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Effect of auxins on stem cutting propagation of three pomegranate genotypes and the relationship of endogenous phenolic compounds with root induction

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Abstract

Objective: The commercial propagation of pomegranates often relies on hardwood stem cuttings taken during the dormant season, and a link has been observed between phenolic compounds in donor plants and the success of adventitious rooting. This study aimed to investigate the effects of seasons, auxin, and genetic variation on pomegranate propagation, as well as the relationship between endogenous phenolic compounds and root induction.

Methods: In this study, the seasonal variation of phenolic compounds in the shoot skins of three pomegranate genotypes: wild (*Punica granatum* cv. spinosa) and two local cultivars (Malas Mumtaz and Shirin Behshahr) was measured. Samples were collected at the end of each season and analyzed for phenolic compounds (gallic acid, caffeic acid, and quercetin) using HPLC, alongside total phenols, flavonoids, and antioxidant activity through spectrophotometric methods. Then, in mid-February, hardwood cuttings procured from the same mother plants were taken, treated with auxin or left untreated, and planted in a sand substrate. Rooting traits were measured, and the relationship of phenolic compounds with rooting traits was assessed.

Results: In all seasons, the highest concentration of flavonoids and caffeic acid belonged to Shirin Behshahr and Malas Mumtaz pomegranate cultivars, respectively. Also, wild pomegranate showed the highest antioxidant capacity (DPPH) on average of four seasons, followed by Shirin Behshahr. The shoots collected during the winter season mainly had lower levels of caffeic acid and the lowest DPPH, but the summer season had the highest antioxidant capacity. Considering the auxin experiment, under no auxin, the Malas Mumtaz variety exhibited significantly higher rooting percentage (93.33%) than Shirin Behshahr and the wild genotype (56.67% and 50%, respectively). Although the auxin treatment at 2000 mg/L had a positive effect on all genotypes (100%, 96.67%, and 86.67% rooting for the wild genotype, Malas Mumtaz, and Shirin Behshahr, respectively), the effect was not significant for the Malas Mumtaz variety because it already had a high rooting percentage (93.33%). The Malas Mumtaz variety also exhibited a higher number of shoots (4.32) than the other two genotypes (4.00 and 3.48 for the wild genotype and the Shirin Behshahr variety, respectively) on average across two auxin conditions. Backward multiple regression showed that rooting percentage was related to quercetin and DPPH. For the mean root length and the longest root length, the remaining variables in the models were DPPH, phenols, and flavonoids. For the number of shoots, the flavonoids, DPPH, quercetin, and gallic acid showed a significant relationship with this trait. DPPH was present in all models with a negative partial regression coefficient. However, a positive relationship of quercetin with rooting percentage and number of shoots was observed.

Conclusion: This study demonstrates that adventitious rooting in pomegranate hardwood cuttings is influenced by seasonal changes of phenolic compounds in the mother plant, genotype, and the application of auxin. The Malas Mumtaz variety demonstrated a high genetic potential for rooting and showed satisfactory rooting, even in the absence of exogenous auxin treatment. Endogenous quercetin and DPPH showed a relationship with the rooting percentage and number of shoots. This finding proved the role of quercetin as a rooting enhancer in pomegranate cuttings.

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Introduction

Propagation through hardwood stem cuttings is widely regarded as the easiest and most effective method for multiplication of pomegranates. According to Holland *et al*. (2009) and Sarkhosh *et al*. (2021), this method is the most commonly used in pomegranate orchards worldwide, where saplings are typically propagated from stem cuttings. Commercial propagation of pomegranate cultivars primarily relies on hardwood cuttings, with layering also being used to a lesser extent (Singh *et al*. 2021). In southern India, air layering is a widely practiced method for propagating pomegranates (Kumar *et al*. 2018). As a result, pomegranate trees are predominantly grown as own-rooted plants in orchards, which has limited the exploration of grafting and budding techniques (Vazifeshenas *et al*. 2009). However, a recent trend in some Iranian pomegranate nurseries and orchards has seen an increased use of grafted saplings; hence, the top grafting techniques in pomegranate orchards are already optimized (Alizadeh and Habibzadeh 2024).

In addition to traditional propagation methods such as cuttings, layering, offshoots, and grafting, mass propagation using plant tissue culture techniques has gained popularity. Consequently, several researchers, including Belkacem *et al*. (2014), Valizadehkaji *et al*. (2013), and Guranna and Huchesh (2018), have tried to develop efficient and reproducible protocols for the direct *in vitro* propagation of pomegranate plantlets, achieving promising results with this approach.

The pomegranate tree is one of the easiest-to-root fruit trees. However, rooting of cuttings is still challenging in some genotypes due to low rooting percentage, weak roots, slow root growth, and high mortality rate. Poor rooting has also been reported in some wild pomegranate genotypes (Alizadeh and Nazari 2021). The percentage of rooting depends on various factors such as the type of cutting, the place and time of cutting, and the age of the branch (Sarkhosh *et al.* 2021).

The optimal type of pomegranate cuttings involves hardwood cuttings that are 25 to 30 cm long and 1 to 1.5 cm in diameter during the dormant season. These cuttings should be sourced from bearing branches, preferably two years old, of high-quality mother plants. Treating the cuttings with auxin can enhance their rooting potential. The timing of cutting preparation significantly influences rooting success, with winter being the best season when the tree is dormant. Research by Owais (2010) indicates that cuttings taken at the end of February have a higher rooting success compared to those collected in early October. Mehta *et al*. (2018) studied the impact of cutting timing on rooting and found that cuttings prepared from December to February yielded the highest number of rooted cuttings, as well as the highest branches and leaves on the new growth, with optimal results occurring in February.

Phenolic compounds, the most abundant secondary metabolites in plants, have been shown to play many significant roles. These compounds are produced by the plants mainly for their growth, development, and protection. These aromatic benzene ring compounds are essential for the plant's interactions with both biotic and abiotic stresses (Pratyusha 2022). It was also documented that phenolics protect auxins from decarboxylation so that after application of phenolics, more auxin is available to induce roots (Wilson and Van Staden 1990). Research indicated a positive correlation between the concentration of phenolic compounds and the number of adventitious roots produced in some plant species (Cheniany *et al.* 2010; De Klerk *et al.* 2011). Phenolic compounds can enhance root formation through their impact on cellular differentiation and signaling. The literature shows that several phenolic compounds, including phloroglucinol, gallic acid, ferulic acid, salicylic acid, quercetin, chlorogenic acid, caffeic acid, phloridzin, and tannins, are involved in adventitious root formation. Phloroglucinol (a triphenol) has been examined most intensively (Hammatt 1994). Gallic acid promotes root formation and possesses antioxidant properties that help plants to withstand stress during rooting. Caffeic acid is associated with enhanced root growth and stimulates root tissue differentiation. Ferulic acid facilitates adventitious root formation (De Klerk *et al.* 2011), particularly in tissue culture systems. As a potent antioxidant, chlorogenic acid helps to mitigate the damage caused by oxidative stress, which is a common byproduct of both biotic and abiotic stressors (Zhong *et al.* 2025). Salicylic acid plays a key role in plant stress responses and promotes rooting in various species (Bagautdinova *et al*. 2022). Quercetin enhances adventitious root development by regulating auxin signaling pathways, while certain tannins are linked to the stimulation of adventitious roots, likely through their effects on growth hormones (do Prado *et al*. 2015).

The presence of phenolic compounds influences physiological processes that regulate root development, highlighting their importance in adventitious root formation (Steffens and Rasmussen 2016). Understanding the relationship between these compounds and adventitious root production can provide valuable insights for improving rooting strategies in plant propagation. Therefore, this project aimed to evaluate the seasonal variation of phenolic compounds in different pomegranate genotypes, investigate the effect of auxin on their rooting, and determine the relationship between the endogenous phenolic compounds in the donor plants and the formation of adventitious roots in their hardwood stem cuttings.

Materials and Methods

The present study was undertaken in the Department of Horticultural Science, Gorgan University of Agricultural Sciences and Natural Resources, Iran. Initially, the pattern of changes in phenolic compounds within the shoot skins of pomegranate mother trees was measured. Three genotypes, including two local cultivars known as Malas Mumtaz and Shirin Behshahr, and a wild pomegranate genotype (*Punica granatum* cv. *spinosa*) were used. Shoot skin samples were collected on the 15th day of the final month of each season, representing four seasons in total. Five pieces of two-year branches of equal diameter were taken from different sides of the tree during this period and transported to the laboratory with proper labeling. The bark and wood of the branches were separated using a knife (Figure 1). To preserve and stabilize the samples until all seasonal collections were completed across the three genotypes, the skin samples were wrapped in aluminum foil and stored in a freezer at -80 °C. The levels of phenolic compounds, including gallic acid, caffeic acid, and quercetin, were measured using the HPLC technique (Tsao *et al.* 2003; Hemmaty *et al.* 2011). Also, the flavonoids (Ebrahimzadeh *et al.* 2008) and DPPH (Prevc *et al.* 2013) were assessed by the spectrophotometric method.

In mid-February, at the time of collecting skin samples for the winter season, 90 hardwood cuttings from two-year-old shoots were also taken from each genotype. These cuttings were then inserted in a fine sand substrate, either treated with indole butyric acid (IBA) (2000 mg/L) or left without auxin treatment. The rooting traits, such as rooting percentage, number of roots, root length, and shoot length, were measured 90 days after planting.

To verify the relationship between caffeic acid and rooting, an initial experiment was also carried out exclusively with the Malas Mumtaz cultivar. In this experiment, 90 standard cuttings, each 25-30 cm long and about 2 cm in diameter, were taken from a mother tree and inserted to a rooting substrate. After 40 days, when leaves began to emerge, the cuttings were treated with a foliar spray of caffeic

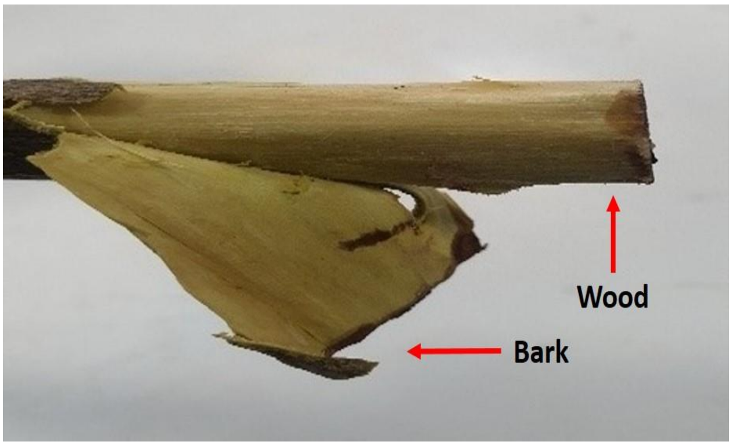

Figure 1. A sample of stem bark tissues of pomegranate utilized for measuring phenolic compounds.

acid extracted from fresh tea leaves at concentrations of 1000 and 2000 mg/L. The extract was prepared by soaking fresh tea leaves in a solvent, following the method of Neyestani and Khalaji (2009). Three apical leaves were used, with 20 grams of fresh tea leaves weighed and crushed. The chopped leaves were mixed with a 1:10 methanol solvent and incubated in the dark for 72 hours. After filtration through filter paper, the liquid was concentrated using a Soxhlet apparatus and then freeze-dried to remove the solvent completely. The dried extract was reconstituted to create caffeic acid solutions at concentrations of 1000 and 2000 mg/L. The caffeic acid solutions were sprayed on the cuttings for three consecutive days under calm weather conditions, while a control group received distilled water. After three months, the rooted plants were carefully removed from the substrate, and the rooting percentage, root length, and number and length of shoots were recorded.

Data collection and analysis

For the evaluation of phenolic compounds, a factorial experiment with two factors based on a completely randomized design (CRD) with three replications was conducted. The factors were season (winter, spring, summer, and autumn) and genotype (Malas Mumtaz, Shirin Behshahr, and a wild genotype). The rooting experiment was also performed as a factorial experiment based on a randomized complete block design (RCBD) with three replications. The factors were auxin (application of auxin and control) and genotype (Malas Mumtaz, Shirin Behshahr, and a wild genotype). Each replication consisted of 30 stem cuttings. Also, the backward multiple regression was used to analyze the relationship of phenolic compounds as independent variables with each of the rooting traits as dependent variables. Before the analysis of variance, the assumption of equality of variances was checked by the Levene’s test for the CRD and by the residual plot for the RCBD. When the assumption of equality of variances was not satisfied through Levene’s test, the Games-

Howell test for comparing means was used. Normality of the errors (residuals) was verified by the normal probability plot (Q-Q plot) and also by the Shapiro-Wilks test. The probable existence of the outlier data was assessed by the Cook's distance, and a distance equal to or lower than unity indicated the lack of an outlier. The data were analyzed with SAS (version 9) and SPSS (version 23) statistical software.

Results and Discussion

The Q-Q plot and the Shapiro-Wilks test for all traits showed the normality of residuals. Except for quercetin, the assumption of equality of variances across treatments was also met, using the Levene test and residual plot. In the case of quercetin, the Games-Howell test was conducted to compare the three pomegranate genotypes to account for the heterogeneous variances. There were no outliers in the data because all Cook's distances were below one (data not shown).

Changes in phenolic compounds in different seasons in three pomegranate genotypes

Phenolic compounds are a diverse class of chemical compounds characterized by the presence of one or more hydroxyl groups (-OH) attached to an aromatic hydrocarbon ring. They are widely distributed in the plant kingdom and play crucial roles in plant physiology, ecology, and human health (Zhang *et al.* 2022). They act as natural protectants against pathogens, pests, and environmental stressors, helping to ensure plant survival and health (Boo 2019; Kisiriko *et al.* 2021).

The assessment of phenolic compounds in three pomegranate genotypes showed significant differences among the genotypes for phenols, flavonoids, caffeic acid, DPPH, quercetin, and gallic acid. Significant variation among the four seasons was also observed for all phenolic compounds, except for quercetin. The genotype × season interaction for phenols, flavonoids, caffeic acid, and gallic acid (Table 1). One reason for the lack of significant seasonal variation as well as genotype × season interaction for quercetin may be the high coefficient of variation obtained for this compound (Table 1).

The results about the phenolic substances reveal that Shirin Behshahr pomegranate had the highest concentration of flavonoids in all seasons, that of gallic acid in the winter and spring, and of phenols in the winter. Meanwhile, the Malas Mumtaz pomegranate contained the greatest amounts of caffeic acid in all seasons, except for the winter season, gallic acid in summer and autumn, and quercetin on average across four seasons. Additionally, wild pomegranate exhibited the highest antioxidant capacity (DPPH) on the average of four seasons, followed by Shirin Behshahr. This wild genotype also showed the highest phenols in the summer and caffeic acid in the winter as compared to other varieties at the same season (Table 2).

The immediate measurement of phenolic substances at the time of cutting (winter season) is crucial. Although there was a significant genotype × season interaction for phenols, flavonoids, caffeic acid, and gallic acid, we can see from Table 2 that shoots collected during the winter season mainly contained lower levels of caffeic acid. Also, the winter season showed the lowest DPPH, but the summer season had the highest antioxidant capacity. Although the phenol content showed somewhat lower variation throughout the year across the studied genotypes, the significant genotype × season interaction suggests that the fluctuations in phenolic levels could be valuable for researchers and food industries looking to extract these compounds from pomegranate tissues.

The shoots and stem tissues of pomegranates are rich in phenolic compounds, which are recognized for their strong antioxidant properties. For example, caffeic acid present in these tissues plays a crucial role in scavenging free radicals, helping to protect the plant’s cells from oxidative damage (Benabdallah *et al.* 2016). Pomegranate skin and shoots contain a variety of phenolic compounds, including quercetin and gallic acid. Quercetin is known for its anti-inflammatory effects, while gallic acid contributes to the overall health benefits of the plant. These phenolics not only protect the plant from environmental stress but also have potential medicinal properties. Abiotic stresses such as drought, high light intensity, nutrient deficiency, and extreme temperature can all stimulate the production of phenolic compounds in plants (Zargoosh *et al.*, 2019). The amount of flavonoid, gallic acid, and phenols is affected by several factors, including genetic diversity, environmental conditions, fruit growth stage, and measurement methods (Schulz *et al.* 2016). High levels of heat and light can also lead to the degradation of phenolic compounds in some cases (Antony and Farid 2022), and this is one reason we observed the highest antioxidant activity in summer and the lowest in winter (Table 2).

Table 1. Analysis of variance of the effect of season on phenolic compounds in the branch bark of three pomegranate genotypes.

SOV	**df**	**Phenols**	**Flavonoids**	**DPPH**	**Caffeic acid**	**Quercetin**	**Gallic acid**
Genotype (G)	2	166.49**	25.02**	0.496**	0.084**	0.001022**	1.390**
Season (S)	3	113.73**	6.18**	0.217*	0.073**	0.000167	0.524**
G × S	6	55.98*	3.50**	0.170	0.039**	0.000210	0.720**
Error	24	17.98	0.326	0.073	0.0007	0.000109	0.0012
CV (%)	-	14.71	13.34	3.53	11.75	80.41	5.87

*,**: Significant at 0.05 and 0.01 probability levels, respectively.

Table 2. The seasonal changes of phenolic substances in bark stem tissues of three pomegranate genotypes.

Season	Genotype	Phenols^{+} (mg GA/g dw)	Flavonoids^{+} (mg/g dw)	DPPH^{++} (%)	Caffeic acid^{+} (mg/g dw)	Quercetin^{++} (mg/g dw)	Gallic acid^{+} (mg/g dw)
Winter	Malas Mumtaz	20.1^{d+++}	2.26^{fg}		0.113^{e}		0.273^{h}
	Shirin Behshahr	35.2^{ab}	4.84^{bc}		0.113^{e}		0.757^{b}
	Wild	23.0^{cd}	3.25^{ef}		0.203^{d}		0.440^{fg}
Spring	Malas Mumtaz	19.5^{d}	2.13^{g}		0.300^{b}		0.500^{ef}
	Shirin Behshahr	28.7^{bc}	5.31^{bc}		0.120^{e}		2.150^{a}
	Wild	29.0^{bc}	3.79^{de}		0.033^{f}		0.230^{h}
Summer	Malas Mumtaz	28.8^{bc}	5.48^{b}		0.563^{a}		0.580^{cd}
	Shirin Behshahr	33.3^{ab}	5.82^{b}		0.143^{e}		0.523^{de}
	Wild	37.3^{a}	4.39^{cd}		0.300^{b}		0.420^{g}
Autumn	Malas Mumtaz	30.4^{abc}	3.18^{ef}		$0.274b^{c}$		0.600^{c}
	Shirin Behshahr	30.5^{abc}	7.77^{a}		0.240^{cd}		0.500^{ef}
	Wild	30.0^{abc}	3.15^{ef}		0.213^{d}		0.230^{h}
Mean (Averaged over seasons)	Malas Mumtaz			7.42^{b}		0.0233^{a++++}	
	Shirin Behshahr			7.72^{a}		0.0054^{b}	
	Wild			7.80^{a}		0.0103^{ab}	
Mean (Averaged over genotypes)	Winter			7.44^{b}			
	Spring			7.66^{ab}			
	Summer			7.82^{a}			
	Autumn			7.67^{ab}			

+: Means in relation to main effects were not compared because the genotype × season interaction was significant; ++: Only the means in relation to main effects were compared because the genotype × season interaction was not significant; +++: Means with different letters in each column are significantly different at the 0.05 probability level according to Duncan's multiple range test; ++++: Based on the Games-Howell test.

Effects of genotype and auxin on rooting

The analysis of variance of data about the effect of genotype and auxin on rooting traits of pomegranate is presented in Table 3. There were significant differences among genotypes for the rooting percentage and the number of shoots. The auxin treatment and auxin × genotype interaction were also significant for the rooting percentage, longest root length, and root length of pomegranate stem cuttings. According to Table 4, when no IBA was applied, the Malas Mumtaz variety showed significantly higher rooting percentage (93.33%) than Shirin Behshahr and the wild genotype (56.67% and 50%, respectively). Although all genotypes responded positively to the auxin treatment at 2000 mg/L, the effect was not significant for the Malas Mumtaz variety because this variety already had a high rooting percentage (93.33%) when no auxin was applied. The data in Table 4 revealed that at 2000 mg/L IBA, the rooting percentage of pomegranate cuttings was high for all three genotypes (100%, 96.67%, and 86.67% for the wild genotype, Malas Mumtaz, and Shirin Behshahr, respectively). Although pomegranates are typically regarded as an easy-to-root species (Sarkhosh *et al.* 2021), the increase in rooting percentages achieved with auxin treatment indicates that applying auxin is a practical and effective approach to further enhance rooting success.

Almost similar results were observed for the root length and the longest root length. Under no IBA, the Malas Mumtaz variety showed higher root length and the longest root length than Shirin Behshahr and the wild genotype. However, auxin significantly enhanced root length and the longest root length of Shirin Behshahr and the wild genotype, while no significant change was observed for the Malas Mumtaz variety.

The Malas Mumtaz variety also showed a higher number of shoots (4.32) than the other two genotypes (4.00 and 3.48 for the wild genotype and the Shirin Behshahr variety, respectively) on average across two auxin conditions (with and without auxin). In total, the Malas Mumtaz variety demonstrated a high genetic potential for rooting because, even in the absence of exogenous auxin treatment, it showed satisfactory rooting characteristics. The significant auxin × genotype interaction may be attributed to differences in genetic factors of the plants, environmental conditions, and phenolic compounds. Studies have shown that certain phenolic compounds, such as flavonoids and phenolic acids, can enhance rooting in cuttings (Curir *et al.* 1993; Denaxa *et al.* 2022). For instance, in olive, the presence of compounds like chlorogenic acid and rutin was linked to increased adventitious root formation (Denaxa *et al.* 2020). Similarly, some of the phenolic compounds, such as ferulic acid, gallic acid, and chlorogenic acid, were also stated to induce rooting in *Malus domestica* (De Klerk *et al.* 2011).

Table 3. Analysis of variance of rooting traits of cuttings of three pomegranate genotypes under auxin treatment.

SOV	df	Mean squares				
		RP	**SRL**	**LRL**	**MRL**	**NS**
Block	2	88.89	0.024	5.42	1.48	0.022
Genotype (G)	2	955.56^{*}	0.086	11.92	2.53	1.06^{*}
Auxin (A)	1	3472.22^{**}	0.387	127.25^{**}	35.38^{**}	0.002
G × A	2	822.22^{*}	0.068	43.84^{*}	11.60^{*}	0.207
Error	10	168.89	0.090	6.89	2.02	0.218
CV (%)	-	16.13	34.09	26.92	26.74	12.20

RP: Rooting percentage; SRL: Shortest root length; LRL: Longest root length; RL: Mean root length; NS: Number of shoots; *,**: Significant at 0.05 and 0.01 probability levels, respectively.

Table 4. The combined effect of genotype and auxin on rooting traits of the pomegranate stem cuttings.

Genotype	Auxin	RP (%)	LRL (cm)	MRL (cm)
Malas Mumtaz	-IBA	93.33^{a}	10.54^{abc}	5.69^{ab}
	+IBA	96.67^{a}	9.62^{bc}	5.29^{ab}
Shirin Behshahr	-IBA	56.67^{b}	6.81^{cd}	3.72^{bc}
	+IBA	86.67^{a}	15.13^{a}	8.00^{a}
Wild genotype	-IBA	50.00^{b}	3.93^{d}	2.33^{c}
	+IBA	100.00^{a}	12.48^{ab}	6.87^{a}

RP: Rooting percentage; LRL: Longest root length; RL: Mean root length; NS: Number of shoots; Means with different letters in each column are significantly different at the 0.05 probability level based on Duncan's multiple range test.

Relationship of phenolic compounds with the rooting of stem cuttings

The results of the backward multiple regression about the relationship of phenolic compounds as independent variables with the rooting traits as dependent variables are shown in Table 5. Only the rooting traits that had a significant relationship with some of the phenolic compounds were presented in the table. In all models, the variance inflation factor (VIF) was less than 10, indicating no multicollinearity among the independent variables. The data showed that some endogenous phenolic compounds present in the stem bark of the pomegranate mother plant have a significant effect on the rooting of the cuttings. For the rooting percentage as the dependent variable, the compounds quercetin and DPPH remained in the model. In the case of mean root length and the longest root length, the remaining independent variables in the models were DPPH, phenols, and flavonoids. This was predicted because there was a very strong correlation (0.999) between mean root length and the longest root length. When the number of shoots was the dependent variable, four compounds, flavonoids, DPPH, quercetin, and gallic acid, showed a significant relationship with this trait. DPPH was present in all models with a negative standardized regression coefficient, indicating its important impact on the rooting capability of pomegranate cuttings. This means that the lower the amount of DPPH in the mother plant, the better the rooting of the cuttings. The act of taking a cutting is considered a form of stress. The plant's response includes a burst of reactive oxygen species and an increase in antioxidant activity to mitigate the damage. A very high DPPH value might be an indicator of a plant under severe stress and, therefore, has a reduced capacity to form new roots (da Costa *et al.* 2013). Gallic acid was also negatively associated with the number of shoots. However, a positive relationship of Quercetin with rooting percentage and number of shoots, and that of phenols with mean root length and the longest root length was observed. This finding is in agreement with the report by Osterc *et al*. (2012) in chestnut, which indicated that clones with satisfactory rooting had higher quercetin content. Furthermore, the positive role of quercetin in Ilex cuttings was documented by Tarrago *et al*. (2005), who observed that quercetin increased the rooting percentage more than three times compared to the control plants. Therefore, these reports support our results established for the role of quercetin as a rooting enhancer in pomegranate cuttings. Finally, the results about flavonoids were not consistent for the rooting characteristics. Flavonoids were negatively associated with mean root length and the longest root length, but showed a positive relationship with the number of shoots.

Although caffeic acid did not enter in any of the models related to rooting, the results of our preliminary experiment, conducted exclusively with the Malas Mumtaz cultivar, as explained in the

Table 5. The results of backward multiple regression about the relationship of phenolic compounds as independent variables with each of the rooting traits as dependent variables.

	Unstandardized partial regression coefficients		**Standardized partial regression coefficients**			
Model	**B**	**Std. Error**	**Beta**	**t**	**p-value**	**VIF**
Intercept	363.031	138.458		2.622	0.039	
Quercetin	974.561	356.594	0.680	2.733	0.034	1.030
DPPH	-41.488	18.687	-0.552	-2.220	0.068	1.030

Dependent variable: Rooting percentage; VIF: Variance inflation factor.

	Unstandardized partial regression coefficients		**Standardized partial regression coefficients**			
Model	**B**	**Std. Error**	**Beta**	**t**	**p-value**	**VIF**
Intercept	81.107	27.515		2.948	0.032	
DPPH	-10.786	4.068	-0.944	-2.651	0.045	1.938
Phenols	0.939	0.366	1.892	2.565	0.050	8.305
Flavonoids	-5.303	1.985	-1.706	-2.672	0.044	6.223

Dependent variable: Longest root length.

	Unstandardized partial regression coefficients		**Standardized partial regression coefficients**			
Model	**B**	**Std. Error**	**Beta**	**t**	**p-value**	**VIF**
Intercept	41.891	14.181		2.954	0.032	
DPPH	-5.522	2.097	-0.936	-2.634	0.046	1.938
Phenols	0.482	0.189	1.880	2. 556	0.051	8.305
Flavonoids	-2.749	1.023	-1.712	-2.688	0.043	6.223

Dependent variable: Mean root length.

	Unstandardized partial regression coefficients		**Standardized partial regression coefficients**			
Model	**B**	**Std. Error**	**Beta**	**t**	**p-value**	**VIF**
Intercept	14.364	2.355		6.099	0.004	
Flavonoids	0.801	0.235	1.555	3.403	0.027	9.498
DPPH	-1.700	0.353	-.898	-4.820	0.009	1.581
Quercetin	44.535	8.751	1.233	5.089	0.007	2.672
Gallic acid	-2.117	0.742	-1.038	-2.855	0.046	6.018

Dependent variable: Number of shoots.

materials and methods section, showed that the exogenous application of caffeic acid at a concentration of 2000 mg/L significantly reduced rooting in the stem cuttings. Singh *et al.* (2009) also reported that caffeic acid inhibits the in vitro formation of roots in mungbean (*Vigna radiata* L.), which aligns with the results of the current study.

The effect of phenols depends on the type of compound and its concentration (Denaxa *et al.* 2022). The influence of phenolic compounds on root induction in stem cuttings has been documented in other plant species. For instance, Abbasi *et al*. (2024) demonstrated that the diversity of phenolic compounds, antioxidant capacity, and mineral content in various fig cultivars can impact rooting success. Their findings indicated that cultivars with high levels of specific phenolic compounds, such as ferulic acid and vanillin, exhibited reduced root formation. Conversely, cultivars with increased phenol oxidase enzyme activity and higher catechin content showed improved rooting performance

Conclusion

This study indicated that the success of adventitious rooting in pomegranate hardwood cuttings is significantly influenced by seasonal biochemical changes in the mother plant, genotype, and the application of auxin. Our findings showed that the phenolic profiles of the mother stock varied considerably across seasons, with the winter period—the optimal time for taking cuttings—being characterized by lower levels of endogenous caffeic acid and DPPH. The application of IBA proved to be a valuable tool for enhancing propagation, as it significantly improved the rooting percentage and root length, particularly for the 'Shirin Behshahr' and wild genotypes. The 'Malas Mumtaz' cultivar, however, exhibited a high inherent rooting ability, showing satisfactory results even without hormonal treatment, which underscores its strong genetic potential in rooting.

Furthermore, a relationship between specific endogenous phenolic compounds and rooting capacity was established. Quercetin was identified as a significant rooting promoter, showing a significant positive relationship with rooting percentage and number of shoots. In contrast, high antioxidant activity (DPPH) was consistently linked to reduced rooting success, possibly indicating that cuttings from stressed mother plants have a lower regenerative capacity. The inhibitory role of caffeic acid was also suggested in a supplementary experiment, where its exogenous application significantly hampered root formation.

In summary, the results highlight that the biochemical status of the mother plant, specifically the balance between rooting promoters like quercetin and inhibitors like caffeic acid, is a critical factor in the vegetative propagation of pomegranate. This knowledge can be practically applied to select superior mother plants and optimize the timing of cutting collection. To further validate these findings, future *in vitro* research is recommended to isolate the effects of these specific phenolic compounds and fully elucidate their mechanisms in adventitious root induction.

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Conflict of Interest

The authors declare that they have no conflict and/or competing interests.

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