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Effects of natural and chemical rooting stimulants on some root traits of two varieties of pomegranate (*Punica granatum* L.) cuttings

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Abstract

The experiment ran from the middle of February until the start of November 2023 under the plant canopy of the Department of Horticulture and Landscape, College of Agriculture, University of Kerbala. The study employed a Randomized Complete Block Design (R.C.B.D.) as a factorial experiment, with two pomegranate varieties (Yamani and Bashkan) as the first factor and various combinations of natural and chemical rootings as the second factor. These included extracts of moringa leaves (M0, M1) and licorice roots (L0, L1) at concentrations of 0 and 15 g L^{-1} each, as well as indole butyric acid at a concentration of 0 and 10 mg L^{-1} (B0, B1). The majority of the root features of the resultant seedlings demonstrated that, overall, the Bashkan variety outperformed the Yamani variety; however, this superiority varied when combined with other rooting combinations. Between the rooting stimulant combinations utilized in the study, the cuttings' dipping results differed. When it came to the quantity, size, and fresh and dry weight of the root system, indole butyric acid in combination with B1M0L0 performed better. It resulted in 250.000 root seedlings^{-1}, 2.270 cm^3, 20,680 g, and 6,650 g, respectively. In contrast, the B0M0L1 licorice root treatment demonstrated superior root length, reaching 38,100 cm. Both the percentage of rooting and the average root diameter were the largest in the combination including both extracts, B0M1L1, reaching 5.170% and 1.603 mm, respectively. Given that natural extracts have outperformed chemical radicals in the majority of the rooting qualities under study—such as licorice root extract and moringa leaf extract—it is possible to conclude from the aforementioned results on their suitability for usage as an alternative.

Keywords: percentage of rooting, natural stimulants, indole butyric acid, Punicaceae.

Introduction

It is thought that pomegranate trees, Punica granatum L., which are native to Iran and northern India, are members of the Punicaceae family [1]. According to [2] Egypt,

Saudi Arabia, Spain, Cyprus, Iraq, Syria, Lebanon, Florida, and California are some of the major producers of pomegranates. In Iraq, around 23 different pomegranate cultivars are produced throughout the country's central and northern regions [3].

In order to produce seedlings that are homogeneous and closely resemble the mother plant in a short amount of time, stem cuttings are one of the most popular and effective methods of vegetative propagation for pomegranates in their growing areas. This is because the wood from which the cuttings are taken varies, so they can be prepared from young ends (less than a year old), which require special conditions for rooting and have a low success rate, or from stem cuttings with mature wood, whose success rate varies depending on the varieties and their carbohydrate content and other factors that promote rooting [4, 5].

Woody pomegranate cuttings can be rooting more successfully, more quickly, more roots than before, and more uniformly if they are treated with natural extracts [6]. One such extract is licorice root extract [7, 8], which is added to cuttings and plays a significant role in growth because it contains sugars, proteins, and certain minerals [9, 10]. [11, 12] report that it also exhibits behavior similar to gibberellin in that it speeds up germination and aids in cell division and elongation, which increases the size of the vegetative portion and improves growth characteristics.

One of the natural extracts that has gained popularity recently is moringa leaf extract [13, 6]. Because this extract is rich in oils, it can be utilized as a natural source of cytokinin to treat horticultural plant cuttings [14, 15]. Moringa leaves are also high in potassium, phenols, carotenoids, and ascorbates. and calcium, which can aid in the development of the brain [16, 17].

One typical method for increasing the success rate and success rate of cuttings is to treat them with artificial auxins. Indole Butyric Acid (IBA) is the most significant of these auxins; it differs from other auxins in that it is less harmful to plant tissue and more efficient in forming roots [18, 19]. IBA is more stable than other rooting chemicals and promotes faster rooting together with larger and more numerous roots, resulting in future seedling success [20, 21, 22].

The aforementioned information served as the basis for this study's testing of several mixes of chemical and natural rooting agents in order to identify the best treatments in terms of root features and the degree to which two distinct pomegranate varieties responded to these mixes.

Materials and Methods

From the middle of February until the start of November 2023, the experiment was conducted under the canopy of the Department of Horticulture and Landscape at the College of Agriculture and University of Kerbala in the Al-Husseiniyah District. Two components made up the study: the first was a combination of the growth regulator Indole Butyric Acid (IBA) and extracts of Moringa leaf (M0, M1) and licorice root (L0, L1), with IBA being used at a concentration of 0 and 10 mg L^{-1} (B0, B1) and the

extracts of Moringa leaf (M0, M1) and IBA at a concentration of 0 and 15 g L^{-1}. The combine treatments as fellow:

1- B0M0L0 without addition
2- B1M0L0 treatment with IBA only.
3- B0M0L1 treatment with licorice root extract only.
4- B0M1L0 treatment with moringa leaf extract only.
5- B1M0L1 treatment with IBA+ licorice root extract.
6- B1M1L0 treatment with IBA + moringa leaf extract.
7- B0M1L1 treatment with licorice root extract + Moringa leaf extract.
8- B1M1L1 treatment with IBA+ licorice root extract + Moringa leaf extract.

Two pomegranate cultivars, Yamani and Bashkan, stood in for the second component. Using a Randomized Complete Block design (R.C.B.D.) and three replications, a factorial experiment (8 ٫ 2) was conducted. There were 480 cuttings total—160 cuttings for each replicate and 10 cuttings for each experimental unit. From one-year-old mother pomegranate tree branches produced on the Holy Imam Hussein Shrine's Fadak farm, 240 wooden cuttings (20–25 cm long) for every variety were chosen. In accordance with the procedures, the cuttings received an hour of soaking.

On February 15, 2023, cuttings were planted in 5 kg plastic bags that were filled with a sandy-mixed soil. Before planting, the soil was treated with a 50 ml per 100 L of water butanol pesticides to sterilize it. In order to remove surplus air, around two thirds of the cuttings were buried and the sidewalls of the soil surrounding them were crushed [18]. All seedlings received normal agricultural service operations until November 20, 2023, when the seedlings were removed. The averages were compared using the LSD test with a probability of 5%, and the findings were statistically analyzed using the Genstate statistical software [23]. Root growth characteristics were measured as follows: percentage rooting (%), average number of roots (root $seedling^{-1}$), average root length (cm), average root volume (cm^3) using water displacement method, average root diameter (mm), average dry weight for the root system (g), the average fresh weight of the root system (g).

Results and Discussion

Rooting percentage (%)

The results presented in Table 1 show that immersing pomegranate cuttings in the mixture of moringa leaf extract and licorice root extract B0M1L1 and B0M0L1 increased the percentage of rooting, yielding the highest rate of 5.170% when compared to the immersion treatment B1M1L1, which yielded the lowest rates of 2.330%. However, no discernible differences were found between the two treatments. Though there were no appreciable variations between the two varieties, the Bashkan variety fared better, yielding 4.790% as opposed to the Yamani variety's 3.790Regarding the bilateral interaction between varieties and treatments, there were notable distinctions. Cut-

tings of the Bashkan variety immersed in combination B1M0L1 performed exceptionally well, yielding the highest rate of 6.670%, whereas the same cuttings immersed in combination B1M1L1 produced the lowest rates, at 2.000%.

Table (1): The impact of various mixes of chemical and natural rooting agents on the rooting percentage (%) of two varieties of pomegranate cuttings.

Average	variety		Combinations *
	Bashkan	Yamani	
3.170	3.670	2.670	B0M0L0
4.500	5.330	3.670	B1M0L0
4.670	4.000	5.330	B0M1L0
5.170	5.000	5.330	B0M0L1
4.670	5.670	3.670	B1M1L0
4.670	6.670	2.670	B1M0L1
5.170	6.000	4.330	B0M1L1
2.330	2.000	2.670	B1M1L1
	4.790	3.790	Average
4.380	1.548	3.097	L.S.D 0.05

*** B0M0L0 without addition, B1M0L0 treatment with IBA, B0M0L1 treatment with licorice root extract, B0M1L0 treatment with moringa leaf extract, B1M0L1 treatment with IBA licorice+ root extract, B1M1L0 treatment with IBA + moringa leaf extract, B0M1L1 treatment with licorice root extract + Moringa leaf extract, B1M1L1 treatment with licorice root extract + Moringa leaf extract + IBA.**

Average number of roots (root seedling $^{-1}$)

Table 2 shows that dipping pomegranate cuttings in the mixture containing B1M0L0 IBA resulted in an increase in the average number of roots and the highest rate of 25.000 root seedling^{-1}. This was in contrast to the cuttings immersion treatment in the combination of IBA, licorice root extract, and Moringa B1M1L1 leaf extract, which produced the lowest rates of 12.200 root seedling^{-1}. Regarding the varieties, there were no discernible variations between the Yamani and Bashkan varieties; the Bashkan variety yielded 18.000 root seedling^{-1}, while the Yamani variety supplied 16.900 root seedling^{-1}. When it came to the binary overlap between mixtures and varieties, the combination B1M0L0 outperformed the others in the Yamani variety, yielding the highest rate of 26.800 root seedling^{-1}. This was in stark contrast to the combination B1M0L1 which produced the lowest rate of 9.900 root seedling^{-1}.

Table (2): The impact of various mixes of chemical and natural rooting agents on the average number of roots (root seedling^{-1}) of two varieties of pomegranate cuttings.

Average	variety		Combinations*
	Bashkan	Yamani	
20.000	15.200	24.900	B0M0L0
25.000	23.300	26.800	B1M0L0
18.000	18.400	17.600	B0M1L0
14.800	16.200	13.300	B0M0L1

16.900	18.200	15.600	B1M1L0
16.200	22.600	9.900	B1M0L1
16.800	18.000	15.600	B0M1L1
12.200	12.500	12.000	B1M1L1
	18.000	16.900	Average
12.30	4.35	8.70	L.S.D 0.05

*** B0M0L0 without addition, B1M0L0 treatment with IBA, B0M0L1 treatment with licorice root extract, B0M1L0 treatment with moringa leaf extract, B1M0L1 treatment with IBA licorice+ root extract, B1M1L0 treatment with IBA + moringa leaf extract, B0M1L1 treatment with licorice root extract + Moringa leaf extract, B1M1L1 treatment with licorice root extract + Moringa leaf extract + IBA.**

Average root length (cm)

According to Table 3's results, immersing pomegranate cuttings in a combination containing only B0M0L1 licorice root extract increased the average root length and produced the highest rate of 38.100 cm. This was in contrast to the treatment that involved immersing cuttings in a combination containing B1M1L1 licorice root extract, IBA, and moringa leaf extract, which produced the lowest rates of 21.200 cm. Regarding the varieties, there were no discernible variations between the Yamani and Bashkan types; the Bashkan variety yielded a higher result, measuring 28.100 cm, while the Yamani variety measured 27.400 cm.

The combination B0M0L1 outperformed the Bashkan variety in the bilateral interaction between treatments and varieties, yielding the highest rate of 41.200 cm. This rate was not significantly different from the treatment of immersing pomegranate cuttings in moringa leaf extract and licorice root extract, B0M1L1, with the same variety, which produced the lowest rate of 18.200 cm.

Table (3): The impact of various mixes of chemical and natural rooting agents on the average root length (cm) of two varieties of pomegranate cuttings.

Average	variety		Combinations*
	Bashkan	Yamani	
22.500	20.600	24.400	B0M0L0
30.400	35.200	25.700	B1M0L0
26.600	23.200	30.100	B0M1L0
38.100	41.200	35.000	B0M0L1
30.300	35.600	25.000	B1M1L0
25.900	30.600	21.100	B1M0L1
27.300	18.200	36.500	B0M1L1
21.200	20.500	21.900	B1M1L1
	28.100	27.400	Average
23.56	8.33	16.66	L.S.D 0.05

*** B0M0L0 without addition, B1M0L0 treatment with IBA, B0M0L1 treatment with licorice root extract, B0M1L0 treatment with moringa leaf extract, B1M0L1 treatment with IBA licorice+ root extract, B1M1L0 treatment with IBA + moringa leaf extract, B0M1L1 treatment with licorice root extract + Moringa leaf extract, B1M1L1 treatment with licorice root extract + Moringa leaf extract + IBA.**

Average root volume (cm^3)

The data in Table 4 shows that dipping pomegranate cuttings in the mixture containing IBA B1M0L0 resulted in an increase in average root volume and the highest rate of 2.270 cm^3. This was a significant difference from the treatment of dipping the cuttings in the mixture containing IBA, licorice root extract, and moringa leaf extract B1M1L1, which produced the lowest rates, which were 1.000 cm^3. Regarding the varieties, no discernible variations were found between the Yamani variety and the Bashkan variety, with the former yielding 1.740 cm^3 and the latter 1.490 cm^3. Regarding the bilateral interaction between treatments and varieties, the combination B1M0L0 gave the Yamani variety the highest rate, 2.440 cm^3, which was significantly higher than the rate of 0.770 cm^3 that was obtained from the treatment of immersing pomegranate cuttings in IBA and the licorice root extract B1M0L1 in the same variety.

Table (4): The impact of various mixes of chemical and natural rooting agents on the average root volume (cm3) of two varieties of pomegranate cuttings.

Average	variety		Combinations *
	Bashkan	Yamani	
1.840	1.770	1.920	B0M0L0
2.270	2.100	2.440	B1M0L0
1.120	0.870	1.370	B0M1L0
1.560	1.850	1.260	B0M0L1
1.450	1.660	1.240	B1M1L0
1.580	2.390	0.770	B1M0L1
2.120	2.230	2.010	B0M1L1
1.000	1.070	0.940	B1M1L1
	1.740	1.490	Average
1.281	0.453	0.906	L.S.D 0.05

*** B0M0L0 without addition, B1M0L0 treatment with IBA, B0M0L1 treatment with licorice root extract, B0M1L0 treatment with moringa leaf extract, B1M0L1 treatment with IBA licorice+ root extract, B1M1L0 treatment with IBA + moringa leaf extract, B0M1L1 treatment with licorice root extract + Moringa leaf extract, B1M1L1 treatment with licorice root extract + Moringa leaf extract + IBA.**

Average of root diameter (mm)

As can be seen from Table 5, immersing pomegranate cuttings in the mixture of licorice root extract and moringa leaf extract B0M1L1 resulted in an increase in average root diameter and the highest rate of 1.603 mm. This was in marked contrast to the treatment of immersing cuttings in the mixture of IBA, licorice root extract, and moringa leaf extract B1M1L1, which produced the lowest rates of 0.883 mm. Regarding the varieties, there were no discernible changes between the Yamani variety and the Bashkan variety; the former yielded 1.279 mm while the latter produced 1.212 mm. The combination B0M1L1 performed exceptionally well with the Yamani variety,

Table (5): The impact of various mixes of chemical and natural rooting agents on the average root diameter (mm) of two varieties of pomegranate cuttings.

Average	variety		Combinations *
	Bashkan	Yamani	
1.079	0.897	1.297	B0M0L0
1.522	1.350	1.693	B1M0L0
1.053	0.910	1.197	B0M1L0
1.362	1.413	1.310	B0M0L1
1.365	1.330	1.400	B1M1L0
1.078	1.503	0.653	B1M0L1
1.603	1.477	1.730	B0M1L1
0.883	0.813	0.953	B1M1L1
	1.212	1.279	Average
0.9833	0.3477	0.6953	L.S.D 0.05

providing it with the highest rate of 1.730 mm in the bilateral interaction between treatments and varieties. This rate was not significantly different from the combination B1M0L1 with the Bashkan variety, which provided the lowest rate of 0.813 mm.

*** B0M0L0 without addition, B1M0L0 treatment with IBA, B0M0L1 treatment with licorice root extract, B0M1L0 treatment with moringa leaf extract, B1M0L1 treatment with IBA licorice+ root extract, B1M1L0 treatment with IBA + moringa leaf extract, B0M1L1 treatment with licorice root extract + Moringa leaf extract, B1M1L1 treatment with licorice root extract + Moringa leaf extract + IBA.**

Average dry weight of the root system (g)

The findings in Table 6 show that immersing pomegranate cuttings in the mixture containing IBA B1M0L0 increased the root system's average dry weight, yielding the highest rate of 6.650 g. This treatment was different from the other one, which yielded the lowest rates, 2.110 g, when the cuttings were immersed in the mixture containing IBA, licorice root extract, and moringa leaf extract B1M1L1. Concerning the varieties, the Yamani variety gave 3.80 g, while the Bashkan variety produced 4.480 g. No discernible variations were found between the two varieties. In terms of the bilateral interaction between treatments and varieties, the B1M0L0 combination outperformed with the Bashkan variety, yielding the highest rate of 7.280 g. This was in stark contrast to the B1M0L1 combination with the same variety, which produced the lowest rate of 1.450 g.

Table (6): The impact of various mixes of chemical and natural rooting agents on the dry weight of the root system (g) of two varieties of pomegranate cuttings.

Average	variety		Combination *
	Bashkan variety	Yamani variety	
3.590	2.950	4.230	B0M0L0
6.650	7.280	6.020	B1M0L0
3.050	2.650	3.460	B0M1L0
5.160	6.400	3.930	B0M0L1
3.710	3.090	4.330	B1M1L0
3.860	6.270	1.450	B1M0L1
5.300	5.130	5.470	B0M1L1
2.110	2.080	2.140	B1M1L1
	4.480	3.880	Average
4.057	1.434	2.869	L.S.D 0.05

*** B0M0L0 without addition, B1M0L0 treatment with IBA, B0M0L1 treatment with licorice root extract, B0M1L0 treatment with moringa leaf extract, B1M0L1 treatment with IBA licorice+ root extract, B1M1L0 treatment with IBA + moringa leaf extract, B0M1L1 treatment with licorice root extract + Moringa leaf extract, B1M1L1 treatment with licorice root extract + Moringa leaf extract + IBA.**

Average fresh weight of the root system (g)

As shown in Table 7, soaking pomegranate cuttings in the mixture containing IBA B1M0L0 led to an increase in the average fresh weight of the root system, delivering rise to the highest rate of 20.680 g. This was in contrast to the treatment where the cuttings were dipped in a mixture that included IBA, licorice root extract, and moringa leaves extract B1M1L1, which made the lowest rates, reaching 5.870 g. Comparing the two varieties, the Yamani variety gave 11.160 g, while the Bashkan variant produced 11.820 g. No discernible variations were found between the two varieties. The combination B1M0L0 outperformed the Bashkan variety in the bilateral interaction between treatments and varieties, yielding the highest rate of 21.980 g. This was in stark contrast to the combination B1M0L1 with the Yamani variety, which produced the lowest rate of 4.490 g.

Table (7): The impact of various mixes of chemical and natural rooting agents on the fresh weight of the root mass (g) of two varieties of pomegranate cuttings.

Average	variety		Combination *
	Bashkan	Yamani	
12.790	9.340	16.240	B0M0L0
20.680	21.980	19.390	B1M0L0
9.480	6.950	12.010	B0M1L0
10.950	13.460	8.450	B0M0L1
8.880	8.880	8.880	B1M1L0
10.010	15.540	4.490	B1M0L1
13.250	12.800	13.700	B0M1L1
5.870	5.600	6.140	B1M1L1
	11.820	11.160	Average
9.517	3.365	6.730	L.S.D 0.05

*** B0M0L0 without addition, B1M0L0 treatment with IBA, B0M0L1 treatment with licorice root extract, B0M1L0 treatment with moringa leaf extract, B1M0L1 treatment with IBA licorice+ root extract, B1M1L0 treatment with IBA + moringa leaf extract, B0M1L1 treatment with licorice root extract + Moringa leaf extract, B1M1L1 treatment with licorice root extract + Moringa leaf extract + IBA.**

The impacts of licorice root extract on a few root development features of two types of pomegranate cuttings are evident from Tables 1 through 7. The increase in some root characteristics of pomegranate cuttings for both varieties can be attributed to the licorice extract containing many chemical compounds that encourage the formation of roots and increasing their growth and number. Also, these substances present in the licorice extract increase the metabolism within the cuttings, which plays an important role. In the rooting process [24]. Some previous studies have shown that licorice extract contains flavonoids, including quercetin, which is considered a stimulant for the formation of roots on cuttings [25]. This was confirmed by [20], who determined that quercetin enhanced the root system of eucalyptus cuttings due to its role in reducing oxidative stress and encouraging cell division. Moreover, licorice extract contains phenols [26], which have been proven to increase root growth on cuttings [27]. The results of the study by [6] showed that the aqueous extraction of many natural plant materials, including licorice extract, proved its efficiency in increasing the length and number of cutting roots. This can be attributed to the presence of a growth regulator IAA, minerals and vitamins in these plant extracts. Comparable findings to those of this study demonstrated the function of licorice extract in elevating indices of root growth for Gardenia plants [8], hardwood cuttings of olive (Olea europaea L.) [24], and Coratina olive cuttings [28].

Additionally, the results showed that most of both types of pomegranate cuttings were significantly affected by combinations including moringa leaf extract (Table 1-7). The explanation might be that moringa leaf extract, which influences the lengths, diameters, and quantity of root growths in addition to the total number of roots, is one of the most significant extracts that boosts plant biomass. The extract's abundance of

nutrients, including sugars and salts, vitamins, minerals, amino acids, ascorbates, and numerous other substances known to promote growth, along with the presence of zeatin—one of the plant's own naturally occurring growth regulators—could be the cause. In addition, it has a high concentration of cytokinins, which are naturally occurring plant hormones that are thought to play a significant role in controlling various aspects of plant growth and development, including lateral root formation, stress tolerance, apical dominance, cell division, and leaf senescence [29, 30].

The outcomes demonstrated that in the majority of root features, the Bashkan variety is inferior to the Yamani type. The cause might be attributed to variations in hormones and nutritional value in the cuttings, which affect promoting root growth, or to anatomical and genetic variances between them [31]. The variations' varying sets of genetic genes, how the genetic structure interacts with the environment, and the degree to which these interactions result in the unique characteristics of the variety all contribute to the variations' varying responses [32].

With the advancement of agriculture, the study's results may pave the way for a reduction in the use of chemicals, as they demonstrated that natural extracts (licorice root extract and moringa leaf extract) outperformed the growth regulator indole butyric acid in certain areas and produced results that were comparable to or better. It is therefore possible to urge those who engage with horticultural plant propagation to use all natural products in order to protect the ecosystem from contamination.

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