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Impact of Rootstock Selection on Fruit Quality and Aril Browning in Pomegranate Cultivars

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ABSTRACT

The impact of rootstock on the pomological and biochemical characteristics of fruits has been investigated across various species of fruit-bearing trees. However, due to its vegetative propagation using cuttings, there are few reports on pomegranate. Also, there is no report so far on the effect of rootstock on aril browning in pomegranate. For this reason, an experiment was designed to examine the influence of rootstock on the pomological and biochemical traits of two distinct pomegranate cultivars, specifically 'Rabab-e-Neyriz' and 'Khafr-e-Jahroom'. The design was completely randomized and encompassed two variables and five replications. Factor included two types of cultivars ('Rabab-e-Neyriz' and 'Khafr-e-Jahroom') and rootstocks in four kinds of cultivars ('Post Ghermaz-e-Aliaghai', 'Gorj-e-Dadashi', 'Gorj-e-Shahvar' and the control group with no grafting). In this study, both quantitative and qualitative parameters of the fruit especially the aril browning percentage included were evaluated. Based on results, the highest amount of aril anthocyanin, aril color, Titratable Acidity (TA), and Total Soluble Solids (TSS) in fruit juice, and also the highest seed width and peel thickness, as well as the minimal percentage of browning were identified in the fruits of 'Rabab-e-Neyriz' cultivar that were grafted onto the rootstock of 'Post Ghermaz-e-Aliaghai'. The highest aril weight, seed thickness, seedlessness percentage, TSS/TA, and Vitamin C of fruit juice, along with the lowest TA and fruit peel thickness, were observed in the fruits of 'Khafr-e-Jahroom' cultivar grafted on 'Gorj-e-Dadashi' rootstock. The findings indicated that the interplay between scion and rootstock was significant in all traits. The findings indicated that the pomegranate fruit's biochemical and pomological traits were influenced by the rootstock type. According to the findings of this investigation, it can be concluded that the selection of rootstock affects the percentage of aril browning, as well as the biochemical and pomological traits of pomegranate fruit.

Introduction

The pomegranate (*Punica granatum* L.) constitutes a significant horticultural crop that has been extensively cultivated in Iran for an extensive duration (Varasteh and Arzani, 2009; Sadeghi Seresht et al., 2023). Fruit trees usually consist of two distinct genotypes, scion and rootstock (Hayat et al., 2021). The rootstock exerts a considerable influence on the development of the scion and the characteristics of the fruit by facilitating the uptake of essential nutrients and regulating hormonal balance. The impact of rootstock on fruit dimensions, fruit quality, growth parameters, and yield of the scion has been investigated across various species of fruit-bearing trees (Amiri et al., 2014; Barry et al., 2004). Despite the existence of the influence of rootstock on pomological and biochemical characteristics of fruit, alongside the nutrient element concentration within leaves in various other fruiting species (Giorgia et al., 2005; Karimi, 2011; Karimi and Farahmand, 2011; Karimi and Hasanpour, 2014; Karimi and Nowrozi, 2017; Eini-Tari et al., 2014; Karimi and Eini Tari, 2015; Eini-Tari et al., 2014; Karimi and Hasanpour, 2016; Karimi et al., 2023), there are few reports about pomegranate due to its vegetative propagation using cuttings. Advances in rootstock breeding for trees focus on enhancing productivity, quality, and resilience against biotic and abiotic stresses (Vahdati et al., 2021). Among the goals of fruit tree rootstock breeding, we can mention resistance to stresses, absorption of nutrients and water, Compatibility, and vigor, which are for ensuring compatibility with various scion cultivars and optimizing vigor for high-density planting are important for economic viability (Vahdati et al., 2021). Some common pomegranate cultivars exhibit sensitivity to aril browning, sunburn, diseases, salinity, and drought which may be mitigated through grafting onto rootstocks exhibiting tolerance (Karimi and Nowrozi, 2017). In recent years, one of the causes of agricultural land being salty has been irrigation with salty and low-quality water (Behzadi Rad et al., 2021). It is possible to reduce the negative effects of salinity in plants, by using calcium, especially in the form of foliar spraying in arid and semi-arid areas (Mohit Rabari et al., 2023).

It has been reported that special rootstocks in pomegranate cultivation can be used to increase the tolerance to all kinds of environmental stress and improve the success rate of grafting (Karimi and Hasanpour, 2014; Karimi and Hasanpour, 2016; Valizadeh Kaji et al., 2020), this approach can be significant for pomegranate production in areas with high environmental stress. In an investigation concerning pomegranate, the impact of rootstock on ecophysiological parameters and growth of two distinct scions was assessed, with findings indicating that optimal growth parameters were achieved in the 'Rabab-e-Neyriz' cultivar when grafted onto the 'Gorj-e-Shahvar' rootstock (Karimi and Nowrozy, 2017).

Karimi et al. (2023) have articulated that the levels of mineral constituents within pomegranate cuttings are predominantly influenced by the characteristics of the root system associated with the rootstock. They also reported that the reason for the higher element concentrations in pomegranate scions grafted on 'Gorj-e-Shahvar' and 'Gorj-e-Dadashi' rootstocks can be attributed to the superior growth capabilities and immense root structure exhibited by these rootstocks. It has been reported that grafting can result in an augmentation of yield alongside a reduction in tree vigor and size within pomegranate cultivation (Vazifeshenas et al., 2009). Anthocyanin content and biological compounds determine the nutritional value of pomegranate. Several studies have reported that the levels of anthocyanin content and bioactive compounds in the fruit of pomegranate are influenced by a multitude of factors, including genotype (Zhao et al., 2013), environmental conditions (Li et al., 2015), orchard management practices (Jafari et al., 2014), irrigation water state (Borochov-Neori et al., 2013), timing of harvest (Fawole and Opara, 2013; Mphahlele et al., 2016), and storage conditions (Varasteh et al., 2012). However, there exists a lack of comprehensive documentation regarding the impact of rootstock on the biosynthesis of these compounds. "Aril browning" represents a physiological disorder that detrimentally influences the quality fruit of pomegranate (Darsana et al., 2016).

Prior investigations have elucidated that aril browning is influenced by diverse effects of cultivars, fruit size, timing of harvest, nutritional status of the orchard, and levels of oxidative stress (Jalikop et al., 2010; Shivashankar et al., 2012). There is no report on the effect of rootstock on aril browning in pomegranate. Therefore, the present study was conducted to elucidate the influence of rootstock on pomological fruit traits, biochemical compounds, and browning disorder of the aril in two distinct cultivars of pomegranate.

Material and methods

This investigation was conducted using two distinct pomegranate cultivars, 'Khafr-e-Jahroom' and 'Rabab-e-Neyriz', grafted on the rootstocks of 'Post Ghermaz-e-Aliaghai', 'Gorj-e-Dadashi', 'Gorj-e-Shahvar' and without grafting (as a control) and with five replications. Grafted plants were propagated in 2014 using omega grafting and arranged at 2 m within rows and 4 m between rows in the experimental field (Karimi and Nowrozy, 2017) (Table 1). During the study, the trees were irrigated on a 6 d cycle. The fruits were harvested at horticultural maturity in November and transferred to the laboratory for parameter measurements.

Evaluation of fruit traits

In this study, both quantitative and qualitative parameters of the fruit include: fruit weight, fresh weight of fruit peel, weight of arils, 100 weight of arils, fresh weight of 100 seeds, 100 dry weight of seeds, aril/peel weight, number of arils in fruit, aril width and length, seed width and length, seed thickness, fruit peel thickness, aril firmness, seed hardness, aril color, fruit peel color, seedlessness percentage and aril browning percentage were evaluated. Fruit weight was measured using a scale, aril length, and width using a digital caliper, and aril firmness, and seed hardness using a hardness tester.

Aril color and peel color were scored based on descriptor (UPOV) in (Table 2) (Jalikop et al., 2010).

Aril browning percentage

The percentage of aril browning was determined through the total scores of the aril browning based on visual evaluation using the color of the arils measured in Table 2. In this way, fruits without aril browning were recorded as of zero score, fruits with a 20% browning score of one, fruits with a 40% browning score of two, fruits with a 60% browning score of three, fruits with 80% browning score fruits with 80% browning of four,, and fruits with 100% browning score five. The percentage of aril browning was computed utilizing the subsequent formula (Kavand et al., 2020).

Ultimately, the proportion of aril browning was determined utilizing the under formula.

$$(\%)\text{Browning} = \frac{\text{Total scores}}{\text{The number of fruits sampled} \times 5} \times 100$$

Table 1. Grafting combination of pomegranate (Karimi and Nowrozy, 2017).

Grafting combination code	Rootstock	Scion
PR	Post Ghermaz-e-Aliaghai (P)	Rabab-e-Neyriz (R)
GDR	Gorj-e-Dadashi (GD)	Rabab-e-Neyriz (R)
GSR	Gorj-e-Shahvar (GS)	Rabab-e-Neyriz (R)
RWG	Rabab-e-Neyriz without graft	-
PK	Post Ghermaz-e-Aliaghai (P)	Khafr-e-Jahroom (K)
GDK	Gorj-e-Dadashi (GD)	Khafr-e-Jahroom (K)
GSK	Gorj-e-Shahvar (GS)	Khafr-e-Jahroom (K)
KWG	Khafr-e-Jahroom without graft	-

Table 2. The Qualitative traits of peel and aril color were measured in grafting combination of pomegranate.

Trait	Grade	Score
Peel color	Light brown (100%)	1
	Light brown (80%) with pink (20%) spots	2
	Light brown (80%) with red (10%) and pink (10%) spots	3
	Light brown (80%) with red (20%) spots	4
	Light orange (80%) with Light brown (20%) spots	5
	Red (80%) with dark brown (20%) spots	6
	Red (80%) with Light brown (20%) spots	7
	Red (80%) with pink (10%) and dark brown (10%) spots	8
	Red (80%) with pink (10%) and Light brown (10%) spots	9
	Pink (100%)	10
	Light red (80%) with white (10%) and pink (10%) spots	11
	Dark red (80%) with pink (10%) and white (10%) spots	12
Aril color	Dark brown (100%)	1
	Light brown (60%) and dark brown (40%)	2
	Dark brown (40%) with red (60%) spots	3
	Light brown (100%)	4
	Light brown (40%) with white (60%) spots	5
	Light brown (40%) with red (60%) spots	6
	White (80%) with red (20%) spots	7
	Light red (80%) with white (20%) spots	8
	Dark red (80%) with white (20%) spots	9
	Red (80%) with pink (20%) spots	10
	Red (100%)	11

Evaluation of biochemical traits of the fruit

Total aril anthocyanin

To measure total aril anthocyanin, five g of aril were weighed and homogenized in a Chinese mortar in the presence of hydrochloric acid-potassium buffers with a concentration of pH 1 and acetate with a concentration of pH 4.5 and the sample was centrifuged for 20 min at a speed of 10,000 rpm. The supernatant was then extracted and diluted with 4 mL of a hydrochloric acid-potassium buffer solution, after which 1 mL of supernatant was again removed and diluted with acetate buffer. Following this procedure, prepared solutions for the light attraction were read at wavelengths of 520 and 700 nm utilizing a spectrophotometer, and the amount of anthocyanin was determined in terms of cyanidin 3-5 diglucoside in 100 mg of aril from the following equations (Wrolstad et al., 1976):

$$A = (A\lambda_{520} - A\lambda_{700})\, pH1.0 - (A\lambda_{520} - A\lambda_{700})\, pH4.5$$

$$(5) TA\ (mg\ g^{-1}) = (\frac{A}{26900})\,(103)\,(310)$$

Molar extinction coefficient = 26900
Conversion factor = 103
Molecular weight = 310
Dilution factor = 5

Vitamin C in fruit juice

To measure the amount of Vitamin C (ascorbic acid) in fruit juice, mix 5 mL of fruit juice with 20 mL of distilled water and 2 mL of 1% starch reagent and mix with iodine solution in potassium iodide until the color changes. It was titrated to gray. Every 1 mL of iodine consumed corresponds to 0.88 mg of Vitamin C. The concentration of Vitamin C in the fruit was computed using the subsequent equation, expressed in terms of mg of Vitamin C 100 mL^{-1} of fruit juice (AOAC, 2005).

$$\text{Mg of Vitamin C in 100 ml of fruit juice} = \frac{\text{Consumption of potassium iodide in volume iodine} \times 0.88}{5} \times 100$$

Total aril phenol content, TSS, and titratable acid of fruit juice

To measure the content of total aril phenol, 5 g of aril was homogenized with 10 mL of phosphate buffer solution in a Chinese mortar and centrifuged for 20 min at 4 °C at a speed of 4800 rpm. 100 mL of supernatant was diluted with 400 mL of phosphate buffer, 2.5 mL of Folin with a ratio of 1:10, and 2 mL of 7.5% sodium carbonate, and then vortex and it will be placed in a hot water bath with a temperature of 50 °C for 5 min. Then, the amount of light absorption was measured with a spectrophotometer at a wavelength of 760 nm (Ayala-Zavala et al., 2004). Total soluble solids were quantified utilizing a manual Refractometer (sugar meter), with results expressed in degrees Brix (%). Also, to measure titratable acid, 3 mL of fruit juice was mixed with 25 mL of distilled water along with 4 drops of 1% phenolphthalein and titrated with 0.2 normal NaOH solutions until the stage of color change (Fawole and Opara, 2013). Also, the pH of the fruit juice was assessed utilizing a pH meter (Mahmoodi Tabar et al., 2009).

Statistical analysis

The experimental design was structured as a factorial arrangement within a completely randomized design (FCRD), incorporating two factors with five replications. The experimental factors included two scion levels (‘Khafr-e-Jahroom’ and ‘Rabab-e-Neyriz’) and four rootstock cultivars (‘Post Ghermaz-e-Aliaghai’, ‘Gorj-e-Dadashi’, ‘Gorj-e-Shahvar’, and a control group without grafting). The analysis of variance for the collected data was performed using SAS 9.0 software (Institute, Inc., USA), and the means were compared using Duncan's multiple range test at a significance level of $P \leq 0.05$ (Tables 3-5).

Results

Pomological traits

Weight of fruit, peel, and aril

The findings derived from the variance analysis indicated that the parameters of fruit weight, aril weight, and the mass of 100 arils were significantly influenced by the rootstock, scion, as well as the interaction between the rootstock and scion. Furthermore, the weight of the fruit peel was also impacted by the rootstock and the interaction of the rootstock and scion (Table 3). Data comparisons revealed that the highest fruit weight in the ‘Khafr-e-Jahroom’ cultivar was observed with GS rootstock. Also, the results showed that GS and GD rootstocks had similar effects on the fruit weight in the ‘Khafr-e-Jahroom’ cultivar so GSK and GDK grafting combinations had more fruit weight than their control (KWG). It was also observed that in the ‘Rabab-e-Neyriz’ cultivar, grafted on the rootstock of GD significantly increased fruit weight compared to its control (RWG). The mean comparison showed that in the ‘Rabab-e-Neyriz’ cultivar, the highest weight of fruit peel was observed with control (RWG). According to the results, the rootstock of the ‘Post Ghermaz-e-Aliaghai’ (P) produced analogous influences in both cultivars concerning fruit peel so that PR and PK grafting combinations both had lower fruit peel weight than non-grafting trees (RWG and KWG). About of aril weight, GDK and GSK compared to their control (KWG). The aril weight was more and had a significant difference with it, which shows that the GD and GS rootstock influenced the scion (K) and caused an increase in its aril weight. The means comparison showed that the GS, GD, and P affected the weight of 100 arils in the ‘Khafr-e-Jahroom’ cultivar so that the weight of 100 arils in the grafting combinations, GSK, GDK, and PK demonstrated a statistically significant enhancement in comparison to the contrast group (KWG). The maximum weight of 100 arils was recorded for the GSK grafting combination. In the ‘Rabab-e-Neyriz’ cultivar, except for the GDR grafting combination which, decreased the weight of 100 arils, the rest of the remaining grafting combinations did not exert a significant influence on the weight of 100 arils (Table 3).

Aril number and aril/peel ratio

The variance analysis revealed that the influences of scion, rootstock, and the interaction between rootstock and scion on the number of arils and aril/peel ratio were significant (Table 3).

Table 3. The ANOVA results for the effect of rootstock on pomological characteristics of the pomegranate cv. Rabab-e- Niriz' and Khafr-e- Jahrom fruit grafted on different rootstocks.

RS	**Fruit weight (g)**		**Peel weight (g)**		**Aril weight (g)**		**100 aril weight (g)**		**Aril/peel weight**		**Aril number**		**Aril browning (%)**		**Peel thickness (mm)**		**Seed thickness (mm)**	
	F Value	***Sig***	***F Value***	***Sig***	***F Value***	***Sig***	***F Value***	***Sig***	***F Value***	***Sig***	***F Value***	***Sig***	***F Value***	***Sig***	***F Value***	***Sig***	***F Value***	***Sig***
Rootstock (A)	88.43	***	21.34	***	47.81	***	91.80	***	23.90	***	43.47	***	246.35	***	18.45	***	14.33	***
Scion (B)	17.77	**	0.26	ns	35.64	***	51.67	***	46.40	***	16.16	**	733.97	***	11.60	ns	12.15	*
A×B	46.50	***	3.38	*	7.19	**	56.02	***	3.92	*	15.32	***	1208.04	***	18.09	***	16.28	***
	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS
Error	20	392.1339	16	468.6310	26	337.6941	16	4.6366	18	0.0050	26	2044.8654	32	3.9375	23	0.0150	19	0.0001
C.V (%)	6.54		16.38		12.46		5.34		6.71		12.66		4.45		9.89		2.98	
Means ± SE.																		
Grafting combination																		
PR	216.45 ± 5.70^{d}		55.44 ± 16.13^{c}		52.38 ± 12.41^{e}		35.183 ± 2.87^{de}		1.03 ± 0.01^{bcd}		115.75 ± 7.65^{c}		0 ± 0^{g}		16.3 ± 0.8^{a}		3.05 ± 0.02^{cd}	
GDR	357.11 ± 4.74^{b}		149.69 ± 13.84^{ab}		164.37 ± 5.56^{bc}		34.053 ± 0.20^{ef}		0.94 ± 0.03^{de}		473.00 ± 31.74^{a}		56 ± 1.51^{c}		11.7 ± 0.5^{bc}		3.05 ± 0.02^{cd}	
GSR	278.39 ± 6.03^{c}		145.59 ± 0.44^{ab}		149.59 ± 10.05^{cd}		41.130 ± 0.49^{bc}		0.99 ± 0.04^{cd}		350.00 ± 11.36^{b}		24 ± 0.70^{f}		11 ± 0.70^{c}		3.28 ± 0.09^{b}	
RWG	294.76 ± 2.67^{c}		168.77 ± 4.44^{a}		142.71 ± 2.73^{cd}		38.203 ± 0.50^{cd}		0.86 ± 0.01^{e}		338.75 ± 28.67^{b}		64 ± 0.70^{b}		11.1 ± 0.4^{c}		3.10 ± 0.05^{cd}	
PK	219.86 ± 6.21^{d}		86.75 ± 4.41^{c}		123.28 ± 2.31^{d}		35.163 ± 0.24^{de}		1.38 ± 0.04^{a}		344.00 ± 13.38^{b}		64 ± 1.22^{b}		13.5 ± 0.5^{b}		3.07 ± 0.04^{cd}	
GDK	345.10 ± 12.83^{b}		158.09 ± 23.89^{ab}		215.89 ± 4.48^{a}		44.003 ± 0.16^{b}		1.13 ± 0.02^{b}		471.60 ± 13.43^{a}		32 ± 0.70^{e}		8.5 ± 0.2^{d}		3.61 ± 0.07^{a}	
GSK	477.31 ± 25.29^{a}		165.98 ± 3.42^{ab}		188.21 ± 14.04^{b}		64.010 ± 1.82^{a}		1.10 ± 0.05^{bc}		292.50 ± 38.43^{b}		68 ± 0.70^{a}		11.6 ± 0.34^{bc}		3.21 ± 0.06^{bc}	
KWG	262.80 ± 8.80^{c}		126.60 ± 13.25^{b}		131.95 ± 4.04^{d}		30.670 ± 0.37^{f}		0.97 ± 0.05de		424.20 ± 16.52^{a}		48 ± 0.7^{d}		16.3 ± 0.8^{a}		3.02 ± 0.01^{d}	

Means within a factor and column followed by the same alphabet are not significantly different at $P = 0.05$ by using the Duncan test. ^{ns} = non-significant effect; significant effects at the $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***).

Table 4. Effect of grafting combination on Phonological characteristics of the pomegranate cv. Rabab-e- Niriz' and Khafr-e- Jahrom fruit grafted on different rootstocks.

RS	Seed length (mm)		Aril length (mm)		Seed width (mm)		Aril width (mm)		100 seed fresh weight (g)		100 seed dry weight (g)		Seed hardness (Kgf)		Aril firmness (Kgf)		Peel color		Aril color	
	F Value	*Sig*	*F Value*	*Sig*	*F Value*	*Sig*	*F Value*	*Sig*	*F Value*	*Sig*	*F Value*	*Sig*	*F Value*	*Sig*	*F Value*	*Sig*	*F Value*	*Sig*	*F Value*	*Sig*
Rootstock (A)	24.17	***	14.64	***	12.94	**	6.92	**	23.38	***	16.79	***	18.03	***	45.33	***	5.87	**	98.26	***
Scion (B)	9.06	**	0.54	ns	5.74	*	6.86	*	279.29	***	87.31	***	2.40	ns	37.86	***	44.56	***	60.74	***
A×B	32.32	***	10.09	**	11.98	**	15.37	***	33.03	***	17.64	***	3.88	*	20.35	***	6.76	**	64.91	***
	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS
Error	16	0.0002	17	0.0013	16	0.0001	16	0.0335	21	0.0042	20	0.0103	16	0.1001	17	0.0013	19	0.9052	18	0.2518
C.V (%)	2.13		3.38		3.72		2.50		1.40		4.14		7.25		12.28		9.58		8.00	
Means ± SE .																				
Grafting combination																				
PR	6 ± 0^{e}		9.67 ± 0.2^{d}		3.83 ± 0. 1^{a}		7.50 ± 0.2^{a}		4.736 ± 0.03^{c}		2.553 ± 0.03^{a}		4.0567 ± 0.04^{cd}		0.426 ± 0.02^{ab}		11.40 ± 0.24^{a}		10.60 ± 0.24^{a}	
GDR	6.93 ± 0.06^{bc}		11.40 ± 0.3^{a}		3.11 ± 0.04^{c}		7 ± 0^{b}		5.075 ± 0.02^{a}		2.690 ± 0.04^{a}		4.4133 ± 0.15^{bc}		0.193 ± 0.01^{d}		10.33 ± 0.66^{a}		3.66 ± 0.33^{e}	
GSR	7.03 ± 0.06^{b}		10.46 ± 0.03^{bc}		3.42 ± 0.01^{b}		7.61 ± 0.07^{a}		4.606 ± 0.006^{d}		2.660 ± 0.02^{a}		5.1033 ± 0.26^{a}		0.090 ± 0.01^{e}		11.66 ± 0.33^{a}		6.33 ± 0.33^{bc}	
RWG	6.95 ± 0.08^{bc}		11.48 ± 0.04^{a}		3.07 ± 0.03^{c}		7.51 ± 0.01^{a}		4.950 ± 0.07^{b}		2.613 ± 0.01^{a}		3.4833 ± 0.19^{d}		0.317 ± 0.01^{c}		10.66 ± 0.33^{a}		5.66 ± 0.33^{cd}	
PK	7.05 ± 0.05^{b}		10.61 ± 0.07^{bc}		3.28 ± 0.04^{bc}		7.43 ± 0.01^{a}		4.627 ± 0.02^{d}		2.650 ± 0.06^{a}		4.0100 ± 0.09^{cd}		0.313 ± 0.02^{c}		8.00 ± 1.00^{b}		5.33 ± 0.33^{d}	
GDK	6.48 ± 0.06^{d}		11.40 ± 0.1^{a}		3.28 ± 0.01^{bc}		7.50 ± 0^{a}		4.327 ± 0.02^{f}		2.200 ± 0.11^{b}		5.0833 ± 0.20^{a}		0.373 ± 0.008^{bc}		7.00 ± 0^{b}		5.00 ± 0^{d}	
GSK	7.40 ± 0.18^{a}		11.01 ± 0.04^{ab}		3.16 ± 0.06^{c}		7.31 ± 0.01^{ab}		4.340 ± 0.02^{f}		2.266 ± 0.06^{b}		4.7067 ± 0.23^{ab}		0.236 ± 0.02^{d}		8.00 ± 1.00^{b}		4.00 ± 0^{e}	
KWG	6.70 ± 0.05^{cd}		10.30 ± 0. 2^{c}		3.23 ± 0.04^{bc}		6.60 ± 0^{c}		4.500 ± 0.04^{e}		1.950 ± 0.02^{c}		4.0567 ± 0.15^{cd}		0.463 ± 0.02^{a}		11.00 ± 0^{a}		6.66 ± 0.33^{b}	

Means within a factor and column followed by the same alphabet are not significantly different at $P = 0.05$ by using the Duncan test. ^{ns} = non-significant effect; significant effects at the $P \leq 0.05$ (*), $P \leq 0.01$ (**) and $P \leq 0.001$ (***).

Table 5. Effects of grafting combinations on seedlessness percentage and biochemical characteristics of the pomegranate cv. Rabab-e- Niriz' and Khafr-e- Jahrom fruit grafted on different rootstocks.

RS	**Seedlessness (%)**		**Vitamin C (mg 100 g^{-1} FW)**		**TSS (Brix)**		**TA (%)**		**TSS/TA**		**TPC (mg GA.g^{-1} FW)**		**Aril anthocyanin (mg 100 g^{-1} FW)**		**pH of fruit juice**	
	F Value	***Sig***	***F Value***	***Sig***	***F Value***	***Sig***	***F Value***	***Sig***	***F Value***	***Sig***	***F Value***	***Sig***	***F Value***	***Sig***	***F Value***	***Sig***
Rootstock (A)	284.72	***	89.52	***	30.11	***	54.24	***	39.91	***	21.69	***	20.13	***	18.64	***
Scion (B)	754.90	***	186.10	***	0.34	ns	9.62	**	21.57	**	2.18	ns	102.85	***	34.25	***
A×B	280.87	***	91.65	***	6.61	**	3.14	*	23.52	***	34.81	***	38.29	***	39.37	***
	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS
Error	32	0.4117	18	0.7022	20	0.514	20	0.0061	17	7.5449	18	36.4886	20	0.1561	21	0.0120
C.V (%)	13.26		8.13		4.81		12.23		11.44		18.78		16.99		3.04	
Means ± SE.																
Grafting combination																
PR	2.00 ± 0.31^{d}		7.92 ± 0.50^{c}		17.65 ± 0.23^{a}		1.015 ± 0.004^{a}		17.565 ± 0.21^{d}		43.507 ± 0.51^{b}		5.12 ± 0.21^{a}		3.42 ± 0.13 cde	
GDR	2.00 ± 0.31^{d}		7.92 ± 0^{c}		13.26 ± 0.48^{d}		0.486 ± 0.01^{d}		24.270 ± 0.40^{cb}		38.060 ± 1.37^{bc}		2.61 ± 0.24^{b}		3.49 ± 0.04 cd	
GSR	2.00 ± 0.31^{d}		7.81 ± 0.06^{c}		13.37 ± 0.38^{d}		0.700 ± 0.01^{b}		19.678 ± 0.75^{cd}		18.873 ± 0.49^{de}		2.45 ± 0.25^{bc}		3.33 ± 0.03 de	
RWG	2.20 ± 0.20^{d}		8.63 ± 0.75^{c}		15.23 ± 0.34^{b}		0.627 ± 0.02^{bc}		25.170 ± 0.86^{b}		23.480 ± 0.77^{de}		1.82 ± 0.27^{cd}		3.74 ± 0.02 b	
PK	4.00 ± 0.31^{c}		8.81 ± 0.01^{c}		15.82 ± 0.17^{b}		0.900 ± 0.05^{a}		16.067 ± 1.11^{d}		29.023 ± 0.29^{cd}		1.29 ± 0.20^{ed}		3.52 ± 0.03 c	
GDK	16.00 ± 0.31^{a}		22.29 ± 0.58^{a}		14.76 ± 0.29^{bc}		0.355 ± 0.02^{e}		43.293 ± 1.68^{a}		18.637 ± 0.71^{de}		1.07 ± 0.16^{e}		4.20 ± 0.02 a	
GSK	0.50 ± 0.13^{e}		8.86 ± 0.06^{c}		13.72 ± 0.57^{cd}		0.519 ± 0.05^{cd}		27.557 ± 2.19^{b}		16.313 ± 0.59^{e}		1.96 ± 0.14^{bc}		3.80 ± 0.03 b	
KWG	10.00 ± 0.31^{b}		11.14 ± 0.58^{b}		14.96 ± 0.38^{b}		0.687 ± 0.05^{b}		19.841 ± 3.22^{cd}		62.210 ± 7.24^{a}		2.20 ± 0.24^{bc}		3.28 ± 0.07 e	

Means within a factor and column followed by the same alphabet are not significantly different at $P = 0.05$ by using the Duncan test. ^{ns} = non-significant effect; significant effects at the $P \leq 0.05$ (*), $P \leq 0.01$ (**) and $P \leq 0.001$ (***).

The mean comparison indicated that in the ‘Khafr-e-Jahroom’ cultivar, P, GD, and GS rootstocks increased the aril/peel ratio, with the maximum aril/peel ratio being observed in the PK grafting combination. In the ‘Khafr-e-Jahroom’ cultivar, grafting combinations of PK, GDK, and GSK had a higher aril/peel ratio than non-grafting trees (KWG). In the ‘Rabab-e-Neyriz’ cultivar, P and GS rootstocks increased the ratio of aril/peel to weight, with the highest aril/peel ratio being noted in grafting combinations of PR and GSR that had significant differences contrast to the contrast group (RWG). Additionally, the results indicated that the GD rootstock increased the number of arils in the cultivar of ‘Rabab-e-Neyriz’. Based on the results, in both cultivars, the highest number of arils was obtained with GD rootstock, however, in the ‘Khafr-e-Jahroom’ cultivar; no significant difference was found between the GD rootstock and the control (Table 3).

Aril browning disorder peels thickness, and Seed thickness

According to the results of variance analysis, aril browning, and seed thickness were influenced by the rootstock, scion, and interaction between rootstock and scion, while the effects of the rootstock and the interaction of the rootstock and scion on the thickness of the fruit peel were significant (Table 3). The means comparison showed that in the ‘Rabab-e-Neyriz’ cultivar, P, GD, and GS rootstocks decreased aril browning percentage compared to the control (RWG) significantly. In the ‘Khafr-e-Jahroom’ cultivar, P and GS rootstocks caused a significant increase and GD rootstock caused a significant decrease in aril browning percentage compared to the non-grafting trees (KWG). In the ‘Rabab-e-Neyriz’ cultivar, the rootstock of P increased the thickness of the fruit peel, while, other rootstocks exhibited no notable impact on this parameter. In the ‘Khafr-e-Jahroom’ cultivar, except for the GDK grafting combination that had a lower thickness of the fruit peel, no substantial differences were observed among the other grafting combinations regarding fruit peel thickness. The comparative analysis of means showed that, except for the GSR grafting combination that had an increase in the seed thickness, no significant distinctions were evident among the other grafting combinations regarding seed thickness (Table 3).

Dimensions of aril and seed

The results derived from the variance analysis of the data indicated that both the width and length of the aril, as well as the width and length of the seed, were influenced by the rootstock and the interaction between the rootstock and scion (Table 4). The comparison of means revealed that both GSR and PR grafting combinations had a decrease in aril length compared to non-grafting trees (RWG), while, GDK and GSK grafting combinations had more aril length than non-grafting trees control (KWG). Aril width, decreased with the GDR grafting combination, while it was increased with PK, GDK, and GSK grafting combinations compared to control (KWG). Based on the comparison of means, ‘Rabab-e-Neyriz’ cultivar, the seed thickness in the GSR grafting combination was significantly augmented in comparison to the control (GSR), whereas no significant differences were identified among the other grafting combinations and the control. In the ‘Khafr-e-Jahroom’ cultivar, the seed thickness was significantly increased in all grafting combinations compared to PK which had not significantly difference from the control. The most considerable seed length was recorded in the grafting combination of GSR. In both cultivars, except for GDK, which exhibited no significant influence on seed length in the ‘Khafr-e-Jahroom’ cultivar, the rest of the grafting combinations resulted from an addition in seed length contrast to the control group (KWG). In ‘Rabab-e-Neyriz’ cultivar, seed width was affected grafting combination except for the GDR which did not have a significant effect on it. No significant difference was observed in the ‘Khafr-e-Jahroom’ cultivar between grafting combinations and the control (KWG) concerning seed width. Based on means compassion the largest seed width was observed in the grafting combination of PR (Table 4).

Fresh and dry seed weight

The variance analysis indicated that both dry and fresh seed weights were influenced by the rootstock, scion, and the interaction between rootstock and scion (Table 4). The maximum of seed fresh weight occurred in the grafting combination GDR. In the PR and GSR grafting combination, seed fresh weight significantly decreased compared to their control (RWG). Also, seed fresh weight increased significantly in the PK grafting combination compared to its control (KWG), while GDK and GSK grafting combinations decreased it. Regarding the dry weight of the seed, grafting combinations (PK), (GDK), and (GSK) led to an increase in dry seed weight when contrast to the control (KWG), while no significant discrepancy was observed among the other grafting combinations (Table 4).

Aril and seed hardness and seedlessness percentage

According to the results of variance analysis, aril hardness, and seedlessness percentage were influenced by the rootstock, scion, and the interaction between rootstock and scion (Tables 4 and 5). The comparative analysis of means indicated that the aril hardness in the grafting combination PR increased compared to the control (RWG), while GSR and GDR decreased it. Also, results showed that three rootstocks P, GS, and GD had a similar effect on the hardness of the aril 'Khafr-e-Jahroom' cultivar and caused its significant decrease. Based on the comparison of the means in both cultivars, it was observed that the P rootstock did not exert a significant impact on seed hardness, while GS and GD rootstocks increased seed hardness compared to their control. The highest seed hardness was observed in GDK and GSR grafting combinations (Table 4). Based on means comparison, GDK grafting combination increased the percentage of seedlessness, and GSK and PK grafting combinations decreased it compared to non-grafting trees (KWG) (Table 5).

Peel and aril color

According to the results of variance analysis, fruit peel color and aril color, were influenced by rootstock, scion, and the interaction of rootstock and scion (Table 4). Means indicated that PK, GDK, and GSK grafting combinations decreased peel color compared to the control (KWG). Aril color was significantly reduced in grafting combinations of PK, GDK, and GSK compared to the control (KWG). The highest and lowest aril color was observed with PR and GDR grafting combinations respectively, which showed that the rootstocks P and GD affected the scion (R) and caused an increased or decreased aril color (Table 4).

Biochemical traits of fruit

Vitamin C, anthocyanin, total phenolic compound

Based on the results of variance analysis, the Vitamin C content in fruit and aril anthocyanin levels were influenced by rootstock, scion, and the interaction of rootstock and scion; moreover, the effects of rootstock and the interaction of rootstock and scion on the total phenolic compounds in the aril were found to be significant (Table 5). The comparison means of grafting combinations showed that in the cultivar of 'Rabab-e-Neyriz', there was no notable discrepancy among the grafting combinations and the control (RWG) regarding the amount of Vitamin C in fruit, while in the 'Khafr-e-Jahroom' cultivar, the GD rootstock was associated with an elevation in Vitamin C levels in the fruits. Total phenolic compounds of an aril in the 'Rabab-e-Neyriz' cultivar, increased with P and GD rootstocks, while no notable discrepancy was noted between the GSR grafting combination and the control. In the 'Khafr-e-Jahroom' cultivar all grafting combinations had a significant decrease in phenolic compounds of aril in contrast to the control (KWG) so the maximum content of the phenolic compound of aril was observed in the control of this cultivar. The comparison of the means showed that the amount of aril anthocyanin in the cultivar 'Rabab-e-Neyriz', increased with grafting combinations PR and GDR in contrast with the control (RWG), however, no notable discrepancy was noted between the control and the GSR grafting combination. In the 'Khafr-e-Jahroom' cultivar, grafting combinations of PK and GDK caused a significant decrease in aril anthocyanin in contrast to the KWG control. The maximum concentration of aril anthocyanin was recorded in the PR grafting combinations (Table 5).

TSS, TA, TSS/TA, and pH of fruit juice

The findings derived from the analysis of variance indicate pH, TA, and TSS/TA of fruit juice were significantly influenced by the rootstock, scion, and their interaction; notably, the impact of rootstock and the interaction between rootstock and scion on TSS was statistically significant (Table 5). Based on the comparison of means, in the cultivar 'Rabab-e-Neyriz', the PR grafting combination increased the TSS of fruit while two grafting combinations GDR and GSR resulted in a statistically notable reduction in TSS of fruit in contrast to the control (RWG). In the 'Khafr-e-Jahroom' cultivar, except for the GSK grafting combination that decreased the TSS of fruit, other grafting combinations had no significant difference compared to the control (KWG) related to the TSS of fruit. The highest TSS was observed in the grafting combination of PR. Based on the comparison of the means, in the 'Rabab-e-Neyriz' cultivar, TSS/TA in the PR and GSR grafting combinations was significantly reduced compared to non-grafting trees (RWG), in contrast, in the 'Khafr-e-Jahroom' cultivar, grafting combinations GSK and GDK increased TSS/TA in contrast to the control (KWG). The GDK grafting combination exhibited the maximum TSS/TA ratio. Based on the results, in the 'Rabab-e-Neyriz' cultivar, all grafting combinations significantly decreased the pH of the fruit juice compared to the control (RWG), while in the 'Khafr-e-Jahroom' cultivar, the pH of the fruit juice was higher in across all grafting combinations than control (KWG). The highest pH of fruit juice was observed with the GDK grafting combinations (Table 5).

Discussion

Although several studies have investigated the pomological and biochemical traits of pomegranate, (Caliskan and Bayazit, 2013; Karimi and Mirdehghan, 2013; Ismail et al., 2014; Khadivi-Khub et al., 2015; Karimi et al., 2020) but the current investigation represents the inaugural comprehensively studying the effect of rootstock on the pomological and biochemical traits of pomegranate fruit. The results of this study indicate that the influence of rootstock on the assessed traits of pomegranate fruit exhibited both enhancement and diminishment. Our findings align with the research conducted by Riesen and Husistein (1998) focused on apples, about the influence of rootstock on fruit quality. Based on the present results, the total phenol content of aril, soluble solid content, and Vitamin C of the juice were influenced by the rootstock and interaction of rootstock and the scion, which confirmed previous reports on apple (Mainla et al., 2011), 'greengage' plum (Reig et al., 2018; Font i Forcada et al., 2019) citrus (Magwaza et al., 2017), lemon (Gil-Izquierdo, 2004), peach and apricot (Scalzo et al., 2005) and cherry (Usenik and Štampar, 2000). It has been documented that the concentration of Vitamin C in fruits is associated with the levels of macro and microelements present in the leaves. Potassium and zinc elements increase Vitamin C in fruits, while high phosphorus and nitrogen decrease it (Lee and Kader, 2000; Dou et al., 2005; Eman et al., 2007).

According to the findings of the current investigation, the PR grafting combination had an aril browning percentage (zero percent), the lowest aril weight, fruit weight, peel weight, and aril and seed length among the grafting combinations and compared to the control (RWG) and also PR had the highest amount of aril anthocyanin, TSS, TA, peel thickness among the grafting combinations and it had a significant increase than control (RWG). In the study, Karimi et al. (2019, 2023), investigated the influence of rootstock and scion on ecophysiological parameters and various growth indices and absorption of nutrients in two pomegranate cultivars 'Rabab-e-Neyriz' and 'Khafr-e-Jahroom', and reported that PR had higher SPAD index, zinc element, and relative water content of leaf than its control. Aril browning disorder in pomegranate is caused by oxidative stress caused by environmental stress, so there is a possibility that the 'Post Ghermaz e Aliaghai' rootstock has saved the relationship water in the scion. Zn element is part of the non-enzymatic antioxidant, thereby mitigating the influence of oxidative stress (Subba et al., 2014). It seems that the reduction of aril browning disorder in the 'Rabab-e-Neyriz' cultivar with 'Post Ghermaz e Aliaghai' (P) due to its higher efficiency in absorbing zinc element and its role element in the synthesis of enzymes involved in the control of oxidative stress. Another reason could be related to increase of the peel thickness fruits in with this rootstock. Several studies have indicated that pomegranate cultivars with thicker fruit peel and higher anthocyanin in arils had lower percentages of aril browning (Meighani et al., 2014; Darsana et al., 2016). Furthermore, another perusal indicated that the anthocyanin concentration in browned arils is significantly lower than that found in non-browning arils (Shivashankar et al., 2004). In the current study, within the PR grafting combination, a higher level of anthocyanin, TSS, TA, and phenolic compounds was observed in aril due to the higher efficiency of this rootstock (P) in absorbing zinc. It reported that, zinc elements role in the synthesis of anthocyanin in fruits of grape and apricot trees (El-Badawy, 2013; Song et al., 2015; Mahdavi et al., 2022). Mahdavi et al. (2022), The impact of the zinc element on grapefruit, with findings reporting a substantial increase in anthocyanin levels in grapefruit upon the application of zinc nano-chelate. In the grafting combination of PR, fruit weight, aril length, and seed were reduced considerably compared to the control which, can be due to photosynthetic parameters, as well as the lower (chlorophyll *b* and F_v/F_m) in this grafting combination. In a prior investigation, Karimi et al. (2019) reported that the rootstock of 'Post Ghermaz-e-Aliaghai' (P) decreases F_v/F_m and chlorophyll *b*, in 'Rabab-e-Neyriz' cultivar. Vazifeshenas et al. (2009) assessed the influence of various grafting combinations across three pomegranate cultivars on vigor, tree size, and fruit quality. They reported that the 'Shavar' cultivar grafted on 'Golnar Farsi' achieved the highest yield while exhibiting the least sucker compared to 'Poost Syah', 'Malas Esfahani', and 'Khafri Poost Sefid Jahrom' rootstocks.

According to the findings of this perusal, the GDK grafting combination had the highest Vitamin C and fruit juice pH and aril weight as well as a grafting combination of GDK, increased fruit weight, the weight of 100 arils, aril dimensions, seed hardness, and decreased aril browning which can be due to its strong root system and higher efficiency it, in the absorption of elements potassium, Magnesium, copper and zinc. Potassium is an essential role in Osmoregulation, enzyme activity, neutralization reactions, and water and nutrients transfer processes (Reddy et al., 2004). Therefore, the reduction of aril browning grafting combination of GDK can be related to the impress of potassium in modulating the plant's water relations and alleviating oxidative stress.

As indicated by the findings of the current investigation, although the GSK grafting combination had the highest fruit weight and weight of 100 arils, it had the highest aril browning percentage among the grafting combinations. In addition, in the grafting combination of GSK, there was a notable enhancement in pH, TSS/TA of fruit juice, seed hardness, aril dimensions, and the aril/peel ratio, coupled with a significant decline in Vitamin C content, TSS, TA, aril color, peel color, and the firmness of the aril, was observed compared to its control (KWG). Increasing the fruit weight, aril dimensions, and TSS/TA, can be related to higher photosynthesis parameters in this grafting combination. It has been reported that in the GSK grafting combinations leaves, the amount of total chlorophyll, chlorophyll *a*, magnesium, copper, and zinc elements increased significantly in contrast to the non-grafting control (Karimi et al., 2019; Karimi et al., 2023). Increasing the photosynthesis parameters in the GSK grafting combination increases the fruit size and the weight of 100 arils. Fruit growth is influenced by the interaction among carbohydrate sources and sinks. A significant correlation exists between fruit as the sink and leaves as the source; additionally, both fruit quality and growth are impacted by leaf characteristics (Smith and Stitt, 2007). A linear correlation between fruit carbohydrates and leaf carbohydrates has been documented in apple trees (Alan and Goffinet, 2013). Fruit size serves as a critical determinant of fruit quality, which is influenced by rootstock attributes. In the 'Navel' sweet orange cultivar, large fruits are produced with sour orange rootstock, and smaller fruits with lemon rootstock (Hartmann et al., 1997). Although the GSK grafting combination had the highest fruit weight, it had a higher aril browning. Correlation between fruit size and aril browning rate in pomegranate was studied and reported that genotypes with small fruits have a lower percentage of aril browning (Kavand et al., 2020). It has been documented that with increasing weight and fruit size of pomegranate, the existence of brown arils is increased (Khodade, 1987).

The grafting combination of GSR had the highest seed hardness and aril browning decreased with this grafting combination. In previous studies, has been reported that in the GSR grafting combination leaves, the amount of zinc, copper, and calcium elements increased significantly in contrast to the control (Karimi et al., 2019; Karimi et al., 2023) which can be one of the reasons on the increasing hardness of seeds. Also, the reduction of aril browning in GSR grafting combination can be related to better water relationship in the 'Rabab-e-Neyriz' cultivar with GS rootstock and the role of calcium and copper in controlling oxidative stress (Meighani et al., 2014; Tadayon, 2021). Straight oxidation of phenolic compounds mediated by peroxidase (POD) and polyphenol oxidase (PPO) enzymes is a principal factor contributing to the browning of fruit tissue (Tomas-Barberan and Espin, 2001), which is caused by oxidative damage to membranes (Shivashankara et al., 2004). Calcium stimulates anthocyanin accumulation and is instrumental in the synthesis of antioxidants (Xu et al., 2014), thereby facilitating the efficient elimination of ROS by the antioxidant system under optimal status (Mittle, 2002).

Under the findings of the current investigation, the GDR grafting combination of anthocyanin and phenolic compounds in arils increased compared to non-grafting trees (RWG), and the percentage of aril browning, of arils was firmness decreased (RWG). In a study, Karimi et al. (2019 and 2023) measured the vegetative and physiological parameters in the grafting combination of GDR and reported that the SPAD index, the RWC, and the concentrations of magnesium, phosphorus, and zinc in leaves of GDR grafting combination, significantly increased and dry weight of sucker decreased compared to the non-grafting trees. Therefore, the reasons for the increase of aril anthocyanin and fruit weight in the GDR grafting combination can be related to the better condition of nutrient elements and leaf water relations. It can also be inferred that the improved water relations of the scion, along with the elevated anthocyanin concentration in the arils, contributed to the mitigation of aril browning. In several studies, it has been reported that pomegranate cultivars with higher anthocyanin content have fewer browning arils due to the neutralization of ROS (Dokhanieh et al., 2016). Based on the present study and previous reports on the grafting combinations present, it can be stated that the higher biochemical parameters measured in the 'Rabab-e-Neyriz' and 'Khafr-e-Jahroom' cultivars grafted onto 'Gorj-e-Shahvar' and 'Gorj-e-Dadashi' rootstocks may be associated with the broader root system of these rootstocks facilitating superior uptake of nutrients and water (Karimi and Nowrozy, 2017).

PK grafting combination increased significantly aril browning and also decreased fruit weight, peel weight, and aril number which can be due to the weak in terms of the root system and less efficient in absorbing minerals. Also, the scions on this rootstock have fewer leaf pigments and low relative water content in leaves, which these traits can be related to it probably is the aril browning disorder in the PK grafting combination. It has been reported that increasing the minerals of the pomegranate tree can reduce aril browning disorder and improve fruit quality (Meighani et al., 2014; Tadayon, 2021).

Conclusion

The outcomes of this research indicated that the traits of pomegranate fruits of grafting cultivars are influenced not only by the rootstock but also by the genetic constitution of the scion. The findings showed that the rootstock can affect the pomegranate fruit's pomological and biochemical traits. Also, the results showed that in the 27 traits studied in this experiment, the interaction between rootstock and scion was significant for all traits. Also, in 27 studied traits, at least one scion cultivar was affected by a rootstock, and the rootstock increased or decreased that trait in the scion. The scion (K) and (R) were affected by at least one rootstock or rootstocks in 26 and 23 traits, respectively, and the rootstock or rootstocks caused increased or decreased traits in them so, scion (K), only the aril width and in scion (R), four traits of Vitamin C, seedlessness percentage, seed dry weight, and peel color were not affected by a rootstock or rootstocks. Moreover, the findings of the current perusal show that traits looking as TSS, vitamin C, total aril phenol content, aril anthocyanin, seed hardness, seed size, and fruit size, which determine the commercial and nutritional value of pomegranate, can be affected by the rootstock. Also, the current results showed that the aril browning problem, which is one of the physiological disorders of pomegranates all over the world, can be controlled by choosing the appropriate rootstock, which suggests more research in this field. Conforming to the findings of the current investigation, it can be asserted that the application of suitable rootstock can be a practical step in overcoming abiotic and biotic stresses and improving the pomological traits and orchard management in pomegranate.

Author Contributions
HK was responsible for the experimental design; AS, SHM, AMM, HI, and KM executed the experiment. All authors have read and agreed to the published version of the manuscript.

Data Availability
All pertinent data are in the manuscript and tables.

Conflict of Interest
The authors indicate no conflict of interest in this work.

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