



## Improving vegetative growth characteristics and mineral content in pomegranate trees c.v. "Wonderful". By spraying Brassinolide, proline and boron

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### ABSTRACT

This study was conducted in the orchard of the Agricultural Research and Experiments Station, College of Agriculture, University of Kirkuk, during the 2024 growing season to investigate the effect of foliar spraying with the growth regulator Brassinolide at three concentrations (0, 2, 4 mg L<sup>-1</sup>), the amino acid proline at three concentrations (0, 100, 200 mg L<sup>-1</sup>), and boron at two concentrations (0, 30 mg L<sup>-1</sup>), as well as their interactions, on some vegetative growth traits and mineral content of pomegranate trees, cultivar Wonderful. A randomized complete block design (RCBD) was used with three factors, four replications, and one tree per experimental unit. The results obtained can be summarized as follows:

Foliar spraying with Brassinolide had a significant effect on vegetative growth traits and mineral content of pomegranate trees, with 2 mg L<sup>-1</sup> affecting (shoot length and shoot diameter) reaching 37.61 cm, 3.32 mm, and 4 mg L<sup>-1</sup> affecting (leaf area, total chlorophyll, and the percentages of nitrogen, phosphorus, and potassium in the leaves) reaching 7.34 cm, 25.29 mg/100g fresh weight (%2.20, % 0.144, % 2.72, respectively). Foliar spraying with the amino acid proline at 200 mg L<sup>-1</sup> led to a clear significant increase in all studied vegetative growth traits (shoot length, shoot diameter, leaf area, total chlorophyll) reaching 38.32 cm, 3.36 mm, 7.54 cm<sup>2</sup>, 24.72 mg/100g fresh weight) respectively and two mineral content traits (percentage of nitrogen and potassium in the leaves) reaching (2.42%, 2.09%). Foliar spraying with boron at 30 mg L<sup>-1</sup> resulted in significant increases in vegetative growth traits and mineral content (shoot length, leaf area, total chlorophyll, and the percentages of nitrogen and potassium in the leaves) reaching (36.64 cm, 7.38 cm<sup>2</sup>, 23.81 mg/100g fresh weight, 2.29%, 2.06%) respectively. A clear significant effect of the two-way and three-way interactions among the study treatments was observed in most of the studied traits.

**Keywords:** Brassinolide, proline, boron, pomegranate, Wonderful.

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### INTRODUCTION

The pomegranate tree (*Punica granatum* L.) ranges from being evergreen to partially deciduous in tropical and subtropical regions, while it is fully deciduous in cold regions. It is widely cultivated in temperate areas, and its original habitat is believed to be Iraq, Iran, and Southwest Asia [1]. The number of fruit-bearing pomegranate trees in Iraq is (6,495,705). Pomegranate ranks second in terms of production compared with other fruit species in Iraq. However, in terms of the number of fruit-bearing trees, Diyala province ranks first with (3,439,563) million trees, followed by Salah al-Din province in second place with (2,513,673) million trees, and Karbala province in third place with (281,784) thousand trees, while Kirkuk province ranked eleventh with (5,780) thousand trees [2]. Recently, researchers have focused on the American cultivar Wonderful due to its strong vegetative growth traits, high yield, and adaptability to Iraqi conditions [3].

The use of some plant growth regulators, such as Brassinolide, can enhance growth, flowering, fruit set, and yield through their effects on carbohydrate assimilation, allocation, and regulation of aquaporin activities [4]. Brassinosteroids appear to coordinate and integrate the diverse processes required for growth, partly through interactions with plant hormones that define the framework for BR responses. Brassinosteroids (BRs) regulate various growth and developmental processes in plants and organ differentiation [5]. In addition, Brassinolide-induced growth

is associated with increased RNA and DNA content, polymerase activity, protein synthesis, carbohydrate fractions, reducing sugars, non-reducing sugars, and starch [6]. [7] Al-Karbolli and Al-Janabi (2024) conducted an experiment on local lemon trees that involved foliar spraying with the plant growth regulator Brassinolide at four concentrations (0, 1.0, 1.5, and 2.0 mg L<sup>-1</sup>). They found that foliar application of Brassinolide at 1.5 mg L<sup>-1</sup> significantly increased leaf area and total chlorophyll content compared to the control treatment.

Proline is one of the free amino acids, containing an amino group within its structure, a feature that distinguishes it from other amino acids. It is a nonpolar amino acid with a side chain that differs from those of other amino acids and is characterized by a unique structural property in which the amino group (NH<sub>2</sub>) is not free [8]. In an experiment on pomegranate trees, cultivar Salimi, [9] reported that foliar spraying with proline at concentrations of (0, 50, 100 mg L<sup>-1</sup>) significantly increased total leaf area (66.94 cm<sup>2</sup>), the increase ratio of shoot length (95.27 cm), annual shoot diameter (9.08 mm), leaf dry matter percentage (65.80%), and single leaf area (6.12 cm<sup>2</sup>).

Boron is one of the essential micronutrients for plants. It has numerous functions, the most important of which are facilitating sugar translocation, contributing to cell wall structure, carbohydrate metabolism, and respiration. It also plays a crucial role in improving flower and fruit fertilization [10]. In addition, it has a significant role in vital physiological activities, including facilitating the movement and transport of photosynthetic products from leaves to active regions of the plant. It also contributes to pollen germination and pollen tube growth [11]. In an experiment, [12] applied foliar spraying of pomegranate trees, cultivar Wonderful, with boron at four concentrations (0, 250, 500, 1000 mg L<sup>-1</sup>). The results showed that the concentration of 1000 mg L<sup>-1</sup> led to a significant increase in leaf content of nitrogen (2.5%), phosphorus (0.7%), potassium (1.9%), and boron (30%).

## Materials and Methods

This study was conducted in one of the orchards of the Agricultural Research and Experiments Station, College of Agriculture, University of Kirkuk, located in the Al-Siyada area, during the period from 15/4/2024 to 31/12/2024, on four-year-old pomegranate trees, cultivar Wonderful. A total of 72 trees were selected to implement the experiment. Suckers were removed, broken and infected branches were pruned, and the field was completely cleaned by plowing, weeding, and removing weeds on 1/3/2024. A drip irrigation system was used for the pomegranate trees.

The trees were sprayed with the growth regulator Brassinolide at three concentrations (0, 2, 4 mg L<sup>-1</sup>) in two applications, starting from 15/4/2024 until complete wetting, with a one-month interval between the first and second spray. Proline amino acid was applied at three concentrations (0, 100, 200 mg L<sup>-1</sup>) in two applications, starting from 16/4/2024 until complete wetting, also with a one-month interval between the two sprays. Boron was applied at two concentrations (0, 30 mg L<sup>-1</sup>) in two applications, starting from 17/4/2024, with a one-month interval between the two sprays. Spraying was carried out at sunset and dawn until complete wetting.

A randomized complete block design (RCBD) was used with three factors, four replications, and one tree per experimental unit. Data were analyzed using the SAS system, and means were compared according to Duncan's multiple range test at the 0.05 probability level.

## Studied Traits

### Vegetative growth traits:

1. **Average increase in shoot length and diameter (cm):**
2. Three shoots were selected from each experimental unit. Their lengths and diameters were measured at the beginning of the experiment on 15/3/2024 and at the end of the experiment on 31/12/2024. The difference between the two measurements represented the increase in shoot length.
3. **Leaf area (cm<sup>2</sup>):**
4. Twenty fully expanded leaves were collected from the middle part of the shoots, from all sides of the tree, and at different heights during the first week of July [13]. The method applied by [14] was followed.
5. **Leaf total chlorophyll content (mg g<sup>-1</sup> fresh weight):**
6. Total chlorophyll in pomegranate leaves was estimated according to the method of [15].

### Mineral content traits:

The mineral content traits were measured on 15/7/2024.

1. **Leaf nitrogen percentage:**
2. Nitrogen was estimated using the Micro-Kjeldahl apparatus, following the method described in [16].
3. **Leaf phosphorus percentage:**
4. Phosphorus was determined using the colorimetric method, with light absorption measured at a wavelength of 410 nm using a UV-1100 spectrophotometer, as described by [17].
5. **Leaf potassium percentage:**
6. Potassium was determined using a Microprocessor Flame Photometer according to [17].

## Results and Discussion

### 1- Average increase in shoot length (cm):

The results in Table (1) indicate that spraying with Brassinolide at a concentration of 2 mg L<sup>-1</sup> gave the highest average increase in shoot length (37.61 cm), while the control treatment recorded the lowest value (33.05 cm). Spraying with proline also showed a significant effect at a concentration of 200 mg L<sup>-1</sup>, giving the highest value of 38.32 cm, compared to the control treatment, which recorded the lowest value of 33.47 cm. The treatment with boron at 30 mg L<sup>-1</sup> outperformed the control, with an average shoot length increase of 36.64 cm compared to 33.89 cm in the control treatment.

Regarding the two-way interaction between Brassinolide and proline, a significant superiority was observed, where the combination of 2 mg L<sup>-1</sup> Brassinolide and 200 mg L<sup>-1</sup> proline gave the highest value (39.75 cm). The two-way interaction between Brassinolide and boron also showed significant superiority at 2 mg L<sup>-1</sup> Brassinolide with 30 mg L<sup>-1</sup> boron, with the highest value of 39.88 cm. Similarly, the two-way interaction between proline and boron was significant, where the combination of 200 mg L<sup>-1</sup> proline and 30 mg L<sup>-1</sup> boron gave 38.55 cm, which was not significantly different from the combination of 200 mg L<sup>-1</sup> proline with 0 mg L<sup>-1</sup> boron. The control treatment recorded the lowest values.

As for the three-way interaction among the treatments, there was a significant increase, where the combination of 2 mg L<sup>-1</sup> Brassinolide, 200 mg L<sup>-1</sup> proline, and 30 mg L<sup>-1</sup> boron achieved the highest value (44.33 cm). However, it was not significantly different from the treatment of 0 mg L<sup>-1</sup> Brassinolide, 200 mg L<sup>-1</sup> proline, and 0 mg L<sup>-1</sup> boron, while the control treatment recorded the lowest values.

Table (1). Effect of foliar spraying with Brassinolide, proline, boron, and their interactions on the increase in shoot length (cm) of pomegranate trees, cultivar Wonderful.

Brassinolide (mg L <sup>-1</sup> )	Proline (mg L <sup>-1</sup> )	Boron (mg L <sup>-1</sup> )		Brassinolide * Proline
		0	30	
0	0	21.90e	33.93c	27.91f
	100	30.16d	33.66c	31.91e
	200	43.16a	35.50bc	39.33ab
2	0	37.50b	37.66b	37.58bc
	100	33.33c	37.66b	35.50d
	200	35.16bc	44.33a	39.75a
4	0	34.00c	35.83bc	34.91d
	100	33.83c	35.33bc	34.58d
	200	35.96bc	35.83bc	35.90cd
Brassinolide (mg L <sup>-1</sup> )				Mean Brassinolide
Brassinolide * Boron	0	31.74c	34.36b	33.05c
	2	35.33b	39.88a	37.61a
	4	34.60b	35.66b	35.13b
Brassinolide (mg L <sup>-1</sup> )				Mean Brassinolide
Brassinolide * Boron	0	31.13c	35.81b	33.47b
	100	32.44c	35.55b	34.00b
	200	38.10a	38.55a	38.32a
Mean Brassinolide		33.89b	36.64a	

Means followed by the same letters within the group are not significantly different according to Duncan's multiple range test at the 0.05 probability level.

**2- Average increase in shoot diameter (mm):**

The results in Table (2) show that spraying with Brassinolide at 2 mg L<sup>-1</sup> resulted in the highest average increase in shoot diameter, reaching 3.32 mm, which was not significantly different from 4 mg L<sup>-1</sup>, while the control treatment recorded the lowest value of 3.11 mm. Spraying with proline had a significant effect on this trait at 200 mg L<sup>-1</sup>, giving 3.36 mm, compared to 3.15 mm in the control treatment. No significant differences were observed between boron treatments for the trait of shoot diameter increase.

Regarding the two-way interaction between Brassinolide and proline, a significant superiority was recorded at 2 mg L<sup>-1</sup> Brassinolide and 200 mg L<sup>-1</sup> proline, with a value of 3.42 mm compared to the control. No significant differences were observed in the two-way interactions between Brassinolide and boron or between proline and boron for this trait. The three-way interaction among Brassinolide, proline, and boron showed a significant increase in shoot diameter at 2 mg L<sup>-1</sup> Brassinolide, 200 mg L<sup>-1</sup> proline, and 30 mg L<sup>-1</sup> boron, reaching 3.54 mm.

Table (2). Effect of foliar spraying with Brassinolide, proline, boron, and their interactions on the increase in shoot diameter (mm) of pomegranate trees, cultivar Wonderful.

Brassinolide (mg L <sup>-1</sup> )	Proline (mg L <sup>-1</sup> )	Boron (mg L <sup>-1</sup> )		Brassinolide * Proline
		0	30	
0	0	2.94b	2.94b	2.94c
	100	3.02ab	3.03ab	3.03bc
	200	3.34ab	3.37ab	3.35ab
2	0	3.29ab	3.23ab	3.26abc
	100	3.29ab	3.29ab	3.29abc
	200	3.31ab	3.54a	3.42a
4	0	3.30ab	3.22ab	3.26abc
	100	3.23ab	3.22ab	3.22abc
	200	3.32ab	3.32ab	3.32ab
	Brassinolide (mg L <sup>-1</sup> )			Mean Brassinolide
Brassinolide * Boron	0	3.10a	3.11a	3.11b
	2	3.30a	3.35a	3.32a
	4	3.28a	3.25a	3.27ab
	Brassinolide (mg L <sup>-1</sup> )			Mean Brassinolide
Brassinolide * Boron	0	3.18a	3.13a	3.15b
	100	3.18a	3.18a	3.18ab
	200	3.32a	3.41a a	3.36a
	Mean Brassinolide	3.22a	3.24a	

Means of each factor or interaction followed by different letters are significantly different at the 5% probability level according to Duncan's multiple range test.

**3- Leaf area (cm<sup>2</sup>):**

The results in Table (3) show that spraying with Brassinolide at 4 mg L<sup>-1</sup> gave the largest leaf area, reaching 7.34 cm<sup>2</sup>, while the control treatment recorded the lowest value of 7.05 cm<sup>2</sup>. Spraying with proline had a significant effect at 200 mg L<sup>-1</sup>, giving the highest leaf area of 7.54 cm<sup>2</sup> compared to 7.10 cm<sup>2</sup> in the control. Spraying with boron at 30 mg L<sup>-1</sup> resulted in a leaf area of 7.38 cm<sup>2</sup>, while the control recorded the lowest value of 7.07 cm<sup>2</sup>.

For the two-way interaction between Brassinolide and proline, significant superiority was observed at 0 mg L<sup>-1</sup> Brassinolide and 100 mg L<sup>-1</sup> proline, giving the highest leaf area of 7.71 cm<sup>2</sup>. The two-way interaction between Brassinolide and boron showed significant superiority with 2 mg L<sup>-1</sup> Brassinolide and 30 mg L<sup>-1</sup> boron, reaching 7.52 cm<sup>2</sup>, which was not significantly different from 4 mg L<sup>-1</sup> Brassinolide and 30 mg L<sup>-1</sup> boron. The two-way interaction

between proline and boron indicated significant superiority at 200 mg L<sup>-1</sup> proline and 30 mg L<sup>-1</sup> boron, with a leaf area of 7.83 cm<sup>2</sup>, while the control recorded the lowest value of 6.92 cm<sup>2</sup>.

The three-way interaction among Brassinolide, proline, and boron showed significant superiority at 2 mg L<sup>-1</sup> Brassinolide, 0 mg L<sup>-1</sup> proline, and 30 mg L<sup>-1</sup> boron, giving the highest leaf area of 8.15 cm<sup>2</sup>.

Table (3). Effect of foliar spraying with Brassinolide, proline, boron, and their interactions on leaf area (cm<sup>2</sup>) of pomegranate trees, cultivar Wonderful.

Brassinolide (mg L <sup>-1</sup> )	Proline (mg L <sup>-1</sup> )	Boron (mg L <sup>-1</sup> )		Brassinolide * Proline
		0	30	
0	0	6.73 d	6.66 d	6.69 b
	100	7.48 b	7.94 a	7.71 a
	200	6.6 d	6.86 d	6.74 b
2	0	6.72d	8.15 a	7.43 a
	100	7.27 c	7.79 ab	7.53 a
	200	7.10 c	6.63 d	6.87 b
4	0	7.32 c	7.04 c	7.18 ab
	100	7.03 c	7.76 ab	7.39 a
	200	7.32 c	7.57 b	7.44 a
Brassinolide (mg L <sup>-1</sup> )				Mean Brassinolide
Brassinolide * Boron	0	6.95 b	7.15 b	7.05 c
	2	7.032 b	7.52 a	7.27 b
	4	7.22 b	7.45 a	7.34 a
Brassinolide (mg L <sup>-1</sup> )				Mean Brassinolide
Brassinolide * Boron	0	6.92 c	7.28 b	7.10 b
	100	7.01 c	7.02 c	7.02 b
	200	7.26 b	7.83 a	7.54 a
Mean Brassinolide		7.07b	7.38a	

Means of each factor or interaction followed by different letters are significantly different at the 5% probability level according to Duncan's multiple range test.

#### 4- Leaf total chlorophyll content (mg 100 g<sup>-1</sup> fresh weight):

The results in Table (4) indicate that spraying the trees with Brassinolide significantly affected leaf total chlorophyll content, with 4 mg L<sup>-1</sup> Brassinolide showing the highest value of 25.29 mg 100 g<sup>-1</sup> fresh weight, while the control recorded the lowest value of 21.61 mg 100 g<sup>-1</sup> fresh weight. Proline at 200 mg L<sup>-1</sup> significantly increased chlorophyll content to 24.72 mg 100 g<sup>-1</sup> fresh weight, compared to 21.81 mg 100 g<sup>-1</sup> fresh weight in the control. Spraying with boron at 30 mg L<sup>-1</sup> also significantly increased chlorophyll content to 23.81 mg 100 g<sup>-1</sup> fresh weight, while the control recorded 23.01 mg 100 g<sup>-1</sup> fresh weight.

Regarding the two-way interaction between Brassinolide and proline, the combination of 4 mg L<sup>-1</sup> Brassinolide and 200 mg L<sup>-1</sup> proline showed significant superiority over other treatments, reaching 26.15 mg 100 g<sup>-1</sup> fresh weight. The two-way interaction between Brassinolide and boron showed that 4 mg L<sup>-1</sup> Brassinolide with 30 mg L<sup>-1</sup> boron was significantly superior, reaching 25.63 mg 100 g<sup>-1</sup> fresh weight. The two-way interaction between proline and boron showed that 200 mg L<sup>-1</sup> proline with 30 mg L<sup>-1</sup> boron achieved 25.01 mg 100 g<sup>-1</sup> fresh weight, significantly higher than other combinations.

The three-way interaction among Brassinolide, proline, and boron significantly increased leaf chlorophyll content at 4 mg L<sup>-1</sup> Brassinolide, 200 mg L<sup>-1</sup> proline, and 30 mg L<sup>-1</sup> boron, reaching 26.34 mg 100 g<sup>-1</sup> fresh weight, compared to other treatments.

Table (4). Effect of foliar spraying with Brassinolide, proline, boron, and their interactions on leaf total chlorophyll content ( $\text{mg } 100 \text{ g}^{-1}$  fresh weight) of pomegranate trees, cultivar Wonderful.

Brassinolide ( $\text{mg L}^{-1}$ )	Proline ( $\text{mg L}^{-1}$ )	Boron ( $\text{mg L}^{-1}$ )		Brassinolide * Proline
		0	30	
0	0	19.75 q	20.23 p	19.99 i
	100	21.34 o	22.74 k	22.04 g
	200	22.44 m	23.15 j	22.79 f
2	0	21.34 o	21.46 n	21.4 h
	100	22.54 l	24.33 h	23.43 e
	200	24.87 f	25.54 d	25.2 c
4	0	23.44 i	24.67 g	24.05 d
	100	25.45 e	25.87 c	25.66 b
	200	25.97 b	26.34 a	26.15 a
Brassinolide ( $\text{mg L}^{-1}$ )				Mean Brassinolide
Brassinolide * Boron	0	21.18 f	22.04 e	21.61 c
	2	22.91 d	23.78 c	23.35 b
	4	24.95 b	25.63 a	25.29 a
Brassinolide ( $\text{mg L}^{-1}$ )				Mean Brassinolide
Brassinolide * Boron	0	21.51 f	22.12 e	21.81 c
	100	23.11 d	24.31 c	23.71 b
	200	24.42 b	25.01 a	24.72 a
Mean Brassinolide		23.01 b	23.81 a	

Means of each factor or interaction followed by different letters are significantly different at the 5% probability level according to Duncan's multiple range test.

#### 5- Leaf nitrogen percentage (%):

The results in Table (5) show that spraying with the growth regulator Brassinolide increased leaf nitrogen content, with  $4 \text{ mg L}^{-1}$  Brassinolide showing the highest value of 2.72%, while the control recorded the lowest value of 1.67%. Spraying with proline significantly affected leaf nitrogen content, where  $200 \text{ mg L}^{-1}$  proline gave the highest value of 2.42%, compared to 2.07% in the control. Spraying with boron at  $30 \text{ mg L}^{-1}$  also significantly increased leaf nitrogen content to 2.29%, while the control recorded 2.19%.

The two-way interaction between Brassinolide and proline showed significant superiority at  $4 \text{ mg L}^{-1}$  Brassinolide and  $200 \text{ mg L}^{-1}$  proline, reaching 2.79%, compared to other treatments. The two-way interaction between Brassinolide and boron showed superiority at  $4 \text{ mg L}^{-1}$  Brassinolide and  $0 \text{ mg L}^{-1}$  boron, reaching 2.74%, which was not significantly different from  $4 \text{ mg L}^{-1}$  Brassinolide and  $30 \text{ mg L}^{-1}$  boron. The two-way interaction between proline and boron showed significant superiority at  $200 \text{ mg L}^{-1}$  proline and  $30 \text{ mg L}^{-1}$  boron, giving 2.49%, compared to the control, which recorded the lowest value.

The three-way interaction among Brassinolide, proline, and boron significantly increased leaf nitrogen content, with most interaction treatments outperforming the control. The highest value was recorded for  $4 \text{ mg L}^{-1}$  Brassinolide,  $200 \text{ mg L}^{-1}$  proline, and  $30 \text{ mg L}^{-1}$  boron, reaching 2.82%, while the lowest value was in the control treatment.

Table (5). Effect of foliar spraying with the growth regulator (Brassinolide) and amino acids (proline and boron) and their interactions on leaf nitrogen percentage (%) of young pomegranate trees, cultivar Wonderful.

Brassinolide (mg L <sup>-1</sup> )	Proline (mg L <sup>-1</sup> )	Boron (mg L <sup>-1</sup> )		Brassinolide * Proline
		0	30	
0	0	1.55 fg	1.66 efg	1.60 d
	100	1.60 fg	1.53 g	1.56 d
	200	1.83 de	1.84 de	1.83 c
2	0	1.77 ef	2.03 d	1.90 c
	100	2.33 c	2.63 ab	2.48 b
	200	2.46 bc	2.81 a	2.64 a
4	0	2.75 a	2.66 ab	2.70 a
	100	2.71 a	2.65 ab	2.68 a
	200	2.76 a	2.82 a	2.79 a
Brassinolide (mg L <sup>-1</sup> )				Mean Brassinolide
Brassinolide * Boron	0	1.66 d	1.67 d	1.67 c
	2	2.19 c	2.49 b	2.34 b
	4	2.74 a	2.71 a	2.72 a
Brassinolide (mg L <sup>-1</sup> )				Mean Brassinolide
Brassinolide * Boron	0	2.02 e	2.11 de	2.07 c
	100	2.21 cd	2.27 bc	2.24 b
	200	2.35 b	2.49 a	2.42 a
Mean Brassinolide		b2.19	a2.29	

Means of each factor or interaction followed by different letters are significantly different at the 5% probability level according to Duncan's multiple range test.

#### 6- Leaf phosphorus percentage (%):

The results in Table (6) indicate that spraying with Brassinolide at 4 mg L<sup>-1</sup> gave the highest leaf phosphorus content, reaching 0.141%, while the control recorded the lowest value of 0.074%. No significant differences were observed between proline and boron treatments for leaf phosphorus content.

The two-way interaction between Brassinolide and proline showed significant superiority at 4 mg L<sup>-1</sup> Brassinolide and 200 mg L<sup>-1</sup> proline, reaching 0.155%, compared to the control, which recorded the lowest phosphorus value. The two-way interaction between Brassinolide and boron resulted in the highest value at 4 mg L<sup>-1</sup> Brassinolide and 30 mg L<sup>-1</sup> boron, reaching 0.145%, which was not significantly different from 4 mg L<sup>-1</sup> Brassinolide with 0 mg L<sup>-1</sup> boron. No significant differences were observed in the two-way interaction between proline and boron.

The three-way interaction among Brassinolide, proline, and boron showed significant superiority at 4 mg L<sup>-1</sup> Brassinolide, 200 mg L<sup>-1</sup> proline, and 30 mg L<sup>-1</sup> boron, reaching 0.158%, while the control recorded the lowest phosphorus content.

Table (6). Effect of foliar spraying with Brassinolide, proline, boron, and their interactions on leaf phosphorus percentage (%) of pomegranate trees, cultivar Wonderful.

Brassinolide (mg L <sup>-1</sup> )	Proline (mg L <sup>-1</sup> )	Boron (mg L <sup>-1</sup> )		Brassinolide * Proline
		0	30	
0	0	0.076 g	0.085 efg	0.080 cd
	100	0.083 fg	0.081 fg	0.082 cd

	200	0.088 defg	0.030 h	0.059 d
	0	0.096 cdefg	0.094 cdefg	0.095 c
2	100	0.102 bcdefg	0.112 abcdefg	0.107 bc
	200	0.123 abcdef	0.131 abcde	0.127 ab
	0	0.134 abcd	0.133 abcd	0.133 ab
4	100	0.141 abc	0.145 ab	0.143 a
	200	0.153 a	0.158 a	0.155 a
	Brassinolide (mg L <sup>-1</sup> )			Mean Brassinolide
Brassinolide * Boron	0	0.082 c	0.065 c	0.074 c
	2	0.107 b	0.112 b	0.110 b
	4	0.143 a	0.145 a	0.144 a
	Brassinolide (mg L <sup>-1</sup> )			Mean Brassinolide
Brassinolide * Boron	0	0.102 a	0.104 a	0.103 a
	100	0.109 a	0.113 a	0.111 a
	200	0.121 a	0.106 a	0.114 a
	Mean Brassinolide	0.104 a	0.103 a	

Means of each factor or interaction followed by different letters are significantly different at the 5% probability level according to Duncan's multiple range test.

#### 7- Leaf potassium percentage (%):

The results shown in Table (7) indicate a significant response to Brassinolide spraying in increasing leaf potassium content at 4 mg L<sup>-1</sup>, reaching 2.20%, while the control recorded the lowest value of 1.94%. Spraying with proline at 200 mg L<sup>-1</sup> significantly increased potassium content to 2.09%, compared to 2.02% in the control. Spraying with boron at 30 mg L<sup>-1</sup> also significantly increased leaf potassium content to 2.06%, while the control recorded 2.04%. The two-way interaction between Brassinolide and proline gave the highest value at 4 mg L<sup>-1</sup> Brassinolide and 200 mg L<sup>-1</sup> proline, reaching 2.24%. The two-way interaction between Brassinolide and boron showed significant superiority at 4 mg L<sup>-1</sup> Brassinolide and 30 mg L<sup>-1</sup> boron, reaching 2.213%. The two-way interaction between proline and boron significantly increased leaf potassium content at 200 mg L<sup>-1</sup> proline and 30 mg L<sup>-1</sup> boron, reaching 2.11% compared to the control.

The three-way interaction among Brassinolide, proline, and boron significantly increased leaf potassium content at 4 mg L<sup>-1</sup> Brassinolide, 200 mg L<sup>-1</sup> proline, and 30 mg L<sup>-1</sup> boron, reaching 2.25% compared to the other treatments.

Table (7). Effect of foliar spraying with Brassinolide, proline, boron, and their interactions on leaf potassium percentage (%) of pomegranate trees, cultivar Wonderful.

Brassinolide (mg L <sup>-1</sup> )	Proline (mg L <sup>-1</sup> )	Boron (mg L <sup>-1</sup> )		Brassinolide * Proline
		0	30	
0	0	1.92 n	1.93 m	1.92 i
	100	1.94 l	1.95 kl	1.95 h
	200	1.96 k	1.97 j	1.96 g
2	0	1.97 j	1.97 ij	1.97 f
	100	1.98 hi	1.99 h	1.99 e
	200	1.99 h	2.12 g	2.06 d
4	0	2.15 f	2.17 e	2.16 c
	100	2.18 d	2.21 c	2.20 b

	200	2.24 b	2.25 a	2.24 a
	Brassinolide (mg L <sup>-1</sup> )			Mean Brassinolide
Brassinolide * Boron	0	1.94 f	1.95 e	1.94 c
	2	1.98 d	2.03 c	2.00 b
	4	2.19 b	2.21 a	2.20 a
	Brassinolide (mg L <sup>-1</sup> )			Mean Brassinolide
Brassinolide * Boron	0	2.01 f	2.03 e	2.02 c
	100	2.04 d	2.05 c	2.04 b
	200	2.06 b	2.11 a	2.09 a
Mean Brassinolide		b2.04	a2.06	

Means of each factor or interaction followed by different letters are significantly different at the 5% probability level according to Duncan's multiple range test.

## Discussion

### Effect of Brassinolide, Proline and Boron on vegetative growth traits and mineral content:

The results presented in Tables (1–7) indicate that foliar spraying with the growth regulator Brassinolide significantly improved the following traits: branch length, branch diameter, leaf area, leaf total chlorophyll content, leaf nitrogen percentage, leaf phosphorus percentage, and leaf potassium percentage. The increase in vegetative growth traits is attributed to the role of Brassinolide in promoting cell division and elongation, as cell elongation is controlled through multiple processes, including changes in mechanical properties, genetic variations, and certain biochemical processes [18,19].

The increased leaf chlorophyll content in trees sprayed with Brassinolide is related to the effect on chlorophyllase, which is responsible for chlorophyll degradation, leading to chlorophyll accumulation in the leaves [20]. Furthermore, spraying pomegranate trees with Brassinolide positively affected leaf mineral content, likely because Brassinolide enhances root absorption of mineral nutrients from the soil [21,22]. These results are consistent with those of [23], who studied apple trees, cultivar Anna; [24], who studied pomegranate trees, cultivar Salimi; and [25], who studied apricot trees, cultivar Canino.

The results from Tables (1–7) indicate that spraying with the amino acid proline significantly improved the following traits: branch length, branch diameter, leaf area, leaf total chlorophyll content, leaf nitrogen percentage, and leaf potassium percentage. This increase is attributed to the important role of proline in plant growth, physiological activity, and metabolic processes, as well as its role in protecting plants from various stress effects by maintaining cellular osmotic balance and stabilizing cell membranes, which in turn positively affects other growth indicators [26].

Proline spraying also increased leaf nutrient content, which enhanced both vegetative and root growth, thereby increasing the plant's ability to absorb sufficient nitrogen and accumulate it, promoting overall growth [27]. These results are consistent with those of [28] and [29] in their studies on pomegranate trees, cultivar Wonderful.

The results presented in Tables (1, 3–5, 7) show that foliar spraying with boron significantly improved branch length, leaf area, leaf total chlorophyll content, leaf nitrogen percentage, and leaf potassium percentage. This increase is attributed to boron being an essential nutrient for plants, as it plays a role in regulating enzymatic activity and auxin transport within the plant [30,31]. Additionally, boron is effective in stabilizing cell membrane systems by forming cis-diol complexes and regulating gene expression related to membrane function [32]. These results are in agreement with [33] who conducted a study on a local pomegranate cultivar Smaqulli. [34] who studied apple trees, cultivar Anna, and [35], who studied apple trees, cultivar Ibrahim.

## Conclusions

The study concludes that foliar spraying with the growth regulator Brassinolide, the amino acid proline, and boron had a significant effect on most vegetative growth traits and leaf mineral content of pomegranate trees. Spraying with Brassinolide at 4 mg L<sup>-1</sup>, proline at 200 mg L<sup>-1</sup>, and boron at 30 mg L<sup>-1</sup> produced the greatest effects and the most effective responses for most of the studied traits under the conditions of the study area.

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## تحسين صفات النمو الخضري والمحتوى المعدني في أشجار الرمان صنف Wonderful برش Brassinolide والبرولين والبورون

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الخلاصة

تمت "هذه الدراسة في بستان محطة البحوث والتجارب الزراعية التابعة لكلية الزراعة - جامعة كركوك ، خلال موسم النمو 2024 لدراسة تأثير الرش الورقي بمنظم النمو *Brassinolide* بثلاثة تراكيز (4،2،0) ملغم لتر-1 والحامض الاميني البرولين بثلاثة تراكيز (200 ، 100، 0) ملغم لتر-1 والبورون بتركيزين (30،0) ملغم لتر-1 وتأثير التداخل بينهما في بعض صفات النمو الخضري والمحتوى المعدني لأشجار الرمان صنف *Wonderful*. استخدم تصميم القطاعات العشوائية الكاملة *RCBD* وبثلاثة عوامل وبأربعة مكررات وشجرة واحدة للوحدة التجريبية الواحدة ،ويمكن تلخيص النتائج التي تم الحصول عليها بما يأتي:

كان للرش الورقي بمنظم النمو *Brassinolide* تأثير معنوي في صفات النمو الخضري و المحتوى المعدني لأشجار الرمان بتركيز 2 ملغم لتر-1 في (طول الافرع، قطر الافرع ) بلغت (37.61سم، 3.32ملم) و بتركيز 4 ملغم لتر-1 في (مساحة الورقة الواحدة ، الكلوروفيل الكلي ، النسبة المئوية للنتروجين،الفسفور،البوتاسيوم في الأوراق) بلغت (7.34 سم، 25.29 ملغم 100غم-1 و وزن طري، 2.72 % و 0.144% و 2.20% ) بالتتابع ( وادى الرش الورقي بالحامض الاميني البرولين عند التركيز 200ملغم لتر-1 الى زيادة معنوية واضحة في جميع صفات نمو الخضري المدروسة(طول الافرع، قطر الافرع، مساحة الورقة الواحدة، الكلوروفيل الكلي) بلغت(38.32سم، 3.36ملم و 7.54سم، 24.72 ملغم 100غم-1 و وزن طري) وصفتي المحتوى المعدني (النسبة المئوية للنتروجين،البوتاسيوم في الأوراق) بلغت ( 2.42% و 2.09%) وادى الرش الورقي بالبورون عند تركيز 30ملغم لتر-1 الى زيادة معنوية في صفات النمو الخضري والمحتوى المعدني (طول الافرع، مساحة الورقة الواحدة ، الكلوروفيل الكلي، النسبة المئوية للنتروجين ، البوتاسيوم في الأوراق) بلغت (36.64سم، 7.38سم، 23.81 ملغم 100غم-1 و وزن طري، 2.29 % و 2.06%) بالتتابع. وهناك تأثير معنوي واضح نتيجة التداخل الثنائي والثلاثي بين معاملات الدراسة في معظم الصفات المدروسة.

الكلمات المفتاحية: *Brassinolide* والبرولين والبورون والرمان و *Wonderful*.