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POMEGRANATE (*PUNICA GRANATUM*) RESPONSE TO NATURAL AND SYNTHETIC GROWTH REGULATORS IN GROWTH TRAITS

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SUMMARY

A study, carried out in 2023 at the University of Kerbala, Iraq, aimed to assess the effects of natural and synthetic growth hormones on the vegetative characteristics of two pomegranate (*Punica granatum* L.) cultivars. The experiment layout had a randomized complete block design (RCBD) with factorial arrangement, two factors, and three replications. The first factor included combinations of natural and chemical growth regulators, while the second comprised stem cuttings of two pomegranate cultivars, Yamani and Bashkan. The results showed cultivar Yamani was superior for vegetative branches (4.110), the number of leaves (341) per seedling, and leaf area (988 cm^2). The triple interaction of indole butyric acid (IBA 0 mg L^{-1}), moringa leaf (M1), and licorice root (L1) (B0M1L1) extracts provided superior performance on the average height and diameter of vegetative shoots (49.500 cm and 2.510 mm, respectively). The interaction of indole butyric acid (IBA 0 mg L^{-1}), moringa leaf (M1), and licorice root (L0) (B0M1L0) extracts also excelled in the average number of branches and chlorophyll content (4.670 branches $seedling^{-1}$ and 0.276 mg g^{-1}, respectively).

Keywords: Pomegranate (*Punica granatum* L.), cultivar stem cuttings, indole butyric acid, moringa leaf extract, licorice root extract, growth and physiological traits

Key findings: The pomegranate cultivar Yamani showed superiority in most traits than the other cultivar Bashkan. The interaction of natural and synthetic growth regulators (B1M1L0) was superior in seedling height, branch diameter, and leaf area. For the number of branches and leaves per seedling, the combinations B1M0L0 and B0M1L1 outshone with the highest averages, while the combination B0M1L0 led for chlorophyll content.

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INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to the family Punicaceae, which seemed to be native to Iran and Northern India. The most influential pomegranate producing countries worldwide are Egypt, Saudi Arabia, Spain, Cyprus, Iraq, Syria, Lebanon, and the USA, specifically Florida and California states (Chandra *et al.,* 2010). Iraq grows 23 cultivars of pomegranates, which spread in the Central and Northern regions. Pomegranate growing is usually for their nutritious values, as their fruits contain protein, fat, carbohydrates, vitamin C, and ash. In addition to the recent use of dried pomegranate seeds and leaf extracts as nutritional supplements in poultry feed, pomegranate leaves and peels could benefit as antibacterial agents (Al-Musodi and Al-Zwean, 2023).

In vegetative propagation, the stem cuttings are one of the most common and successful methods of pomegranate propagation in its cultivation areas to obtain homogeneous seedlings and similar to the mother plant quickly. The wood as source of the cuttings differs, hence, preparing the cutting can come from young ends (less than a year old). These cuttings need special conditions for development because the rooting has a low success rate. In pomegranate stem cuttings with mature wood, the success rate also varies depending on the cultivars and their carbohydrate content and other factors that aid rooting (Aryan *et al.,* 2023).

In increasing the success rate of rooting in woody pomegranate cuttings and enhancing the number and length of uniformed roots, they need treatment application with some natural extracts, including the licorice root extract. The licorice root extract has an essential role in growth when added to cuttings because it contains sugars, proteins, and some minerals. Moreover, it has a similar behavior to gibberellins in stimulating the speed of germination and assisting in cell division and elongation, causing increased growth and development of young seedlings of horticultural plants (Rajan and Singh, 2021).

Similarly, moringa leaf extract is a considered natural extract, used widely for years. The different cuttings of horticultural plants can accept treatments with the moringa leaf extract because it is rich in oils, making it effective as a natural source of cytokinins. Additionally, moringa leaves are rich in ascorbates, carotenoids, phenols, potassium, and calcium, which can promote plant growth and development in date palm (Abdulrahman *et al.,* 2023).

By treating the stem cuttings with artificial auxins is one of the common processes to make them successful and enhance their success rate. The most prominent of these auxins is the indole butyric acid (IBA), which is distinct from other auxins as more effective in root formation and less toxic to tissues of mulberry cuttings (Sourati *et al.,* 2022). IBA is more stable than other rooting compounds and provides a rapid rooting process, resulting in more large roots that further strengthen the seedlings of *Conocarpus erectus* stem cuttings (Abdelrahman *et al.,* 2020). Based on the above discussion, this study aimed to determine the effects of different combinations of natural and synthetic growth substances and verify their suitable combinations on vegetative characteristics of two pomegranate cultivars.

MATERIALS AND METHODS

This study aimed to assess the effects of natural and synthetic growth hormones on the vegetative characteristics of two pomegranate (*Punica granatum* L.) cultivars, conducted in 2023 at the University of Kerbala, Kerbala, Iraq. The experiment lay out had a randomized complete block design (RCBD) with factorial arrangement, two factors, and three replications. The first factor comprised the growth regulator indole butyric acid (IBA), leaf extract of moringa, and licorice root extract. The growth regulator IBA had the concentration of 0 and 10 mg L^{-1} (B0 and B1, respectively). Moringa leaf (M0 and M1) and licorice root extracts had the concentration of 0

and 15 g L^{-1} (L0 and L1, respectively). The second factor was the two cultivars of pomegranate, i.e., Yamani and Bashkan.

In the experiment, each experimental unit used 10 stem cuttings of pomegranate, 160 cuttings for each replication, making the total number of cuttings reach 480. The 240 stem wood cuttings (20–25 cm long) for each cultivar came from one-year-old branches of mother pomegranate trees grown on the Fadak Farm at the Holy Imam Hussein Shrine, Iraq. The stem cuttings received all the treatments by immersion for one hour.

The planting of pomegranate stem cuttings began in mid-February 2023 in polyethylene bags with a capacity of 5 kg, filled with sandy mixed soil. Before planting, sterilizing the soil first used the pesticide Butanol at the concentration of 50 ml 100 L^{-1} of water. Approximately two-thirds of the cuttings reached being buried, compressing the sides with the soil surrounding the cuttings to remove excess air. All the field operations proceeded regularly for all the seedlings until uprooting the seedlings at the end of November 2023.

Data recorded

The data recorded on the vegetative growth characteristics comprised seedling height (cm), average diameter of vegetative shoots (mm), average number of vegetative branches (branches $seedling^{-1}$), average number of leaves (leaves $seedling^{-1}$), average leaf area (cm^2), and average leaf chlorophyll content (mg g^{-1} fresh weight).

Statistical analysis

All the recorded data for various traits underwent the analysis of variance (ANOVA) as per the randomized complete block design. The least significant difference ($LSD_{0.05}$) test served to compare and separate further the treatment means (Al-Mohammadi and Al-Mohammadi, 2012). All the analyses' processing employed the computer software GenStat-12.

RESULTS AND DISCUSSION

Seedling height

The results revealed a significant effect of the natural and synthetic growth regulators on the pomegranate seedling height (Table 1). The interaction of the indole butyric acid (IBA 0 mg L^{-1}), moringa leaf (M1), and licorice root (L1) (B0M1L1) extracts excelled by giving the highest average of the seedling height (49.50 cm). But, it did not differ significantly from the two interactions (B1M0L0 and B0M0L1), which gave the average values of 45.20 cm and 48.20 cm, respectively, compared with the control treatment (B0M0L0 = 32.30 cm)**.** This may be because the licorice root extract is a rich source of phenolic compounds that are extremely vital for plants due to its ability to eliminate free radicals by the presence of the hydroxyl group. The licorice extract is vital in promoting plant growth with its various rich biological structures of protein necessary for plant growth (Wanas and Khamis, 2021).

Licorice with antioxidant and antimicrobial components also enhances the bioactive qualities of plant parts generated in the tissues (Al-Mukhtar, 2022). Moreover, the said extract contains various nutrients, such as, sugars, salts, minerals, amino acids, ascorbates, and other compounds known for their ability to stimulate plant growth. It also contains zeatin, considered one of the natural growth regulators that the plant produces (Argueso *et al.,* 2009).

The moringa leaf extract is rich with cytokinins, which are natural plant hormones serving as crucial growth regulators for cell division, apical dominance, stress tolerance, lateral root formation, and hormonal balance, positively reflecting in the seedling height (Nouman *et al.,* 2012). The results further showed nonsignificant difference between the two pomegranate cultivars for the seedling height (Table 1). The bilateral interaction between treatments and cultivars, and the interaction combination B0M0L1 with cultivar Bashkan excelled by displaying the tallest

Table 1. Effect of different combinations of natural and synthetic substances on the seedling height in pomegranate.

Combinations	Cultivars		Means (cm)
	Yamani	Bashkan	
B0M0L0	34.00	30.70	32.30
B1M0L0	46.00	44.50	45.20
B0M1L0	37.60	37.40	37.50
B0M0L1	41.70	54.70	48.20
B1M1L0	33.10	44.10	38.60
B1M0L1	22.00	49.00	35.50
B0M1L1	52.80	46.20	49.50
B1M1L1	33.70	26.30	30.00
Means (cm)	37.60	41.60	
$LSD_{0.05}$ Combinations: 5.20, Cultivars: N.S., Combination × Cultivar interactions: 7.33			

Table 2. Effect of different combinations of natural and chemical rooting substances on the diameter of branches in pomegranate.

Combinations	Cultivars		Means (mm)
	Yamani	Bashkan	
B0M0L0	2.25	1.38	1.82
B1M0L0	2.44	2.23	2.34
B0M1L0	1.88	2.03	1.95
B0M0L1	2.37	2.63	2.50
B1M1L0	1.75	2.28	2.01
B1M0L1	1.09	2.25	1.67
B0M1L1	2.83	2.19	2.51
B1M1L1	1.68	1.16	1.42
Means (mm)	2.04	2.02	
$LSD_{0.05}$ Combinations : 0.34, Cultivars: N.S, Combinations × Cultivars interactions: 0.42			

seedling height (54.70 cm). Meanwhile, the interaction of B1M0L1 with pomegranate cultivar Yamani revealed the least value for the said trait (22.00 cm).

Branches' diameter

For the average diameter of the branches in pomegranate cultivars, the natural and synthetic growth regulators enunciated noteworthy differences (Table 2). The interaction of the indole butyric acid (IBA 0 mg L^{-1}), moringa leaf (M1), and licorice root (L1) extracts (B0M1L1) appeared with the maximum average branch diameter (2.51 mm). However, the said combination did not differ significantly from the two other interactions, i.e., B1M0L0 and B0M0L1, with values of 2.34 mm and 2.50 mm, respectively, compared with the control treatment B0M0L0 (1.82 mm)**.** The increase in the average diameter of the branches was due to the positive role of the plant extracts, containing various nutrients, such as potassium, sodium, calcium, phosphorus, magnesium, iron, zinc, and copper. Moreover, these contained amino acids, vitamins, amides, and other minerals that have collectively contributed to raising the efficiency of vital processes within the plants. The process of carbon metabolism also stimulates an increase in the dry matter accumulation, manifesting in the branches' increased diameter (Quintana *et al.,* 2019).

The moringa leaf extract has essentially become a natural source of cytokinins, which stimulates the production of protein, carbohydrates, and acids. It also promotes the enzymes responsible for photosynthesis, in addition to containing the growth regulators, such as, gibberellins and

auxins. Similarly, it is a natural source of some nutrients, viz., nickel, phosphorus, magnesium, iron, and sodium. Therefore, the natural substances contained in the extract contribute to increasing growth rates, and eventually, enhance the average diameter of branches (Nouman *et al.,* 2012). The results further showed no significant difference existed between the two pomegranate cultivars for branch diameter (Table 2). However, the combination of natural and synthetic growth regulators (B0M0L1) with the cultivar Bashkan excelled, displaying the supreme branch stem diameter (2.63 mm). The combination B1M0L1 in interaction with the cultivar Yamani revealed the lowest value for the said trait (1.09 mm).

Branches per seedling

The outcomes indicated substantial variations among the natural and synthetic growth regulators for the average number of branches per seedling in pomegranate cultivars (Table 3). The combination B0M1L0 excelled with an average of 4.67 branches seedling^{-1}, which had no remarkable difference from the other combination B1M0L0 (4.49 branches seedling^{-1}), compared with the control treatment B0M0L0 (4.26 branches seedling^{-1}). The increase in the number of branches may be due to the effect of growth regulators, including the indole butyric acid, which is crucial in increasing the number of branches per plant. The IBA works by stimulating the growth of lateral roots and the formation of new branches, as well as, promoting the formation of secondary vascular tissues in the plant, supporting the development of more branches per plant (Arora *et al.,* 2020). The licorice extract is also rich in cytokinins, being the most important plant hormones vital in escalating cell division in the effective growth areas, stimulating the progress of lateral shoots (Hussein *et al.,* 2021). Likewise, the moringa leaf extracts positively affected an increase in the number of branches. The reason is it contains substances that encourage growth, namely, thiamine, niacin, riboflavin, vitamin B12, and folic acid, raising the level of efficiency of the plant's vital processes, reflecting in more number of branches (Wahba *et al.,* 2022).

The results further showed a considerable difference between the two pomegranate cultivars for the number of branches in said cultivars (Table 3). The cultivar Yamani had an average of 4.11 branches seedling^{-1}, while the cultivar Bashkan indicated an average of 3.82 branches seedling^{-1}**.** This may be due to the anatomical and genetic differences between the two cultivars and the response of the cultivars to hormones with the genotypes' differences in genetic make-up (Therios, 2009). As for the bilateral interaction between the combinations of natural and synthetic growth regulators and pomegranate cultivars, the combination B0M1L0 in the cultivar Yamani exhibited the

Table 3. Effect of different combinations of natural and chemical rooting substances on the average number of branches in pomegranate.

Combinations	Cultivars		Means (branches seedling^{-1})
	Yamani	Bashkan	
B0M0L0	4.47	4.04	4.26
B1M0L0	4.67	4.31	4.49
B0M1L0	5.13	4.20	4.67
B0M0L1	4.20	3.64	3.92
B1M1L0	3.13	3.60	3.37
B1M0L1	2.58	4.32	3.45
B0M1L1	4.72	3.57	4.14
B1M1L1	4.00	2.89	3.44
Average (branches seedling^{-1})	4.11	3.82	
$LSD_{0.05}$ Combinations : 0. 34, Cultivars: 0.11, Combinations × Cultivars interactions: 0.59			

Table 4. Effect of different combinations of natural and chemical rooting substances on the leaves per seedling in pomegranate.

Combinations	Cultivars		Means (leaves seedling^{-1})
	Yamani	Bashkan	
B0M0L0	124.00	176.00	150.00
B1M0L0	296.00	249.00	272.00
B0M1L0	155.00	195.00	175.00
B0M0L1	341.00	269.00	305.00
B1M1L0	269.00	374.00	321.00
B1M0L1	127.00	245.00	186.00
B0M1L1	394.00	237.00	316.00
B1M1L1	176.00	112.00	144.00
Means (leaves seedling^{-1})	235.00	232.00	
$LSD_{0.05}$ Combinations : 6.80, Cultivars: 2.61, Combinations × Cultivars interactions: 8.10			

most number of branches per seedling (5.13). Meanwhile, the combination B1M0L1 with the same cultivar gave the fewest branches seedling^{-1} (2.58).

Leaves per seedling

The results enunciated the existence of significant differences among the natural and synthetic growth regulators for the average number of leaves per seedling in pomegranate cultivars (Table 4). The interaction of B1M1L0 showed the utmost number of leaves per seedling (321.00), which did not vary radically from the other combination B0M1L1, at 316.00 leaves seedling^{-1}, compared with the control treatment B0M0L0 (150.00 leaves seedling^{-1}). The increase in leaf number may be due to the use of butyric acid, which is a natural organic acid found in plants, bacteria, and fungi. It seemed to stimulate the growth of plant tissues, the formation of buds, and the development of leaves, by activating cell division processes and enhancing the plant hormones' activity responsible for growth, such as, cytokinins and auxins (Kumar *et al.,* 2021).

The licorice extract considerably affects positively plant growth, with its rich contents of plant hormones, vitamins, and minerals. These could enhance plant tissue growth and stimulate metabolic processes and vegetative expansion, and thus, increase the number of leaves per plant (Al-Sereh *et al.,* 2020). In addition, the moringa leaf extracts contain a wide range of nutrients and beneficial plant compounds (vitamins, minerals, amino acids, enzymes) and active plant compounds. These components can also stimulate plant growth and boost their development, including raising the number of leaves per plant (Aljabary *et al.,* 2024).

The results further revealed a significant difference between the pomegranate cultivars for the number of leaves in said cultivars (Table 4). The cultivar Yamani had an average of 235.00 leaves seedling^{-1}, while the cultivar Bashkan yielded an average of 232.00 leaves seedling^{-1}. The difference in the number of leaves indicates the genetic diversity and adaptation that can lead to the emergence of variations between the cultivars (Aziz *et al.,* 2023). As for the bilateral interaction between the combinations of synthetic growth regulators and cultivars, the combination B0M1L1 with cultivar Yemeni showed the highest average (394.00 leaves seedling^{-1}). The interaction of combination B1M1L1 and cultivar Bashkan gave the least number of leaves per seedling (112.00).

Leaf area

The interaction of natural and synthetic growth regulators revealed notable variations for average leaf area in pomegranates (Table 5). The combination B0M0L1 displayed the widest average of leaf area (1484.00 cm^2), compared with the control treatment (B0M0L0), with an average of 726.00 cm^2**.** The indole butyric acid is a plant growth stimulant, believed to stimulate cell growth and reproduction, as reflected in an enlargement in the leaf area

Table 5. Effect of different combinations of natural and chemical rooting substances on the leaf area in pomegranate.

Combinations	Cultivars		Means (cm^2)
	Yamani	Bashkan	
B0M0L0	601.00	850.00	726.00
B1M0L0	1331.00	1190.00	1261.00
B0M1L0	510.00	552.00	531.00
B0M0L1	1930.00	1038.00	1484.00
B1M1L0	1142.00	924.00	1033.00
B1M0L1	354.00	1281.00	817.00
B0M1L1	1583.00	875.00	1229.00
B1M1L1	453.00	381.00	417.00
Means (cm^2)	988.00	886.00	
$LSD_{0.05}$ Combinations : 70.60, Cultivars: 53.80, Combinations × Cultivars interactions: 123.70			

Table 6. Effect of different combinations of natural and chemical rooting substances on the leaf chlorophyll content in pomegranate.

Combinations	Cultivars		Means (mg g^{-1})
	Yamani	Bashkan	
B0M0L0	0.185	0.250	0.217
B1M0L0	0.241	0.250	0.246
B0M1L0	0.319	0.232	0.276
B0M0L1	0.229	0.226	0.227
B1M1L0	0.134	0.318	0.226
B1M0L1	0.193	0.169	0.181
B0M1L1	0.184	0.212	0.198
B1M1L1	0.308	0.146	0.227
Means (mg g^{-1})	0.224	0.225	
$LSD_{0.05}$ Combinations : 0.011, Cultivars: N.S, Combinations × Cultivars interactions: 0.016			

size (Ausari *et al.,* 2023). Likewise, the licorice root and moringa leaf extracts can stimulate the cell growth and elongation as a result of the availability of natural plant compounds and hormones, stimulating cell growth and proliferation, boosting size and surface area of the leaves (Aljabary, 2023).

As for cultivars, the results showed the cultivar Yamani was superior, with an average leaf area of 988.00 cm^2, while the cultivar Bashkan gave an average of 886.00 cm^2**.** The reason for the difference in the response of cultivars to growth regulators might be due to the disparities in the genetic make-up and the extent of their gene expression between the cultivars, which are responsible for increased leaf area (Zarei, 2017; Adiba *et al.,* 2021). It indicated the occurrence of significant interaction effects, and the combination B0M0L1 with the cultivar Yamani providing the maximum leaf area (1,930.00 cm^2), while the combination B1M1L1 with the cultivar Bashkan emerged with the least leaf area (381.00 cm^2).

Leaf chlorophyll content

In pomegranate cultivars for leaf chlorophyll content, the interactions of natural and synthetic growth regulators revealed remarkable differences (Table 6). The combination B0M1L0 appeared superior in performance, with an average chlorophyll content (0.276 mg g^{-1}) versus the control treatment B0M0L0 (0.217 mg g^{-1}). This may refer to the positive role of the Indole butyric acid, helping to activate photosynthesis efficiently in the plant, boosting chlorophyll production (Koteswara *et al.,* 2020). Equally, the licorice extract may enhance some vital processes in plants, increasing the chlorophyll content because it contains numerous nutritional elements. Correspondingly, the

effect of moringa extract helps enhance the chlorophyll content in crop plants with its rich nutrient and vitamin contents. Hence, those nutrients and active compounds may indirectly augment the chlorophyll content in leaves (Medan, 2023; Al-Mayahi *et al*., 2024).

The findings further implied no significant difference appeared between the pomegranate cultivars for the leaf chlorophyll content (Table 6). The interaction of the B0M1L0 with the cultivar Yamani revealed the maximum average of leaf chlorophyll content (0.319 mg g^{-1}), while the combination B1M1L0 and the cultivar Yamani showed the minimum value for the said trait (0.134 mg g^{-1}).

CONCLUSIONS

In pomegranate, the promising findings could open new horizons for reducing chemical uses in light of agriculture development. The plants' extracts (moringa leaf and licorice roots) showed the superior performance compared with the synthetic growth regulator indole butyric acid. Thus, in propagating horticultural plants, the natural products can be applicable to preserve the ecosystem from pollutants.

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