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Biostimulant-induced drought tolerance in grapevine is associated with physiological and biochemical changes

Hosein Irani[1], Babak ValizadehKaji[1*] and Mohammad Reza Naeini[2]

Abstract

Background: In this research, the effects of exogenous application of certain biostimulants [amino acid (AA), humic acid (HA), fulvic acid (FA), and seaweed extract (SE)] on the fruit yield and quality, leaf mineral contents, and some critical physio-chemical characteristics of grapevine (*Vitis vinifera* L.) cv. 'Yaghouti' were investigated under well-watered (WW) and drought-stressed (DS) conditions.

Results: Drought stress caused a remarkable reduction in the weight of 20 berries and fruit yield, and meanwhile a marked increase in the titratable acidity (TA) and total soluble solid (TSS) content of fruits. Application of biostimulants, especially SE, enhanced the weight of 20 berries, fruit yield, and TSS content, and decreased TA in fruits of DS vines. Although drought stress had a negative effect on the chlorophyll content of grapevine, this effect was alleviated by the application of biostimulants, especially SE. Moreover, drought stress made the accumulation of abscisic acid (ABA), proline, total phenol, and soluble carbohydrates, the level of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), as well as the activity of guaiacol peroxidase (GPX) and catalase (CAT) enzymes increased in leaves. Application of biostimulants, especially SE, further increased the accumulation of ABA, proline, total phenol, and soluble carbohydrates and the activity of the antioxidant enzymes, but reduced the level of MDA and H_2O_2 in DS vines. Under drought stress conditions, concentrations of N, P, and K increased, and concentrations of Fe and Zn decreased; however, DS grapevines treated with biostimulants and especially SE accumulated a higher level of these mineral nutrients than CON vines.

Conclusion: In sum, as evidenced by the study results, biostimulants have a high potential for promoting fruit yield and quality of grapevine in drought-prone regions.

Keywords: ABA, Antioxidant enzymes, Fruit yield and quality, Water deficit

Background

Grapevine is a famous fruit species in the temperate regions of the world, which is cultivated for numerous valuable byproducts. Iran with the total area of 141,914 ha under grapevine cultivation and annual production of about 1,866,340 tonnes, ranks 10th and 11th in the world, respectively [20]. Nevertheless, there are some limitations on the yield of vineyard, resulting in severe adverse economic impacts on producers including water deficiency as a critical problem of grapevine. In Iran, where the average annual precipitation is less than 250 mm [46], drought management is a critical requirement.

Drought stress is one of the leading agricultural problems limiting the growth and production of plants in most arid and semiarid regions of the world, whose effects are expected to intensify as global temperature increases [19]. Numerous morphological, physiological, and biochemical functions of plants are affected by

*Correspondence: b-valizadehkaji@araku.ac.ir; valizadehkaji@yahoo.com
[1] Department of Horticultural Sciences, Faculty of Agriculture and Natural Resources, Arak University, 38156-8-8349 Arak, Iran
Full list of author information is available at the end of the article

drought stress in fruit trees [11]. The change in the level of soluble carbohydrates, soluble proteins, proline, MDA, H_2O_2, antioxidant activities, ABA, and phenolic compounds are among the critical metabolic defenses during vine exposure to drought stress [37]. Drought stress may also slow tree growth or reduce yield and fruit quality [37, 52]; therefore, it is imperative to find approaches to address this problem. Genetic improvement is a difficult and time-consuming method to increase tree drought tolerance. Also, it has encountered with insufficient success due to the lack of appropriate genes such as genes conferring tolerance to drought in the germplasm. Application of biostimulants (AA, HA, FA, and SE) for their effectiveness, high performance, and low cost is newly considered as an alternative technique of increasing tree drought tolerance [13, 15]. Plant biostimulants contain different organic and inorganic substances or microorganisms which can increase plant growth, crop quality, nutrient uptake, and tolerance to biotic and abiotic stresses [13].

Humic acids and fulvic acids are natural components of soil organic matter, resulting from not only the decomposition process of plants, animals, and microbial residues, but also the metabolic activity of soil microbes [44]. These substrates are plant growth stimulants that lead to an increase in membrane permeability, respiration, photosynthesis, absorbance and transport of nutrients or a decrease in the uptake of toxic elements [4]. Foliar application of humic substances improves tolerance to abiotic stresses due to increased photosynthesis, carbohydrate, and rubisco activity [32].

Amino acids are a mixture of amino acids, peptides, polypeptides, and denatured proteins that can be obtained by hydrolysis of proteins (chemical, enzymatic, thermal or their combination), derived from both plant and animal sources. Amino acids play a role in tree growth and protection against abiotic stresses. These substrates can impact upon the physiological activities of plants. They are essential to the metabolism of nitrogen and biosynthesis of chlorophyll [13].

Seaweed extracts are a vast group of macroscopic, multicellular marine algae that can be brown, red, and green. These substrates contain organic matter and fertilizer nutrients. They can increase plant growth, photosynthetic activity, and tolerance to biotic and abiotic stresses, thereby improving the fruit yield and quality [13, 49]. Seaweeds contain various hormones as well as several active mineral and organic compounds that contribute to the growth and development of plants [6].

There are many reports regarding the effect of various biostimulants on quantitative and qualitative characteristics of grapevine [29, 45, 47, 51]; however, there is no information on mechanisms of biostimulant-induced drought tolerance of grapevine. Moreover, it has been shown that biostimulants can effectively promote the drought stress endurance of some plants [13, 15]. In the current study, for the first time, the effects of biostimulants on fruit yield, quality, and leaf mineral contents of 'Yaghouti' grape cultivar were investigated. In particular, the study aimed to evaluate how the application of biostimulants affect ABA, proline, phenolic compounds, soluble carbohydrate, soluble protein, H_2O_2, and MDA levels as well as antioxidant activity in leaves of 'Yaghouti' grapevine under WW and DS conditions.

Materials and methods

Vineyard selection and treatments

This research was done in 2018 and 2019, on 10-year-old 'Yaghouti' grapevines grown in a commercial vineyard located at Qom province (latitude 34° 40′; longitude 51° 0′ E; altitude 936 m above sea level), Iran. In this climatic zone, rainfall and relative humidity are very low, while the temperature is very high (Table 1). The vines were grown on their root in a sandy clay loam soil under a drip irrigation system, spaced 2×3 m, and pruned on 1st March to 6 canes with 10 buds besides 8 renewal spurs. Some physical and chemical traits of soil associated with this research are presented in Table 2.

The experiment was carried out as a split-plot in a randomized complete block design with three replications, and included five grapevine plants per experimental unit. The main plot was subjected to two irrigation treatments, including irrigation after 60 and 100 mm evaporation from pan evaporation as WW and DS, respectively. In our preliminary experiment, different irrigation regimes were employed and irrigation after 100 mm evaporation was chosen as the appropriate treatment to simulate drought stress. The irrigation treatments were applied from early April to mid-October in two consecutive seasons 2018 and 2019. The evaporation rate from the pan was measured daily and irrigation of each treatment was performed after reaching the evaporation rate to the desired value. After irrigation treatments, every vine was irrigated with 120 L. This quantity of water was previously determined to be optimal for vine growth in this region in early spring when the experiment was conducted.

The sub-plot was assigned to nutrient treatments as follows:

1. Control (CON) [application of distilled water with 0.2% Tween 20 as a foliar spray in two times (millet-sized berry and 2 weeks later)].
2. Application of AA as a foliar spray at a concentration of 0.5% with 0.2% Tween 20 in two times (millet-sized berry and 2 weeks later).

Table 1 Climatological data during the experiment period

Months	Avearage high temperature (°C)	Average low temperature (°C)	Average humidity (%)	Average rainfall (mm)	Average daylight (h)	Average sunshine (h)
January	10.2	− 1.9	66	25.4	10.1	6
February	13.6	0.6	58	20.5	11	6.9
March	19.1	5	48	27.7	12	7.1
April	26	10.5	42	20.2	13.1	7.8
May	31.8	15.4	33	10.4	14	9.6
June	37.9	20.2	24	2.3	14.5	11.7
July	40.3	23.4	23	0.7	14.2	11.4
August	39.4	21.2	24	0.3	13.4	11.2
September	34.9	15.6	26	0.8	12.4	10.3
October	27.7	10.3	38	6.2	11.3	8.5
November	18.9	4.1	52	14.3	10.4	6.8
December	12.2	− 0.1	66	19.4	9.9	5.6

Table 2 Soil mineral contents, physical and chemical properties of the experimental vineyard

Soil texture	Clay (%)	Silt	Sand	Organic matter	EC (ds m^{-1})	pH	N (ppm)	P	K	Zn	Mn	Fe	Cu
Sandy clay loam	12	22	66	3.3	2.4	7.6	0.17	17	300	0.67	2.8	2.7	0.68

3. Application of HA with irrigation water at a concentration of 20 g per vine two times (bud swell and millet-sized berry).
4. Application of FA as a foliar spray at a concentration of 0.5% with 0.2% Tween 20 two times (millet-sized berry and 2 weeks later).
5. Application of SE as a foliar spray at a concentration of 0.5% with 0.2% Tween 20 two times (millet-sized berry and 2 weeks later).

The characteristics of biostimulants used in this study are given in Table 3.

The nutrient solutions were sprayed to the runoff on each vine in 2018–2019. In a preliminary study, the vines were treated with different concentrations of biostimulants. It was found that treatment with the levels mentioned above significantly improved drought tolerance and fruit yield and quality of vines, and these concentrations, therefore, were used in the following experiments. It is worth noting that all vines received similar chemical fertilizers according to soil analysis.

Fruit quality and yield per vine

At the commercial ripening stage, clusters per vine were enumerated and weighted to conclude total yield per vine. A random sample of 100 berries for each replication was taken to determine the weight of 20 berries, TSS, and TA contents of berry juice.

TSS concentration of berry juice was determined with a refractometer (Atago, PAL-1, Japan) at 25 ± 1 °C, and results expressed as the means of % (Brix). TA was determined by titration with 0.1 N NaOH up to pH of 8.1, using 1 mL of diluted juice in 25 mL distilled water, and results expressed as g tartaric acid equivalent per liter.

Chlorophyll contents

Leaf samples were collected in mid-July 2019 from each vine. Approximately 0.5 g of the leaf samples was used for chlorophyll extraction using acetone (80% v/v). The absorbance of the upper solution was assayed using a UV–visible spectrophotometer (Cary Win UV 100; Varian, Sydney, Australia) according to Lichtenthaler [31]. Concentrations of chlorophyll were determined at wavelengths of 646.8 and 663.2 nm for chlorophyll assays, and all pigments were expressed as mg g^{-1} FW (fresh weight) leaf.

ABA

Leaf samples were collected in mid-August, and the concentration of ABA was determined based on the technique of Beheshti Rooy et al. [8]. Separation and

Table 3 Active ingredient of biostimulants employed in the study

Biostimulants	Active ingredient W/W %
AA	Total amino acids 14.4%; L-amino acids 12%; total nitrogen 7%
HA	K_2O 10%; HA 55%; FA 15%
FA	Total nitrogen 3%; K_2O 10%; FA 50%; chlorine 4.3%
SE	Alginic acid 16%; mannitol 4–6%; amino acid 0.5%; total nitrogen 1.2%; P_2O_5 1%; K_2O 17%; cytokinin and gibberellin 600 ppm; vitamins and other hormones 5000 ppm; sulfur 1.5%; calcium 0.4%; magnesium 0.2%; iron 200 ppm; zinc 100 ppm; manganese 10 ppm; copper 6 ppm; boron 20 ppm; molybdenum 5 ppm

determination of ABA were performed with a Crystal 200 series HPLC pump (ATI Unicam, Cambridge, UK) fitted out with an SPD UV–Vis detector (Philips, Cambridge, UK) and a Diamonsil-C_{18} Column (5 µm, 250 mm × 4.6 mm i.d.) from Hichrom (Berkshire, UK).

Proline

Proline concentration was assessed according to Bates et al. [5] method with slight modification. Leaves were ground in liquid nitrogen, and 0.5 g of ground tissue was homogenized in 10 mL of 3% (w/v) aqueous sulfosalicylic acid. Then homogenate was filtered via a Whatman No. 1 filter paper. Two mL of filtered extract plus 2 mL ninhydrin and 2 mL glacial acetic acid were taken for the analysis. The reaction mixture was incubated in a boiling water bath for 1 h, and the reaction was completed in an ice bath. Four mL of toluene was added to the mixture, and the organic phase was extracted. While toluene was used as a blank, absorbance was measured at 520 nm spectrophotometrically (Cary Win UV 100, Varian, Australia). The concentration of proline was calculated using a calibration curve and exhibited as µmol g^{-1} FW.

Total phenol

Total phenolic content was determined colorimetrically using Folin–Ciocalteu reagent as described by Beheshti Rooy et al. [8] with some modification. In brief, fresh leaves tissue (0.5 g) was homogenized in methanol (85%) and centrifuged at 6000×*g* for 10 min. Afterward, 0.3 mL of each diluted methanolic extract (10%) and 1 mL of Folin–Ciocalteu reagent (10%) were mixed and vortexed. A volume of 1 mL from 7% sodium carbonate solution was added to the mixture after 5 min. Shaking the final solution for 90 min at ambient temperature, the absorbance was spectrophotometrically measured at 765 nm. Total phenolic values were measured by applying a calibration curve drawn for the gallic acid standard solution and expressed as mg gallic acid g^{-1} FW.

Soluble carbohydrates

Soluble carbohydrates were estimated according to the approach presented by Beheshti Rooy et al. [8] with some modification. Until analysis, leaves were oven-dried for 3 days at 80 °C. Soluble carbohydrates were extracted three times from 1 g of ground tissue with 5 mL of 80% ethanol and centrifuged for 15 min at 9000 rpm. One mL of 0.2% anthrone reagent (2 g anthrone in 1 L of 72% sulfuric acid) was added to 100 µL of the extract of ethanolic. The reaction mixture was heated in a boiling water bath for 10 min and then rapidly cooled on ice. Using a Cary WinUV 100 spectrophotometer at 620 nm, the extract absorbance was measured. Glucose was used as a blank sample. Using a calibration curve, the concentration of soluble carbohydrates was finally measured and exhibited as mg g^{-1} FW.

Total soluble proteins

Total soluble proteins were extracted from leaves, and their content was determined by measuring the absorbance at 595 nm using the colorimetric approach of Bradford [12], considering bovine serum albumin as the standard. Total soluble protein values were represented as mg g^{-1} FW.

H_2O_2

After reaction with potassium iodide, H_2O_2 concentration was spectrophotometrically measured based on Velikova and Loreto [55] method. One gram of fresh leaf tissue was ground and homogenized in a mortar, including 10 mL of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 6000×*g* for 15 min, where 0.5 mL of the supernatant, 0.5 mL of 10 mM potassium phosphate buffer, pH 7.0, and 0.1 mL of reagent were mixed (0.1 M KI in double-distilled fresh water). The supernatant absorbance was read at 390 nm. In the absence of bud extract, a blank sample was provided using 0.1% (w/v) TCA. From a standard curve of known H_2O_2 concentrations, the concentration of H_2O_2 was obtained and expressed as µmol g^{-1} FW.

MDA

Lipid peroxidation of the membrane was observed for 100 mg of each leaf sample, which was homogenized in 5 mL of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 6000 rpm for 5 min. The supernatant was collected, and lipid peroxidation was measured in terms of MDA concentration based on Heath and Packer [26].

Antioxidant activities

About 0.5 g of leaf tissue was weighed and then macerated in a mortar with liquid nitrogen until a fine powder was obtained. From each sample, 100 mg of the frozen leaf powder was homogenized in 1.0 mL of sodium phosphate buffer (0.5 M, pH 7.8), including 1 mM EDTA and PVP-40 (2% w/v). Samples were homogenized and centrifuged at 10,000×*g* for 20 min at 4 °C, and the activity of all enzymes was assessed using the supernatant.

According to Herzog and Fahimi [27], the GPX activity measurement was done following guaiacol oxidation by H_2O_2 at 470 nm. The 1 mL volume of each crude flower enzyme extract was added to 3 mL volume of the reaction mixture, including 0.8 µL guaiacol (25 Mm) and 1.3 µL H_2O_2 (30% v/v) in a volume of 3 mL sodium phosphate buffer (50 mM, pH 7.0). The GPX activity unit was defined as the amount of enzyme that oxidizes 1.0 µmol guaiacol mL^{-1} min^{-1}. The specific activity of the enzyme is defined as unit mg^{-1} protein.

The activity of CAT was calculated by measuring the drop of H_2O_2 absorbance at 240 nm wavelength [10]. The 3 mL volume of each reaction mixture included sodium phosphate buffer (0.05 M, pH 7.0) with H_2O_2 (3% v/v) and EDTA (1.0 mM). The drop in absorption at 240 nm occurred for 3 min. One unit of CAT activity was determined by the enzyme amount, which resulted in 1.0 µmol of degraded H_2O_2 mL^{-1} min^{-1}. The specific activity of the enzyme is displayed as unit mg^{-1} protein.

Leaf mineral contents

Leaf samples were collected in mid-July 2019 from each vine, and their N, P, K, Zn, and Fe concentrations were measured. Three leaves were taken from the middle of growing shoots. Petioles samples were oven-dried at 65 °C for 48 h in a forced-air oven, then ground to a powdery texture, and 0.2 g was taken to determine the aforementioned elements. Total N was determined by the Kjeldahl method. P was determined using a spectrophotometer. K was flame photometrically determined. The sample extracts were analyzed for Zn and Fe using an atomic absorption spectrophotometer (Varian, 220).

Statistical analysis

The data were analyzed using the GLM procedure of SAS software (Version 9.1), and significant differences were tested at $P \leq 0.05$ using Duncan's multiple ranges. Before statistical analysis, the expressed data as percentages were subjected to arcsine transformation, and the original values of all transformed data were presented.

Results

The experiment was done for 2 years (2018–2019), and the results are the outcome of a 2-year attempt.

Fruit quality and yield per vine

The weight of 20 berries decreased significantly under drought stress conditions by 29.47%. However, the application of AA, HA, FA, and SE caused a significant increase in the weight of 20 berries under drought stress conditions. The effect of SE was dramatically higher than that of other biostimulants. With SE application, the weight of 20 berries was 87.50% higher than CON under drought stress. On the other hand, the weight of 20 berries increased by 45.46% with the application of AA under WW conditions, and this effect was superior to that of other biostimulants (Table 4).

Yield per vine declined significantly under drought stress when no treatment was used. This decline was considerably lower in vines supplied with AA, HA, FA, and SE. Thus, the application of biostimulants can significantly improve yield per vine under drought conditions. The highest benefit was found with SE application under drought stress conditions, improving yield per vine by 38.20%. In contrast, under sufficient water conditions, the highest yield per vine (11.63 kg) was obtained from the application of AA, which led to an increase of 22.42% compared with the CON (9.50 kg) (Table 4).

Biostimulants significantly affected TA of berries under both WW and DS conditions. Vines treated with biostimulants had significantly lower TA than untreated vines. Under both WW and DS conditions, the application of SE led to the maximum decrease in TA of berries (25.59% and 28.69%, respectively) (Table 4).

Under both WW and DS conditions, fruits of vines treated with biostimulants had higher levels of TSS than fruits treated with CON treatment (Table 4). The TSS rose significantly under drought stress conditions by 14.76%. Under drought stress, the application of SE led to an increase of 15.22% in the TSS of fruits, and this effect was superior to that of other biostimulants except for FA. In contrast, under sufficient water conditions, the application of AA led to the maximum increase in the TSS level (up to 20.92%) (Table 4).

Chlorophyll contents

Content of chlorophyll *a* decreased significantly under drought stress conditions by 17%; however, SE-treated vines had higher chlorophyll content (up to 15%) than

Table 4 Influence of biostimulants on fruit yield and quality of 'Yaghouti' grapevine under WW and DS conditions

Irrigation regimes	Biostimulants	Weight of 20 berries (g)	Yield vine^{-1} (kg)	TA (g L^{-1})	TSS (Brix)
WW	CON	42.32 ± 1.52 cd	9.50 ± 0.43 c	5.43 ± 0.20 b	16.73 ± 0.63 f
	AA	61.56 ± 1.45 a	11.63 ± 0.35 a	4.66 ± 0.15 c	20.23 ± 0.34 cd
	HA	52.23 ± 2.30 b	10.43 ± 0.40 b	4.11 ± 0.44 e	19.31 ± 0.16 de
	FA	53.45 ± 2.08 b	10.76 ± 0.32 b	4.10 ± 0.10 e	19.37 ± 0.28 de
	SE	55.41 ± 0.57 b	10.86 ± 0.32 b	4.04 ± 0.10 e	18.72 ± 1.80 e
DS	CON	24.00 ± 2.64 g	6.70 ± 0.34 f	5.75 ± 0.11 a	19.70 ± 0.52 de
	AA	33.73 ± 3.56 f	7.63 ± 0.70 e	4.81 ± 0.17 c	21.20 ± 0.20 bc
	HA	39.83 ± 0.76 de	8.10 ± 0.36 de	4.70 ± 0.41 c	21.41 ± 0.27 b
	FA	38.33 ± 1.55 e	8.40 ± 0.52 de	4.46 ± 0.16 d	21.70 ± 0.20 ab
	SE	45.00 ± 2.60 c	9.26 ± 0.37 c	4.10 ± 0.15 e	22.70 ± 0.34 a
Significance					
Irrigation regime		**	*	*	**
Biostimulants		**	**	**	**
Irrigation regime × biostimulants		**	**	*	**

Mean values followed by the similar letters within a column are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test). * and ** are significant at $P \leq 0.05$ and at $P \leq 0.01$, respectively. Values are means of three replicates ± SD

untreated vines under drought stress. Under WW conditions, application of biostimulants did not affect chlorophyll *a* (Table 5).

The content of chlorophyll b decreased markedly under drought stress conditions by 28.61%. The decline in chlorophyll b content was significantly less in vines treated with biostimulants than those untreated; the highest benefit was related to AA treatment, increasing chlorophyll b content by 36.73%. Also, the application of AA significantly increased chlorophyll b content by 13.60% under WW conditions (Table 5).

Total chlorophyll content reduced significantly under drought stress conditions; however, vines treated with biostimulants had higher total chlorophyll content than untreated vines. On the other hand, the application of AA caused an increase of 9.45% in total chlorophyll content under WW conditions. However, no significant difference was found between this treatment and the other biostimulants regarding this trait (Table 5).

ABA

The concentration of ABA rose significantly under drought stress conditions by 93%. DS vines treated with biostimulants accumulated a higher level of ABA than CON vines. The effect of SE was significantly higher than the other biostimulants. With SE application, the concentration of ABA was 13.97% higher than CON under drought stress. On the other hand, the application of biostimulants had no significant effect on ABA concentration under WW conditions (Table 6).

Proline

The proline content was significantly increased by 191.93% due to drought stress. Application of AA, HA, FA, and SE significantly increased proline content of DS vines, where the effect of SE was higher than AA, HA, and FA. Besides, vine treatment with biostimulants increased proline content under WW conditions (Table 6).

Total phenol

The levels of total phenol increased significantly under drought stress conditions by 100%. Vines treated with biostimulants had significantly higher total phenolic content than untreated vines under drought stress conditions. The effect of SE tended to be higher than AA, HA, and FA on total phenol in vines under drought stress. Compared to the CON treatment, total phenolic content was 3.32%, 4.07%, 4.68%, and 11.32% higher for the AA, HA, FA, and SE treatments under drought stress, respectively. In addition, biostimulant-treated vines had higher total phenolic content than untreated vines under WW conditions (Table 6).

Soluble carbohydrates

Drought stress noticeably increased the content of soluble carbohydrates by 51%. DS vines fed with AA, HA, FA, and SE had a higher content of soluble carbohydrates than untreated vines (about 17.32–29.75%), where the effect of SE on soluble carbohydrates was higher than the other biostimulants. In addition, soluble carbohydrates significantly increased with the application of biostimulants under WW conditions (Table 6).

Table 5 Influence of biostimulants on chlorophyll content of grapevine leaves cv. 'Yaghouti' under WW and DS conditions

Irrigation regimes	Biostimulants	Chlorophyll a (mg g FW^{-1})	Chlorophyll b (mg g FW^{-1})	Total chlorophyll (mg g FW^{-1})
WW	CON	10.53 ± 0.28 ab	7.13 ± 0.10 c	17.67 ± 0.33 b
	AA	11.24 ± 0.22 a	8.10 ± 0.09 a	19.34 ± 0.15 a
	HA	10.70 ± 0.26 ab	7.77 ± 0.13 ab	18.47 ± 0.15 ab
	FA	10.93 ± 0.45 ab	7.82 ± 0.17 ab	18.75 ± 0.28 ab
	SE	10.91 ± 0.07 ab	7.58 ± 0.08 b	18.50 ± 0.10 ab
DS	CON	8.73 ± 0.75 d	5.09 ± 0.10 g	13.83 ± 0.85 d
	AA	9.03 ± 0.85 d	6.96 ± 0.15 cd	16.00 ± 0.91 c
	HA	9.33 ± 0.70 cd	6.73 ± 0.25 de	16.06 ± 0.95 c
	FA	9.63 ± 0.47 cd	6.43 ± 0.32 ef	16.06 ± 0.70 c
	SE	10.03 ± 0.30 bc	6.16 ± 0.47 f	16.20 ± 0.75 c
Significance				
Irrigation regime		**	**	*
Biostimulants		**	**	**
Irrigation regime × biostimulants		**	*	**

Mean values followed by the similar letters within a column are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test). * and ** are significant at $P \leq 0.05$ and at $P \leq 0.01$, respectively. Values are means of three replicates ± SD

Table 6 Influence of biostimulants on concentrations of ABA, proline, total phenol, soluble carbohydrate and soluble protein of grapevine leaves cv. 'Yaghouti' under WW and DS conditions

Irrigation regimes	Biostimulants	ABA concentration (nmol g FW^{-1})	Proline (μmol g FW^{-1})	Total phenol (mg g FW^{-1})	Soluble carbohydrates (mg g FW^{-1})	Soluble proteins (mg g FW^{-1})
WW	CON	18.32 ± 0.54 d	1.24 ± 0.08 e	3.31 ± 0.03 e	26.26 ± 0.83 f	4.44 ± 0.26 d
	AA	18.20 ± 0.87 d	1.88 ± 0.10 d	4.91 ± 0.11 d	37.76 ± 1.56 de	5.85 ± 0.18 ab
	HA	18.26 ± 0.51 d	1.74 ± 0.11 d	4.63 ± 0.41 d	35.90 ± 0.62 e	5.35 ± 0.20 c
	FA	18.26 ± 0.53 d	1.72 ± 0.12 d	4.67 ± 0.15 d	36.60 ± 0.45 e	5.47 ± 0.20 bc
	SE	17.85 ± 0.37 d	1.83 ± 0.13 d	4.87 ± 0.15 d	37.06 ± 0.92 e	5.99 ± 0.10 a
DS	CON	35.36 ± 1.51 c	3.62 ± 0.10 c	6.62 ± 0.10 c	39.66 ± 1.52 d	2.36 ± 0.04 f
	AA	38.06 ± 2.00 b	3.80 ± 0.15 b	6.84 ± 0.04 bc	49.03 ± 1.95 b	3.65 ± 0.47 e
	HA	36.46 ± 1.50 bc	3.86 ± 0.17 b	6.89 ± 0.13 bc	46.56 ± 1.20 c	3.33 ± 0.13 e
	FA	36.06 ± 0.90 c	3.94 ± 0.16 b	6.93 ± 0.12 b	46.53 ± 0.83 c	3.34 ± 0.17 e
	SE	40.30 ± 0.70 a	4.29 ± 0.19 a	7.37 ± 0.16 a	51.46 ± 1.92 a	3.45 ± 0.30 e
Significance						
Irrigation regime		**	**	*	**	**
Biostimulants		**	**	**	**	**
Irrigation regime × biostimulants		**	**	**	**	*

Mean values followed by the similar letters within a column are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test). * and ** are significant at $P \leq 0.05$ and at $P \leq 0.01$, respectively. Values are means of three replicates ± SD

Soluble proteins

The concentration of soluble proteins decreased significantly under drought stress conditions by 46.84%. DS vines treated with biostimulants accumulated a higher level of soluble proteins than CON vines. In addition, biostimulant-treated vines had higher soluble proteins than untreated vines under WW conditions, where the effect of SE was significantly higher than the other biostimulants except for AA (Table 6).

H_2O_2

The levels of H_2O_2 increased significantly under drought stress conditions by 59.72%; however, vines treated with biostimulants had significantly lower H_2O_2

content than untreated vines. Under drought stress, the application of SE led to a decrease of 44.78% in H_2O_2 content, and this effect was superior to that of other biostimulants. In addition, biostimulant-treated vines had lower H_2O_2 content than untreated vines under WW conditions (Table 7).

MDA

The level of MDA rose significantly under drought stress conditions by 46.10%. Treated DS vines with biostimulants had a lower concentration of MDA than CON vines. The effect of SE was significantly higher than the other biostimulants. With the application of SE, the concentration of MDA was 40.44% lower than CON under drought stress. In contrast, the application of AA caused a decrease of 27.27% in MDA content under WW conditions. However, no significant difference was found between this treatment and the other biostimulants regarding this trait (Table 7).

Antioxidant activities

The activity of GPX increased under drought stress conditions by 92.82%. Compared to untreated vines, GPX activity of DS vines treated with AA, HA, FA, and SE was significantly improved, and the effect of AA and SE was more considerable than HA and FA. In addition, GPX activity significantly increased with the application of AA, HA, FA, and SE under WW conditions (Table 7).

Drought stress markedly increased the activity of CAT by 138.94%. DS vines fed with AA, HA, FA, and SE had higher CAT activity than untreated vines, where the effect of AA and HA was higher than FA and SE. In addition, WW vines treated with AA, HA, and FA showed a marked increase in CAT activity in comparison with untreated vines (Table 7).

Leaf mineral contents

Under drought stress conditions, concentrations of N, P, and K increased, and concentrations of Fe and Zn decreased. DS vines treated with AA, HA, FA and SE accumulated a higher concentration of N, P, K, Fe, and Zn than CON vines. The effect of SE was significantly higher than that of other biostimulants. Application of SE, respectively, increased concentrations of N, P, K, Fe, and Zn by 27.08%, 125%, 59.82%, 29.93%, and 53.83% under drought conditions (Table 8). In addition, SE-treated vines had higher concentrations of N, P, K, Fe, and Zn than untreated vines under WW conditions (Table 8).

Discussion

Grapevine has a relatively high tolerance to drought; however, the yield and quality of grapes were unfavorably affected by drought stress [37]. According to this study, drought stress sharply reduced the weight of 20 berries and fruit yield of 'Yaghouti' grapevine plants. The low fruit weight may be due to water deficiency for cell growth [58]. However, there was a lower reduction in the weight of 20 berries and fruit yield in the presence of externally applied AA, HA, FA, and especially SE. Therefore, the application of biostimulants could improve fruit yield under drought stress conditions (Table 4). Increases in yield with exogenous use of some biostimulants under

Table 7 Influence of biostimulants on H_2O_2 and MDA concentrations and GPX and CAT activities of grapevine leaves cv. 'Yaghouti' under WW and DS conditions

Irrigation regimes	Biostimulants	H_2O_2 concentration (μmol g FW^{-1})	MDA concentration (μmol g FW^{-1})	GPX activity (units mg^{-1} protein)	CAT activity (units mg^{-1} protein)
WW	CON	1.44 ± 0.12 c	1.54 ± 0.19 bc	2.37 ± 0.14 e	0.95 ± 0.05 e
	AA	1.01 ± 0.11 d	1.12 ± 0.04 d	3.75 ± 0.36 d	1.30 ± 0.09 d
	HA	0.98 ± 0.09 d	1.34 ± 0.16 cd	3.44 ± 0.19 d	1.16 ± 0.05 d
	FA	0.91 ± 0.08 d	1.33 ± 0.12 cd	3.44 ± 0.14 d	1.22 ± 0.09 d
	SE	0.79 ± 0.13 d	1.20 ± 0.07 d	3.63 ± 0.30 d	1.12 ± 0.02 de
DS	CON	2.30 ± 0.09 a	2.25 ± 0.14 a	4.57 ± 0.12 c	2.27 ± 0.05 c
	AA	1.70 ± 0.22 b	1.73 ± 0.26 b	5.91 ± 0.06 a	3.24 ± 0.11 a
	HA	1.71 ± 0.16 b	1.68 ± 0.14 b	5.46 ± 0.12 b	3.11 ± 0.12 a
	FA	1.76 ± 0.13 b	1.76 ± 0.14 b	5.52 ± 0.18 b	2.92 ± 0.09 b
	SE	1.27 ± 0.08 c	1.34 ± 0.02 cd	6.11 ± 0.21 a	2.75 ± 0.25 b
Significance					
Irrigation regime		*	**	*	**
Biostimulants		**	**	**	**
Irrigation regime × biostimulants		**	**	**	**

Mean values followed by the similar letters within a column are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test). * and ** are significant at $P \leq 0.05$ and at $P \leq 0.01$, respectively. Values are means of three replicates ± SD

Table 8 Influence of biostimulants on leaf mineral contents of 'Yaghouti' grapevine under WW and DS conditions

Irrigation regimes	Biostimulants	N (%)	P (%)	K (%)	Fe (ppm)	Zn (ppm)
WW	CON	1.37 ± 0.12 c	0.31 ± 0.06 e	1.07 ± 0.04 d	166.43 ± 1.52 g	17.30 ± 0.98 ef
	AA	1.42 ± 0.11 c	0.41 ± 0.04 cd	1.34 ± 0.02 c	183.62 ± 1.34 de	22.70 ± 0.60 c
	HA	1.43 ± 0.11 c	0.42 ± 0.03 cd	1.36 ± 0.04 c	187.56 ± 2.51 cd	23.03 ± 0.30 c
	FA	1.43 ± 0.12 c	0.42 ± 0.06 cd	1.37 ± 0.05 c	189.45 ± 1.44 c	20.96 ± 0.75 d
	SE	1.62 ± 0.09 b	0.44 ± 0.06 c	1.46 ± 0.08 c	200.33 ± 1.46 a	26.83 ± 0.28 a
DS	CON	1.44 ± 0.10 c	0.36 ± 0.09 de	1.12 ± 0.05 d	157.62 ± 4.04 h	16.03 ± 0.95 f
	AA	1.65 ± 0.09 b	0.71 ± 0.07 b	1.64 ± 0.04 b	179.32 ± 1.15 ef	20.68 ± 1.21 d
	HA	1.64 ± 0.11 b	0.74 ± 0.06 b	1.73 ± 0.13 ab	186.01 ± 2.00 cd	18.56 ± 1.19 e
	FA	1.69 ± 0.12 b	0.69 ± 0.04 b	1.66 ± 0.14 b	177.10 ± 1.73 f	20.13 ± 0.51 d
	SE	1.83 ± 0.14 a	0.81 ± 0.06 a	1.79 ± 0.09 a	195.34 ± 5.03 b	24.66 ± 0.57 b
Significance						
Irrigation regime		*	**	*	*	*
Biostimulants		**	**	**	**	**
Irrigation regime × biostimulants		*	**	**	*	**

Mean values followed by the similar letters within a column are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test). * and ** are significant at $P \leq 0.05$ and at $P \leq 0.01$, respectively. Values are means of three replicates ± SD

drought stress conditions have been reported in different plants, including roselle [18], wheat [25], and cherry tomato [40]. However, to the best of our knowledge, this is the first report on the effectiveness of biostimulants in drought damage of vines. Furthermore, the application of some biostimulants was previously reported to be effective in the yield of grapevine under WW conditions [42, 47]. The positive impact of biostimulants on yield can be attributed to the point that most of them contain different hormones, vitamins, and minerals, which might have a role in the orientation and translocation of metabolites from leaves into reproductive tissues. Furthermore, these substrates can play a role in the synthesis of nucleic acids and proteins and decrease their degradation, which might result in the enhancement of yield [13, 26].

The results of this work indicated that drought stress increased TA and TSS content of fruits, and the application of biostimulants, especially SE, enhanced TSS but decreased TA content in DS vines (Table 4). There are various reports on the effect of drought stress on TA by the researchers. Some researchers described that TA was not affected by drought stress [30], while some others showed that TA was decreased by drought stress [54], which is inconsistent with the results of this study. Increases in TSS content of fruits under drought stress conditions have been reported in different fruit species, including strawberry [21], table grape [22], and apple [56]. These findings are in agreement with the results detected in the current study (Table 4). Since TSS could adjust the osmotic pressure of plant cells, drought stress stimulated the increase of TSS in fruits. Furthermore, our findings are in line with those obtained by Kok and Bal [29], reporting that the application of some biostimulants decreased TA and enhanced TSS content of grapevine fruits under WW conditions. In contrast, Sabir et al. [45] indicated that the biostimulants did not influence the acidity of grapevine berries.

Compared to unstressed vines, drought stress adversely impacted the chlorophyll content of grapevine leaves. However, the negative effect of drought stress on the chlorophyll content was lower in vines treated with AA, HA, FA, and especially SE, indicative of increased chlorophyll content of DS vines by biostimulants (Table 5). In this regard, it was found that the application of biostimulants increased the chlorophyll content in DS faba bean [17], tomato [24], and wheat [33]. This increase can be justified regarding the reduction in chloroplast biogenesis, decrease in chlorophyll degradation, and delay in senescence [28, 41]. Furthermore, the application of SE was previously reported to achieve a significant increase in the chlorophyll content of grapevine under WW conditions [45], which is in agreement with the results achieved in the current study (Table 5).

ABA is a well-known stress-response hormone and plays a vital role in plant responses to water stress [59]. In this study, accumulation of ABA took place in the leaves of vines subjected to DS conditions, and a further enhancement in the level of ABA occurred in the leaves of water-stressed vines by the application of SE and AA (Table 6). Therefore, it can be claimed that biostimulants protect vines against drought stress by enhancing the accumulation of ABA. Similar results on the positive effects of biostimulants on the ABA content have been obtained for soybean [50] and maize [57] plants under

drought stress conditions. There was no report regarding the effect of biostimulants on endogenous ABA in fruit trees under drought stress.

The obtained results in this study revealed that drought stress increased the proline content of grapevine leaves, and the application of biostimulants, especially SE, caused a further increase in proline content of DS vine leaves (Table 6). In line with the results revealed in the present study, increases in proline content of leaves under drought stress conditions have been reported in different plants, including tomato [24], wheat [26], and German chamomile [36] (Table 6). Proline, as an antioxidant of AA, responds to abiotic stresses, stabilizes proteins, prevents lipid peroxidation, and acts as an osmolyte in the permeability of cell membranes. Therefore, proline can be assumed as a leading indicator of abiotic stress [9, 23]. Furthermore, our findings confirm previous reports that the exogenous application of some biostimulants improved proline content in DS plants [1, 18, 24, 26, 36].

In this work, total phenolic content significantly increased by the exposure of grapevine leaves to drought stress, and further increased by the application of SE (Table 6). Similarly, Murtic et al. [40] observed that exogenous application of SE promoted total phenolic content in cherry tomato under drought conditions. Phenolic compounds are a group of non-enzymatic antioxidant systems activated in plants under abiotic stresses [3]. These compounds by cellular osmotic regulation lead to an increase in membrane integrity and act as a scavenger of free radicals [3, 43].

The results of the current study indicated that drought stress enhanced soluble carbohydrates in grapevine leaves, and the application of biostimulants, especially SE, caused a further enhancement in the leaves of DS vines (Table 6). Increases in soluble carbohydrates of leaves under drought stress conditions have also been reported in different plants, including wheat [34], and German chamomile [36]. Since soluble carbohydrates are vigorously involved in osmoregulation, biological membrane stabilization, protection of enzyme structure, and protection against hydroxyl radicals [48], high levels of these substrates are assumed influential in drought tolerance. In addition, our findings are in agreement with previous reports that the exogenous application of some biostimulants increased soluble carbohydrates in DS plants [23, 26, 36].

Drought stress negatively impacted soluble proteins of grapevine leaves. However, the negative effect of drought stress on soluble proteins was lower in vines treated with AA, HA, FA, and SE, indicating that biostimulants increased soluble proteins of DS vines (Table 6). Reductions in soluble proteins of plant leaves could be one of the indicators of oxidative stress continually detected in plants under drought stress [39]. Furthermore, our findings support those obtained by Hammad et al. [26] on wheat and Masoudi Sadaghiani et al. [36] on German chamomile, reporting that the application of some biostimulants enhanced soluble proteins of the DS plant.

Drought stress induces oxidative processes mediated by reactive oxygen species (ROS). APX, CAT, POD, and SOD, as important antioxidant enzymes, are effective scavengers of ROS. Moreover, the ROS accumulation would induce lipid peroxidation and harm membrane structure [2, 38]. It has been suggested that the development of plant drought tolerance is concerned with the enhancement of antioxidant enzyme activity [14]. Biostimulants not only induce drought tolerance and alleviate drought damage, but also enhance antioxidant enzyme activity [14, 35]. The results of the current study also revealed that under drought stress, biostimulants significantly improved the activity of GPX and CAT (Table 7). Because increased antioxidant enzyme activity could improve the ability of tissue to remove H_2O_2, the level of H_2O_2 was lower in the leaves of vines treated with biostimulants (Table 7).

Under drought stress conditions, a specific sign of membrane lipid damage is MDA accumulation. In this study, a rise in MDA concentration of the leaves was observed under drought stress conditions, but this rise was lessened by the application of biostimulants, especially SE (Table 7). Furthermore, H_2O_2 concentration showed similar trends to MDA concentration, i.e., H_2O_2 concentration of leaves increased with the decrease in water supply, but the application of biostimulants, especially SE, significantly reduced this increase (Table 7). Our findings are in agreement with previous reports that the exogenous application of some biostimulants decreased the accumulation of MDA in DS plants [7, 16, 24].

Previous research showed that higher concentrations of leaf nutrients occurred to alleviate the negative effects of drought stress [34]. In the present study, concentrations of N, P, and K increased, and concentrations of Fe and Zn decreased under drought stress (Table 8). Increases in N, P, and K and decreases in Fe and Zn concentrations under drought stress conditions have been reported in several plants [36, 53]. Furthermore, our findings are consistent with those of previous research that the exogenous application of some biostimulants increased concentrations of N, P, and K in DS plants [26]. No evidence was found regarding the effect of biostimulants on concentrations of Fe and Zn in plant leaves under drought stress conditions.

Conclusion

The application of biostimulants, especially SE, noticeably improved the yield of 'Yaghouti' grapevine under drought stress. The results of this study highlight the role of biostimulants in regulating the drought-stress response of grapevine, suggesting that biostimulants are involved in physiological and biochemical activities. The results showed that the application of biostimulants, especially SE, stimulated the accumulation of ABA, proline, total phenol, and soluble carbohydrates as well as the activity of the antioxidant enzymes, whereas reduced the level of MDA and H_2O_2 in DS vines. Thus, the appropriate application of biostimulants could result in improved production of grapevine, especially in dryland regions.

Abbreviations

AA: Amino acid; HA: Humic acid; FA: Fulvic acid; SE: Seaweed extract; CON: Control; WW: Well-watered; DS: Drought-stressed; TA: Titratable acidity; TSS: Total soluble solid; MDA: Malondialdehyde; H_2O_2: Hydrogen peroxide; GPX: Guaiacol peroxidase; CAT: Catalase; ABA: Abscisic acid.

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Authors' contributions

BV and MRN designed the experiment. HI and MRN performed the experiments. BV and HI conducted the laboratory measurements. BV analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Author details

[1] Department of Horticultural Sciences, Faculty of Agriculture and Natural Resources, Arak University, 38156-8-8349 Arak, Iran. [2] Horticulture Crops Research Department, Qom Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.

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