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Apple seed stocks affected scion tree vigor and performance based on maternal self(in)compatibility

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Abstract: In a breeding program to increase uniformity of apple saplings size, shape and vigor the genetic improvement of seed rootstocks was considered as a key point. The Half-Sib seeds of two selected native crab parents, self-compatible "Morabbaei" and self-incompatible "Azayesh", both dwarf, were considered as the main sources of variances into the six grafted scions. The inductive effects of the two seed masses were assessed on vegetative traits of 6 and 7-year-old scion trees. According to the results, shoot length, shoot thickness and internode length as main components of tree vigor showed significant differences at 1% level in all the six scions. The shoot length mean of the grafted scions on "Azayesh" seed progeny was higher than "Morabbaei" seed stock. The grafted scions on self-compatible "Morabbaei" seed stock gave place to the shoots with higher thickness than the same grafted on "Azayesh" seed source. Combinations of "Red Delicious"-"Morabbaei" and "Braeburn"-"Azayesh" demonstrated max and min values of internodes length during two consecutive years. Both rootstock and scion trunk diameters on self-incompatible "Azayesh" seeds resulted higher than seed progeny of self-compatible "Morabbaei". Shoot number was not affected by rootstock type, whereas the effect of rootstock × scion, scion and year resulted significant.

1. Introduction

Malus genus is characterized by great phenotypic and genetic diversity. High heterozygosity is reinforced by dominant self-incompatibility besides inbreeding depression (Korban and Skirvin, 1984). Among 6000 documented apple cultivars, a large part of commercial production still relies on few cultivars including "Golden Delicious", "Granny Smith", "Fuji", "Gala" and Delicious group (O'Rourke, 2003). Rootstocks can influence vigor, habit and cropping of the scion cultivar as well as tolerance to unfavorable climatic or edaphic conditions (Webster, 2005). Rootstock performance is highly correlated with the genetic potential of rootstock to provide anchorage, adaptaion to pedo-climatic conditions and resist to abiotic stress efficiently (Fazio, 2014). Trees with limited height are requested due to facilitate orchard management. Smaller trees are also

easier to target with sprays, reducing undesirable spray drift and increasing efficiency of usage (Jobir, 2016). Good performance, dwarfness, precociousness, scion compatibility, free from suckers and burrknots are some of the attributes of an ideal rootstock (Wertheim and Webster, 2005). Clonal rootstocks are being developed rapidly while seed rootstocks have gradually decreased. Still today, many nurseries in Canada, Belarus and Finland use apple seed rootstocks. "Antonovka" was selected as a seed source due to the freeze resistance and its cytoplasmic heritability (Żurawicz and Lewandowski, 2014), the system for which some traits are transmitted to the next generation by maternal parent (Lorenzetti and Ceccarelli, 1980). Seed rootstocks improvement is applied for dwarfing effect, and tolerance to dry soil conditions (Brown and Maloney, 2005). In Iran seed rootstocks are used because of the alkaline and dry soils and also due to bad topography and exaggerated fragmentations. In absence of a specialized sector for pure seed production, the mixture of recycled seeds of processing industry characterized by high genetic diversity are used in saplings production (Hajnajari and Tarrahi, 2009; Hajnajari, 2010). Increasing genetic purity of seed rootstocks will enhance scion tree size and uniformity by taking advantage of their neglected aspects (Hajnajari and Mizani, 2015). Similarly, almond trees are raised on wild peach and bitter almond rootstock (Sharma *et al.*, 2004). "Mazzard" seedlings followed by "Mahaleb" are the most widely used rootstocks for cherry tree production (Demirsoy *et al.*, 2017). Each open pollinated seed has its specific hormonal status due to the different genetic provenience different from the rest which will cause a gradient of tree growth habits and vigor within the same scion cultivar. While tree vigor is provoked by hybrid heterosis (Lorenzetti *et al.*, 2011), the mandatory inbreeding ruling the self-compatible cultivars as maternal seed source may cause vigor decline of scion trees. It was suggested that the inbred F1 seed stocks could weaken the vigor of scion. Vigorous trees with higher canopy size need increased planting distance (Kosina, 2010). Selection of proper parents as seed source was the unique pass to way out from the emblematic situation (Hajnajari, 2018). Cultivar evaluation program led to selection of few native dwarf crabs "Morabbaei", "Zinati" and "Azayesh" as seed source parents (Hajnajari, 2010). Contemporaneous field screening program for determination of self-compatibility level of the 92 cultivars followed by fluorescent microscope studies showed complete self-compatibility of "Morabbaei", "Zinati" and "Azayesh" as self-incompatible (Forughikia *et al.*, 2014; Hajnajari and Moradi, 2014). Using such maternal parents that profit high level of self-compatibility accompanied with dwarfness can minimize seed genetic variability leading to production of the F1 seed progeny marked by high genetic purity. Both traits of dwarfism and low vigor within the seedlings can be used for production of uniform saplings. The same parents were used for production of clonal rootstocks resistant to the crown rot disease (Hajnajari *et al.*, 2012). The effects of two seed masses, as F1 progeny of "Azayesh" (self-incompatible) and "Morabbaei" (self-compatible) on growth characteristics of six apple scion cultivars were investigated. Practically, the two open pollinated seed masses derived from two different maternal parents are assumed as two family's genetically, diverse within the lots and among them, however two various sister masses belonging to different families. Nevertheless, both parents as crab apples carry jointly dwarfness trait and are native to Iranian habitat. Here is shown that the seed rootstocks bred by self- compatible parent incur more morphological uniformity and control of tree vigor in scion trees compared with the rival sister seeds produced by heterozygote parent "Azayesh" which afford more vigorous and less homogenous tree architecture due to the need of self-incompatible parent for foreign pollen.

2. Materials and Methods

The mature apple trees, 6 and 7-year-old ("Golden Delicious", "Red Delicious", "Granny Smith", "Braeburn", "Gala" and native "Golab-e Kohanz") pruned in spindle form, grafted on two selected seed masses were studied to understand the effects of seed sources on vegetative characteristics of the scion cultivars. Genetically, the two seed lots were identified as F1 Half-Sib progenies derived from two different maternal parents including the self-compatible cultivar "Morabbaei" and the other self-incompatible "Azayesh", revealed as homozygote and heterozygote, correspondingly. The two seed lots were obtained from the maternal parents exposed equally to open pollination, so forming two genetically diverse seed masses as two families considered as the main treatments. The investigated traits included annual diametric growth of scion

trunk, graft point and rootstock. For each tree, 4 annual shoots were selected and labelled, and internodes number, internodes length, shoot length, shoot thickness and lenticels number were assessed. Also, shoot numbers per tree and crotch angle were studied. The entire comparative inter and intra varietal measurements were achieved to distinguish the influences of the two seed rootstocks as the main treatments. Interaction of scion per rootstock was recorded to study also the role of scion tree vigor against dwarfing effect of the seed source. Six trees, 3 × 3 m, were assigned for each combination of "Scion cv-Seed progeny" within six replications, large enough to explain seed source inductive effects. Growth traits of grafted scions were measured in two successive vegetative seasons (2014 and 2015). The experimental orchard was established in Meshkin-Abad Station under Temperate and Cold Fruit Research Center located in Karaj, Alborz Province (Iran). The experiment was set as according to a Completely Randomized Blocks Design (RCBD) with six replications for statistical analysis. ANOVA was performed by combining the analysis of the collected data regarding all growth traits in exam, both seed rootstocks and cultivars. All statistical analyses were carried out using the general linear model (GLM) procedure of the SAS version 9.0. The Duncan' multiple range test ($P \leq 0.01$) was used to evaluate differences between treatments.

3. Results

Shoot length and thickness

Shoot length and shoot thickness were affected significantly by rootstock, scion and year interactions (Table 1). All variables are measured at annual shoots.

Shoot length

In 2014, "Red Delicious"-"Azayesh" combinations showed the longest shot lenght, while "Golden Delicious" on "Morabbaei" had the lowest value, in 2015. Comparing the treatments, no significant differences were observed for effects of the two seed progenies "Azayesh" and "Morabbaei" on the single cultivars, at the end of the first growing season. In the second year, significant differences were observed by different sources of seed rootstocks for grafted cultivars (Table 2). In 2014, it was found that shoot length value for all scions of "Golab-e Kohanz", "Gala", "Granny Smith" and "Braeburn" combined with "Morabbaei" seed rootstocks showed lower values than "Azayesh" F1 progeny; albeit "Braeburn" demonstrated significant difference, "Golden Delicious" and "Red Delicious" showed no significant differences on the 2 seed sources for shoot length. These results indicate that the maternal self-incompatible "Azayesh" gave place to more vigorous seed progeny. Whereas in 2015, the entire scions had lowered values of Shoot length showing significant difference affected by seed source (Table 2). Considering the prevailing traits of dwarfness associated with homozygosis carried by "Morabbaei" it becomes clear how these are transmitted to the relative F1 seed progeny whom could control the vigor of the combined scion trees. Though both parents may produce seeds which reinforced vigor control of scion trees but the "Morabbaei" seed progeny showed the capability both to induce dwarfism and uniformity in size and shape into the scion cultivars (Table 2). Parallel researches of artificial self-pollination followed by florescent microscopy studies put in evidence how the pollen tubes of "Morabbaei" could penetrate to its own ovary confirming the total self-compatibility of selected maternal parent (Forughikia, *et al.*, 2014). Future investigations are

Table 1 - Analysis of variance regarding the effects of year, rootstock and scion on vegetative traits

Source	DF	Mean Square									
		Shoot length	Shoot thickness	Internode number	Internode length	Rootstock diameter	Graft diameter	Trunk diameter	Shoot number	Branch angle	Lenticels number
Year (Y)	1	44376.9 **	3.92 **	7.65 **	1.14 **	1912.11 **	4460.61 **	0.22 **	0.25 *	67.79 NS	0.007 NS
Block (year)	10	4982.37 **	0.04 **	0.17 **	0.07 **	223.66 NS	108.48 NS	0.01 NS	0.03 NS	420.71 **	0.07 *
Rootstock (R)	1	39402.37 **	0.67 **	0.04 NS	0.56 **	1005.01 *	911.83 *	0.03 NS	0.03 NS	3679.76 **	1.29 **
Scion (S)	5	10396.58 **	0.063 **	0.24 **	0.12 **	28.27 NS	111.47 NS	0.02 NS	0.14 *	833.27 **	0.24 **
R × S	5	6614.24 **	0.067 **	0.06 NS	0.23 **	409.78 *	143.15 NS	0.02 NS	0.17 **	1749.75 **	0.76 **
Y × R	1	17580.61 **	0.003 NS	0.05 NS	0.56 **	56.99 NS	31.91 NS	0.03 NS	0.006 NS	667.52 *	0.10 NS
Y × S	5	7960.64 **	0.06 **	0.12 **	0.08 **	82.02 NS	79.06 NS	0.02 NS	0.10 NS	440.02 *	0.20 **
Y × R× S	5	11061.31 **	0.07 **	0.12 **	0.06 **	52.05 NS	46.51 NS	0.01 NS	0.01 NS	668.91 **	0.37 **
CV (%)		25.62	12.16	16.52	10.74	24.007	23.11	7.63	22.32	16.80	14.83

**= Significant differences at 1% level, *= Significant differences at 5% level, NS= Not significant.

required to elucidate the mechanisms and biochemical pathways by which a self-compatible cultivar can accept insider pollen leading to fertilization, within a self-incompatible species.

Shoot thickness

Combination of "Red Delicious"-"Morabbaei", in the first year, showed the highest shoot thickness, while both combinations of "Golden Delicious" and "Red Delicious" on "Azayesh", in the second year, showed the lowest rates. It could be inferred that low vigor of "Morabbaei" F1 progeny inhibited Shoot length growth rising inversely shoot thickness. In 2014, Converse growth rhythm was registered in "Golden Delicious" and "Braeburn" on heterozygote seeds of "Azayesh" inducing significant differences, while "Golden Delicious", "Red Delicious" and "Gala" were affected significantly by "Azayesh" F1 progeny for the measured variables in 2015. Moreover, in 2014 "Morabbaei" seeds induced significant differences between "Golden Delicious" and "Granny Smith". Similar differences were observed among "Golden Delicious", "Red Delicious" and "Gala", in 2015 (Table 2). Such differences in successive years within the combinations are attributed to the normal annual growth.

Internode number

Year and scion main effects and year × scion and year × rootstock × scion interaction significantly affected internode number (Table 1). The highest number of internode was found in 2014 in combination of "Golden Delicious"-"Morabbaei" confirming the role of homozygosity on lowering vegetative growth, changes in metabolic pathways and relative hormonal modifications at intra-cellular level. However, the lowest expression of the genes for internode number was found in "Gala"-"Azayesh" combination, in 2015. As a reason, the latter low vigor of "Gala" occurred in "On" year could influence such result. In 2014, there were no significant differences among the cultivars for internode number on "Azayesh" seeds, while in the second year significant differences were observed caused by annual growth. Contrarily, "Morabbaei" seed stocks induced significant differences in "Gala", "Golden Delicious" and "Granny Smith", in 2014, which confirms relatively lower vigor of "Gala", whilst in 2015 significant differences was observed among the grafted cultivars (Table 2). In 2014, there were no significant differences between rootstocks for each scion combination, but in 2015, "Golden Delicious" and "Red

Table 2 - The interaction effects of year × scion × rootstock on some vegetative traits of the apple scion cultivars

Year	Seed stock	Scion	Shoot length mm	Shoot thickness mm	Internodes number	Internodes length mm	Rootstock diameter mm	Graft diameter mm	Trunk diameter mm	Shoot number	Crotch angle	Lenticels number
2014	Azayesh	'Golden Delicious'	179.24 b-g	5.70 ef	21.06 ab	14.66 b-e	45 a	43 a	30.2 a	7.75 a	61.67 cd	16.25 ij
		'Red Delicious'	208.22 a	6.43 de	19.26 a-d	17.20 bc	43.6 a	47.2 a	38.5 a	10.2 a	68.33 bcd	24.23 cde
		'Granny Smith'	177.39 b-h	6.87 bcd	20.17 abc	17.19 bc	57.5 a	58.25 a	52.25 a	17.66 a	73.75 a-d	33.12 a
		'Braeburn'	175.25 b-h	5.10 ghi	18.08 b-e	15.54 b-e	49.4 a	56 a	40.5 a	11.16 a	83.33 a-d	14.53 j
		'Golab-e Kohanz'	168.75 b-h	6.80 cd	19.40 a-d	14.66 cde	47.33 a	51.66 a	50.83 a	8.5 a	87.50 ab	10.22 k
		'Gala'	178.45 b-g	6.86 bcd	18.16 b-e	17.83 b	62.83 a	60.83 a	47.66 a	10.5 a	85.00 abc	18.16 e-i
	Morabbaei	'Golden Delicious'	193.63 abc	6.18 de	22.86 a	16.83 bcd	47 a	43.66 a	35.83 a	14.5 a	82.17 a-d	21.21 c-f
		'Red Delicious'	195.06 ab	8.00 a	19.46 a-d	20.33 a	56 a	41 a	51 a	17.16 a	95.00 a	21.94 c-g
		'Granny Smith'	174.61 b-h	6.36 de	20.20 abc	13.73 b-e	40.25 a	49.6 a	41.8 a	13 a	90.00 ab	21.40 c-h
		'Braeburn'	159.70 e-i	7.63 ab	19.58 a-d	15.75 b-e	43.4 a	49.6 a	43.4 a	9.33 a	60.00 d	23.80 bcd
		'Golab-e Kohanz'	165.74 c-h	7.41 abc	19.56 a-d	15.30 b-e	44.6 a	54.5 a	43.16 a	6.33 a	82.50 a-d	29.27 ab
		'Gala'	171.44 b-h	7.86 ab	15.57 d-g	15.03 b-e	41.2 a	44.5 a	36 a	8.00 a	73.33 a-d	15.50 hij
2015	Azayesh	'Golden Delicious'	176.64 b-h	3.94 k	13.39 fgh	13.24 ef	69.33 a	70.33 a	58.66 a	6.33 a	68.33 bcd	14.18 ij
		'Red Delicious'	149.59 hi	4.03 k	9.52 hi	11.36 fg	51 a	62 a	52.33 a	10.16 a	70.00 bcd	22.65 c-g
		'Granny Smith'	164.23 d-h	4.47 h-k	14.17 efg	14.89 b-e	63.2 a	70.2 a	58.8 a	10.66 a	69.00 bcd	19.34 c-h
		'Braeburn'	181.71 a-f	4.31 jk	11.32 ghi	9.97 g	62.16 a	69.16 a	61 a	9.4 a	59.00 d	16.30 hij
		'Golab-e Kohanz'	187.42 a-e	4.30 ijk	14.11 efg	10.23 g	60.33 a	68.66 a	58 a	7.5 a	76.67 a-d	18.22 d-i
		'Gala'	185.61 a-e	4.63 g-j	8.25i	10.36 g	68.33 a	68.5 a	57.5 a	9.16 a	83.33 a-d	17.28 ghi
	Morabbaei	'Golden Delicious'	143.23i	6.10 de	9.11i	14.67 cde	53 a	58.33 a	45.83 a	10.00 a	90.00 ab	21.08 c-g
		'Red Delicious'	191.06 a-d	4.33 h-k	16.10 c-f	21.24 a	60 a	62 a	53 a	17.16 a	95.00 a	22.56 bc
		'Granny Smith'	116.40 j	4.63 g-j	14.70 efg	13.79 ef	44.6 a	57.5 a	44.66 a	7.33 a	85.00 abc	17.26 hij
		'Braeburn'	151.71 ghi	4.68 g-j	11.31 ghi	16.79 bc	51.66 a	64 a	50 a	6.66 a	76.67 a-d	23.19 bc
		'Golab-e Kohanz'	156.16 f-i	4.97 gh	14.43 efg	15.61 b-e	58.25 a	65.75 a	53 a	7.83 a	73.33 a-d	19.94 c-h
		'Gala'	150.88 ghi	5.24 fg	11.82 f-i	11.50 fg	44 a	57.66 a	44 a	9.33 a	83.33 a-d	17.50 f-i

In each column, means with similar letters are not significantly different at the 1% probably level using Duncan multiple range test.

Delicious" showed significant differences on "Morabbaei" and "Azayesh" seed sources (Table 2).

Internode length

Internode length was affected by year, scion cultivar and rootstock main effects and their interactions (Table 1). The longest internode was found in "Red Delicious" on "Morabbaei" rootstocks in both years and the lowest value was registered in combination of "Braeburn"-"Azayesh", in 2015 (Table 2). In the first year, among all the scions combined with "Azayesh" rootstocks, there was significant difference between "Golab-e Kohanz" and "Gala" and among the grafted cultivars on "Morabbaei" sole "Red Delicious" showed significant difference. "Golab-e Kohanz" is the most vigorous Iranian cultivar early and "Gala" with moderate tree vigor. Tree vigor, denoted as potential of vegetative growth, is measured based on height and spread of adult trees self-rooted, or relative to reference cultivars on the same rootstock in apple descriptors (Watkins and Smith, 1997). "Golden Delicious" and "Red Delicious" were used as reference cultivars to assess rootstocks efficiency for tree vigor, the trait that encompasses other components as shoot length, shoot thickness, internode number, internode length, shoot number, and crotch angel by which the tree overall size, shape and architecture will be reflected. Fazio (2014), assumed that rootstock performance is highly correlated with the genetic potential to impart positive architectural properties to the scion like vigor control. Considering the ruling genetic purity carried by "Morabbaei" seed rootstocks, it imparted uniformity in the size of internode length in all the scion cultivars. In the second year, "Golden Delicious" and "Granny Smith" showed significant differences against other scions grafted on "Azayash", while "Red Delicious" and "Gala" had significant difference relative to the other scions on "Morabbaei" (Table 2). Such differences are attributed to the different genetic vigor of scion materials.

Rootstock, graft point and scion diameter

According to the Table 1, the effect of year was significant for rootstock, graft union and scion trunk diameters. Overall, rootstock diameter, graft and trunk were higher in the second year. Rootstock and graft point were affected equally by rootstock factor. Interaction of rootstock × scion was significant for rootstock (Table 1). Interaction effect of scion × rootstock on rootstock demonstrated that combination of "Granny Smith" on "Azayesh" and "Morabbaei" rootstocks endured modifications on the trait, for example 60.66 mm to 42.66 mm, respectively. There was only significant difference between "Red Delicious" (47.30 mm) and "Gala" (65.58 mm) for rootstock limiting factor to "Azayesh" rootstock. Similar comparison within seed lots of "Morabbaei" revealed significant difference in combination with "Granny Smith" (42.66 mm) and "Gala" (60.22 mm). Surprisingly, both seed rootstocks influenced "Gala" increasing rootstock to the highest values of 65.58 mm and 60.22 mm (Table 3). Moreover, significant difference was observed between the combinations of "Granny Smith"-"Azayesh" and "Granny Smith"-"Morabbaei" for the effect of rootstock type on each scion for rootstock, while no difference was observed between seed rootstocks for other combinations (Table 3). This result is assumed positive for uniform apple tree saplings production on the improved seed rootstock.

Shoot number

Shoot number was not affected by rootstock type, while the effects of year, scion and rootstock × scion were significant (Table 1). In 2014, more lateral branches were grown than 2015. Among scions-stocks combinations, "Morabbaei" seed stock showed both max. and min. shoot number, on "Red Delicious" (17.16) and on "Golab-e Kohanz" (7.08), respectively (Table 3). No significant difference was found among the six scion cultivars for shoot number on "Azayesh", whilst "Morabbaei" rootstock induced significant differences of shoot number on "Golden Delicious" and "Red Delicious".

Crotch angle

In this study crotch angle was affected by year,

Table 3 - The interaction effect of scion × rootstock on rootstock diameter and shoot number

Rootstock	Scion	Rootstock diameter (mm)	Shoot number
'Azayesh'	'Golden Delicious'	55.43 abc	7.14 c
	'Red Delicious'	47.30 bc	10.18 bc
	'Granny Smith'	60.66 ab	13.00 bc
	'Braeburn'	56.36 abc	10.36 bc
	'Golab-e Kohanz'	53.83 abc	8.00 bc
	'Gala'	65.58 a	9.83 bc
'Morabbaei'	'Golden Delicious'	50.27 abc	12.25 ab
	'Red Delicious'	58.00 abc	17.16 a
	'Granny Smith'	42.66 c	10.16 bc
	'Braeburn'	46.50 bc	8.00 bc
	'Golab-e Kohanz'	50.67 abc	7.08 c
	'Gala'	60.22 ab	9.25 bc

In each column, means with similar letters are not significantly different at the 1% probably level using Duncan multiple range test.

rootstock and scion interaction (Table 1). The combination of "Red Delicious"-"Morabbaei" showed the widest value of crotch angle probably as a result of heavy cropping, while light bearing trees in the combination of "Braeburn"-"Azayesh" had the lowest crotch angle rates, in both years. In general, the scion cultivars grafted on "Morabbaei" had wider crotch angle than on "Azayash" progeny (Table 2). Except partial genetic role of the scions regarding growth habit, this effect might be result of higher tree vigor control by self-compatible parent "Morabbaei" rootstocks and subsequent decrement of woody parts in favor of flower bud initiation and fruit set (Wertheim and Webster, 2005). Clearly, bearing shoots under higher fruit weight were bent downward increasing crotch angle.

Lenticels number

Lenticels number was affected by year × rootstock × scion interaction (Table 1). The max and min values of lenticels number were found in "Granny Smith" and "Golab-e Kohanz" both on Half-Sib seed stocks of "Azayesh", in the first year. It can be deduced that the increased leaf lenticels number of the "Golab-e Kohanz" as the most vigorous scion is a normal physiological trend appeared trough interaction of the grafting with as much vigorous seed stock "Azayesh" in the year. More vegetative growth induced by vigorous seeds demands higher respiration rate for which a higher lenticels number equilibrate the defined biological status. In both years, significant difference was observed among the fruit scions on "Azayesh" and "Morabbaei". This confirms significant effect of rootstock seed masses for lenticels number. In 2014, comparisons of rootstocks for each cultivar showed that "Golden Delicious", "Granny Smith", "Braeburn" and "Golab-e Kohanz" were affected by seed stocks for lenticels number. Also in 2015, "Golden Delicious" and "Braeburn" were affected for this trait (Table 2). The comparison between two seed sources put in evidence of high dominant uniformity of lenticels number trait for scions grafted on the seed stocks and originated from self-compatible "Morabbaei".

4. Discussion and Conclusions

According to the results, shoot length, shoot thickness and internode length as 3 main components of tree vigor showed significant difference in all the six cultivars grafted on two seed masses. Dominant homozygosity carried out by self-compatible "Morabbaei" and relative progeny overcame heterosis effects which induced by "Azayesh" seeds and tree vigor interaction. Our results regarding control of tree vigor "Morabbaei" seed stocks demonstrated that it could reduce shoot length; infact this trait decreased in all the cultivars except for "Golden Delicious", in 2014. This cultivar showed the same opposite rhythm for all growth traits like crotch angle, internode thickness, internode number, roostock, and scion diameter on "Morabbaei" seed stock. Also, other four scion cvs. decreased shoot length value and were affected by dwarfing seeds in the range of 13 to 3 mm for "Red Delicious" and "Golab-e Kohanz", 7 and 3 mm for "Gala" and "Granny Smith", respectively. These results confirm the reciprocal influences of two genomes, scion and seed rootstock within the same genus, each one specialized differently for which were selected in the selection procedure. There is no clear reason for reaction of "Golden Delicious" for measured trait when compared with five scion cultivars grafted on the same seed pure line progeny. This may not be related to the distant geographical origin of two genomes, Iran-USA. With studying scion tree vigor was suggested that J-TE-E as an acceptable rootstock for "Rubin" among 14 rootstocks (Kosina, 2010). The scion cultivars on "Morabbaei" seeds showed higher shoot thickness than the same scions on "Azayesh" seed progeny. Increased shoot thickness as the diametric growth of annual shoots on "Morabbaei" seeds indicated accumulation of carbohydrates for productive functions. However the final canopy size is determined genetically by scion-rootstock interaction. Higher dwarfing efficiency raised in "Morabbaei" than "Azayesh" progeny is in agreement with Tworkoski and Miller (2007 a), who indicated that Malling rootstocks have different size-controlling effects on different apple scions. The lowest interned number (8.25) was found in "Gala"-"Azayesh" combination, in 2014, while the highest (22.86) was found in combination of "Golden Delicious"-"Azayesh", in 2015. The biennial comparisons evidenced that the higher mean of internode number was induced by "Morabbaei" in both years, 22.86 and 9.11, related to "Azayesh" equal to 21.06 and 8.25. These finding confirmed stronger dwarfing effects of the self-compatible parent. Dwarfing mechanism acted through an increase of internode length and contrarily shortening internode length. Highest value of interned length was found in "Red

Delicious"-"Morabbaei" combination for 2014 and 2015 and the lowest in "Braeburn" on "Azayesh" in 2015 (Table 2). Others reported that wild peach rootstocks influenced shoot growth and leaf area for the scions only during the first year (Sharma *et al.*, 2004). In general, in the first year, the internode number and internode length were higher than the second year. It can be attributed also to the difference between orchard management during two years. Interesting results were obtained in 2015 comparing effects of two seed stocks on major part of the scions. Dwarfing property of "Morabbaei" seed stocks overcame in the next season which caused shoot length shortening in appropriate growth rhythm and shoot thickness increasing and internode number and decreasing rootstock and scion diameter. A part of less expected behavior of "Red Delicious" on "Morabbeai" stock which shoot length and internode length was both increased, but other traits like shoot thickness, internode numbe , and scion diameter followed normal dwarfing trend. All the other growth traits assessed in the rest of four scion cultivars were highly affected by dwarfing effect of "Morabbeai" seed progeny, without exception (Table 2). Weibel *et al. (*2003) indicated that differences in peach shoot length were related primarily to the internode length rather than the node number, whereas Seleznyova *et al.* (2003) attributed the differences in apple branch size to the reduction in neoformed internode length and node number. Mean of internode length depended on node number. It means that shorter internodes are caused by lower node number (Costes and Garcia-Villanueva, 2007). Measured diametric values of graft union and scions on self-incompatible "Azayesh" seed stocks showed higher than those on self-compatible "Morabbaei" F1 seed progeny. Scion-Rootstock discontinuity may lead to Burrknot formation and high diametric difference between rootstock and trunk that may be a site where xylem may be affected by modified cell diameters or cell disorganization between different scions and rootstocks (Atkinson *et al.*, 2003; Tworkoski and Fazio, 2011). Such morphological differences may in turn affect hydraulic conductivity, root hormones production, or nutrient transport and explain the differences in growth. Tworkoski and Fazio (2015) reported that the scion cultivars "Gala", "SM.9", and "SM.27" reduced rootstock of "G.41" and "G.11" compared with "Fuji" scion. In the second year, the most of fruit scions on "Morabbaei" seed had smaller diameter than "Azayesh" without significant difference, except for "Red Delicious" (Table 2). This implies that rootstocks may affect scion. So, shoot number was not affected by rootstock type, whereas the effect of rootstock×scion, scion and year resulted significant. "Red Delicious" and vigorous "Golab-e Kohanz" on "Morabbaei" seed rootstock had the highest and lowest BrN, respectively (Table 3). "Morabbaei" F1 seeds induced more new shoots than "Azayesh" seed stocks. Generally, the mandatory cut back of leader in Spindle pruning method activates the latent buds, forming new lateral shoots due to removal of apical dominance. This practice however was performed equally on the leader of all combinations. Thus, it may be concluded that while, on one side, homozygosis affects canopy size and decreases shoot length, on the other side, the reserved carbohydrates might be used in activation of latent buds in absence of apical dominance. Obviously, winter pruning obliges crossing out of adventitious shoots for flower initiation purposes. Across all scions, the dwarfing M.9, and seedling rootstock induced the lowest and the highest tree height and scion diameter, respectively. Significant interactions indicated that effects of size-controlling rootstock on components of shoot growth vary with apple tree growth habit (Tworkoski and Miller, 2007 a). In this study all the six scions carried the spread habit, for which crotch angle was investigated. Meantime, it might be attributed to the hormonal factor due to the genetic factor derived from seed stocks, scions and relative interaction. Seedling rootstock might affect growth habits by increasing the Auxin/Cytokinin ratio. Changing in hormonal ratio might be a factor regulating the development of growth habit in apple scions, and rootstock might modify the hormone concentrations in shoot tips (Tworkoski and Miller, 2007 b). As prerequisite, crotch angle is mentioned as a stable trait and used in cultivar differentiation and cultivar/progeny evaluation (Watkins and Smith, 1997), and less affected by external factors which is agreed with our results of crotch angle among five scions on two diffrent stocks, except for "Red Delicious". In general, the grafted scions on "Morabbaei" seeds showed wider crotch angle than "Azayash" progeny, probably for higher fruit weight as a result of higher set. The significant differences of crotch angle between "Golden Delicious" and "Granny Smith" on "Azayesh" seed rootstocks, in the first year, can be attributed to the stability of this trait, whereas lack of differences in the second year would be justified by pruning effect. It is however very important that the single cultivars on "Azayesh" didn't show significant differences in

terms of crotch angle with the same cultivar on "Morabbaei", except for "Red Delicious" (Table 2). However, endogenous growth regulators mainly auxins and cytokinins play a major role in the control of lateral bud development (Sachs and Thimann, 1967). There are numerous reports of growth-regulating chemicals affecting branch crotch angle (Verner, 1938; Williams and Billingsley, 1970; Elfving and Forshey, 1977). Warner (1991) reported that rootstock affects primary scaffold branch crotch angle of apple trees, which is consistent with our results. Also lenticels number of annual shoots unit, defined as stable trait like crotch angle, was affected by rootstocks and year, and interaction of rootstock × scion × year. Comparisons of rootstocks for each cultivar, in 2014, showed that "Golden Delicious", "Granny Smith", "Braeburn" and "Golab-e Kohanz" were affected by seed stocks for lenticels number, and in 2015, "Golden Delicious" and "Braeburn" were affected for this trait (Table 2). However, the use of lenticels number may be successfully applied for cultivar/hybrid evaluations when more cultivars are grafted on similar rootstocks.

The self-compatible parents are able to supply seeds characterization with high genetic purity. Furthermore, the improved seed rootstocks seem to be the preferred solution to combat genetic variability, suitable for heavy and alkaline soils and inappropriate slopes. Conventional breeding remains the main method to release new clonal or seed rootstock (Wertheim and Webster, 2005). Seed rootstock improvement program was started to impede use of a mixture of seeds with unknown origin and with high genetic variability and evolved into adequate genetic purity, which is the basic element for standard apple tree production.

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