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Optimizing Pomegranate Nutrient Uptake, Yield, and Fruit Quality using Growth Stimulants under Varying Irrigation Conditions

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ABSTRACT

The utilization of growth stimulants (GS) offers a potential strategy to mitigate the adverse impacts of drought stress. This study hypothesized that GS could enhance both the quantitative and qualitative traits of pomegranate fruit and improve nutrient absorption under varying irrigation regimes. A split-plot experiment was conducted in a randomized complete block design over two growing seasons (2020–2022). Two main irrigation treatments, which provided 100% ETC and 70% ETC for pomegranate, were established as main plots. Seven GS treatments were assigned to subplots including a control, foliar application of amino acid (Aa), fulvic acid (Fa), and seaweed extract (Se); soil application of humic acid (Ha) and arbuscular mycorrhizal fungus (AMF); and combined consumption of GS (Cc). Providing 70% ETC reduced fruit yield, fruit count, total acidity (TA), and nutrient absorption, while increasing total soluble solids (TSS). Under 100% ETC, the AMF, Se, and Cc treatments increased fruit yield by 17.9, 15.1, and 28.5%, respectively, compared to the control, whereas under 70% ETC, the corresponding increases were 26.6, 29.7, and 40.8%. At 70% ETC, the Cc treatment reduced TA by 9.8%, whereas at 100% ETC, Aa, Fa, and Cc reduced TA significantly by 10.9, 13.0, and 13.0%, respectively. Applying the Cc treatment at 70% ETC maximized quality while causing minimal yield loss. Overall, most GS treatments effectively improved leaf nutrient concentrations under both irrigation regimes. The enhanced efficiency of GS in improving fruit yield and nutrient absorption under drought conditions highlights its potential as a sustainable solution for pomegranate production in arid regions.

Abbreviations: Growth stimulants (GS), Foliar application of amino acid (Aa), Foliar application of fulvic acid (Fa), Foliar application of seaweed extract (Se), Soil application of humic acid (Ha), Arbuscular mycorrhizal fungus (AMF), Combined consumption of growth stimulants (Cc), Fruit total acidity (TA), Fruit total soluble solids (TSS), Irrigation regimes (IR)

Introduction

Global warming, climatic changes, and water scarcity are among the critical challenges facing agriculture nowadays. In Iran, which lies within an arid and semi-arid region, agricultural production is predominantly constrained by the quantity and quality of available irrigation water. Drought stress significantly reduces crop yield by altering physiological traits and limiting nutrient absorption (Sabagh et al., 2019; Ebrahimi et al., 2021). Furthermore, drought conditions disrupt photosynthetic processes and intensify oxidative stress in plants (Loreto and Centritto, 2008). Previous studies suggest that drought tolerance can be enhanced through modifications in physiological and biochemical mechanisms (Attarzadeh et al., 2020). Moreover, enhanced nutrient absorption under drought stress contributes to higher photosynthetic efficiency, thereby strengthening plant resilience to adverse conditions (Attarzadeh et al., 2019).

In recent years, GS have emerged as a promising approach to mitigate the adverse impacts of drought stress on plants. These substances are naturally derived and are applied to reduce reliance on chemical fertilizers, enhance growth, improve quality traits, and bolster tolerance to environmental stresses (Halpern et al., 2015; Bulgari et al., 2019). Unlike conventional chemical fertilizers, GS promote agricultural productivity while preserving natural resources and minimizing environmental harm (Aamir et al., 2020). Substances such as amino acids, seaweed extract, humic acid, fulvic acid, and arbuscular mycorrhizal fungi (AMF) are notable examples of GS that have been extensively studied (Battacharyya et al., 2015; Canellas et al., 2015).

Research indicates that the application of amino acids enhances plant tolerance to environmental stresses by regulating ion transport, modulating stomatal activity, and functioning as osmolytes (Anjum et al., 2014). Amino acids such as glycine and glutamine play important roles in plant growth and metabolism. Foliar application of these amino acids at moderate concentrations has been reported to enhance shoot growth, leaf area, chlorophyll content, and nutrient accumulation, suggesting a practical approach to improve crop performance (Aghaye Noroozlo et al., 2020).

Humic acid enhances nutrient absorption and boosts photosynthesis, leading to significant improvements in crop yield (Rashedy et al., 2022). The growth-promoting effects of humic acid and fulvic acid are largely attributed to their ability to stimulate ATPase-H^+ enzyme activity, thereby enhancing energy transfer and nitrate uptake efficiency in plants (Canellas et al., 2015). Seaweed extract, rich in vital vitamins, amino acids, and plant hormones like cytokinin and auxin, also exerts a significant positive influence on plant growth and development (Battacharyya et al., 2015). Moreover, integrated applications of seaweed extract and AMF in combination with chemical fertilizers have been reported to improve yield, ascorbic acid, anthocyanin, protein, and mineral content in pomegranate (Maity et al., 2024).

Pomegranate, recognized for its nutritional benefits and antioxidant-rich juice, is widely cultivated in Asia, the Mediterranean Basin, and the United States (Holland et al., 2009; Mellisho et al., 2012). Iran, considered the primary origin of pomegranate, yields over one million tons annually (Iran Ministry of Agriculture, 2016). However, drought stress poses a significant challenge to pomegranate cultivation in Iran. The efficacy of GS in mitigating drought stress has been well documented in horticultural crops, including pomegranate. For example, biofertilizers have been found to enhance pomegranate growth under drought conditions (Aseri et al., 2008). Similarly, studies by Khattab et al. (2012) demonstrated improved vegetative growth, flowering, and fruit yield when lower irrigation levels were supplemented with higher doses of humic acid or amino acids. While numerous studies have examined the effects of GS on pomegranate under optimal moisture conditions (Abubakar et al., 2013; Harhash et al., 2021; Maity et al., 2024), limited research has been conducted on their impact under drought stress.

Therefore, this study aimed to evaluate the effects of selected GS on yield, fruit quality traits, and nutrient absorption in pomegranate under both normal and drought stress conditions. A moderate water stress was intentionally applied to reflect the typical drought conditions encountered by farmers, allowing the results to provide realistic and practical insights into plant responses under field-relevant conditions rather than under extreme or unrepresentative stress levels.

Materials and Methods

This study was conducted in the Kohmera Sorkhi region, Shiraz city, Fars province, Iran (latitude: 29°46'48.49" N; longitude: 52°18'33.12" E; altitude: 1,900 meters above sea level). This region is characterized by a semi-arid climate with mild winters and hot summers. The annual averages are 300 mm of rainfall, 1,800 mm of evaporation, and an average temperature of 20 °C. The experiment was carried out over two consecutive years (2020–2021 and 2021–2022) in a pomegranate orchard containing 10-year-old, fruit-bearing trees of the ‘Berit’ early-ripening cultivar. The trees were planted at a spacing of 3 × 4 meters and managed using a pressurized drip irrigation system.

Prior to the initiation of the experiment, soil samples were collected from two depths (0–30 cm and 30–60 cm) and analyzed for physicochemical properties. These properties are summarized in Table 1. The gravimetric water content of the soil was 23.4% at field capacity ($\psi s = -0.33$ MPa) and 9.5% at the permanent wilting point ($\psi s = -1.5$ MPa). Rainfall data for the two experimental years are presented in Table 2. The crop coefficient (Kc) and irrigation applied (m^3 ha^{-1}) to pomegranate trees exposed to different irrigation treatments for the two growing seasons of the experiment are presented in Table 3.

Table 1. Soil physical and chemical properties of the orchard under study.

Year	Depth	Texture	O.C	pH	TNV	EC	Total N	P	K	Fe	Mn	Zn	Cu
	(cm)		%		%	(dS m^{-1})	%	mg kg^{-1}					
2020–2021	0-30	Clay Loam	1.31	7.5	34.5	2.45	0.13	8.6	308	9.5	9.1	1.5	1.6
	30-60	Loam	1.03	7.7	34.0	2.32	0.10	5.6	238	10.4	7.6	1.1	1.5
2021–2022	0-30	Clay Loam	1.36	7.6	33.8	2.31	0.14	9.1	310	10.1	9.5	1.3	1.4
	30-60	Loam	1.08	7.5	33.4	2.22	0.10	5.2	218	10.0	6.9	1.1	1.2

Table 2. The amount of rainfall (mm) in the years of the experiment.

Year	October	November	December	January	February	March	April	May	June	July	August	September	Total
2020-2021	0.9	12	30	32	28	26	22	6	0	0.2	0.6	0	157.7
2021-2022	0.6	14	32	28	22	30	31	15	0.2	0.4	0	0	174.4

Table 3. The crop coefficient (Kc) and irrigation applied (m^3 ha^{-1}) to pomegranate trees exposed to different irrigation treatments for the two growing seasons of the experiment.

Month	K_C	100%		70%	
		2020-2021	2021-2022	2020-2021	2021-2022
March	0.32	752	698	526	489
April	0.41	889	842	622	589
May	0.47	819	791	573	554
June	0.68	2025	2020	1418	1414
July	0.77	2342	2287	1639	1601
August	0.79	2410	2415	1687	1691
September	0.71	2085	2032	1460	1422
October	0.62	1928	1965	1350	1376
Total		13250	13050	9275	9135

Fertilizers were applied based on soil analysis, and animal manure (5 kg $tree^{-1}$) was incorporated annually into pits dug around each tree. In both years, urea fertilizer was applied in three stages, before flowering, during nut fruit development, and in mid-August, at a total rate of 200 kg ha^{-1}. The experiment followed a split-plot design within a completely randomized block layout, consisting of two main factors and seven sub-factors. In each replication (block), two main irrigation plots were established, and within each main plot, seven growth stimulant treatments were applied. Consequently, each replication contained 14 experimental units, with four trees assigned to each unit. Two rows of trees served as buffers between the main plots and between blocks.

Main plot treatments

Irrigation in the main plots was scheduled according to the Penman–Monteith equation (Allen et al., 1998). Crop evapotranspiration (ET_C) was calculated using $ET_C = K_C \times ET_O$, where K_C is the crop coefficient and ET_O is the reference evapotranspiration. Throughout the growing season, pomegranate trees were irrigated via drip lines equipped with four emitters (4 L h^{-1}) per tree. A volumetric water meter was installed for each main plot (six meters in total) to precisely monitor irrigation amounts before water entered the plots. Irrigation was applied every 7–10 d based on crop evapotranspiration (Table 3). Two irrigation regimes were imposed during both growing seasons: full irrigation (100% ET_C) and deficit irrigation (70% ET_C). For the second regime, sustained deficit irrigation (SDI) was employed, in which irrigation was uniformly reduced throughout the entire growing season. This strategy enabled the gradual development of moderate water stress under realistic field conditions; therefore, instead of targeting a specific phenological stage, stress was consistently maintained across all growth stages (Volschenk, 2021).

The sub-plot treatments consisted of seven GS applications. The first treatment served as the control and involved only chemical fertilizers based on soil test results. The second treatment included the control plus a foliar spray of amino acids (Aa), while the third consisted of the control combined with soil application of humic acid (Ha). The fourth treatment involved the control with a foliar spray of fulvic acids (Fa), and the fifth applied the control together with a foliar spray of seaweed extract (Se). The sixth treatment comprised the control plus soil application of arbuscular mycorrhizal fungi (AMF). Finally, the seventh treatment involved the combined application of treatments 2, 3, 5, and 6 (Cc).

All growth stimulants were sourced from commercially approved products recognized by the Soil and Water Research Institute of Iran. The humic acid contained 52.95% humic acid, the amino acid formulation included 36.79% free amino acids, the fulvic acid contained 22.1% fulvic acid, and the seaweed extract contained 10% alginic acid. Foliar treatments were prepared at a concentration of 5 kg stimulant per 1,000 L of water and applied twice during the season, once after fruit formation and again one month later, with 10 L tree^{-1} sprayed each time to ensure uniform and complete canopy coverage. Humic acid was applied in two stages, at the beginning of vegetative growth and during fruit set, with 20 g tree^{-1} applied at each stage. The AMF inoculum, validated by Iran's Soil and Water Research Institute, consisted of spores, hyphal fragments, and colonized root pieces of *Glomus mosseae*, *Glomus intraradices*, and *Glomus etunicatum*. It was applied at 1 kg tree^{-1}, as recommended for mature pomegranate trees (~10 years old) with extensive root systems and large surrounding soil volumes. Approximately 90% of the inoculum consisted of the carrier substrate (expanded clay and organic compost), with the remainder containing active fungal propagules. AMF was incorporated into fertilizer pits dug around the trees at the beginning of the growing season.

In mid-July of each experimental year, 50 healthy leaves were collected from non-fruiting branches grown during the current season for nutrient analysis. The leaves were washed with distilled water, oven-dried at 70 °C for 48 h, and then ground. One gram of the ground material was placed in a furnace at 500 °C to obtain ash, which was subsequently extracted with 2 N hydrochloric acid. Potassium (K) concentration in the extract was measured using a flame photometer (Jenway 7 model, Germany). Leaf phosphorus (P) was determined colorimetrically with the molybdate–vanadate reagent using a spectrophotometer (Vis 2100 model) at 420 nm. Nitrogen (N) content was measured after digesting the leaf sample and titrating the distillate using a Kjeldahl apparatus (model V40) following the method of Lang (1958). The concentrations of iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) were quantified using a Shimadzu AA-6400 atomic absorption spectrophotometer.

Harvesting was performed in mid-October, and data were recorded on fruit number and yield per tree. Total soluble solids (TSS) were measured using a GMK-701AC refractometer, and titratable acidity (TA) was determined by titration with 0.2 N sodium hydroxide and expressed as citric acid percentage (Alamo et al., 1993). All laboratory analyses were carried out at the Fars Agricultural and Natural Resources Research and Education Center, Shiraz, Iran.

Statistical analyses were conducted using SAS software. Assumptions of the mixed-effects model were carefully evaluated: residuals were tested for normality, and error variances were examined for homogeneity across groups. Bartlett's test was applied to all measured traits to confirm variance homogeneity. When error variances across years were homogeneous (i.e., not significantly different), combined analysis of variance was performed, followed by mean comparisons using Duncan's multiple range test at the 5% significance level.

Results

Fruit yield and fruit count of pomegranate trees

The effects of year, irrigation regimes (IR), GS, and the interaction between IR and GS on pomegranate fruit yield were statistically significant (Table 4). Drought stress reduced pomegranate fruit yield. In most GS treatments, the fruit yield of pomegranate trees under 100% ET_C was higher than under 70% WR (Table 5). Under 70% ET_C, the application of Ha, Se, AMF, and Cc significantly increased pomegranate fruit yield by 13.4, 26.6, 29.7, and 40.8%, respectively, compared to the control (Table 5). Although Aa and Fa treatments improved pomegranate fruit yield compared to the control, these increases were not statistically significant (Table 5). Under 100% ET_C, only the Cc treatment significantly enhanced pomegranate fruit yield compared to the control. In both IR treatments, the highest fruit yield was observed with Cc application; however, this was not significantly different from AMF and Se treatments (Table 5). Data indicated that the effect of these treatments on increasing fruit yield was higher under 70% than under 100% ET_C. Specifically, the 100% ET_C, AMF, Se, and Cc treatments increased fruit yield by 17.9, 15.1, and 28.5%, respectively. However, under 70% ET_C, the corresponding increases were 26.6, 29.7, and 40.8%, respectively (Table 5). Additionally, fruit yield in the second year was significantly higher than in the first year (Table 6).

Table 4. Combined analysis of variance for the effects of year, irrigation regimes, and growth stimulants on the studied traits of pomegranate.

Source of variation	DF	FY tree^{-1}	FN tree^{-1}	TSS	TA	Leaf N	Leaf P	Leaf K	Leaf Zn	Leaf Mn	Leaf Cu	Leaf Fe
Year	1	1359**	2400^{ns}	13.0**	0.0157*	0.45**	0.002**	1.08**	8.05^{ns}	1.71^{ns}	0.012^{ns}	4144^{ns}
Block (year)	4	2.84^{ns}	1373^{ns}	0.76^{ns}	0.0091**	0.0162^{ns}	0.0002*	0.032**	9.85**	52.6*	0.17^{ns}	5592*
IR	1	235.8*	7261*	20.1**	0.002*	0.0016^{ns}	0.000001^{ns}	0.038**	6.68*	92.2*	0.11^{ns}	13075*
Year × IR	1	14.3^{ns}	63.4^{ns}	0.82^{ns}	0.0006^{ns}	0.0029^{ns}	0.0001^{ns}	0.025**	21.0**	105.2*	0.012^{ns}	1829^{ns}
IR × Block (year)	4	140.6^{ns}	645.8^{ns}	0.48^{ns}	0.0087*	0.0204^{ns}	0.0003**	0.002^{ns}	2.11^{ns}	74.0**	0.024^{ns}	2240^{ns}
GS	6	494.6**	3089^{ns}	4.89**	0.0075*	0.0498**	0.0001^{ns}	0.01*	4.79*	32.5^{ns}	0.26^{ns}	2516*
IR × GS	6	82.2*	618^{ns}	0.24^{ns}	0.006*	0.0487**	0.00002^{ns}	0.003*	5.22*	15.4^{ns}	0.19^{ns}	7273**
Year × GS	6	31.5 ^{ns}	1800*	1.05*	0.0024^{ns}	0.0249*	0.00006^{ns}	0.011**	5.41*	37.5*	0.32 ^{ns}	3469*
Year × IR× GS	6	4.0^{ns}	643^{ns}	0.47^{ns}	0.0012^{ns}	0.0109^{ns}	0.00006^{ns}	0.002^{ns}	5.47^{ns}	42.3^{ns}	0.15^{ns}	6893^{ns}
Error	48	86.5	1686	0.99	0.0024	0.0167	0.00005	0.003	5.45	16.06	0.25	1920
C.V. (%)		15.1	17.8	6.7	11.7	7.7	5.4	4.5	8.4	9.7	7.9	9.36

*, ** and ^{ns}, significant at probability level of 5%, 1%, and Not-significant respectively. (IR): irrigation regimes; (GS): growth stimulants; (FY tree^{-1}): fruit yield per tree; (FN tree^{-1}): fruit number per tree; (TSS): total soluble solids; (TA): titratable acidity.

Table 5. Mean comparison for the interaction of irrigation regimes and growth stimulants on FY, TA and leaf concentration of N, K, Zn and Fe in pomegranate.

IR	GS	FY tree^{-1} (kg)	TA (%)	N (%)	K (%)	Zn	Fe
						(mg kg^{-1})	
70%	Control	50.55±3.2^{c}	0.41±0.011^{b}	1.50±0.023^{d}	1.22±0.011^{c}	16.84±1.3^{c}	415.5±4.5^{c}
	Aa	55.58±1.6^{bc}	0.40±0.021^{b}	1.64±0.034^{bc}	1.25±0.023^{b}	18.83±1.6^{ab}	463.5±3.3^{b}
	Ha	57.80±4.2^{b}	0.41±0.022^{b}	1.69±0.014^{b}	1.21±0.021^{c}	18.17±2.1^{b}	464.7±5.1^{b}
	Fa	55.05±3.2^{bc}	0.38±0.026^{bc}	1.73±0.035^{ab}	1.26±0.017^{ab}	18.00±2.4^{b}	483.7±3.7^{ab}
	Se	64.04±1.8^{ab}	0.40±0.024^{b}	1.70±0.023^{b}	1.22±0.015^{c}	18.50±3.1^{ab}	463.4±3.5^{b}
	AMF	65.56±1.6^{ab}	0.41±0.021^{b}	1.66±0.024^{b}	1.28±0.013^{ab}	18.67±2.7^{ab}	423.4±3.4^{bc}
	Cc	71.17±2.2^{a}	0.36±0.020^{c}	1.70±0.034^{b}	1.26±0.017^{b}	19.60±1.7^{a}	474.4±3.1^{ab}
100%	Control	56.89±2.5^{b}	0.46±0.020^{a}	1.58±0.034^{c}	1.22±0.021^{c}	18.34±2.5^{b}	445.2±4.8^{bc}
	Aa	59.68±1.8^{b}	0.41±0.022^{b}	1.61±0.023^{b}	1.27±0.013^{b}	18.67±2.7^{ab}	488.4±3.1^{ab}
	Ha	62.64±2.7^{b}	0.43±0.021^{ab}	1.73±0.027^{ab}	1.29±0.022^{ab}	17.67±1.8^{b}	457.4±3.4^{b}
	Fa	58.41±3.1^{b}	0.40±0.023^{b}	1.52 ±0.028^{cd}	1.29±0.014^{ab}	19.00±3.2^{ab}	508.7±3.5^{a}
	Se	67.09±2.5^{ab}	0.44±0.023^{ab}	1.76±0.032^{ab}	1.27±0.017^{b}	18.5±2.4^{b}	444.0±2.4^{bc}
	AMF	65.50 ±2.4^{ab}	0.43±0.022^{ab}	1.75±0.027^{ab}	1.30±0.027^{ab}	18.83±2.7^{ab}	506.2±2.7^{a}
	Cc	73.21±2.7^{a}	0.40±0.024^{b}	1.85±0.027^{a}	1.35±0.012^{a}	20.40±1.6^{a}	513.9±3.5^{a}

In each column means followed by the same letters have no significant difference at the probability level of 5% based on the Duncan's multiple range test. (IR): irrigation regimes; (GS): growth stimulants; (Aa): amino acid; (Ha): humic acid; (Fa): fulvic acid; (Se): seaweed extract; (AMF): arbuscular mycorrhizal fungi; (Cc): combined consumption; (FY tree-1): fruit yield per tree; (TA): Titratable acidity.

Table 6. Effect of year on fruit yield, titratable acidity and leaf P concentration of pomegranate.

Year	FY tree^{-1} (kg)	TA (%)	P (%)
(2020-2021)	62.0±2.2^{b}	0.431±0.001^{a}	0.122 ±0.001^{b}
(2021-2022)	65.7±1.8^{a}	0.404±0.002^{b}	0.132±0.001^{a}

In each column means followed by the same letters have no significant difference at the probability level of 5% based on the Duncan's multiple range test. (FY tree-1): fruit yield per tree; (TA): Titratable acidity.

The IR and the interaction between year and GS treatments had a significant effect on the number of fruits per pomegranate tree (Table 4). Examination of the main effect of IR showed that under 100% ET_C, the number of fruits per tree was 8.4% higher than under 70% ET_C (Table 7). In the first year of the experiment, all GS treatments except Aa significantly increased the number of fruits per tree compared to the control (Table 8). In the first year, the increases in fruit number for Ha, Fa, Se, AMF, and Cc treatments were 21.5, 18.6, 23.2, 17.1, and 18.8%, respectively. In the second year, only Ha and Cc treatments significantly increased the number of fruits per tree, by 13.4 and 13.1%, respectively, compared to the control, with no statistically significant difference between them (Table 8).

Table 7. Effect of irrigation regimes on FN, TSS and TA of pomegranate.

IR	**FN tree^{-1}**	**TSS (%)**
70%	221.3 ± 4.1^{b}	15.5 ± 1.8^{a}
100%	239.9 ± 3.4^{a}	14.5 ± 1.3^{b}

In each column, mean values followed by the same letters have no significant difference at the probability level of 5% based on Duncan's multiple range test. (IR): irrigation regimes; (FN tree^{-1}): fruit count per tree; (TSS): total soluble solids.

Table 8. Mean comparison for the effect of growth stimulants on FN, TSS, and leaf concentration of N, K, Zn, Mn, Cu, and Fe of pomegranate in each year.

Year	**GS**	**FN tree^{-1}**	**TSS**	**N**	**K**	**Zn**	**Mn**	**Fe**
				%			**mg kg^{-1}**	
(2020-2021)	Control	199.2 ± 2.5^{c}	13.88 ± 0.4^{c}	1.45 ± 0.030^{d}	1.21 ± 0.020^{c}	17.34 ± 2.7^{c}	39.34 ± 2.8^{c}	$426.2\ \pm5.8^{c}$
	Aa	184.6 ± 4.7^{d}	14.52 ± 1.3^{b}	1.54 ± 0.021^{c}	1.27 ± 0.011^{b}	17.50 ± 1.3^{c}	38.67 ± 1.7^{c}	482.5 ± 8.3^{ab}
	Ha	242.0 ± 2.1^{ab}	13.91 ± 1.6^{c}	1.71 ± 0.027^{b}	1.25 ± 0.012^{b}	18.00 ± 1.9^{bc}	40.16 ± 1.3^{bc}	449.1 ± 4.0^{b}
	Fa	236.3 ± 3.5^{b}	14.50 ± 0.7^{b}	1.58 ± 0.032^{c}	1.34 ± 0.023^{a}	18.49 ± 2.1^{b}	39.83 ± 2.6^{c}	528.2 ± 7.3^{a}
	Se	245.5 ± 4.1^{ab}	15.31 ± 1.3^{ab}	1.64 ± 0.028^{b}	1.36 ± 0.030^{a}	18.33 ± 1.7^{b}	40.33 ± 1.7^{bc}	456.7 ± 4.7^{b}
	AMF	233.3 ± 3.5^{b}	14.31 ± 0.8^{bc}	1.60 ± 0.022^{bc}	1.24 ± 0.025^{bc}	19.50 ± 1.0^{ab}	42.67 ± 1.4^{ab}	465.5 ± 5.8^{b}
	Cc	236.6 ± 2.0^{b}	15.8 ± 0.7^{ab}	1.67 ± 0.024^{b}	1.37 ± 0.021^{a}	19.67 ± 0.89^{a}	44.17 ± 1.5^{a}	483.9 ± 9.3^{ab}
(2021-2022)	Control	219.5 ± 1.8^{bc}	14.45 ± 1.3^{b}	1.71 ± 0.025^{b}	1.23 ± 0.027^{c}	17.8 ± 0.78^{bc}	41.83 ± 1.7^{b}	434.7 ± 3.5^{c}
	Aa	234.2 ± 4.3^{b}	$16.05\ \pm3.3^{a}$	1.70 ± 0.011^{b}	1.26 ± 0.023^{b}	20.00 ± 1.3^{a}	$40.66\ \pm1.3^{bc}$	469.4 ± 6.7^{b}
	Ha	249.0 ± 2.4^{a}	15.37 ± 4.4^{ab}	1.70 ± 0.021^{b}	1.26 ± 0.027^{b}	$17.84\ \pm1.8^{bc}$	41.00 ± 3.5^{b}	473.0 ± 5.4^{b}
	Fa	221.6 ± 5.3^{bc}	14.71 ± 1.0^{b}	1.67 ± 0.017^{b}	1.21 ± 0.021^{c}	18.50 ± 2.2^{b}	41.17 ± 3.7^{b}	464.0 ± 6.3^{b}
	Se	244.6 ± 3.4^{ab}	15.35 ± 0.6^{ab}	1.76 ± 0.032^{ab}	1.23 ± 0.014^{c}	18.67 ± 2.7^{ab}	40.67 ± 1.5^{bc}	450.7 ± 4.5^{bc}
	AMF	224.0 ± 5.1^{b}	15.45 ± 0.7^{ab}	1.80±0.024ab	1.24 ± 0.017^{bc}	18.00 ± 1.9^{bc}	$42.84\ \pm3.4^{ab}$	464.0 ± 5.6^{b}
	Cc	248.3 ± 3.1^{a}	16.42 ± 1.4^{a}	1.87 ± 0.024^{a}	1.24 ± 0.023^{bc}	20.33 ± 3.9^{a}	43.00 ± 1.7^{a}	504.4 ± 6.1^{a}

In each column, mean values followed by the same letters have no significant difference at the probability level of 5% based on Duncan's multiple range test. (GS): growth stimulants; (Aa): amino acid; (Ha): humic acid; (Fa): fulvic acid; (Se): seaweed extract; (AMF): arbuscular mycorrhizal fungi; (Cc): combined consumption; (FN tree-1): fruit number per tree; (TSS): total soluble solid.

Fruit quality attributes

The effects of year, IR, GS, and the interaction between year and GS on TSS of pomegranate fruit juice were significant (Table 4). The results indicated that drought stress increased the TSS of pomegranate juice. The 70% ET_C treatment led to a 6.9% increase in TSS compared to the 100% ET_C (Table 7). Regarding all GS treatments, TSS levels were higher in the second year than in the first year (Table 8). In the first year, Aa, Fa, Se, and Cc treatments significantly increased TSS compared to the control. Although Ha and AMF also increased TSS in the first year, these increases were not statistically significant. In the second year, only Aa and Cc treatments resulted in significant increases in TSS compared to the control. Across both years, the highest TSS values were observed in the Cc treatment, with increases of 13.6 and 13.8% in the first and second years, respectively, compared to the control, although these differences were not statistically significant (Table 8).

The effects of year, IR, GS, and the interaction of IR and GS on the TA of pomegranate fruit juice were also significant (Table 4). Drought stress decreased TA levels. While TA under the 70% ET_C treatment was lower than under 100% ET_C across all GS treatments, significant differences between the two IR levels were observed only in the control and Cc treatments (Table 5). Under 70% ET_C, the Cc treatment caused a significant 9.8% decrease in TA compared to the control. Under 100% ET_C, Aa, Fa, and Cc treatments significantly reduced TA by 10.9%, 13.0%, and 13.0%, respectively (Table 5). These results suggest that GS were more effective in reducing TA under 100% ET_C than under 70% ET_C. Furthermore, TA levels were 6.8% higher in the first year compared to the second year (Table 6).

Leaf macro- and micronutrients concentration

The effects of GS, year, the interaction between IR and GS, and the interaction between GS and year on the N concentration of pomegranate leaves were significant (Table 4). Under 70% ET_C, all GS treatments significantly increased the N concentration in pomegranate leaves compared to the control, with no significant differences observed among the GS treatments (Table 5). Under 100% ET_C, all GS treatments, except Fa, significantly increased leaf N concentration, with the highest value recorded under the Cc treatment, which increased leaf N by 17.1% compared to the control (Table 5). Across all GS treatments, leaf N concentrations were higher in the second year than in the first year (Table 8). In the first year, all GS treatments significantly improved leaf N concentration compared to the control, while in the second year, only the Cc treatment resulted in a statistically significant increase (Table 8). The P concentration in pomegranate leaves was significantly affected by the year, but no significant effects were observed from other factors or their interactions (Table 4). Leaf P concentration in the second year increased by 8.2% compared to the first year (Table 6).

Significant effects of year, IR, GS, the interaction between IR and GS, IR and year, and GS and year were observed for the K concentration in pomegranate leaves (Table 4). Drought stress reduced leaf K levels. For all GS treatments, except for the control, leaf K concentrations were numerically higher under 100% ET_C than under 70% ET_C (Table 5). The highest K concentration was recorded in the Cc treatment under 100% ET_C, representing a 10.7% increase compared to the control (Table 5). Under 100% ET_C, all GS treatments significantly increased leaf K concentration compared to the control, while under 70% ET_C, only the Aa, Fa, AMF, and Cc treatments caused significant increases. IR had no significant impact on leaf K concentration in the first year, but drought stress in the second year significantly decreased this trait (Table 9). The impact of GS treatments on leaf K concentration was stronger in the first year compared to the second year, with all GS treatments causing significant increases in this year, while in the second year, only Aa and Ha treatments resulted in significant changes (Table 8).

Table 9. Mean comparison for the effect of irrigation regimes on leaf concentration of K, Zn, and Mn of pomegranate in each year.

Year	IR	K (%)	Zn	Mn
			mg kg^{-1}	
(2020-2021)	70%	1.37±0.011^a	18.47±0.52^b	41.38±1.12^b
	100%	1.38±0.025^a	18.04±1.21^b	41.24±1.32^b
(2021-2022)	70%	1.11±0.021^c	18.1±1.04^b	39.43±0.98^c
	100%	1.20±0.027^b	19.67±0.98^a	43.76±1.21^a

In each column, mean values followed by the same letters have no significant difference at the probability level of 5% based on Duncan's multiple range test. (IR): irrigation regimes.

The effects of IR, GS, their interaction, and dual interactions involving IR, year, and GS on the Zn concentration in pomegranate leaves were significant (Table 4). Under 70% ET_C, all GS treatments significantly increased leaf Zn concentration compared to the control, whereas under 100% ET_C, only the Cc treatment produced a significant increase (Table 5). The effect of GS in increasing leaf Zn concentration was greater under 70% ET_C than 100% ET_C. For instance, the Cc treatment enhanced Zn concentration by 16.4% under 70% ET_C and by 11.2% under 100% ET_C. Similar trends were observed for other GS treatments (Table 5). IR had no significant effect on

Zn concentration in the first year, but drought stress in the second year significantly reduced Zn levels (Table 7). In the first year, Fa, Se, AMF, and Cc treatments significantly increased Zn concentration compared to the control, whereas in the second year, only Aa and Cc achieved similar results (Table 8).

The effects of IR, the interaction between IR and year, and the interaction between GS and year on Mn concentration were significant (Table 4). In the first year, drought stress had no significant effect on leaf Mn concentration, whereas in the second year, it significantly reduced this trait (Table 9). In the first year, AMF and Cc treatments significantly increased leaf Mn concentration compared to the control, whereas in the second year, only the Cc treatment achieved this effect. The highest leaf Mn concentration in both years was recorded with the Cc treatment (Table 8).

Leaf Cu concentration was not significantly affected by any experimental factors or their interactions. However, the effects of IR, GS, their interaction, and interaction involving GS and year on leaf Fe concentration were significant (Table 4). Under 70% ET_C, all GS treatments, except AMF, significantly increased leaf Fe concentration compared to the control. Under 100% ET_C, Fa, AMF, and Cc significantly enhanced leaf Fe concentration (Table 5). In the first year, all GS treatments increased Fe concentration relative to the control, while in the second year, all except Se showed significant improvements. The highest Fe concentrations in both years were observed with the Cc treatment, showing increases of 13.5% and 16% in the first and second years, respectively (Table 8).

Discussion

Drought stress reduces the relative water content of leaves, leading to stomatal closure. Consequently, intracellular carbon dioxide levels decline, disrupting photosynthesis and reducing plant yield (Wang and Bughrara, 2007; Barzana et al., 2014). Insufficient water during the pomegranate growth period similarly reduces flowering, fruit set, and overall yield. Selahvarzi et al. (2017) observed that withholding water until the end of fruit set, followed by irrigation based on 100% ETc, decreased lateral flowers in seven-year-old ‘Shavar’ pomegranate trees by 38% compared to continuous irrigation with 100% ETc throughout the season. Furthermore, deficit irrigation (60% ETc) during fruit growth and ripening reduced yields of ‘Mollar de Elche’ and 'Wonderful' pomegranate cultivars by 24.1% and 41%, respectively (Cano-Lamadrid et al., 2018). In this study, GS application improved fruit yield in both IR treatments. Harhash et al. (2021) reported that foliar spraying of pomegranate trees with various concentrations of humic acid, fulvic acid, yeast extract, and Moringa leaf extract, or their combinations, increased fruit set, weight, size, and yield. Similarly, the simultaneous application of commercial bio-stimulant products containing seaweed extract and AMF, alongside chemical fertilizers, significantly enhanced both fruit yield and number. The highest yield (51.6% higher than chemical fertilizers alone) was achieved through soil application of seaweed extract and AMF products, combined with foliar spraying of seaweed extract products at rates of 625 mL ha^{-1}, 5 kg ha^{-1}, and 2 g L^{-1}, respectively (Maity et al., 2024).

Bio-stimulants improve plant growth by enhancing nutrient uptake, chlorophyll content, growth hormone production, anti-stress compound synthesis, osmotic regulation, and the physical, chemical, and microbial properties of the soil (Du Jardin, 2015). As a plant growth regulator, fulvic acid facilitates nutrient uptake, boosts chlorophyll and photosynthesis, and reduces stomatal opening and transpiration. Fulvic acid stimulates the production of antioxidants, IAA, GA_3, cytokinin hormones, and vitamins, thereby enhancing vegetative growth in plants (Harhash et al., 2021). The study conducted on pomegranate demonstrated that foliar application of seaweed extract positively affected both aerial and root growth, resulting in increased leaf number, leaf area, root length, seedling height, and dry matter accumulation (Radhi and Ridha, 2022). Foliar application of the commercial organo- mineral fertilizers including Siapton rich in amino acids and peptides including proline, hydroxychlorine, glycine, and arginine, and Allibio-rad, containing humic and fulvic acids derived from worm compost, significantly promoted both aerial and root growth of “Wonderful” pomegranate seedlings, leading to increases in leaf number and area, root length, seedling height, trunk diameter, and dry matter accumulation. Under stress-free conditions, plants can easily absorb water and nutrients, bypassing the need for anti-stress compounds while progressing through natural growth stages. However, under stress conditions, anti-stress compounds play a critical role in sustaining plant growth. Thus, in addition to their benefits under normal conditions, GS help plants in coping with stress by stimulating the production of anti-stress compounds (Bulgari et al., 2019).

In this study, drought stress increased TSS but decreased TA in pomegranate juice. Regarding TSS, Larbi et al. (2013) reported that even small changes in the Brix index can significantly alter fruit taste. Research on the effects of deficit irrigation on TSS and TA in pomegranate juice has produced varying results (Volschenk, 2021). Some studies have found that severe deficit irrigation reduces TSS (Ghosh et al., 2015) and increases TA (Pena-Estevez et al., 2015). However, Adiba et al. (2022) observed that sustained severe deficit irrigation at 50% ETc influenced TSS and TA differently among pomegranate cultivars, significantly reducing TSS and increasing TA in 4–5 out of 11 varieties investigated, compared to fully irrigated trees at

100% ETc. In contrast, experiments on the 'Rabab' cultivar showed that severe water stress (50% ETc) increased TSS compared to full irrigation and other irrigation strategies. Additionally, alternate-side irrigation under deficit conditions decreased TA compared to full irrigation (Parvizi and Sepaskhah, 2015). Meanwhile, Martinez-Nicolas et al. (2019) reported that withholding irrigation during flowering and fruit set did not affect TSS or TA at harvest. These findings suggest that the effects of water stress on fruit TSS and TA depend on genotype, drought application methods, drought intensity, and experimental conditions.

The beneficial effects of GS on fruit quality, including increased TSS and reduced TA under varying soil moisture conditions, have been documented in several studies. El-Boukhari et al. (2020) demonstrated that seaweed extract improved yield and fruit quality by enhancing TSS, vitamin C, fructose, anthocyanins, antioxidant activity, and total phenol content. Similarly, Al-Musawi (2018) showed that seaweed extract improved fruit size, peel thickness, juice percentage, ascorbic acid content, and TSS. Khattab et al. (2014) reported that the use of 48 g of humic acid or 16 g of amino acids per tree per year increased TSS under deficit irrigation (7–9 m^3 $tree^{-1}$ $year^{-1}$) compared to the control (11 m^3 $tree^{-1}$ $year^{-1}$) and reduced TA across all combinations. Foliar application of 0.2% fulvic acid, as well as combinations of 0.2% humic acid, 0.2% fulvic, 0.2% yeast extract, and 0.2% Moringa leaf extract, increased TSS and the TSS/TA ratio in the ‘Wonderful’ pomegranate cultivar over two seasons (Harhash et al., 2021). Furthermore, Maity et al. (2024) found that TA was significantly reduced when seaweed extract and seaweed extract + AMF combinations were applied alongside chemical fertilizers, compared to the control and sole application of chemical fertilizers.

The transport of mineral nutrients from the soil solution to plant roots is highly dependent on soil moisture. Under drought stress, in addition to the severe limitations for plant growth and development, disruption in the absorption of nutrients appears as a secondary side effect. (Farooq et al., 2012). Under drought stress, roots cannot uptake many nutrients from the soil solution due to low ion diffusion and water movement. In addition, nutrients mineralization is affected by drought due to the reduction of microorganisms and enzyme activity (Dubey and Pessarakli, 2001). Reduced transpiration rates under drought stress also diminish nutrient absorption and translocation in the xylem (Waraich et al., 2011). Furthermore, drought-induced stomatal closure reduces transpiration and consequently limits nutrient uptake (Silva et al., 2009). Marathe et al. (2016) observed that improper irrigation intervals, causing either waterlogging or drought, restricted nutrient absorption in pomegranate. Similarly, Hamdy et al. (2016) reported that reducing soil moisture from 100% to 40% of field capacity decreased N, P, K, Fe, Mn, Zn, and Cu concentrations in pomegranate seedlings under greenhouse conditions.

In this study, GS treatments positively influenced macro- and micronutrient concentrations in pomegranate leaves under both irrigation regimes. Humic compounds improve cation exchange capacity, enhance nutrient availability, and prevent phosphorus precipitation as calcium phosphate (Du Jardin, 2015). Constituents of seaweed extract, including sterols, betaines, and hormones, further promote plant growth (Craigie, 2011). AMF symbiosis improves nutrient efficiency and water stress tolerance (Gianinazzi et al., 2010; Rouphael et al., 2015). Exogenous application of GS such as amino acids, humic acid, fulvic acid, and seaweed extract in grapevines under varying water regimes demonstrated improved N, P, K, Fe, and Zn concentrations compared to the control (Irani et al., 2021). In pomegranate, integrating seaweed extract and AMF with chemical fertilizers enhanced root growth, leaf macro- and micronutrient concentrations, as well as nutrient use efficiency, leading to better fruit quality and increased exportable yield (Maity et al., 2024). Zaid et al. (2022) tested three P rates with biofertilizers on three pomegranate cultivars. Root inoculation with AMF + PDB (phosphate-dissolving bacteria) notably increased soil P availability, plant P concentration and fruit quality. Application of vesicular arbuscular mycorrhiza tablets containing *Rhizophagus fasciculatus* and *Rhizophagus intraradices* significantly enhanced nutrient uptake in pomegranate plants. Treated trees showed up to 2.32 times higher fruit yield compared to uninoculated controls, highlighting the formulation’s effectiveness in improving micronutrient absorption and boosting fruit productivity (Mistry et al., 2023).

Conclusion

The results of this study demonstrated that the application of growth stimulants, in combination with chemical fertilizers adjusted according to soil test recommendations, can effectively enhance yield, fruit quality, and nutrient absorption under both normal and drought stress conditions. Under drought stress, nutrient uptake is often hindered; however, growth stimulants help sustain plant growth by supplying essential nutrients or improving their utilization efficiency. Thus, the beneficial effects of growth stimulants, particularly under water stress, are partly attributable to their ability to enhance plant nutrition. In addition, growth stimulants can provide stress-relieving compounds or stimulate plants to synthesize them. Because plants require higher amounts of such substances to maintain optimal growth under stress than under non-stress conditions, growth stimulants play a crucial role in mitigating drought-induced physiological constraints.

This study showed that the Cc treatment, when applied alongside chemical fertilizers, enabled pomegranate trees under deficit irrigation to achieve fruit yields comparable to those under full irrigation, while also improving fruit quality. This improvement is linked to the ability of growth stimulants to enhance nutrient uptake and supply stress-relieving compounds, thereby supporting plant growth and maintaining productivity under water-limited conditions.

Given these findings, future research should aim to identify optimal deficit irrigation levels that conserve water while enhancing fruit quality and sustaining plant growth without compromising yield. Furthermore, the superior efficiency of growth stimulants in improving fruit yield and nutrient absorption under drought stress compared to normal moisture conditions highlights their potential as a promising strategy for pomegranate production in arid regions.

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Author Contributions

Conceptualization, AM, MR, and MN; methodology, AM; software, MR; validation, AM, MR, and MN; formal analysis, MR; investigation, AM; resources, AM and MR; data curation, AM and MR; writing—original draft preparation, AM and MR; writing—review and editing, AM and MR; visualization, MR; supervision, MN; project administration, AM; funding acquisition, MR. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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