ECOLOGICAL AND PHYSIOLOGICAL PERFORMANCE OF WHITE BEAN (*PHASEOLUS VULGARIS* L.) AFFECTED BY ALGAE EXTRACT AND SALICYLIC ACID SPRAYING UNDER WATER DEFICIT STRESS

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Abstract. In order to investigate the effects of salicylic acid and seaweed extract foliar on photosynthetic pigments of white bean grown under water deficit stress, a split-split plot experiment was conducted based on randomized complete block design with three replicates. The experimental factors consisted of irrigation regimes at three levels, seaweed extract at four levels and salicylic acid foliar application at two levels. In both places of the experiment, application of 150 $g.ha^{-1}$ seaweed extract along with salicylic acid increased leaf relative water content in plants grown under water deficit stress. The maximum chlorophyll a content (15.15 $mg.g^{-1}$ FW) was observed when 150 $g.ha^{-1}$ seaweed extract was applied under no water deficit stress condition. By contrast, the minimum chlorophyll a content (8.34 $mg.g^{-1}$ FW) was related to 50 $g.ha^{-1}$ seaweed extract. In addition, the minimum proline content (79 $mg.g^{-1}$ FW) was found when no salicylic acid foliar was applied under water deficit stress. However, the maximum proline content (123.2 $mg.g^{-1}$ FW) was obtained when 100 $g.ha^{-1}$ from seaweed extract was applied under severe water deficit stress. The results indicated that the maximum seed yield (2343.3 kg ha^{-1}) was achieved when 100 $g.ha^{-1}$ seaweed extract was applied and there was no water deficit stress.
Keywords: *anthocyanin, proline, seed yield, RWC, total chlorophyll*

Introduction

Protein deficiency in diet causes several problems for human health. Besides animal proteins, plant proteins such as legumes are also an important source of proteins. With growing population in the world, protein requirement and consumption of plant resources are increasing. Among various crops, beans are used as one of the most important and valuable sources of plant proteins (Hosseini, 2008) and have been used in human diet for centuries. Beans are a rich source of protein (20-30%) and carbohydrates (50-60%) and a relatively good source of minerals and vitamins (Rehman and Shah, 2004). In most parts of the world, water is a limiting factor for agricultural productions. Drought stress is one of the major threats to successful crop production across the world. Drought stress reduces crop yield by 50% or more (Bai et al., 2006). According to the FAO reports (2010), around 90% of Iran with arid and semi-arid climate conditions is covered with sparse vegetation.

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One of the effects of environmental stresses is lipid peroxidation (Bai et al., 2006) and degradation of proteins and nucleic acids (Kovacik et al., 2014).

Salicylic acid is a phenolic compound known as a plant hormone or growth regulator, which plays a key role in defense mechanisms against biotic and abiotic stress factors including drought tolerance. It has a significant role in reducing oxidative damage caused by different stresses in plants and developing an anti-stress mechanism in plant cells. Studies have shown that, under drought stress conditions, salicylic acid improves plant growth, transpiration, stomatal regulation, photosynthesis and ion absorption and transfer (Hayat and Ahmad, 2007). Algae have been used to reduce physiological disturbances caused by mineral deficiency and thus improved grain production and resistance to frost (Sridhar and Rengasamy, 2011). In addition to supplying nitrogen and mineral elements, algae regulate plant growth by releasing plant regulating hormones. The presence of plant hormones such as auxin, gibberellin and cytokinin has been shown in the extract of brown algae. Therefore, application of seaweed extract as a fertilizer, increases the growth and production of plants (Erulan et al., 2009). Inorganic extract of *A. nodosome* contains nitrogen, phosphorus, potassium, calcium, iron, magnesium, zinc, sodium and sulfur. Various types of seaweeds stimulate different responses of plants for example, increase in yield, increase in nutrients uptake, changes in the composition of plant tissues, increase in cold resistance, increase in pathogen resistance and increase in fruits quality and germination rate (Sridhar and Rengasamy, 2011). Seaweed extract is produced by hydrolysis under high pressure, however the process differs from species to species. The final concertation of the extract depends on its use as high concentrations could be harmful for plants. Seaweed extract is suitable for those products in which growth hormones such as IAA, IBA, cytokinins, vitamins and amino acids are used (Kaoaua et al., 2013). This study was conducted to investigate the effects of seaweed extract and salicylic acid on white bean performance, grown under water deficit stress condition.

Materials and Methods

The current study was carried out to investigate the effects of salicylic acid and seaweed extract foliar application on photosynthetic pigments, biochemical characteristics and yield of white bean (*Phaseolus vulgaris* L.) grown under water deficit stress condition in the Agricultural Research Station, Khorramabad, Iran, in 2016 and 2017 growing seasons. The soil characteristics, meteorology data and geographical location of the study site are presented in *Tables 1 and 2*.

The experiments were conducted as a split-split plot experiment based on a randomized complete block design with three replicates. The experimental factors consisted of irrigation regimes (main plots) at three levels, (irrigation after 60 (no water deficit stress), 90 (mild water deficit stress) and 120 mm (severe water deficit stress) evaporation from class A evaporation pan), seaweed extract (*Ascophyllum nodosum*) (sub-plots) at four levels (0, 50, 100 and 150 $g.ha^{-1}$) and salicylic acid foliar application (sub-sub plots) at two levels (with and without). Chlorophyll a, b and total chlorophyll were measured according to Porra method (Porra, 2002). Anthocyanins and carotenoids were measured based on the methods described by Lichtenthaler and Wellburn (1983). Leaf samples (500 mg) were rest in 5 ml 80% acetone and centrifuged at 13000 rpm at 4 °C for 15 min. The supernatant made up to 10 ml with 80% acetone and used for spectrophotometry.

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Table 1. *Soil physicochemical properties*

Depth	Total N (%)	P(AVO) P.P.M.	K(AVO) P.P.M.	C.E.C. Meq/mg	Neutralized percentage	pH	EC*10	Saturation percentage s.p.	Organic carbon O.C%
0-20	13	9.2	640	30.8	15.2	7.6	0.73	54	1.26
60-20	6	2.8	380	30.2	17.5	7.7	0.49	55	0.63
95-60	3	2.4	240	28.0	24.5	7.8	0.44	52	0.29
125-95	4	2.8	220	28.0	26.0	7.8	0.48	51	0.38

Table 2. *Meteorological and geographical properties of the study site*

Longitude	Latitude	Altitude	Average annual rainfall	Average temperature	Absolute max temperature	Absolute min temperature
47° 26′	34° 8′	1346 m	538 mm	10.5 °C	41°C	-28.8 °C

Different types of leaf chlorophyll amount were determined based on Arnon (1949) approach. Leaf samples of 0.1 g, from each experimental unit (pots) were obtained from youngest leaves. Samples were grounded and placed in 10 ml of 80% acetone. Resulting extract was centrifuged in 3000 rpm for 10 minutes and the obtained supernatant was placed in cuvette. The absorbance of the solution was recorded by spectrophotometry at 645 and 663 nm wavelengths. Arnon equations were used to estimate the chlorophyll a and b as below:

$$\text{Cholorophyll a (mg/ml)} = [(12/7 \times A663) - (2/69 \times A645)] \qquad \text{(Eq.1)}$$

$$\text{Cholorophyll b (mg/ml)} = [(22/9 \times A645) - (4/68 \times A663)] \qquad \text{(Eq.2)}$$

$$\text{Cholorophyll a+b (mg/ml)} = \text{Chla} + \text{Chlb})) \qquad \text{(Eq.3)}$$

$$\text{Carotenoid } (\mu g/ml) = (1000(A470)-1.8(Chla) -85.02(Chlb))/198 \qquad \text{(Eq.4)}$$

Proline content was determined using Bates et al. (1973) method. Briefly, 500 ml leaf sample was placed in 10 ml of 3% sulfosalicylic acid and then the homogenate was filtered using filter paper. Then 2 ml of the sample was mixed with 2 ml ninhydrin acid (25.1 g ninhydrin plus 30 ml glacial acetic acid) and 2 ml glacial acetic acid in test tubes. The test tubes were heated at 100 °C for 1 h and then cooled down at 4 °C for 30 min before adding 4 ml toluene. The samples were vortexed and left on the bench for 10 min. The upper layer was used for spectrophotometry at 520 nm. The following equations were used to calculate proline content.

$$\frac{\mu g}{mL}\, Proline \times mL\ toluene) \times \frac{\mu mol}{115/5\mu g} \times \frac{5}{sample\ g} = \mu mol proline\ per\ g\ fresh\ sample \qquad \text{(Eq.5)}$$

$$(\frac{\mu g}{mL}\, Proline \times mL\ toluene) \times \frac{5}{sample\ g} = \mu g\ proline\ per\ g\ fresh\ sample \qquad \text{(Eq.6)}$$

The relative water content was determined using (Diaz-Perez et al., 2006) method. Young leaves were detached and equal leaf discs were prepared and then weighted (fresh weight). The discs were soaked in distilled water for 24 h and weighed again to

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determine saturated weight. Finally, the samples were dried in an oven for 48 h to determine dry weight. The relative water content was calculated according to the following equation.

$$RWC= (FW - DW)/(TW - DW) *100 \quad \text{(Eq.7)}$$

where: F_w: fresh weight, D_w: dry weight and T_w saturated weight.

Leaf electrolytes leakage was measured according to Flint (1967). Equal leaves were taken and then were cut into discs. The discs were washed using distilled water and then put into tests tubes containing 5 ml deionized distilled water. After 24 h, the electrical conductivity (EC) of the samples was measured using EC meter (Jenway, 4010). To assess the photosynthetic pigments quartz spectrometer cell (UV_160A_SHIMADO model, made in Japan) was used. Electrical conductivity Multi-range (model H18733) was used to measure the EC. The samples were put in the freezer at -20 C for another 24 h and then the EC was measured again. The value of electrical leakage was calculated using the following equation:

$$\frac{Ec_1}{Ec_2} \times 100 \quad \text{(Eq.8)}$$

Before exposing data to ANOVA and statistical analysis, SPSS was used to perform test for errors and data normality. Data were analyzed by SPSS as split-plot factorial based on randomized complete block design and Duncan’s test was used for mean comparison. Interactive effects of means were signified by Duncan’s test by using MSTATC app and MS Excel was used for plots.

Results and Discussion

This study results indicated that the effects of water deficit stress, seaweed extract, and salicylic acid foliar applications were significant on all the studied traits. However, none of the traits were affected by place. Although the interaction between each factor and place was not significant, all interaction between experimental factors was significant. The interaction between place and other experimental factors was only significant on RWC.

Relative Water Content (RWC)

The results showed that main effects of water deficit stress, seaweed extract, and salicylic acid were significant on RWC. Significate interactions are shown in *Table 3*. The results indicated the minimum RWC (36.41%) was due to mild water deficit stress and salicylic foliar application treatment in the first place. In addition, in both sites of the experiment, salicylic acid increased RWC under severe water deficit stress condition and seaweed extract application. Salicylic acid seems to increase the RWC through the ability of the plant to maintain the leaf water potential. It has been reported that spraying salicylic acid during flowering would increase the RWC, osmotic potential and leaf turgidity (Hussain et al., 2008). Ramroudi and Khamar (2013) showed increase of RWC in basil by application of salicylic acid.

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Table 3. *Analysis of variance on bean traits*

Source of variation	df	Carotenoid	Anthocyanin	Total chlorophyll	Chlorophyll a	Chlorophyll b	Electrolyte leakage	RWC	proline	yield
(P) place	1	32.3ns	1995.4ns	20.1ns	0.702ns	10.2ns	4.8ns	160.4ns	45.6ns	985.4ns
(r/P)	4	57.6	410.2	5.7	0.665	2.71	33.8	47.1	76.5	546.4
a	2	437.3**	1434.9ns	208.8**	11.9**	101.7**	1508.2**	2941.5**	567.78**	409.5**
P × a	2	2.5ns	1304.8ns	1.8ns	0.994ns	10.5ns	3.4ns	16.8ns	6.5ns	909.5ns
error	8	3.74	478.6	2.30	0.432	1.60	44.1	22.8	7.87	321.4
b	3	37.4*	1054.5**	194.8**	13.2**	112.6**	693.3**	3138.4**	43.0**	1209.5**
P × b	3	7.38ns	2.147ns	0.039ns	0.182ns	0.053ns	0.113ns	3.5ns	11.34ns	108.5ns
a × b	6	151.0**	121.3**	9.6**	0.493ns	6.6**	74.2**	70.4**	165.5**	231.5**
P × a × b	6	4.59ns	18.99ns	0.163ns	0.103ns	0.048ns	1.4ns	28.2*	6.87ns	430.7ns
error	36	9.64	18.35	1.18	0.395	0.636	6.83	10.9	12.23	187.3
c	1	84.7**	114.7**	49.1**	1.5**	24.0**	123.1**	332.4**	109.4**	654.3**
P × c	1	8.03ns	1.13ns	0.012ns	0.020ns	0.295ns	0.941ns	3.4ns	12.87ns	121.2ns
a × c	2	79.3**	26.3ns	14.2**	0.758*	9.8**	120.5**	81.4**	87.9ns	221.2ns
P × a × c	2	0.313ns	0.102ns	0.404ns	0.018ns	0.218ns	0.021ns	20.3**	1.213ns	108.6ns
b × c	3	124.2**	88.9**	16.4**	7.3**	4.2**	13.4*	21.7**	232.2ns	88.9ns
P × b × c	3	0.866ns	12.4ns	0.164ns	0.136**	0.027ns	0.741ns	4.2ns	2.346ns	78.4ns
a × b × c	6	116.7**	97.8**	5.4**	3.5**	3.1**	8.5ns	51.1**	187.6ns	112.0ns
P × a × b × c	6	2.34ns	11.7ns	0.075ns	0.060ns	0.144ns	0.574ns	21.9**	4.870ns	234.7ns
Error	48	10.71	14.3	0.547	0.212	0.473	4.35	4.4	12.65	67.3
C.V(%)	-	15.5	8.7	4.7	9.7	6.2	9.0	3.7	11.2	12.3

*, ** and ns significant at 5%, 1% and no significant, respectively

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Electrolytes leakage

The results (*Table 3*) showed that the maximum electrolyte leakage (38.3%) was related to severe water deficit stress treatment without any foliar application. The minimum value was observed when no water deficit stress was imposed and 150 g ha^{-1} seaweed extract was applied (*Table 4*). The interaction between water deficit stress and salicylic acid showed that the maximum electrolyte leakage (31.3%) was monitored under severe water deficit stress and salicylic acid foliar application treatments. By contrast, the minimum value (17.3%) was achieved when no water deficit stress was induced but salicylic acid was applied on the plants (*Table 5*). The interaction between seaweed and salicylic acid indicated that the maximum electrolyte leakage (29.3%) was related to control treatment whereas the minimum value (17.2%) was observed when 150 g ha^{-1} seaweed extract and salicylic acid were applied (*Table 6*).

Table 4. *Interaction between water deficit stress and seaweed extract*

Treatments		Yield	Proline	Electrolyte leakage
a_1	b_1	1902.2bc	79k	21.17cd
	b_2	2090.6b	79.6h	19.17de
	b_3	2343.3a	82.2h	16.35fg
	b_4	2176.6ab	87.6h	16g
a_2	b_1	1208.1de	89.5b	28.03b
	b_2	1408.8d	98.8ef	21.79c
	b_3	1592.2cd	102.2cde	20.41cde
	b_4	1790.6c	108.6d	18.29ef
a_3	b_1	902.5h	110.5cd	38.28a
	b_2	1090.3g	121.4d	28.03b
	b_3	1132.4fg	123.2fg	28.91b
	b_4	1200.2de	111.2e	21.72c

Means with the same letter are not significantly different from each other (P>0.05 ANOVA followed by DMRT).
Treatment a drought stress at 3 levels.
Treatment of algae b at 4 levels.

Table 5. *Interaction between water deficit stress and salicylic acid*

Treatments		Electrolyte leakage
a_1	c_1	17.29f
	c_2	19.05e
a_2	c_1	23.69c
	c_2	20.57d
a_3	c_1	31.33a
	c_2	27.14b

Means with the same letter are not significantly different from each other (P>0.05 ANOVA followed by DMRT).
Treatment a drought stress at 3 levels.
Salicylic acid treatment at 2 levels.

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Table 6. *Interaction between seaweed extract and salicylic acid*

Treatments		Electrolyte leakage
b_1	c_1	29.27a
	c_2	29.05a
b_2	c_1	23.80b
	c_2	22.20c
b_3	c_1	23.21bc
	c_2	20.56d
b_4	c_1	20.13d
	c_2	17.22e

Means with the same letter are not significantly different from each other (P>0.05 ANOVA followed by DMRT).
Treatment of algae b at 4 levels.
Salicylic acid treatment at 2 levels.

Under abiotic stresses, such as salinity, drought, high and low temperatures, plants produce reactive oxygen species, molecules which result in oxidative stress through affecting cellular components and plant metabolism. The reactive oxygen species cause considerable damages to the cells lipid membrane and lead to dis-organisation of the lipid matrix (membrane fluidisation), which in turn decrease cell permeability. Therefore, maintaining cell membrane integrity has an important role in increasing stress resistance (Shim et al., 2003). The effect of salicylic acid on plant growth is mainly due to increased cell division in meristem areas and cell growth. Salicylic acid may also impose its effects through other plant hormones (Shakirova et al., 2003).

Chlorophyll a

The results showed that the main effect of water deficit stress, seaweed extract and salicylic acid and some interactions between them were significant on chlorophyll a concentration (*Table 3*). The maximum chlorophyll a concentration (15.15 mg g FW^{-1}) was obtained when 150 $g.ha^{-1}$ seaweed extract was applied to non-stressed plants. By contrast, the minimum value (8.43 mg g FW^{-1}) was recorded under severe water deficit stress and application of 50 g ha^{-1} seaweed extract (*Table 7*). In this study, water deficit stress reduced photosynthetic pigments content (chlorophyll and carotenoid). Reduction in photosynthetic pigments under water deficit stress can be attributed to the destruction of chloroplast and photosynthetic apparatus, photo-oxidation of chlorophyll, degradation of precursors of chlorophyll and the suppression of chlorophyll biosynthesis as well as the activation of chlorophyll degrading enzymes and finally hormonal disorders (Orcutt and Nilsen, 2000). However, accumulation of sodium and chloride ions in leaves under salt stress condition also has a negative effect on chlorophyll concentration. Additionally, stress disrupts absorption of certain essential elements such as iron and magnesium, which are essential for chlorophyll synthesis (Neocleous and Vasilakakis, 2007). Lipoxygenase has been reported as one of the enzymes involved in chlorophyll catabolism, while lipoxygenase is one of the enzymes involved in lipid peroxidation too (Farooq et al., 2009). Amino acid and seaweed extract foliar application could significantly increase plant height, photosynthetic pigments, potassium content,

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phosphorus content, and yield, fresh and dry weight in celery. Seaweed extracts contain large amounts of cytokines, auxins and betaine that increase chlorophyll content of leaves (Shehata et al., 2011).

Table 7. *Interaction between water deficit stress, seaweed extract and salicylic acid*

Treatments			Carotenoid	Anthocyanin	Total chlorophyll	Chlorophyll b	Chlorophyll a
a_1	b_1	c1	14.65i	39.37fg	12.70mn	3.65klm	9.04ij
		c2	14.26i	33.34i	18.28ef	5.97b	12.26f
	b_2	c1	19.22defgh	28.34j	15.36hi	5.36cd	9.60i
		c2	27.22ab	40.89efg	17.11g	4.41fghi	12.55f
	b_3	c1	17.35fghi	48.02cd	18.64ef	5.04de	13.32de
		c2	15.08hi	45.12cde	19.69cd	6.04b	13.42cd
	b_4	c1	19.67defg	53.42ab	20.33bc	6.10ab	14.05bcd
		c2	16.11ghi	49.29bc	21.30a	5.70bc	15.15a
a_2	b_1	c1	23.56bcd	43.27def	11.92n	3.31lm	8.94ij
		c2	24.25bc	40.62efgh	14.90ij	4.25hij	10.68h
	b_2	c1	29.48a	48cd	13.14lm	4.04ijk	9.61i
		c2	18.11efghi	47.98cd	13.90kl	4.95def	8.79ij
	b_3	c1	21.03cdef	54.52a	15.99h	4.53efghi	12.00fg
		c2	17.11fghi	48.80c	17.76fg	5.22cd	12.55ef
	b_4	c1	20.91cdef	56.21c	20.74ab	6.63a	14.20bc
		c2	17.75fghi	55.61a	19.06de	4.38fghi	14.66ab
a_3	b_1	c1	22.40cde	39.86fg	12.12n	3.14m	8.75ij
		c2	29.09a	33.67hi	12.77mn	3.73jkl	9.07ij
	b_2	c1	17.32fghi	39.24fg	12.69mn	4.34fghi	8.43j
		c2	23.55bcd	33.32i	13.08lm	4.37fghi	8.90ij
	b_3	c1	29.75a	39.85fg	14.42jk	4.76defgh	9.60i
		c2	22.38cde	38.03gh	13.78kl	4.28ghij	9.55i
	b_4	c1	27.54ab	41.55efg	15.39hi	4.90defgh	11.19gh
		c2	19.53defg	43.57def	15.81hi	4.92def	10.96h

Means with the same letter are not significantly different from each other (P>0.05 ANOVA followed by DMRT).
Treatment a drought stress at 3 levels.
Treatment of algae b at 4 levels.
Salicylic acid treatment at 2 levels.

Chlorophyll b

This study results showed that the main effect of water deficit stress, seaweed extract and salicylic acid and some interactions between them were significant on chlorophyll b concentration (*Table 3*). The maximum chlorophyll b concentration (6.63 mg g FW^{-1}) was obtained when 150 g ha^{-1} seaweed extract and salicylic acid were applied on plants grown under mild water deficit stress. By contrast, the minimum value (3.14 mg g FW^{-}

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^{1}) was related to severe water deficit stress without seaweed or salicylic acid application (*Table 7*). Salicylic acid application on plants increases chlorophyll (as one of the main components of the photosynthetic apparatus affecting dry weight) and carotenoid content in control and stressed plants, indicating the ability of salicylic acid to improve plant growth. Similarly to the results of this experiment, it has been reported that salicylic acid increased chlorophyll and carotenoid content in barley, wheat, spinach, canola, tomato and pea (El-Tayeb, 2005). Increase in fresh weight, dry weight, root length, stem length and chlorophyll content on the account of seaweed extract has been reported previously (Sridhar and Rengasami, 2011).

Total chlorophyll

The results showed that the main effect of water deficit stress, seaweed extract and salicylic acid and some interactions between them were significant on total chlorophyll concentration (*Table 3*). The maximum total chlorophyll concentration (21.3 mg g FW^{-1}) was obtained when 150 $g.ha^{-1}$ seaweed extract was applied on plants grown under no water deficit stress. By contrast, the minimum value (11.9 mg g FW^{-1}) was related to mild water deficit stress without seaweed or salicylic acid application (*Table 7*). Seaweed-based fertilizers improve plants growth by providing more nitrogen, phosphorus, and potassium, as well as supplying micronutrients and secondary metabolites (Karthick et al., 2013). Studies on cucumber, after using seaweed extracts (red and green algae) indicated that fresh and dry weight and leaf area increased probably due to increased nitrogen concentration and improving soil physical conditions through providing more energy for microorganisms helps to improve availability and absorption of mineral nutrients. Increase in yield may also be related to some nutritional elements, especially iron, zinc and manganese in compost and potassium, calcium, magnesium, sulfur, and iron in seaweed extract. These elements can stimulate vegetative growth, chlorophyll biosynthesis, and photosynthesis, which in turn affect flowering and fruit production (Ahmed and Shalaby, 2012).

Anthocyanin

The results indicated that the main effect of water deficit stress, seaweed extract and salicylic acid and some interactions between them were significant on anthocyanin concentration (*Table 3*). The maximum anthocyanin concentration (56.2 mmol g FW^{-1}) was obtained when 150 $g.ha^{-1}$ seaweed extract and salicylic acid were applied on plants grown under mild water deficit stress. By contrast, the minimum value (28.3 mmol g FW^{-1}) was related to 50 $g.ha^{-1}$ seaweed extract and salicylic acid treatment on plants grown under no water deficit stress (*Table 7*). Flavonoids, flavones, and anthocyanins have anti-oxidant properties and it has been proven that their production and gene expression increase under stress conditions (Tang et al., 2006). In general, it has been shown that seaweeds affect antioxidant activity and chemical composition of plants grown under environmental stresses (Van Alstyne, et al., 2007).

Carotenoids

The results indicated that the main effect of water deficit stress, seaweed extract and salicylic acid and some interactions between them were significant on carotenoids content (*Table 3*). The maximum carotenoids content (29.7 mg g FW^{-1}) was obtained when 100 $g.ha^{-1}$ seaweed extract and salicylic acid were applied on plants grown under

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severe water deficit stress. By contrast, the minimum value (14.3 mg g FW^{-1}) was related to non-stressed plants without any seaweed extract or salicylic acid application (*Table 7*). The induction of carotenoid biosynthesis under stress conditions might be due to their protective role in photosynthetic systems. These pigments are responsible for neutralizing reactive oxygen species and preventing lipid peroxidation and ultimately oxidative stress. Carotenoids release large amounts of energy from photosystems (I) and (II) in the form of heat or chemical reactions which can maintain chloroplast membranes (Koyro, 2006). In a study carried out by Sivasankari et al. (2006), application of 20% seaweed liquid fertilizer (SLF) could increase shoot length, root length, fresh weight, dry weight, chlorophyll, carotenoid, shoots and root protein content, shoots and root amino acids content, alpha amylase and beta amylase activity. The improvement of growth parameters due to salicylic acid might be due to its effect on the photosynthetic apparatus, photosynthesis rate, rubisco enzyme activity, photosynthetic pigments concentration, stomatal conductance, antioxidant defense system, reduction of oxidative stress and ion leakage. An increase in cell membrane integrity, nitrogen metabolism, and mineral nutrition are also mentioned in various studies (El-Tayeb, 2005).

Proline

The results indicated that the main effect of water deficit stress, seaweed extract and salicylic acid and some interactions between them were significant on proline content (*Table 3*). The minimum proline content (79 mg g FW^{-1}) was obtained when no seaweed extract was applied on plants grown under no water deficit stress. By contrast, the maximum value (123.2 mg g FW^{-1}) was related to severe water deficit plants treated with 100 g.ha^{-1} seaweed extract (*Table 4*). In all stress levels, application of 100 or 150 g.ha^{-1} seaweed extract resulted in proline accumulation. Increasing in proline accumulation on the account of a particular treatment, such as seaweed extract, leads to increased resistance to drought stress, followed by higher yields (Koyro, 2006). Water stress cause chlorophyll degradation, and glutamate, which is a precursor of chlorophyll and proline, is transformed into proline, resulting in a reduction in the chlorophyll content (Lawlor and Cornic, 2009). Proline is known as an osmotic regulator that increases in response to salinity stress. One of the most important mechanisms in higher plants grown under salinity conditions is the accumulation of compounds such as proline. Proline accumulation is a primary defense response to maintaining osmotic pressure in cells. It has been reported that proline has a key role in osmotic regulation, protection of cell structures and neutralization of reactive oxygen species as well as malondialdehyde and the ascorbate peroxidase (Ashraf and Foolad, 2007). Another reason to increase the proline content in plants grown under water deficit stress is chlorophyll degradation. As a result, the concentration of these substances increases under stress conditions. In this study, in the plants grown under water deficit stress, the total chlorophyll content decreased whereas proline leaf concentration increased, which is consistent with the above hypothesis.

Seed yield

The results indicated that the main effect of water deficit stress, seaweed extract and salicylic acid and interaction between water deficit stress and seaweed extract was significant on seed yield (*Table 3*). The maximum seed yield (2343.3 kg ha^{-1}) was obtained when 100 g ha^{-1} seaweed extract was applied on non-stressed plants. By

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contrast, the minimum seed yield (90.2.5 kg ha^{-1}) was related to severe water deficit plants without seaweed extract application. In all stress levels, application of 100 or 150 g ha^{-1} seaweed extract could increase seed yield. Numerous studies have shown the positive effects of seaweed and biological fertilizers on growth and yield of plants. It is likely that seaweed extract application increases, absorption and storage of nutrients in different parts of the plants, including leaves and stems, which in turn results in increased yield (Ahmad and Khalili, 2006). In addition, it seems that increase in stress, reduces plant growth and nutrient content. Therefore, reduction in vegetative growth due to the reduction of osmotic potential, reproductive growth and finally seed yield is not surprising. Environmental stresses decrease water uptake, transpiration and stomatal closure, which lead to a decrease in growth (Ben-Asher et al., 2006). The results have shown that the application of green and red seaweed extracts and commercial extract of seaweed with compost, improve vegetative growth, dry and fresh weight and yield of cucumber (Ahmed and Shalaby, 2012). Increase in fresh and dry weight might be due to nitrogen availability and improving soil physical properties as well as improved oil microorganisms' activity on the account of seaweed application. Increase in yield may also be related to some nutritional elements, especially iron, zinc and manganese in compost and potassium, calcium, magnesium, sulfur and iron in seaweed extract. These elements can stimulate vegetative growth, chlorophyll biosynthesis and photosynthesis, which in turn affect flowering and fruit production (Ahmed and Shalaby, 2012). Seaweed-based fertilizers improve plant growth through providing more nitrogen, phosphorus and potassium, as well as supplying micronutrients and secondary metabolites. In a study carried out by (Ahmed and Shalaby, 2012) seaweed extract application could increase vegetative growth and fruit yield of cucumber.

Conclusion

Application of salicylic acid and seaweed extract by increasing the activity of anti-oxidant enzymes (including catalase, ascorbate peroxidase and peroxidase) and plant proline, have an important role in the reduction of oxygen radicals (hydroxyl, peroxide hydrogen, and super oxide) that were produced due to water stress. Using salicylic acid and seaweed extract enhance the biosynthesis and protection of photosynthetic pigments under water stress and result in higher chlorophyll concentration. Thus it seems that foliar application of salicylic acid under water stress can promote water saving which is very critical at current situation of water scarcity for agriculture and can mitigate the damages of water stress which may finally result in higher crop yield.

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