly record yield, average fruit weight, and other fruit quality attributes (Janick et al., 1996; Naschitz and Naor, 2005).

Research on existing cultivars suggests that there is a strong consumer preference for apples with exceptional firmness, sweet to acidic taste, crispness, and juiciness (Hampson et al., 2000; Cliff et al., 2015). These sensory fruit attributes present primary targets for consumer-focused plant breeding in apples (Amyotte et al., 2017).

For this study, through a 15-year breeding program, 108 apple cultivars were evaluated and classified based on dwarfism, tolerance to leaf chlorosis, high fruit set, seed set, long blooming period, and high percent of flowering density. Finally, the five apple cultivars/genotypes were opted and used as maternal seed sources. Once the seeds (obtained from the selected maternal parents) germinated and developed to seedlings, the four scions were grafted onto them. The present investigation was held at the latest phase.

The main goals of this investigation were selecting the best seed rootstock sources by examining the effects of F1 half-sib seedling rootstocks on grafted commercial cultivars' pomological and organoleptic traits, as well as a comparative study of the self-compatible ('Morabbaei' and 'Zinati') with the self-incompatible seed rootstocks treatments.

Materials and Methods

Plant materials

This study was carried out in Meshkin Abad Horticultural Research Station (35°45'04.5 "N 50°57'17.4"E) in Alborz province, affiliated to the Temperate Cold Fruit Research Centre, Iran.

The open-pollinated seed lots were collected from selected maternal parents, including self-compatible Morabbaei ('Mor') and Zinati ('Zin'), self-incompatible Azayesh ('Aza'), all three dwarf cultivars with long flowering period and high flowering density, as well as Northern Spy ('NS') (carrying woolly aphid resistance gene) and Golden Karaj ('GoK') genotype, as the main treatments, along with the controls (Thesis 1 ('T1') as the 1st and Thesis 2 ('T2') is the 2nd quality seed source in commerce) (Fig. 1-9).

Fig. 1. The maternal parent 'Morabbaei' as the self-compatible crab seed source treatment. Characteristics: very dwarf, self-compatible, long flowering period, high flowering density, high seed set, and high fruit set

Fig. 2. The maternal parent 'Zinati' as the self-compatible crab seed source treatment. Characteristics: very dwarf, self-compatible, long flowering period, high flowering density, high seed set, and high fruit set

Fig. 3. The maternal parent 'Azayesh' as the crab seed source treatment.
Characteristics: dwarf, self-incompatible, long flowering period, high flowering density, medium seed set, and medium fruit set.

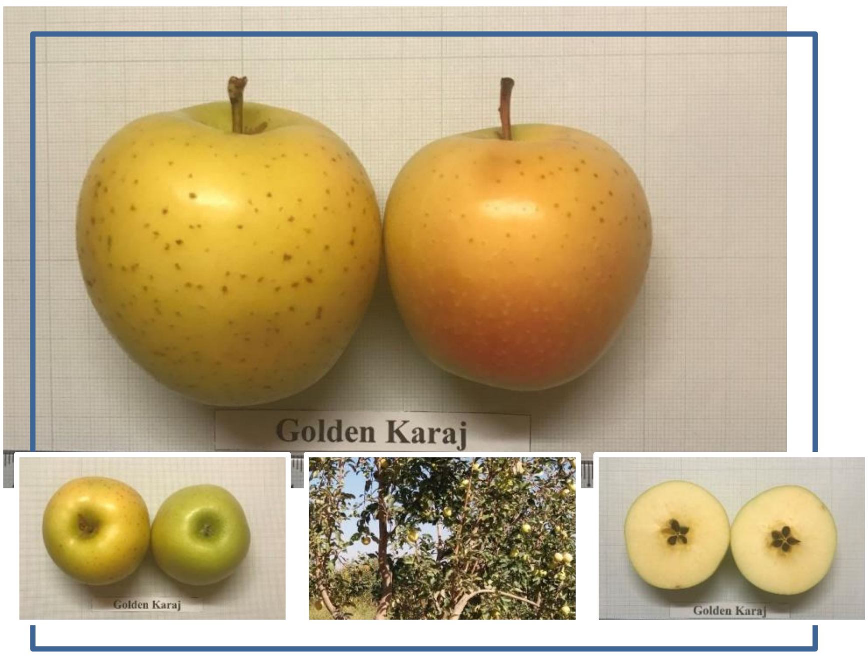

Fig. 4. The maternal parent 'Golden Karaj' as the seed source treatment.
Characteristics: medium-high vigour, low self-compatibility, excellent flowering density, excellent seed set, and excellent fruit set.

Fig. 5. The maternal parent 'Northern Spy' as the seed source treatment.
Characteristics: standard vigor, self-incompatible, carrying woolly aphid resistance gene, parent of vegetative rootstocks (MM111 and MM106), and very good fruit set.

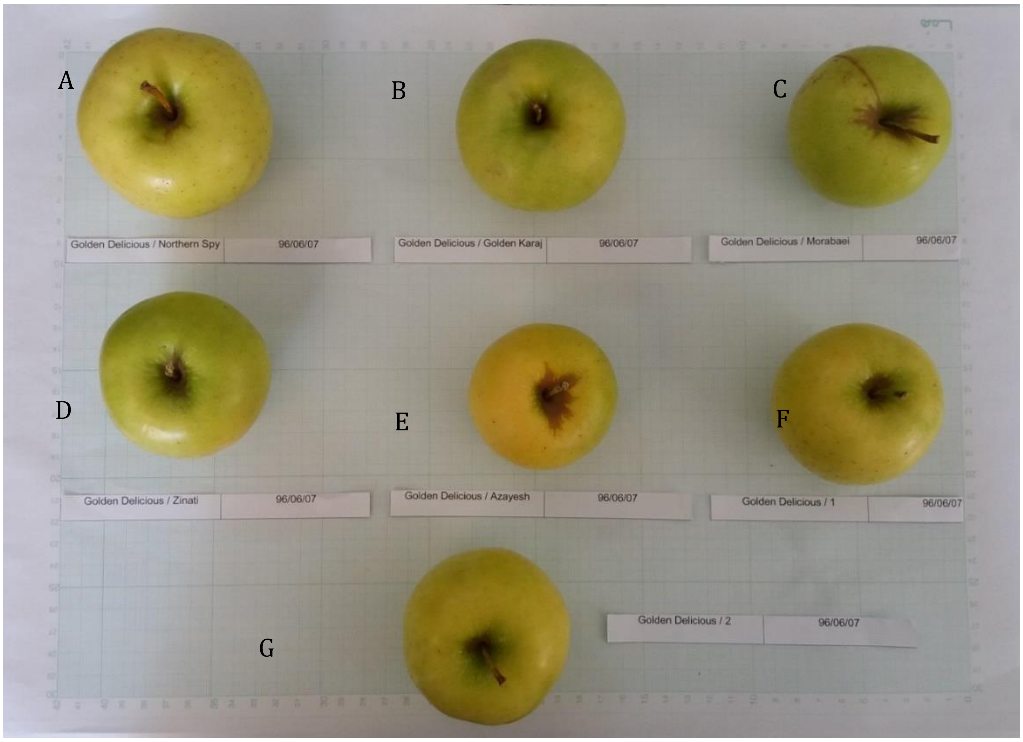

Fig. 6. Golden Delicious grafted onto the selected seed rootstocks.
A: Northern Spy, B: Golden Karaj, C: Morabbaei, D: Zinati, E: Azayesh, F: Thesis 1, G: Thesis 2.

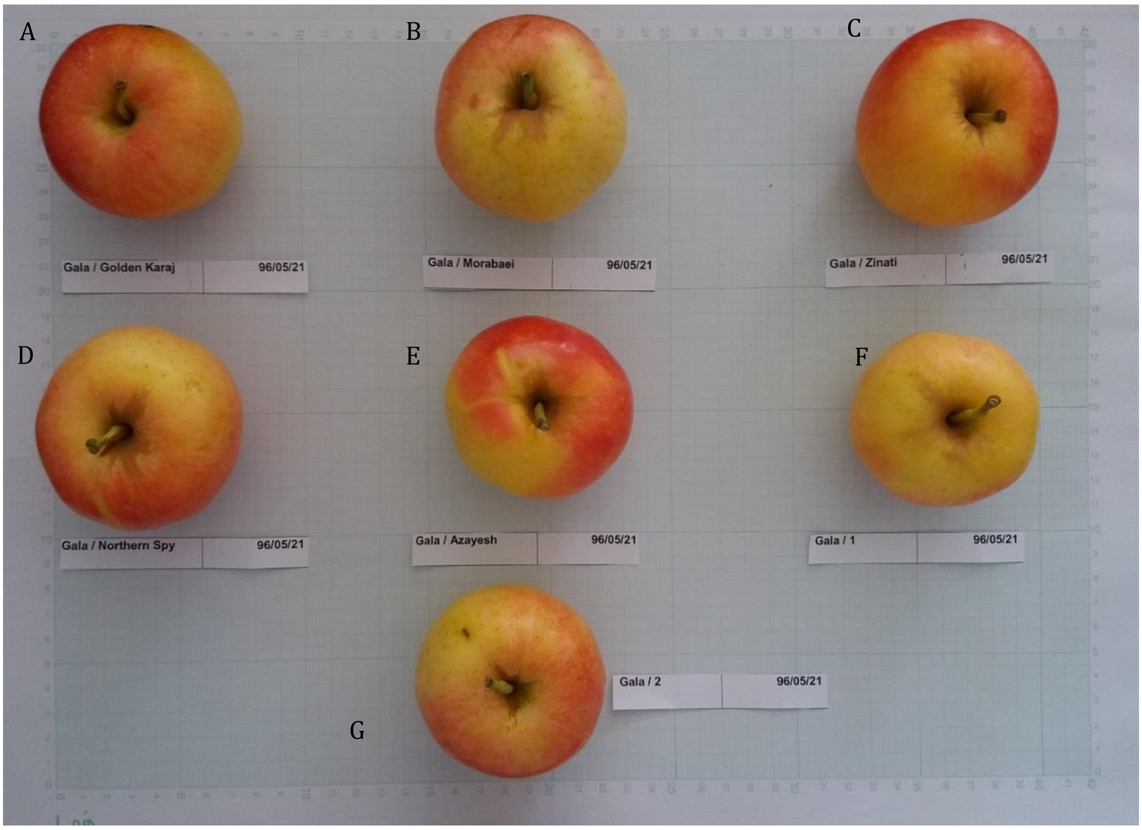

Fig. 7. Gala grafted onto the selected seed rootstocks.
A: Golden Karaj, B: Morabbaei, C: Zinati, D: Northern Spy, E: Azayesh, F: Thesis 1, G: Thesis 2.

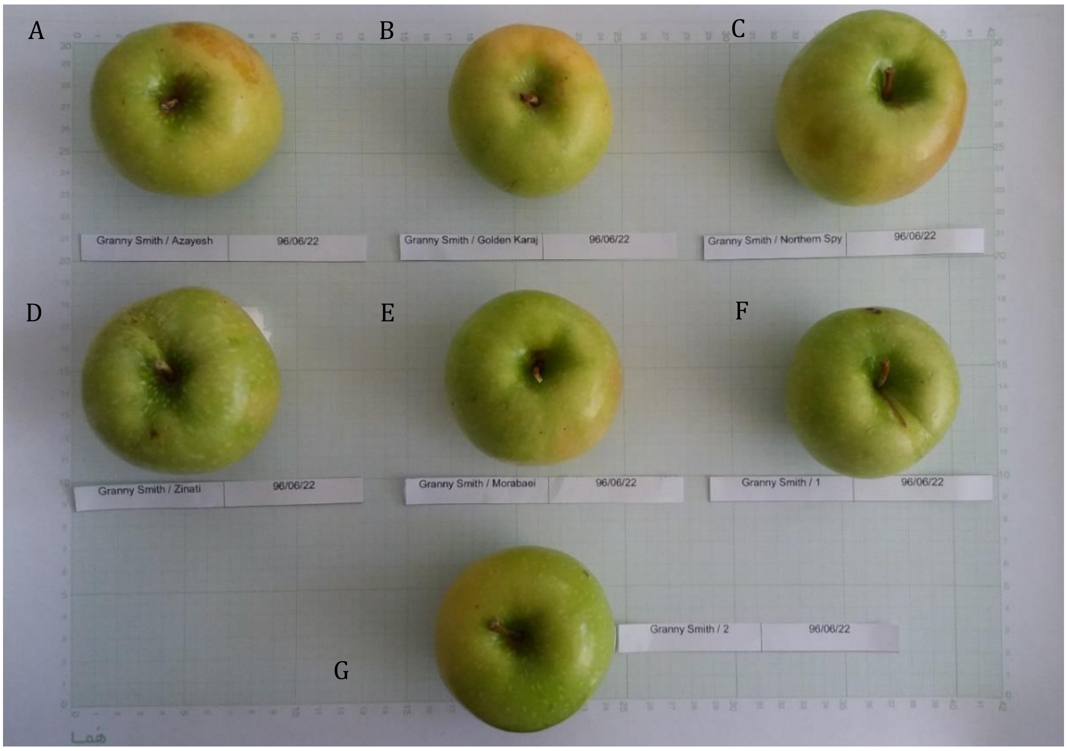

Fig. 8. Granny Smith grafted onto the selected seed rootstocks.
A: Azayesh, B: Golden Karaj, C: Northern Spy, D: Zinati, E: Morabbaei, F: Thesis 1, G: Thesis 2.

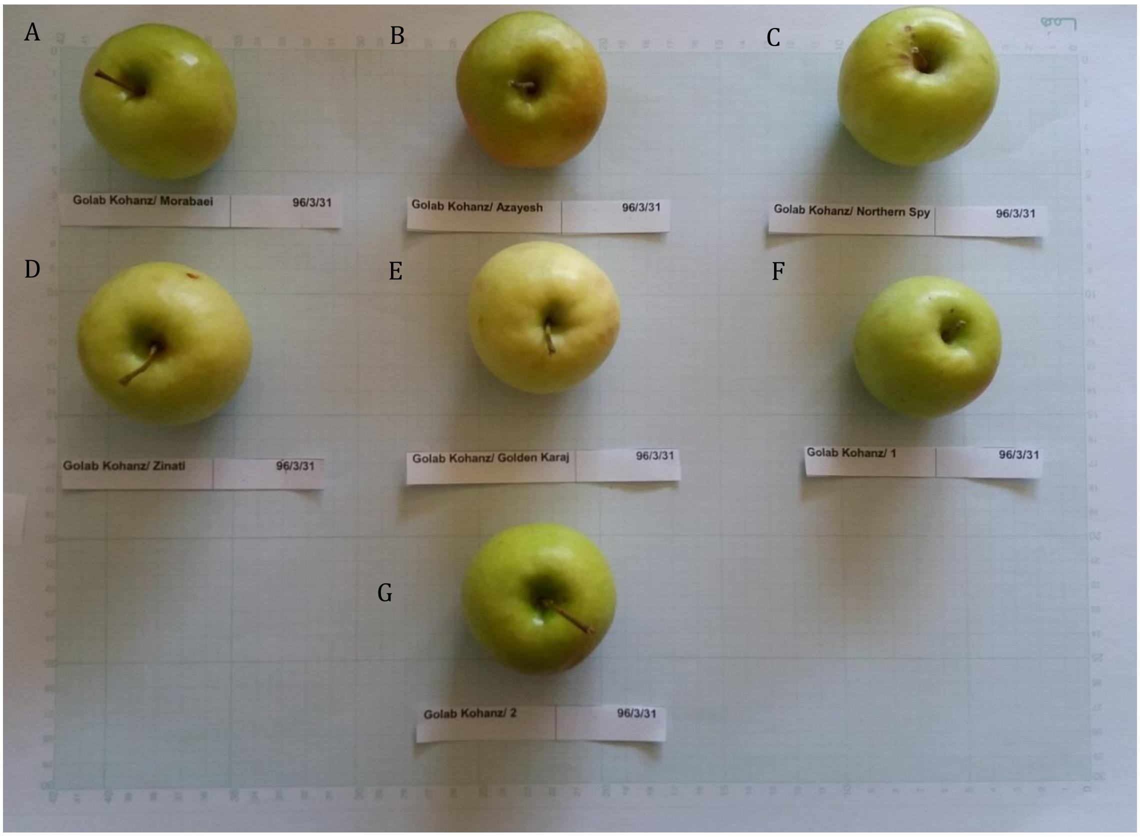

Fig. 9. Golab Kohanz grafted onto the selected seed rootstocks. A: Morabbaei, B: Azayesh, C: Northern Spy, D: Zinati, E: Golden Karaj, F: Thesis 1, G: Thesis 2.

The 4- and 5-year-old trees of early very vigorous Golab Kohanz ('GK'), the mid-late semi-vigorous Golden Delicious ('GD'), vigorous mid-ripen Gala ('GA'), and the late mid-vigorous Granny Smith ('GS'), planted in 3.5×4 m and pruned in spindle form, were established in the large experimental orchard (~1 ha).

The trees were distributed in four distinct parcels (nests), each nest containing a single cultivar on 7 (5+2) seedling treatments. Per combination, three accidental replicates were planned with numerous units, varied from 12 to 24 (Table 1). Fruits per cultivar were harvested at the ripening stage, representing a wide range of harvest time from late August to mid-June (Table 2). Then, they were placed in cold storage to lower fruit susceptibility to internal breakdown. The seed treatments were assessed for their inductive effects on pomological and organoleptic traits. All of the examined Scion×Rootstock combinations reached the standards for the edible quality of apples.

Table 1. The number of Scion×Rootstock combinations in each nest

Nest (Scion)	Rootstock						
	Morabbaei	Zinati	Azayesh	Golden Karaj	Northern Spy	T1	T2
Golden Delicious	15	23	30	22	24	23	23
Gala	16	34	30	27	23	18	18
Granny Smith	14	28	19	18	15	23	24
Golab Kohanz	13	25	26	25	16	30	34

T1 (Thesis 1) as the 1st and T2 (Thesis 2) is the 2nd quality seed source in commerce.

Note: the values represent the number of the different Scion×Rootstock combinations in each nest.

Table 2. Ripening time of the scions affected by climatic oscillations

Cultivar	2017							2018						
	RT	MiT	MaT	AT	MiRH	MaRH	ARH	RT	MiT	MaT	AT	MiRH	MaRH	ARH
Golden Delicious	26-Aug	15.4	32.9	25	12	72	33.25	5-Aug	22.1	39.5	31	11	58	29
Gala	12-Aug	17.5	34.5	26	17	77	40.63	6-Aug	20.1	38.6	29	13	73	39.13
Granny Smith	6-Sep	18.1	36.3	28	8	43	21	14-Aug	16.8	35.3	25	13	68	42.5
Golab Kohanz	10-Jun	15.4	35.7	28	12	59	29.25	26-Jun	17.7	36.3	28	13	62	26.88

RT: ripening time; MiT: minimum temperature (°C); MaT: maximum temperature (°C); AT: average temperature (°C); MiRH: minimum relative humidity; MaRH: maximum relative humidity; ARH: average relative humidity (Meteorological traits obtained from Karaj Agricultural Station-99373).
Note: the values show different meteorological traits of the ripening time (day) for each cultivar.

Pomological and biochemical assessments

Ten fruits per 28 Scion×Rootstock (S×R) combinations were randomly selected. They were assessed for pomological characteristics, including fruit length and diameter, length to diameter ratio, stalk length and diameter, stalk cavity depth and width, eye cavity depth and width, fruit weight, and flesh firmness. A small skin area was removed from the equatorial region on the least blushed side of the fruit by a hand Penetrometer (Fruit-Tester, Penetometro, Italy) as apple fruits have variable sensory properties so that one side can differ from the other (Dever et al., 1995). Additionally, the biochemical properties of fruit extracts, such as TSS (Master Reflectometer, Atago, Canada), TA, and pH, were measured.

Sensory assessments

Five assessors of the Temperate Fruit Research Centre carried out the sensory assessments (aged 25-45 years) who are generally available for sensory assessment panels. A week before the study's onset, the assessors took part in training sessions to be familiarized with the reference standards and provide feedback on scoring of apple samples to develop consistency among assessors. Five fruits per 28 S×R combinations were randomly distributed among the panellists. The evaluation was performed in three days, ensuring that assessors were not overwhelmed or fatigued by the number of samples needed to be tasted. The first author, who was not engaged in the tasting procedure, arranged the samples and coded them for presentation to diminish the assessors' prior knowledge of the samples. The organoleptic traits and their definitions are presented in Table 3.

Table 3. Organoleptic traits and their definitions

Trait	definition
Odour/Scent/Aroma	Volatile compounds, including esters, aldehydes, ketones, alcohols, hydrocarbons, etc.
Taste/Flavor	Combination of acids, sugars, tannin, and aromatic substances.
Sweetness	One of the basic tastes (e.g., sucrose).
Sourness	One of the basic tastes (e.g., citric acid).
Juiciness	Amount of juice released on mastication.
Hardness/Firmness	Amount of force required to bite entirely through a sample placed between teeth.
Skin Thickness	Amount of force needed to bite through apple skin.

Data analysis

Factorial experiment was conducted in a Completely Randomized Block Design (RCBD) in each nest. Analysis of variance (ANOVA) was performed by SAS software (version 9.1) and mean comparisons by Duncan's test.

Results

Pomological traits

Significant scion and rootstock effects (except flesh firmness) and S×R interactions (except length/diameter ratio) were observed for all pomological traits (Table 4). Amongst the scions, 'GK' had the lowest fruit length (51.32 mm). On the other hand, the highest fruit diameter and length/diameter ratio belonged to 'GA' (69.83 mm) and 'GD' (0.93). Furthermore, the longest stalk was seen in 'GD' and 'GA'. The highest stalk diameter, stalk cavity depth, stalk cavity width, eye cavity width, and fruit weight (155 g), all belonged to 'GA'. Flesh firmness and eye cavity depth were higher in 'GD' (6.49 kg cm^2) and 'GK', respectively (Table 5).

Table 4. Analysis of variance of the pomological traits

S.O.V	DF	Fruit length (mm)	Fruit diameter (mm)	Length/diameter ratio	Stalk length (mm)	Stalk diameter (mm)	Stalk cavity depth (mm)	Stalk cavity width (mm)	Eye cavity depth (mm)	Eye cavity width (mm)	Flesh firmness (kg cm^2)	Fruit weight (g)
Block	9	150.18**	229.69**	0.0025^{ns}	57.29^{ns}	0.2^{ns}	12.36**	18.17**	3.5**	10.93**	0.45^{ns}	7767.88**
Scion	3	2002.58**	1922.74**	0.0344**	2106.7**	16.1**	315.21**	316.84**	16.36**	144.15**	37.38**	67645.92**
Rootstock	6	237.76**	221.91**	0.0062*	274.34**	0.4472**	10.27**	30.56**	5.47**	16.89**	0.4^{ns}	5896.19**
Scion× Rootstock	18	101.23**	105.64**	0.0033^{ns}	130.41**	0.3549**	7.69**	28.4**	3.31**	25.13**	1.68**	2713.72**
Error	243	14.48	13.44	0.0022	31.12	0.1141	2.61	2.74	1.09	3.01	0.36	312.1
(%) CV		6.41	5.57	5.26	22.19	14.44	14.79	9.15	18.32	9.26	10.47	14.04

The P-value for each trait is indicated with its significance (ns: non-significant; *: $P<0.05$; **: $P<0.01$).
S.O.V: source of variation; DF: degrees of freedom; CV (%): coefficient of variation (%).

Table 5. Mean comparisons of pomological traits (Scions)

Scion	Fruit length (mm)	Fruit diameter (mm)	Length/diameter ratio	Stalk length (mm)	Stalk diameter (mm)	Stalk cavity depth (mm)	Stalk cavity width (mm)	Eye cavity depth (mm)	Eye cavity width (mm)	Flesh firmness (kg/cm^2)	Fruit weight (g)
Golden Delicious	62.21^{a}	66.93^{c}	0.93^{a}	29.79^{a}	2.39^{b}	11.7^{b}	18.92^{b}	5.32^{b}	17.69^{c}	6.49^{a}	130.93^{b}
Gala	61.72^{a}	69.83^{a}	0.88^{c}	27.93^{a}	2.97^{a}	13.56^{a}	19.86^{a}	5.68^{b}	20.82^{a}	5.67^{c}	155.003^{a}
Granny Smith	62.11^{a}	68.3^{b}	0.91^{b}	17.35^{c}	2.16^{c}	9.33^{c}	18.61^{b}	5.39^{b}	18.45^{b}	5.98^{b}	135.48^{b}
Golab Kohanz	51.32^{b}	58.14^{d}	0.88^{c}	25.47^{b}	1.83^{d}	9.08^{c}	15.01^{c}	6.38^{a}	17.93^{bc}	4.75^{d}	81.91^{c}

Means of columns followed by similar letters are not significant based on Duncan's test.

Within the seed rootstock treatments, 'NS' induced the highest fruit length (62.36 mm) and diameter (68.62 mm), whereas the highest length/diameter ratio was recorded for 'Zin' (0.92). Stalk length was the longest in 'T2' and the shortest in 'Zin'. The highest and lowest stalk diameter were recorded for 'Zin' and 'T1', respectively. 'NS' treatment caused the highest value for stalk cavity depth and width, as well as eye cavity depth. 'Mor' and 'T2' both showed the highest eye cavity width. Regarding fruit weight, 'NS' (142.15 g) and 'GoK' (106.31 g) showed the highest and lowest values (Table 6).

The 'GS-Zin' combination (68.99 and 73.46 mm) showed the highest fruit length and diameter. The longest stalk length was recorded for the 'GD-GoK'. 'GA' performed the highest stalk diameter and stalk cavity depth on all of the selected rootstocks. The maximum stalk cavity width, eye cavity depth, and eye cavity width were observed in 'GD-NS', 'GK-Aza', and 'GA-Zin'. In addition, the combination of 'GD-NS' (7.12 kg/cm^2) and 'GA-NS' (172.84 g) demonstrated the highest flesh firmness and fruit weight (Table 7). Such fascinating results related to 'NS' seedling treatment may be attributed to the woolly aphid resistance genes carried by the maternal source and F1 progeny, causing the improved use of carbohydrates for fruit growth in the absence of pest damage.

Table 6. Mean comparisons of pomological traits (Rootstocks)

Rootstock	Fruit length (mm)	Fruit diameter (mm)	Length/diameter ratio	Stalk length (mm)	Stalk diameter (mm)	Stalk cavity depth (mm)	Stalk cavity width (mm)	Eye cavity depth (mm)	Eye cavity width (mm)	Fruit weight (g)
Morabbaei	60.52^{bc}	67.35^{ab}	0.8972^{bc}	23.57^{c}	2.33^{b}	10.93^{b}	18.17^{b}	5.51^{bc}	19.18^{a}	131.85^{bc}
Zinati	61.61^{ab}	66.88^{b}	0.9222^{a}	20.53^{d}	2.51^{a}	10.73^{b}	18.15^{b}	5.94^{ab}	18.99^{ab}	133.97^{b}
Azayesh	59.08^{c}	66^{b}	0.8952^{bc}	24.34^{bc}	2.38^{ab}	10.98^{b}	17.27^{c}	5.98^{ab}	18.72^{ab}	124.65^{c}

Rootstock	Fruit length (mm)	Fruit diameter (mm)	Length/diameter ratio	Stalk length (mm)	Stalk diameter (mm)	Stalk cavity depth (mm)	Stalk cavity width (mm)	Eye cavity depth (mm)	Eye cavity width (mm)	Fruit weight (g)
Golden Karaj	56.07^{d}	61.76^{d}	0.9075^{abc}	26.28^{ab}	2.26^{bc}	10.17^{b}	16.79^{c}	5.38^{c}	17.45^{c}	106.31^{e}
Northern Spy	62.36^{a}	68.62^{a}	0.909^{ab}	25.67^{bc}	2.35^{b}	11.88^{a}	19.49^{a}	6.28^{a}	19.14^{ab}	142.15^{a}
T1	56.31^{d}	63.56^{c}	0.885^{c}	26.96^{ab}	2.17^{c}	10.77^{b}	18.26^{b}	5.45^{c}	18.32^{b}	114.41^{d}
T2	59.43^{c}	66.44^{b}	0.893^{bc}	28.59^{a}	2.36^{ab}	10.97^{b}	18.58^{b}	5.31^{c}	19.26^{a}	127.47^{bc}

Means of columns followed by similar letters are not significant based on Duncan's test.
T1 (Thesis 1) as the 1st and T2 (Thesis 2) is the 2nd quality seed source in commerce.

Table 7. Scion×Rootstock interactions (Pomological traits)

Rootstock	Scion	Fruit length (mm)	Fruit diameter (mm)	Stalk length (mm)	Stalk diameter (mm)	Stalk cavity depth (mm)	Stalk cavity width (mm)	Eye cavity depth (mm)	Eye cavity width (mm)	Flesh firmness (kg/cm^2)	Fruit weight (g)
Morabbaei	Golden Delicious	65.44^{abcd}	71.1^{abc}	29.57^{bcde}	2.4^{cdef}	12.75^{bc}	18.88^{cd}	4.69^{hi}	17.43^{hij}	5.92^{efg}	150.96^{bcd}
	Gala	61.51^{defg}	69.28^{bcd}	25.88^{cdef}	3.07^{a}	12.28^{bcd}	19.04^{cd}	5.46^{bcdefghi}	19.4^{defg}	6.07^{cdefg}	152.53^{bcd}
	Granny Smith	64.7^{bcd}	71.62^{abc}	17.5^{hij}	2.06^{fghi}	9.97^{efghi}	20.37^{bc}	5.93^{bcdefg}	20.79^{abcd}	6.05^{defg}	146.99^{bcde}
	Golab Kohanz	50.45^{kl}	57.39^{hi}	21.35^{fgh}	1.81^{ij}	8.73^{hijk}	14.4^{g}	5.95^{bcdefg}	19.11^{defgh}	4.9^{klm}	76.92^{jk}
Zinati	Golden Delicious	62.58^{cdef}	66.07^{de}	24.91^{def}	2.58^{bcd}	10.48^{efg}	19.11^{cd}	5.33^{cdefghi}	18.29^{fghi}	6.85^{ab}	127.62^{fg}
	Gala	62.77^{cdef}	71.55^{abc}	27.46^{cde}	3.06^{a}	14.48^{a}	21.28^{b}	6.41^{bc}	22.3^{a}	6.25^{cdef}	164.35^{ab}
	Granny Smith	68.99^{a}	73.46^{a}	15.49^{ijk}	2.4^{cdef}	10.03^{efghi}	19.84^{bc}	5.9^{bcdefg}	18.94^{efghi}	6.07^{cdefg}	164.6^{ab}
	Golab Kohanz	52.12^{jkl}	56.46^{i}	14.25^{jk}	2.02^{ghij}	7.95^{jk}	12.39^{h}	6.14^{bcde}	16.45^{jk}	4.5^{mn}	79.31^{jk}
Azayesh	Golden Delicious	60.42^{efg}	64.78^{ef}	28.22^{bcde}	2.21^{efgh}	11.55^{cde}	16.94^{ef}	5.88^{bcdefg}	17.42^{hij}	6.65^{abcd}	118.72^{gh}
	Gala	59.14^{fgh}	69.59^{bcd}	26.54^{cdef}	3.08^{a}	13.27^{ab}	19.17^{cd}	5.26^{defghi}	20.4^{bcde}	5.45^{ghijk}	149.88^{bcd}
	Granny Smith	61.83^{defg}	67.99^{cde}	18.48^{hij}	2.24^{defgh}	8.72^{hijk}	17.23^{ef}	4.84^{ghi}	17.82^{ghij}	5.92^{efg}	131.6^{efg}
	Golab Kohanz	54.95^{ij}	61.64^{fg}	24.14^{efg}	2^{ghij}	10.37^{efgh}	15.73^{fg}	7.93^{a}	19.26^{defg}	4.95^{klm}	98.4^{i}
Golden Karaj	Golden Delicious	56.28^{hi}	60.96^{gh}	35.8^{a}	2.11^{fghi}	10.82^{def}	16.65^{ef}	5.11^{efghi}	15.4^{k}	6.67^{abc}	100.88^{i}
	Gala	62.69^{cdef}	70.06^{abc}	29.29^{bcde}	2.88^{ab}	13.82^{ab}	20.13^{bc}	5.67^{bcdefgh}	20.31^{cde}	5.3^{hijkl}	156.3^{abc}
	Granny Smith	55.07^{ij}	59.18^{ghi}	11.33^{k}	2.08^{fghi}	7.5^{k}	15.55^{fg}	5^{fghi}	15.11^{k}	5.92^{efg}	92.24^{ij}
	Golab Kohanz	50.23^{kl}	56.85^{i}	28.7^{bcde}	1.98^{ghij}	8.56^{ijk}	14.85^{g}	5.75^{bcdefgh}	18.98^{defghi}	4.85^{klm}	75.82^{jk}
Northern Spy	Golden Delicious	67.06^{ab}	71.66^{abc}	30.31^{bcd}	2.61^{bc}	13.5^{ab}	23.14^{a}	6.23^{bcde}	19.07^{defgh}	7.12^{a}	155.63^{abcd}
	Gala	64.42^{bcd}	72.46^{ab}	28.56^{bcde}	3.14^{a}	14.62^{a}	20.37^{bc}	5.79^{bcdefgh}	21.29^{abc}	5.12^{ijkl}	172.84^{a}
	Granny Smith	66.19^{abc}	72.65^{ab}	19.16^{ghij}	2.24^{defgh}	10.29^{efgh}	19.69^{bc}	6.56^{b}	18.95^{efghi}	5.82^{fgh}	160.73^{abc}
	Golab Kohanz	51.77^{jkl}	57.7^{hi}	24.65^{def}	1.4^{k}	9.11^{ghijk}	14.75^{g}	6.53^{b}	17.24^{ij}	4.8^{lm}	79.4^{jk}
T1	Golden Delicious	64.14^{bcde}	69.36^{bcd}	28.26^{bcde}	2.49^{cde}	11.23^{cde}	20.57^{bc}	5.28^{cdefghi}	19.97^{cdef}	6.52^{bcde}	144.39^{cdef}
	Gala	58.72^{gh}	66.26^{de}	29.81^{bcde}	2.52^{cde}	13.14^{ab}	18.85^{cd}	5.52^{bcdefghi}	20.04^{cdef}	5.92^{efg}	137.35^{def}
	Granny Smith	53.91^{ijk}	62.53^{fg}	18.53^{hij}	1.98^{ghij}	9.44^{fghij}	17.86^{de}	5^{fghi}	17.74^{ghij}	5.97^{efg}	103.09^{hi}
	Golab Kohanz	48.47^{l}	56.08^{i}	31.24^{abc}	1.7^{j}	9.26^{fghij}	15.75^{fg}	5.99^{bcdef}	15.53^{k}	4.17^{n}	72.8^{k}
T2	Golden Delicious	59.57^{fgh}	64.56^{ef}	31.47^{abc}	2.32^{cdefg}	11.58^{cde}	17.17^{ef}	4.73^{hi}	16.28^{jk}	5.67^{fghi}	118.28^{gh}
	Gala	62.81^{cdef}	69.65^{abcd}	28.01^{cde}	3.06^{a}	13.34^{ab}	20.21^{bc}	5.65^{bcdefgh}	22.03^{ab}	5.57^{ghij}	151.77^{bcd}
	Granny Smith	64.07^{bcde}	70.65^{abc}	20.94^{fghi}	2.14^{fghi}	9.4^{fghij}	19.72^{bc}	4.51^{i}	19.82^{cdef}	6.07^{cdefg}	149.11^{bcd}
	Golab Kohanz	51.29^{jkl}	60.89^{gh}	33.94^{ab}	1.93^{hij}	9.56^{fghij}	17.22^{ef}	6.37^{bcd}	18.93^{efghi}	5.07^{jklm}	90.72^{ij}

Means of columns followed by similar letters are not significant based on Duncan's test.
T1 (Thesis 1) as the 1st and T2 (Thesis 2) is the 2nd quality seed source in commerce.

Organoleptic traits

The organoleptic traits were significantly affected by scion, rootstock, and S×R interactions (except sourness and juiciness; not affected by rootstock and Scion×Rootstock, respectively) (Table 8).

'GA' remarkably registered the highest scent, flavor, sweetness, juiciness, flesh hardness, and overall acceptance. However, as expected, the least sweetness, the most sourness, and skin thickness were recorded for 'GS' (Table 9).

Table 8. Analysis of variance of the organoleptic traits

S.O.V	DF	Scent	Flavor	Sweetness	Sourness	Juiciness	Flesh hardness	Skin Thickness	Overall acceptance
Block	4	273.1^{ns}	104.28^{ns}	186.53^{ns}	47.2^{ns}	114.84^{ns}	66.62^{ns}	219.94^{ns}	71.43^{ns}
Scion	3	1521.79**	2262.86**	18382.21**	15880.05**	7730.6**	8560.03**	24233.02**	3299.05**
Rootstock	6	808.98**	654.52**	961.12**	167.98^{ns}	631.88**	628.94**	587.78**	566.43**
Scion×Rootstock	18	308.79*	543.41**	362.83**	456.29**	210.82^{ns}	357.83*	365.19**	556.27**
Error	108	157.62	200.95	141.56	125.32	175.01	202.49	144.97	155.87
CV (%)		24.51	26.67	29.64	24.74	30.44	30.92	22.93	22.99

The P-value for each trait is indicated with its significance (ns: non-significant; *: P<0.05; **: P<0.01).
S.O.V: source of variation; DF: degrees of freedom; CV (%): coefficient of variation (%).

Table 9. Mean comparisons of the organoleptic traits (Scions)

Scion	Scent	Flavor	Sweetness	Sourness	Juiciness	Flesh hardness	Skin Thickness	Overall acceptance
Golden Delicious	47.71^{b}	46.29^{b}	42.71^{c}	37.57^{c}	37.23^{b}	46.57^{b}	47.14^{b}	50.29^{b}
Gala	60.86^{a}	64.57^{a}	59.43^{a}	54.06^{b}	65.71^{a}	64.29^{a}	51.71^{b}	66.86^{a}
Granny Smith	50^{b}	52.57^{b}	7.32^{d}	69.43^{a}	35.71^{b}	47.14^{b}	87.43^{a}	56^{b}
Golab Kohanz	46.34^{b}	49.14^{b}	51.09^{b}	19.95^{d}	35.18^{b}	26.06^{c}	23.73^{c}	44^{c}

Means of columns followed by similar letters are not significant based on Duncan's test.

The highest fruit scent, flavor, and sweetness were observed for 'Zin' and 'Aza'. In addition, 'Aza' registered the highest juiciness and flesh hardness, as well as the lowest skin thickness. Furthermore, the highest value of skin thickness was recorded for 'Zin' and 'T1'. Overall, 'Zin' and 'Aza' rootstocks were the favorite seedling treatments promoting panellists' general acceptability (Table 10).

The highest scent belonged to the combination of 'GS-Zin'. In addition, 'GS-Zin' and 'GS-Aza' had the best flavor and registered the highest sweetness. The combination of 'GS-Mor' had the highest sourness. 'GA' and 'GS' onto all rootstocks (except 'NS' for flesh hardness) performed the highest flesh hardness and skin thickness. Eventually, 'GA-Zin' and 'GS-GoK' combinations achieved the highest scores (Table 11).

Table 10. Mean comparisons of the organoleptic traits (Rootstocks)

Rootstock	Scent	Flavor	Sweetness	Juiciness	Flesh hardness	Skin Thickness	Overall acceptance
Morabbaei	48^{b}	50^{b}	32^{c}	37^{b}	42.5^{bc}	47^{bc}	52.5^{abc}
Zinati	61^{a}	60^{a}	49.45^{a}	45^{b}	46^{abc}	57.37^{a}	61^{a}
Azayesh	59^{a}	62^{a}	49.1^{a}	54.67^{a}	54^{a}	44.5^{c}	60^{a}
Golden Karaj	48^{b}	50^{b}	37^{bc}	43.65^{b}	38.75^{c}	51.95^{abc}	58^{ab}
Northern Spy	50.1^{b}	50.5^{b}	38^{bc}	39.5^{b}	51.35^{ab}	53.75^{ab}	50^{bc}
T1	43.5^{b}	46.5^{b}	33.9^{bc}	42.9^{b}	48.5^{abc}	60^{a}	47.5^{c}
T2	49^{b}	53^{ab}	41.5^{b}	41.5^{b}	41^{c}	52.95^{ab}	51^{bc}

Means of columns followed by similar letters are not significant based on Duncan's test.
T1 (Thesis 1) as the 1st and T2 (Thesis 2) is the 2nd quality seed source in commerce.

Table 11. Scion×Rootstock interactions (Organoleptic traits)

Rootstock	Scion	Scent	Flavor	Sweetness	Sourness	Flesh hardness	Skin Thickness	Overall acceptance
Morabbaei	Golden Delicious	36^{hi}	30^{h}	14^{gh}	26^{lmno}	26^{fg}	32^{fghi}	40^{fgh}
	Gala	58^{abcdef}	62^{abcd}	54^{abcde}	60^{cdefgh}	68^{a}	46^{efg}	64^{abcde}
	Granny Smith	58^{abcdef}	62^{abcd}	0.012^{h}	82^{a}	54^{abcd}	90^{ab}	64^{abcde}
	Golab Kohanz	40^{fghi}	46^{cdefgh}	60^{abcd}	15.2^{no}	22^{g}	20^{i}	42^{fgh}
Zinati	Golden Delicious	46^{defghi}	38^{fgh}	56^{abcde}	35^{jklm}	42^{cdefg}	48^{ef}	44^{fgh}
	Gala	68^{ab}	68^{ab}	68^{a}	47.5^{fghij}	68^{a}	60^{de}	76^{a}
	Granny Smith	72^{a}	74^{a}	9.8^{h}	70^{abcd}	52^{abcd}	94^{ab}	74^{ab}
	Golab Kohanz	58^{abcdef}	60^{abcde}	64^{ab}	29.8^{klmn}	22^{g}	27.5^{hi}	50^{defgh}
Azayesh	Golden Delicious	$56^{abcdefg}$	58^{abcdef}	55^{abcde}	38^{ijkl}	60^{abc}	44^{efgh}	64^{abcde}
	Gala	66^{abc}	66^{abc}	64^{ab}	45.2^{hijk}	70^{a}	54^{de}	70^{abc}
	Granny Smith	64^{abcd}	76^{a}	27.4^{fg}	62^{cdefg}	52^{abcd}	60^{de}	66^{abcd}
	Golab Kohanz	$50^{bcdefghi}$	48^{bcdefg}	50^{abcde}	17.8^{no}	34^{defg}	20^{i}	40^{fgh}
Golden Karaj	Golden Delicious	$48^{cdefghi}$	42^{defgh}	40^{ef}	30^{klmn}	42^{cdefg}	52^{de}	42^{fgh}
	Gala	66^{abc}	66^{abc}	58^{abcde}	60^{cdefgh}	54^{abcd}	56^{de}	70^{abc}
	Granny Smith	40^{fghi}	40^{efgh}	0.01^{h}	66^{bcde}	34^{defg}	80^{bc}	76^{a}
	Golab Kohanz	38^{ghi}	52^{bcdefg}	50^{abcde}	16^{no}	25^{fg}	19.8^{i}	44^{fgh}
Northern Spy	Golden Delicious	$50^{bcdefghi}$	52^{bcdefg}	44^{cdef}	34^{jklm}	50^{abcde}	44^{efgh}	54^{cdefg}
	Gala	60^{abcde}	66^{abc}	62^{abc}	52.5^{efghi}	62^{abc}	48^{ef}	66^{abcd}
	Granny Smith	38^{ghi}	38^{fgh}	0.01^{h}	80^{ab}	66^{ab}	94^{ab}	36^{gh}
	Golab Kohanz	$52.4^{bcdefghi}$	46^{cdefgh}	46^{bcde}	13.36^{o}	27.4^{fg}	29^{ghi}	44^{fgh}
T1	Golden Delicious	44^{efghi}	48^{bcdefg}	40^{ef}	54^{defghi}	60^{abc}	48^{ef}	52^{cdefgh}
	Gala	$52^{bcdefghi}$	58^{abcdef}	52^{abcde}	50^{efghij}	68^{a}	48^{ef}	58^{abcdef}
	Granny Smith	34^{i}	34^{gh}	0.01^{h}	76^{abc}	36^{defg}	96^{ab}	34^{h}
	Golab Kohanz	44^{efghi}	46^{cdefgh}	43.6^{def}	20^{mno}	30^{efg}	30^{ghi}	46^{efgh}
T2	Golden Delicious	$54^{abcdefgh}$	56^{abcdef}	50^{abcde}	46^{ghij}	46^{bcdef}	44^{efgh}	56^{bcdef}
	Gala	$56^{abcdefg}$	66^{abc}	58^{abcde}	63.2^{cdef}	60^{abc}	50^{de}	64^{abcde}
	Granny Smith	44^{efghi}	44^{defgh}	14^{gh}	50^{efghij}	36^{defg}	98^{a}	42^{fgh}
	Golab Kohanz	42^{efghi}	46^{cdefgh}	44^{cdef}	27.5^{lmno}	22^{g}	19.8^{i}	42^{fgh}

Means of columns followed by similar letters are not significant based on Duncan's test.
T1 (Thesis 1) as the 1^{st} and T2 (Thesis 2) is the 2^{nd} quality seed source in commerce.

Biochemical properties

'GK' had a slightly higher pH value on all rootstocks than any other scions, and the highest pH belonged to 'GK-GoK' (4.2) (Fig. 10). 'GS' registered the highest TA values, which was slightly higher in 'GS' onto 'NS' (0.096), 'Mor', (0.094), and 'T2' (0.092) (Fig. 11). The highest TSS was recorded for 'GA' onto 'Mor' (17.5) and 'NS' (17.5) seed lot treatments. Overall, 'GK' on the selected rootstocks registered the lowest values of TSS (Fig. 12).

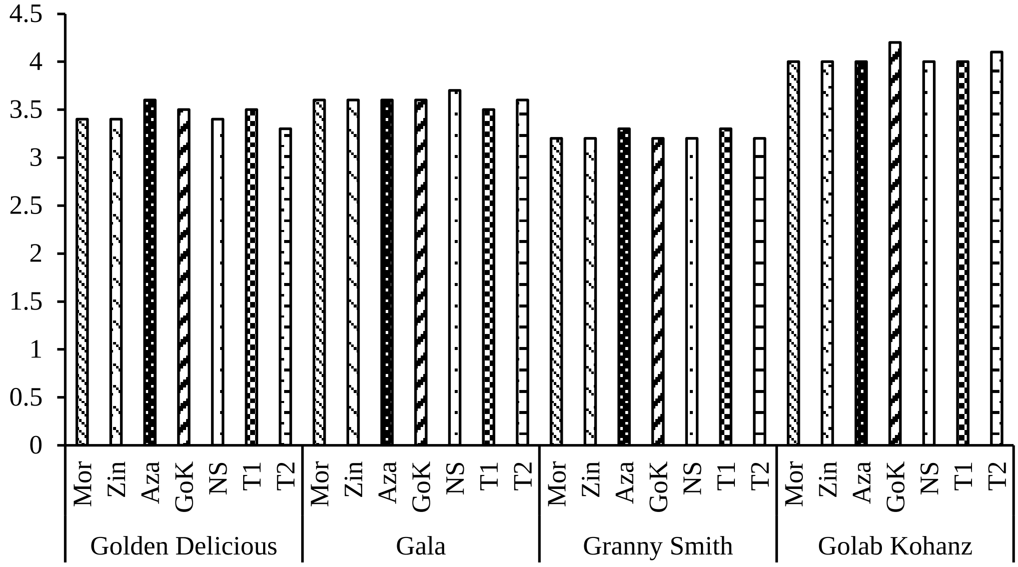

Fig. 10. Mean comparisons of pH (Scion×Rootstock combinations)
Rootstocks; Mor: Morabbaei, Zin: Zinati, Aza: Azayesh, GoK: Golden Karaj, NS: Northern Spy; T1 (Thesis 1) as the 1st and T2 (Thesis 2) is the 2nd quality seed source in commerce.

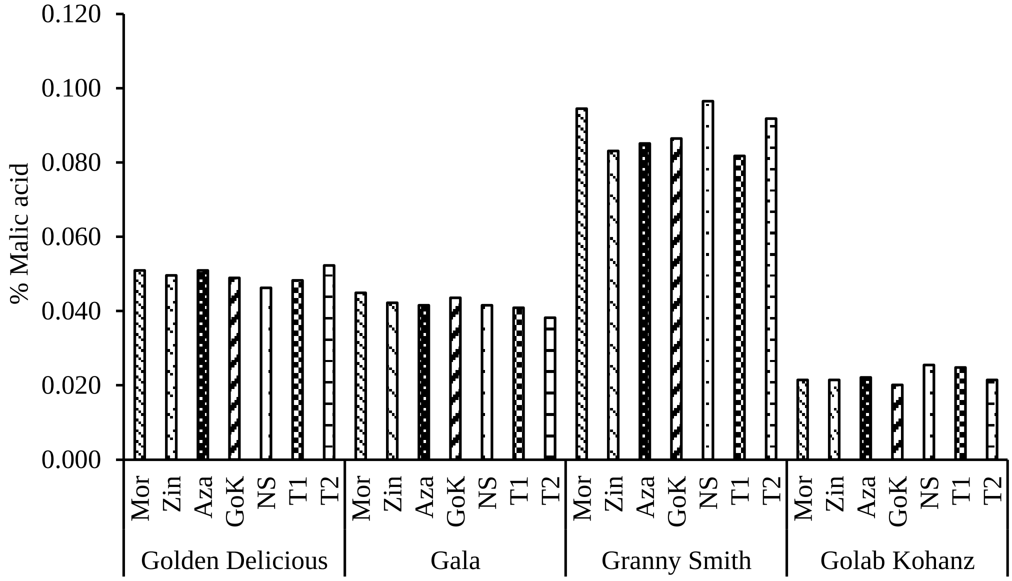

Figure 11. Mean comparisons of TA (Scion×Rootstock combinations)
Rootstocks; Mor: Morabbaei, Zin: Zinati, Aza: Azayesh, GoK: Golden Karaj, NS: Northern Spy;
T1 (Thesis 1) as the 1st and T2 (Thesis 2) is the 2nd quality seed source in commerce.

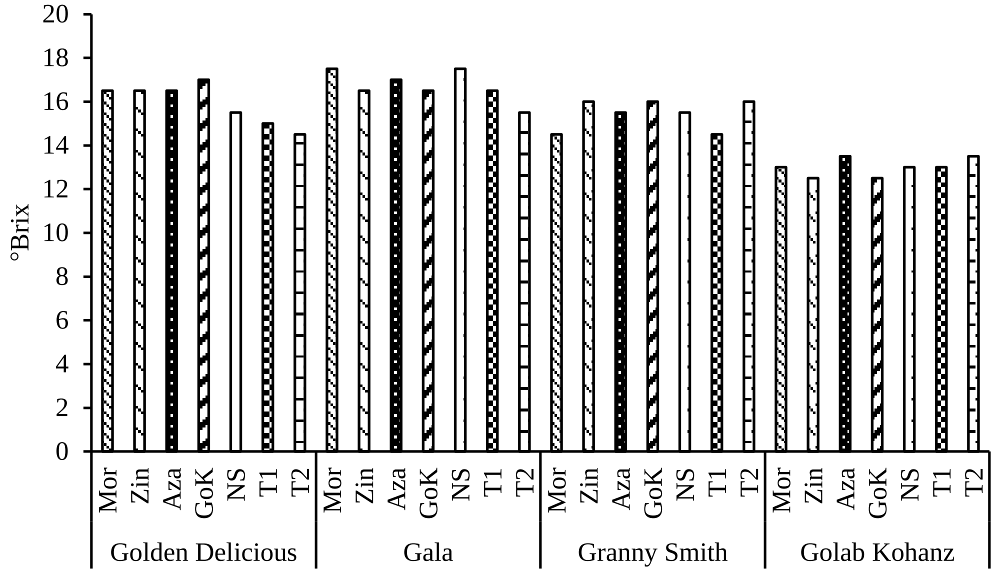

Fig. 12. Mean comparisons of TSS (Scion×Rootstock combinations)
Rootstocks; Mor: Morabbaei, Zin: Zinati, Aza: Azayesh, GoK: Golden Karaj, NS: Northern Spy;
T1 (Thesis 1) as the 1st and T2 (Thesis 2) is the 2nd quality seed source in commerce.

Discussion

Pomological traits

As a moderately variable character, fruit size can be influenced by environmental conditions, primarily by fruit load, which is one of the most critical attributes for apple seedlings selection (Janick et al., 1996; Hoehn et al., 2003; Naschitz and Naor 2005). Generally, apple fruit size is influenced by crop load and other factors but not by rootstock (Al-Hinai and Roper, 2004).

Fruit firmness is a reflection of its storage capability, but it is not the only factor. Differences between rootstocks in terms of fruit firmness were not in consistent with the results of other studies (Holger Daugaard and Callesen, 2002). However, Skendrović Babojelić et al. (2007) reported a significant difference in firmness between the scions and 'Pink Lady' had the highest firmness with an average value of 7.3 kg cm^2, followed by 'Granny Smith' (6.4 kg cm^2) and 'Idared' (4.5 kg cm^2).

Regardless of the scions, 'NS' followed by 'Zin' and 'Mor' performed much better than other rootstocks in terms of fruit length, diameter, and weight. Besides, 'Zin' achieved the highest flesh firmness.

Organoleptic traits

In the panel test, Fuji fruits grown on seed stocks had higher quality for aroma, flesh firmness, sweetness, juiciness, and general acceptability. Overall, seed rootstocks positively affected fruit quality traits for fresh consumption (Hajnajari and Mizani, 2015). Furthermore, rootstock is essential in obtaining high yield and good fruit quality of the green-coloured apple cultivar Mutsu (Daugaard and Callesen, 2002). Significant differences were recorded among cultivars in terms of firmness, flavour, and sweet/sour balance (Skendrović Babojelić et al., 2007)

In this study, scent, flavour, and sweetness were the most critical factors from the panellists' point of view, and accordingly, 'Zin' and 'Aza' received the highest score from panel members.

Biochemical properties

The flavour constituents are complex combinations of acids, sugars, tannin, and aromatic substances (Karlsen et al., 1999). The basis of apple taste and flavour is acidity and sweetness and balance between these traits, regardless of aroma, primarily determines the fruit's acceptability. The acid in the mature fruit is almost entirely malic acid and is measured either as a percentage of malic acid in the fruit juice or as the pH of the juice. The main sugars are fructose, sucrose, and glucose, conveniently measured by refractive index as a percentage of total sugars in the fruit juice (Janick et al., 1996).

Traits such as TSS, pH, and flesh firmness, which play essential roles in the ripening time, could influence the type of fruit's application. For instance, cultivars with higher flesh firmness are sent to distant areas, and those with higher TSS are used in conversion industries. Besides, TSS and pH are good indicators of fruit's sweetness and taste, respectively (Nour et al., 2010).

Rootstock does not strongly affect TSS, TA, and pH, and the reported results are inconsistent (Kviklys et al., 2014; Karbalaei Khiavi and Pirayesh, 2017). However, these traits are under cultivars' influence (Alizadeh, 2014). In a study, 'Pink Lady' had the highest TSS (16.36 °Brix), followed by 'Idared' (15.36 °Brix), while the lowest average value belonged to 'Granny Smith' (12.48 °Brix) (Skendrović Babojelić et al., 2007). In terms of TA, 'Granny Smith' had the highest acidity (0.69%) followed by 'Pink Lady' (0.54%), while Idared had the lowest amount (Skendrović Babojelić et al., 2007).
In our study, pH, TA, and TSS were influenced by scion. However, rootstocks within each cultivar demonstrated minor effects.

Conclusion
Based on the results obtained from present study, F1 seedling rootstocks of 'NS', 'Zin', 'Mor', and 'Aza' were able to enhance fruit quality in terms of fruit length, diameter, weight, flesh firmness, and organoleptic traits. They showed higher qualities when compared with the controls ('T1' and 'T2').

Acknowledgments
The authors would like to thank the Temperate Fruit Research Centre of Karaj for providing facilities and technical assistance.

Conflict of interest
The authors declare no conflict of interest.

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