Effects of transpiration reducers on white beans under drought stress conditions

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Abstract

The effects of seaweed fertilizer and salicylic acid (SA) as a transpiration reducer on the yield and physiological traits of white beans (the Almas variety) were investigated in a split-split plot experimental design with three replications in two locations, West Islamabad and Solaymanieh, in the crop year 2017-2018. The main plots were the application of three stress levels (60, 90, and 120 mm of surface evaporation from a Class A evaporation pan). In the subplots, treatments were foliar application of seaweed fertilizer at 0, 50, 100, and 150 g/ha, and two levels of foliar application and no SA foliar application were applied in the sub-subplots. The results showed that the two selected experimental locations were significantly different in climatic characteristics (average temperature and rainfall). The highest grain yield was obtained from the Islamabad region. Drought stress caused a decrease in grain yield and its related traits, as well as an increase in oxidative damage and ion leakage from membranes. Drought stress activated the antioxidant defense system and reduced water use efficiency. Moreover, drought stress reduced both the photosynthetic rate and chlorophyll content, and seaweed and SA spraying increased these two traits. Proline content, ion leakage, and the activity of antioxidant enzymes (catalase, ascorbate peroxidase, and superoxide dismutase) increased due to drought stress. SA application increased the activity of antioxidant enzymes, but the response to SA was not similar in different seaweed concentrations. Drought stress also increased proline content and the ion leakage rate from the membrane and decreased grain yield.

Keywords: *white bean, drought stress, seaweed, salicylic acid*

Introduction

As a major agricultural plant worldwide, bean (*Phaseolus vulgaris* L.) is used for people's nutritional consumption. The global cultivated area and production of beans are 27 million hectares and 15.5 million tons, respectively, with an average yield of 568 kg/ha. In Iran, the area under bean cultivation is equal to 89,000 ha, with a production of 140,000 tons.

Salicylic acid (SA) is a phenolic compound that regulates the biological and biochemical processes of plants (Khan et al., 2003), including the regulation of transpiration, stomatal closure, membrane permeability, growth, and photosynthesis (Khan et al., 2003; Shakirova et al., 2003). In general, previous studies show that this compound can be effective from plant germination to aging and increase the photosynthesis efficiency and the final crop. Evidence indicates the undeniable effect of SA and other salicylate derivatives on many physiological and morphological processes of plants. The range of these changes varies considerably and may be stimulating in some reactions, accelerating in a number, and inhibiting in others (Rivers, 2008). According to the literature, the use of SA in crops improves yield and yield components (Zhao et al., 1995), resulting from the effect of SA on plant physiological processes.

Seaweeds are used as food for humans and animals, organic fertilizers, and fossil fuel production (Koyro et al., 2006). An example of these biofertilizers includes heterocyst-bearing blue-green algae. The characteristics of these algae include increasing soil pores due to their fibrous structure and producing viscous substances, secreting growth-stimulating substances (e.g., hormones, vitamins, and amino acids), preventing the formation of toxic substances in the soil by photosynthesis and O_2 production, increasing soil water-holding capacity (WHC) due to the gel structure, reducing soil salinity, adding biomass to the soil after death and decomposition, preventing weed growth, and increasing soil phosphate levels by releasing organic acids (Saadat Nia et al., 2018).

As mentioned above, the supplementary use of biological fertilizers is of special importance in soil fertility and structure preservation, vital activity, and WHC in the soil. The results of different cultivation systems, related ecological studies, and the use of organic fertilizers show positive results of using organic or biological sources in the framework of plant nutrition systems (Milady Lari, 2010). Very limited research is available on the cultivation of cereals under stress conditions. The importance of research in this field is further highlighted since dry areas cover most of our country. Therefore, this project aimed to study the effect of transpiration reducers on water use efficiency (WUE), agrophysiological characteristics, and biomolecular traits of white beans under drought-stress conditions.

Methodology

This project was implemented in a split-split plot experiment with a randomized complete block design. The length and width of each main plot were 12 m and 3 m, respectively, and the length and width of subplots were 3 m and 3 m, and the sub-subplots measured 1.5 × 3 m. The main plots included the application of drought stress at three levels (60, 90, and 120 mm of surface evaporation from a Class A evaporation pan). In the subplot treatments, seaweed fertilizer spraying was applied at 0, 50, 100, and 150 g/ha, and two levels of foliar application and no SA foliar application were applied in the sub-subplots. A white bean variety called Almas, was used in this experiment. Physiological and agro-morphological traits were investigated in the samples.

After ensuring the normality of data, the data obtained from the two studied sites were subjected to a mixed analysis of variance (ANOVA) using SAS 9.4 statistical software. The means of the studied traits were compared with Duncan's test at the 5% level. Figures were drawn using Excel software.

Results

1. Morphological traits

The results of ANOVA showed that the main effect of location and the interaction effect of irrigation regime × seaweed were significant on plant height at the 1% probability level (Table 1). Foliar amino acid and seaweed extract spraying on a celery species significantly increased plant height, photosynthetic pigments, potassium/phosphorus content, yield, and fresh/dry weight of leaves (Shehata et al., 2011). Soil application of seaweed extract increased tomato plant height from 75 cm to 79 cm (Hua et al., 2010).

The main effects of location and irrigation regime, as well as the interaction effect of location × irrigation regime, were significant on the number of pods per plant at the 1% probability level (Table 1).

Table 1. The mean squares obtained from the analysis of variance for the effects of the experimental factors on the studied traits

Sources of variation	df	Plant height	No. of pods/plant	Shell weight	No. of seeds/plant	Seed weight/plant	Pod weight/plant	Stem/leaf DW	Biological yield	Grain yield	Harvest index
Location (L)	4#	4553,66--#	565<,9:--#	587,7qv#	45<9,3qv#	556:,5<--#	95;,66--#	4834,89--#	57<99,79qv#	79;987,6-#	7:;8,:--#
L × replication (R)	5#	45<6,85#	6;5,54#	:74,;8#	789<,35#	9<4,9;#	4<,9;#	584,73#	4;84;;,6#	53;4<7,3<#	468,97qv#
Irrigation regime (IR)	5#	4<::,54qv#	77<;,:6--#	4<;8,36-#	4<<:6,4;--#	7936,;6--#	595,85--#	5554,55--#	8:7;:8,<--#	99988;,6--#	668,8-#
L × IR	5#	7,4;qv#	456;,9:--#	43,4<qv#	8;,;5qv#	::5,<<-#	4<6,<3--#	465<,:;--#	6:73<8,36--#	6596,4<qv#	993,<--#
Error a	;#	;34,6;#	755,5:#	:;8,69#	6<<4,9:#	7<4,7;#	77,53#	698,3;#	96;56,43#	6;:855,5#	55;,<7#
Seaweed (SW) × L	6#	754,93--#	:9,<9qv#	4868,49-#	;476,53-#	575,4;qv#	6<,;8qv#	43;,<3qv#	484676,34--#	<;;896,4--#	<9,48qv#
SW	6#	3,93qv#	589,<4qv#	46,4<qv#	57,54qv#	8:;,96-#	:;,9;qv#	<3,<8qv#	;64<8,85qv#	4754,7qv#	88,57qv#
IR × SW	9#	558,;;--#	5;8,6;qv#	549:,<<--#	48;39,:9--#	793,<6-#	479,48--#	544,89qv#	:5:9:,67-#	57<67<,3:-#	463,7;qv#
L × IR × SW	9#	:,:6qv#	466,:6qv#	59,45qv#	43,54qv#	497,99qv#	8:,;:qv#	487,88qv#	4;;<6,85qv#	:5;,<6qv#	444,;<qv#
Error b	69#	<8,44#	53<,;5#	973,<:#	6445,::#	575,9#	79,88#	663,65#	<<93,<:#	46<834,3#	99,48#
SA × L	4#	;;,69qv#	57,66qv#	<,<5qv#	954,9:qv#	46;,78qv#	468,66qv#	4577,<--#	443;5,7<qv#	599847,3qv#	;;,66qv#
SA × IR	4#	;,35qv#	97,86qv#	464,43qv#	3,86:qv#	8,46qv#	89,:8qv#	8;;,39-#	56:::,98qv#	;358,4:qv#	446,55qv#
SA × IR × L	5#	97,34qv#	634,7;qv#	7<:,68qv#	4759,85qv#	435,67qv#	47,73qv#	65:,;;qv#	6<76,<qv#	489<85,3;qv#	85,:3qv#
SA × IR × L	5#	59,5:qv#	449,;5qv#	4579,9qv#	7;87,83qv#	53;,<9qv#	74,<8qv#	496,;<qv#	;5:8,3<qv#	:378<,35qv#	84,7;qv#
SA × SW	6#	7;,5;qv#	4<5,5;qv#	89,6;qv#	987,83qv#	5:9,36qv#	433,8<-#	84,85qv#	45436,84qv#	456;8<,;<qv#	;;,43qv#
SA × SW × L	6#	64,;3qv#	573,;6qv#	4584,94qv#	4;;5,87qv#	9:,8;qv#	5:,39qv#	75,64qv#	55945,3;qv#	474;<,8qv#	44,94qv#
SA × SW × IR	9#	7:,56qv#	43<,;4qv#	5:<,;<qv#	4<:7,;:qv#	549,9<qv#	65,:3qv#	<3,38qv#	68595,:9qv#	7949;,:8qv#	;<,63qv#
L × SA × SW × IR	9#	87,46qv#	466,;8qv#	;99,;5qv#	5:56,98qv#	59:,;6qv#	66,65qv#	8<,;6qv#	68595,:9qv#	78593,;qv#	98,67qv#

Error c	7;#	79,:5#	4;9,:#	864,56#	5;44,:7#	49<,:8#	67,:9#	465,59#	5;8;9,6#	<8469,<#	:4,:<#
C.O.V		:,44#	56,59#	54,<#	54,57#	4<,89#	4:,4:#	48,<9#	49,96#	47,:7#	53,45#

According to the results of ANOVA, the main effects of irrigation regime and seaweed use, as well as the interaction of irrigation regime × seaweed, were significant on the number of pods per plant (Table 1). In a study, the use of seaweed liquid fertilizer at different concentrations (0, 25.0, 1.5, 1.5, and 2%) increased fresh weight, dry weight, and root and stem length in several types of tumbleweeds at a concentration of 1.5 (Zhang et al., 2011).

2. Agronomic traits: yield and its components

The results of ANOVA indicated that the main effects of irrigation regime and seaweed application, as well as the interaction effect of irrigation regime × seaweed, were significant on the number of seeds per plant (Table 1). Drought stress application during the flowering stage reduces the number of fertile flowers due to the fall of flowers, which occurs from the fall of flowers due to the shortage of water and nutrients needed by flower buds, thereby reducing the number of seeds in the pod (Neocleous and Vasilakakis, 2007; Naveed et al., 2014).

Based on the results of ANOVA, seed weight per plant was significantly affected by the main effects of location and irrigation regime as well as the interaction effects of location × irrigation regime, location × seaweed, and irrigation regime × seaweed (Table 1). Rio et al. (2000) believe that drought stress from pollination to physiological maturity, especially when accompanied by high temperatures, reduces the seed filling period and filling rate, resulting in a decrease in the average seed weight. Foliar seaweed extract spraying on soybean plants under rainfed conditions significantly increased yield components. Grain yield (with a concentration of 12.5%) was improved by 57% compared to the control (46%) (Basra et al., 2007).

The results of ANOVA indicated the significant main effects of location and irrigation regime as well as the interaction effects of location × irrigation regime, irrigation regime × seaweed, and seaweed × SA on the weight of pods per plant (Table 1). Senaranta et al. (2002) reported similar results of SA resistance induction against heat, cold, and drought stresses in tomatoes and cowpeas.

The results of ANOVA showed that the main effects of location, irrigation regime, and SA, as well as the interaction effects of location × irrigation regime and the interaction effect of location × SA, were significant on the dry weight of the plant's stem and leaves (Table 1). Arfan et al. (2006) reported that SA foliar application could effectively increase wheat growth and yield under salinity stress. Moreover, SA foliar application positively affected cucumber plant growth (Yadav et al., 2010).

The results of ANOVA showed that the main effects of irrigation regime and seaweed, as well as the interaction effects of location × irrigation regime and the interaction effect of irrigation regime × seaweed, were significant on biological yield (Table 1). Shepherd et al. (2003) reported that amino acid and seaweed extract foliar spraying on a celery species significantly increased plant height, yield, and fresh/dry weight of leaves.

According to the results of ANOVA, the main effects of location, irrigation regime, and seaweed, as well as the interaction effect of irrigation regime × seaweed, were significant on grain yield (Table 1). A decrease in grain yield as a result of drought stress and increased irrigation intervals was also reported by Du et al. (1998). The use of seaweed liquid extract (20%) led to an increase in the germination percentage, growth, and yield (the number of seeds and seed dry weight) in wheat (Kafi, 2009).

3. Physiological traits

Based on the results of ANOVA, the canopy temperature was significantly influenced by the main effect of location and irrigation regime and the interaction effect of location × irrigation regime at the 5% probability level (Table 2).

Table 2. The mean squares obtained from the variance analysis for the effects of experimental factors on the physiological traits of white beans

Sources of variation	df	Canopy temperature	Leaf temperature	PAR	Photosynthetic rate	Respiration rate	Leaf surface CO_2	Sub-stomatal CO_2	Mesophyll conductance	Relative humidity	WUE
Location (L)	4#	43,78-#	;;,7;--#	4;376<5<--#	45,7:--#	4,<7--#	77558<;--#	--973<45#	3,33333:6--#	46:3,:9--#	7,44--#
L × replication (R)	5#	53,38#	4:,<8#	<9996#	5,55#	3,63#	453;;#	;:78#	3,33333;8#	43;,43#	3,:7#
Irrigation regime (IR)	5#	8,6;-#	4,96qv#	:4664-#	9,93--#	3,4:qv#	7658:qv#	4485:#	3,333358;--#	564,78#	6,7;--#

L × IR	5#	8,;9-#	5,46-#	5::<7qv#	5,;3--#	3,6:qv#	6956:qv#	69<#	3,33334:6--#	5<:,93#	4,8:--#
Error a	;#	;34,6;#	755,5:#	:;8,69#	6<<4,9:#	7<4,7;#	77,53#	698,3;#	96;56,43#	6;:855,5#	55;,<7#
Seaweed (SW) × L	6#	3,57#	3,63qv#	55;36qv#	4,6<-#	3,5:qv#	:58:3qv#	8;53#	3,333334;qv#	48,7<#	4,88--#
SW	6#	4,9<#	3,87qv#	47556qv#	6,:6--#	3,4<qv#	44457;qv#	:399#	3,333354:--#	7:,86#	6,;<--#
IR × SW	9#	4,6;#	3,4;qv#	5669:qv#	6,84--#	3,5;qv#	499:6qv#	494;3#	3,333339<--#	8,;4#	4,<8--#
L × IR × SW	9#	4,3;#	3,54qv#	:368qv#	6,:7--#	3,5:qv#	6;7<:qv#	:757#	3,33334<<--#	4;,67#	6,7:--#
Error b	69#	5,48#	3,<5#	588:8qv#	3,66#	3,59#	43;585#	4<9:6#	3,333333;#	<6,94#	3,54#
SA × L	4#	3,;<#	3,336qv#	763qv#	3,4:qv#	3,33qv#	655qv#	7;67#	3,3333335qv#	47,59#	3,34#qv#
SA × IR	4#	3,65#	3,83qv#	53;44qv#	8,;;-#	3,36qv#	;<76qv#	4:<6<#	3,3333639--#	:4,<4#	8,73--#
SA × IR × L	5#	4,4;#	3,35qv#	:;6<qv#	8,3:--#	3,45qv#	46<37qv#	-63<73#	3,333373;--#	<,;;#	6,63--#
SA × IR × L	5#	3,69#	3,37qv#	7;6;qv#	3,75qv#	3,44qv#	94;8qv#	48<5#	3,3333343qv#	;,46#	3,5<#qv#
SA × SW	6#	3,:8#	3,36qv#	9868qv#	3,<7qv#	3,54qv#	::6qv#	;4::#	3,333337;qv#	5,3<#	3,57#qv#
SA × SW × L	6#	3,55#	3,3<qv#	699;<qv#	3,:8qv#	3,48qv#	4873<qv#	4378<#	3,333337<qv#	54,:3#	3,:8#qv#
SA × SW × IR	9#	3,6;#	3,3;qv#	4;<<6qv#	4,3:qv#	3,39qv#	5355;qv#	93:7#	3,3333383qv#	5;,<<#	3,::#qv#
L × SA × SW × IR	9#	3,44#	3,35qv#	4774<qv#	4,9<qv#	3,46qv#	45488qv#	<884#	3,333344:-#	5:,87#	4,<9--#
Error c	7;#	3,;6#	3,55#	4<:85#	3,;:#	59,3<#	494:;#	:49<#	3,333336;#	53,33#	3,93#
C.O.V	#	45,6<#	44,67#	43,86#	54,68#	3,45#	4;,<7#	48,5:#	59,99#	;,75#	651::#

The results of ANOVA revealed the significant main effect of location on leaf temperature (Table 2). Drought stress did not significantly affect the leaf temperature trait. The significant effect of location shows that atmospheric factors (rainfall rate, minimum/maximum air, and soil temperature) and geographical factors (soil physical and chemical properties, latitude and longitude, and altitude above the sea level) have caused differences in the studied trait. According to the experimental results, the Solaymanieh region was more affected than the Karaj region. SA and seaweed foliar spraying did not significantly influence leaf temperature.

The results of ANOVA revealed that only the main effect of location was significant on the amount of photosynthetically active radiation (PAR) (Table 2). A higher average amount of PAR was measured in Solaymanieh than in Karaj.

According to the results of ANOVA, the photosynthetic rate was significantly affected by the main effects of location, irrigation regime, and seaweed, as well as the interaction effects of location × irrigation regime, location × seaweed, irrigation regime × seaweed, location × SA, and irrigation regime × SA (Table 2). Zhu et al. (2002) observed that water deficit stress reduced grain filling duration, net photosynthesis, and stomatal conductance in rice plants and accelerated leaf senescence. When the plants are under stress during the vegetative and reproductive stages, the photosynthetic rate decreases in all parts of the wheat plant.

The results of ANOVA indicated that only the main effect of location was significant on the transpiration rate at the 1% probability level (Table 2). The transpiration rate in Solaymanieh was higher than that in Karaj. Atmospheric (rainfall rate and minimum/maximum air and soil temperatures) and geographic (soil physicochemical properties, latitude, longitude, and altitude above sea level) factors caused fluctuations in the studied trait.

Based on the results of ANOVA, only the main effect of location was significant on leaf surface CO_2 levels at the 1% probability level (Table 2). According to the comparison results, a higher average CO_2 concentration on the leaf surface was recorded in Solaymanieh than in Karaj.

The results of ANOVA showed that the main effects of location and the interaction effects of irrigation regime × SA were significant on the sub-stomatal CO_2 concentration (Table 2). The comparison of the average main effect of location on sub-stomatal CO_2 concentrations showed a higher level of this trait in the Karaj region. Stomatal closure occurs in the plant at the time of drought stress, resulting in the decreased release of CO_2 from the atmosphere to the carboxylation site, thereby reducing sub-stomatal CO_2 concentrations and photosynthesis (Peeva and Cornic, 2009).

The results of ANOVA indicated that leaf mesophyll conductance was significantly influenced by the main effects of location and irrigation regime as well as the interaction effects of location × irrigation regime, location × seaweed, irrigation regime × seaweed, location × SA, irrigation regime × SA, and the interaction effect of location × irrigation regime × seaweed × SA (Table 2).

According to the results of ANOVA, only the main effect of location was significant on the relative humidity percentage at the 1% probability level (Table 2). The mean comparison showed higher relative humidity in the Karaj region than in the Solaymanieh region.

The results of ANOVA revealed the significant main effects of location and irrigation regime as well as their interaction effect on leaf relative water content (RWC) (Table 2). In general, decreased leaf RWC in response to drought stress was reported in most studies (Fu and Huang, 2001; Shi and Zhu, 2008), suggesting that it could be a suitable index for drought tolerance.

The results of ANOVA showed that WUE was affected by the main effect of location as well as the interaction effects of irrigation regime × seaweed and irrigation regime × SA (Table 2). The average main effect of location on WUE was higher in the Solaymanieh region.

The use of SA in crops improved yield and yield components (Xie et al., 2014), resulting from the effect of SA on the physiological processes in the plant. There are multiple and varied plant responses to seaweed application, including higher yield, greater WUE, increased nutrient uptake, changes in plant tissue composition, increased resistance to frost and fungal diseases, insect attacks, extended shelf life of fruits, and better seed germination.

Foliar application of amino acid and seaweed extract significantly increased plant height, photosynthetic pigments, potassium, phosphorus content, yield, and leaf fresh and dry weight in a species of celery (Shehata et al., 2000).

Table 3. The mean squares obtained from the variance analysis for the effects of experimental factors on the pigments and antioxidants of white beans

Sources of variation	df	Chl. a	Chl. b	Anthocyanin	Ion leakage	Carrot.	Proline	CAT	SOD	APX
Location (L)	4‡	43,5# qv#	3,: 35#qv#	4<<817# qv#	7,;# qv#	651 6#qv#	78,9# qv#	3,3 --<#	3, --47#	8935, 4<--#
L × Replication (R)	5‡	5,:4#	3,9 98#	74315#	66,;#	8:1 9#	:9,8#	3,3 3#	3, 33#	6:,97#
Irrigation regime (IR)	5‡	434, :--#	44, <--#	47671<# qv#	483 ;--#	76: 16#--#	89:. :--#	3,3 --<#	3. 55--#	7595, 57--#
L × IR	5‡	4,8# qv#	3,< <7#qv#	46371;# qv#	6,7# qv#	518# qv#	9,8# qv#	3,3 --4#	3, 4<--#	4349, 7<--#
Error a	;‡	;4,6 ;#	5,5 :#	:;8,69#	6<4, 9:#	7<4 ,7;#	77,5 3#	98, 3;#	6, 43#	6;5,5#
Seaweed (SW) × L	6‡	445, 9--#	46, 5--#	438718# --#	9<6, 6--#	6:1 7#-#	76,3 --#	3,3 36qv#	3, 35qv#	94;,; 8--#
SW	6‡	3,38 6#qv#	3,4 ;5#qv#	5147:#qv#	3,44 6#qv#	:16 ;#qv#	44,6 7#qv#	3,3 4--#	3, 35-#	453,: 4-#
IR × SW	9‡	9,9- -#	3,7 <6#qv#	45416# --#	:7,5 --#	484 13#--#	498, 8--#	3,3 4--#	3, 37--#	<76,5 6--#
L × IR × SW	9‡	3,37 ;#qv#	3,4 36#qv#	4;1<<#qv#	4,7# qv#	718 <#qv#	9,;:# qv#	3,3 6--#	3, 34qv#	44:3, ;8--#
Error b	6 9#	3,96 9#	3,6 <8#	4;168#	9,;6#	<19 7#	45,5 6#	3,3 3#	3, 33#	68,45#
SA × L	4‡	57,3 --#	4,8 --#	4471:# --#	456, 4--#	;71 :#--#	43<, 7qv#	3,3 :--#	3, 48--#	3,34qv#

SA × IR	4ǂ	3,5< 8#qv#	3,3 53#qv#	4146#qv#	3,<7 4#qv#	;13 6#qv#	45,; :#qv#	3,3 9--#	3, 37qv#	8<,95 qv#
SA × IR × L	5ǂ	<,;- -#	3,: 8;-#	5916#qv#	453, 8--#	:<1 6#--#	;:,<# qv#	3,3 4qv#	3, 48--#	54;,7 5qv#
SA × IR × L	5ǂ	3,54 ;#qv#	3,3 4;#qv#	31435#qv#	3,35 4#qv#	316 46#qv#	4,54# qv#	3,3 5--#	3, 49--#	<6,3< qv#
SA × SW	6ǂ	7,5- -#	:,6 --#	;;1< --#	46,7 -#	457 15 --#	565, 5##qv#	3,3 5--#	3, 37-#	;76,9 7--#
SA × SW × L	6ǂ	3,35 :#qv#	3,4 69#qv#	4517#qv#	3,:7 4#qv#	31; 99#qv#	5,67# qv#	3,3 4-#	3, 3;--#	5853, 65--#
SA × SW × IR	9ǂ	6,4- -#	6,8 --#	<:1;#--#	;,8##	449 1:#--#	4;:, 9#qv#	3,3 4--#	3, 36--#	687,3 4-#
L × SA × SW × IR	9ǂ	3,47 7#qv#	3,3 93#qv#	441:#qv#	3,8: 7#qv#	516 7#qv#	7,;:# qv#	3,3 4qv#	3, 3:--#	4677, :4--#
Error c	7 ;#	43,5##	3,5 45#	4716#	7,68#	431 :4#	45,9 8#	3,3 3#	3, 34#	44:,9 7#
C.O. V		5,:4#	<,:#	;1:#	<,3#	481 8#	44,5#	48, 9<#	4; ,94#	49,;6#

The results of ANOVA showed that the main effects of irrigation regime, seaweed, and SA, as well as the interaction effects of seaweed × SA, irrigation regime × SA, and the three-way interaction effect of irrigation regime × seaweed × SA, were significant on chlorophyll b content. Additionally, total chlorophyll content was significantly influenced by the main effect of irrigation regime, seaweed, and SA, the interaction effects of irrigation regime × SA, irrigation regime × location, and the interaction effect of irrigation regime × seaweed (Table 3).

As indicated by the results of ANOVA, the main effects of location, seaweed, and SA, the interaction effects of irrigation regime × SA, irrigation regime × location, and the three-way interaction effect of irrigation regime × seaweed × SA were significant on anthocyanin content (Figure 1).

Altogether, drought stress increased the anthocyanin content. Anthocyanins are synthesized at the end of the flavonoid biosynthetic pathway and play a protective role against ultraviolet rays, drought, cold, and heavy metals in plants (Soltanloo and Hadian, 2017). The reduction of stress effects by flavonoids is attributed to the binding of phenolic compounds to heavy metal ions (Banon et al., 2001). Flavonoids, flavones, and anthocyanins have antioxidant properties, and their production rate and the expression of genes related to their synthesis increase under stress conditions (Tang et al., 2006).

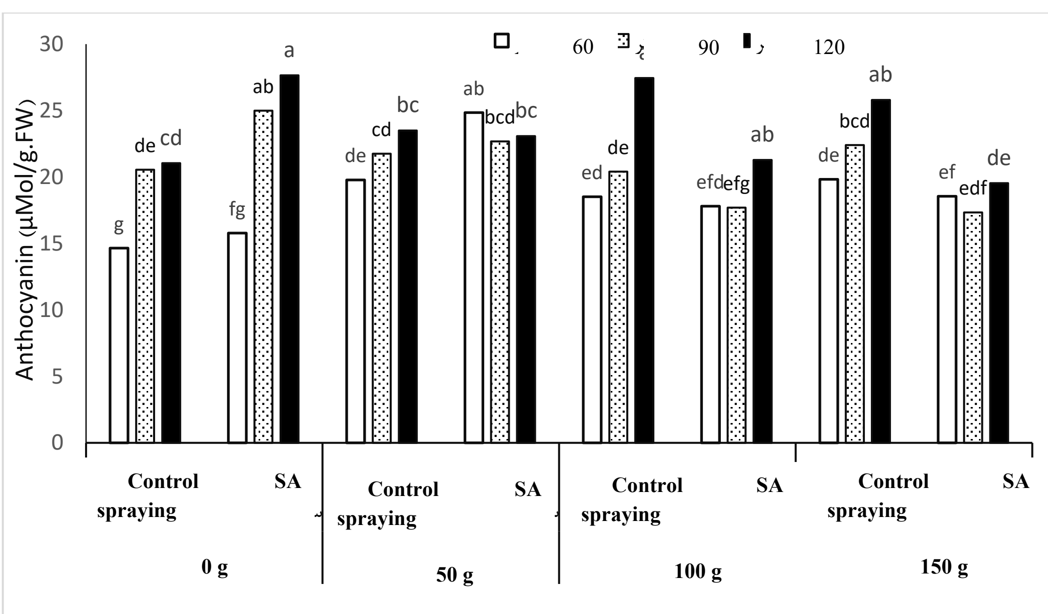

Figure 1. Comparison of the average three-way interaction effect of irrigation regime × seaweed × salicylic acid on anthocyanin content

The results of ANOVA revealed that the main effects of seaweed and SA, the interaction effects of irrigation regime × SA, irrigation regime × location, and the three-way interaction effect of irrigation regime × seaweed × SA were significant on the ion leakage rate (Table 3). The stimulating effects of SA on growth may be due to reasons such as increasing the division rate in meristem areas and cell growth, thereby increasing growth. Besides, SA is known to positively affect other plant hormones (Shakirova et al., 2003).

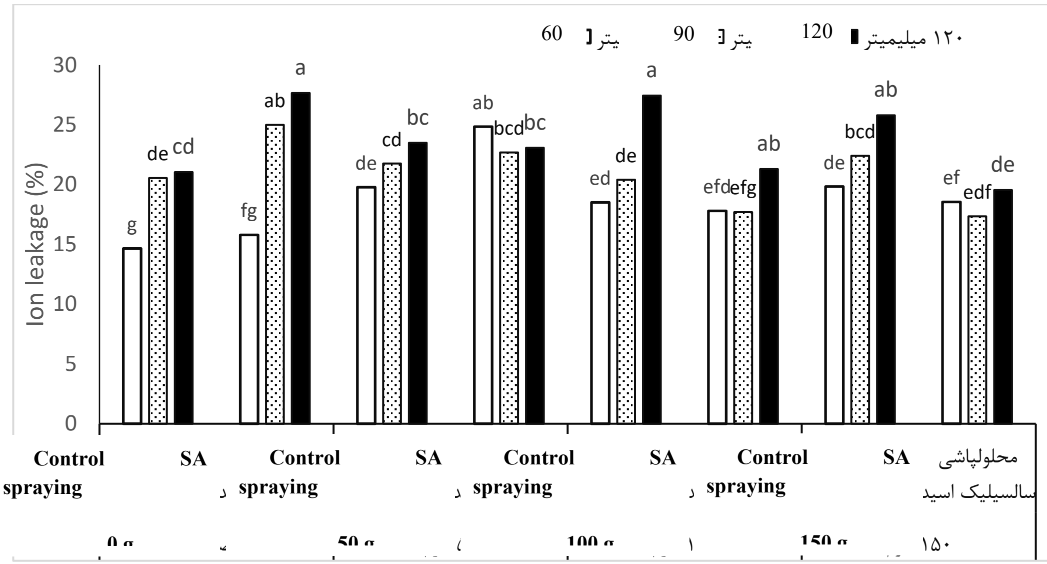

Figure 2. Comparison of the average three-way interaction effect of irrigation regime × seaweed × salicylic acid on the ion leakage

The results of this study showed that carotenoid content was significantly influenced by the main effect of drought stress, seaweed, and SA, the interaction effect of drought and seaweed, the interaction effect of drought stress and SA, the interaction effect of location, drought stress, and SA, the effect of SA and seaweed, the interaction effect of drought stress, seaweed, and SA, and the interaction effect of year, drought stress, seaweed, and SA (Table 3).

Table 4. Comparison of the average three-way interaction effects of the studied traits

Total evaporation from the evaporation pan (mm)	Seaweed ($kg.ha^{-1}$)	SA (mM)	APX (H_2O_2/g.DMμ)	SOD (unit mg/Dm)	CAT (H_2O_2/g.DMμ)	Carrot.
60	3#	3#	84.45ef	3،67ijk#	3،59jk#	14.65 g
		4#	93،<5gh#	3،6;ij#	3،67hi#	14.26 g
	83#	3#	84،77ghi#	3،64k#	3،67hi#	19.22 de
		4#	94،:9gh#	3،7<hi#	3،68ghi#	27.22 ab
	433#	3#	76،8;hi#	3،6:ij#	3،58jk#	17.35 ef
		4#	94،35gh#	3،78hij#	3،65hi#	15.08 fg
	483#	3#	5<،9;i#	3،73ij#	3،57k#	19.67 de
	#	4#	73،3;hi#	3،98ef#	3،59jk#	16.11 efg
90	3#	3#	:3،;3efg#	3،85ghi#	3،66hi#	23.56 bc
	#	4#	93،<5gh#	3،87gh#	3،75fg#	24.25 bc
	83#	3#	94،<3gh#	3،8:vgh#	3،64hij#	29.48a
	#	4#	:9،<9ef#	3،94fg#	3،78ef#	18.11 def
	433#	3#	:4،59efg#	3،7<hi#	3،57k#	21.03 cde
	#	4#	98،:9fg#	3،:4d#	3،6:gh#	17.11 ef
	483#	3#	9:،39fg#	3،6;ij#	3،63ij#	20.91 cde
	#	4#	:6،<3ef#	3،8;fgh#	3،74fg#	17.75 ef
	3#	3#	98،67fg#	3،99e#	3،79ef#	22.40 cd
	#	4#	:7،<7ef#	3،9:e#	3،7<de#	29.09 a
	83#	3#	97،3;fgh#	3،93fg#	3،74fg#	17.32 ef

120	#	4#	:9,59ef#	3,98ef#	3,84d#	23.55 bc
	433#	3#	:<,<5de#	3,93fg#	3,83d#	29.75 a
	#	4#	<3,45d#	3,:4d#	3,7<de#	22.38 cd
	483#	3#	;3,97de#	3,8;fgh#	3,7:ef#	27.54 ab
	#	4#	:7,;3ef#	3,98ef#	3,83d#	19.53 de

The comparison of the average three-way effects showed an increase in carotenoid content with increasing drought stress levels. SA application also increases carotenoid accumulation, but the response of this trait is variable in different fertilizer levels. In total, the highest carotenoid content was obtained from the treatment with 120 mm, no use of seaweed, and SA foliar spraying (Table 4).

The results of this study revealed that the main effect of irrigation regime and seaweed, as well as the effect of irrigation regime × seaweed, were significant on proline content (Table 3).

Based on the results of ANOVA, CAT activity was significantly influenced by the main effects of location, irrigation regime, and the three-way interaction effects of irrigation regime × seaweed × SA (Table 3). Moreover, the main effects of location, irrigation regime, and SA, as well as the three-way interaction effects of irrigation regime × seaweed × SA, were significant on SOD activity (Table 3).

The results of ANOVA showed that the main effects of location, irrigation regime, and seaweed, as well as the three-way interaction effects of irrigation regime × seaweed × SA, were significant on ascorbate peroxidase activity (Table 3).

Table 5. Comparison of the average interaction effects of irrigation regime × seaweed on proline content

Irrigation regime	Seaweed	Proline (mg/g FW)
120 mm	3#	110.5 fg#
	83#	45:17#g#
	433#	45615#ij#
	483#	44415#h#
90 mm	3#	;<18#e#
	83#	<;1;#hi#
	433#	43515#fgh#
	483#	43;19#g#
120 mm	3#	:<13#h#
	83#	:;19#k#
	433#	;515#k#
	483#	;:19#k#

Conclusion

The results of this research showed that the two selected experimental locations were significantly different in terms of climatic characteristics (average temperature, rainfall, etc.), which affected the studied traits differently. Significant differences were also observed in yield, yield components, and the studied physiological traits. The highest grain yield was obtained from the Karaj region. However, the Solaymanieh region was superior in terms of grain yield reduction due to drought stress. Decreased grain yield and its related traits, as well as increased oxidative damage and ion leakage from membranes, were observed because of drought stress. Drought stress activated the antioxidant defense system and reduced WUE in this research. Grain yield improved with the use of seaweed.

According to the evaluation results of the internal SA content in the plant and its effect on the content of hormones such as abscisic acid, ethylene, etc., it is recommended to carry out experiments separately for seaweed and SA.

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Conflict of interest:

The authors declare that there is no conflict of interest regarding the publication of this article. The research was conducted impartially, and the authors have no financial or personal relationships with any individuals or organizations that could potentially bias the interpretation of the results. No external funding or financial support was received for this research.

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Drought stress resulted in a reduction in grain yield and related traits.

Increased oxidative damage and ion leakage from membranes were observed under drought conditions.

Activation of the antioxidant defense system occurred in response to drought stress.

Drought stress led to a decrease in water use efficiency.

Proline content, ion leakage, and the activity of antioxidant enzymes increased under drought stress.

SA application increased the activity of antioxidant enzymes, but the response to SA varied with different seaweed concentrations

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