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The Effects of Indole Butyric Acid and Seaweed (*Posidonia oceanic*) and their Mixture in Improving Photosynthetic Pigments of Salt-Stressed Wheat Cultivar (Marjawi)

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A B S T R A C T

Salt stress is one of the most limiting factors in the production of agricultural crops. This study was conducted to test the effect of different salinity levels at concentrations (0.0, 100, 200, and 300 mM) of sodium chloride on the photosynthetic pigments content of *Triticum aestivum* (Marjawi cultivar), and attempting to treat using several transactions of different treatments, include: spraying at 100 mg/L of Indole3- Butyric Acid (seedlings 2 weeks old), adding crude powder of seaweed *Posidonia oceanica* 25 g/pot (before agriculture), and (mixture) of a crude powder of *P.oceanic* 25 g/pot + spraying IBA, with three replications according to a completely randomized design. The results showed a significant decrease in the content of chlorophyll (a, b), carotenoids, and Total pigments with increasing NaCl concentrations, compared to a control. Moreover, spraying with (IBA) decrease significantly the negative effect of salinity. Also result indicated that adding crude powder of *P.oceanica* was not successful in reducing salt stress, in addition, the result showed that the mixture was superior in recording the best rates in improving the photosynthetic pigments content of wheat salt-stressed, This study concluded that harmful effects of salinity can be mitigated using the mixed treatment.

1 Introduction

Photosynthesis is a sensitive process to various abiotic and biotic stresses in plants, damaged photosynthesis system causes a decrease in the rate of photosynthesis and impairs the productivity of agricultural crops (Sharma *et al*., 2022). These changes were associated with the production of reactive oxygen species (ROS), resulting from these pressures (Pandey *et al*., 2022). Salt stress is one of the more abiotic stresses that poses a major threat to the desertification of arable land all across the globe. induces osmotic stress, and ionic stress, both of which impair plant growth and metabolism (Wang *et al*., 2022; Kapadia *et al*., 2022). In addition produce oxygen species and reactive nitrogen species (RNS), which is an imbalance in the homeostasis redox of the cell (Singh *et al*., 2022). Wheat is considered one of the food crops that are physiologically affected by salt stress, which inhibits the process of nutrient uptake (Ca, Mg, Fe, N, K, P, and Zn), plant water uptake, photosynthesis, protein synthesis, and enzymatic activity, (Kesh *et al*., 2022; Lata *et al*., 2022). *Posidonia oceanica* (L.) is classified as an endemic seaweed to libyan coasts (Ezziany *et al*., 2015), distributed at sea level and reaches a depth of 38 m (Bay,1984). The dead leaves are accumulated on the beaches in huge quantities as waste material, causing great environmental and economical problems (Dural *et al*., 2011). Seaweed has been reported as a bio-fertilizer in agricultural fields, especially in salt soils, because it contains macro- and micro-nutrients that

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stimulate seed germination and improve water and nutrient absorption by rebalancing the ionic and metabolic status (Nabti *et al*., 2017; Khan *et al*., 2022). Plant hormones improve tolerance to abiotic stresses by inhibiting the accumulation of reactive (ROS), and stimulating the expression of stress-specific genes (Rachappanavar *et al*., 2022). For example, chlorophyll (a, b), total chlorophyll, and carotenoids content were enhanced in a wheat plant salt-stressed 150 mM of NaCl, through the application of salicylic acid and gibberellic acid (Iqbal *et al*., 2022). Also, Abbas *et al*., (2022) noted an increase in stomatal conductance and chlorophyll (a, b), and total chlorophyll contents, of wheat SARC1 and SARC5 genotypes, using a combined application of potassium and humic acid. Moreover, spraying foliar ascobin up to 1000 ppm promoted increased chlorophyll, protein, relative water content, and cell membrane stability (Dadrwal *et al*., 2022).

Therefore, our aims were to:

Evaluation effect of different salinity levels on the content of photosynthetic pigments (chlorophyll a, b and carotenoids) in leaves of wheat.

investigate whether (indole-3-butyric acid or Posidonia oceanica or a mixture) applications are more effective in alleviating salinity stress in cultivar wheat Marjawi.

2 Materials and Methods

2.1 Seed Selection:

Seeds of wheat cultivar Marjawi were obtained, From the Department of Crops / faculty of Agriculture / Omar Al-Mukhtar University, were cleaned of impurities, and viability was tested by soaking in distilled water to get rid of empty seeds floating on the surface, were soaked in 1% sodium hypochloride solution for 3 minutes, and washed with distilled water (Dafaallah *et al*., 2019).

2.2 Collection and Preparation of Seaweed Samples:

Fresh *Posidonia oceanica* (leaves and rhizomes) were collected from the coastline of Al-Hamamah, north of Al-Bayda city / Al-Jabal Al-Akhdar / Libya, and classified in the Department of Biology / Faculty of Education / Omar Al-Mukhtar University, They were washed and rinsed with distilled water in order to eliminate sand and plankton, after that, they were dried at room temperature, ground by an electric grinder and kept until use.

2.3 Preparation of the Used Solutions:

The brine was prepared using sodium chloride salt NaCl (100, 200, and 300), as follows:

1m Mol = molecular weight of the solute / 1000 * concentration

100 m Mol=58.5 / 1000* 100

100 m Mol=5.85 g/L

take weight 5.85g of NaCl salt, then dissolve it in a standard flask of capacity 1000 ml and complete the volume with distilled water to the mark and the same steps for the rest of the concentrations.

Then prepare the concentration of 100 mg / L of indole3-butyric acid (IBA).

2.4 Pot Experiments:

Sterilized seeds germinated in sterile petri dishes containing a damp sterile filter paper, sterile distilled water was added at intervals to keep the paper and germinated seeds wet, dishes were incubated at 30 ºC for 2-3 days or until the radicals length were 2-3 cm. Ten germinated seeds were planted into each pot. Five kilograms of dried clay-sandy soil were put into pots. the ratio of 2:1 (weight to weight), ten germinated seeds were planted into each pot. When the growing plants were about 12 cm in length. They were thinned down to five per pot. Pots were divided into four groups, and each of them was irrigated with different concentrations of saline solutions (0.0, 100, 200 and 300 m M NaCl) from the beginning of agriculture to 60 days:

- The first group: was irrigated with the saline solution to reach the salinization level.

- The second group: spraying the seedlings (2 weeks old) three times with 100 mg/L of Indole3- Butyric Acid (IBA) (10 cm3 per pot)+ (saline solution).

- The third group: was treated by adding 25g/pot of *Posidonia oceanica* crude powder + (saline solution).

- The fourth group: a mixture (the crude powder 25g / pot of *Posidonia oceanica* + spraying the seedlings IBA) + (saline solution).

2.5 Photosynthetic Pigment:

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were extracted from fresh weight of leaves wheat Marjawi in 85% aqueous acetone to a certain concentration for spectrophotometric measurements. were determined spectrophotometrically according to (Metzner *et al*., 1965).The pigments extract was measured against a black of pure 85% aqueous acetone at three wavelengths of 452.5, 644 and 663 nm. After 60 days of sowing. using following equations:

Chlorophyll = 10.3* 663 – 0.918 *644 = mg/ml

Chlorophyll b = 19.7 *644 – 3.87 *663 = mg/ml

Carotenoids = 4.2 *452.5– 0.0264 *chl. a+ 0.4260 *chl. b

Statistical Analysis:

The study experiences were designed according to the complete random design (CRD). Statistical analysis was performed using Minitab 17 program and ANOVA variance analysis tables. The averages were compared using Tukey's test at P <0.05 (Salih and Abdulrraziq, 2021).

3 Results and Discussion

3.1 An effect of Salinity Levels on Photosynthetic Pigments of Wheat.

Current work shows in table (1) the effect of salinity levels (0.0, 100, 200, and 300mM) on photosynthetic pigments of the cultivar wheat (Marjawi). Chlorophyll content (a, b), carotenoids, and total pigments decreased compared to a control, after 60 days of sowing. The concentration of 100 mM caused a decrease of chlorophyll (a, b), carotenoids, and total pigments from (100%) of a control to (79.0%) of chlorophyll (a, b), (74.3%) of carotenoids and total pigments by up to (78.3 %). The rates of decrease of photosynthetic pigments increased with increasing salinity. The concentration of 200 mM recorded a decrease in chlorophyll (a, b), carotenoids, and total pigments with (60.4%, 58.8%, 51.3% and 58.2 %) respectively, while the harmful effect of saline stress was clear at a concentration of 300mM, where recorded the highest inhibition rates of chlorophyll (a, b), carotenoids, and Total pigments with (34.8%, 46.5%, 32.8%, and 37. 5%) respectively. The detrimental effects of salt stress on photosynthetic pigments were reported in studies on wheat (Ahanger *et al*., 2019; Kesh *et al*., 2022). This was probably due to an inhibition of ribulose-1,5-bisphosphate enzyme or formation of proteolytic enzymes such as chlorophyllase, Responsible for chlorophyll synthesis (Santos, 2004), thus resulting in a reduction of Calvin cycle enzymes, and inactivation of photosystem II (PSII) reaction centers (Ma *et al*., 2020).

Table (1): Effect of salinity levels and different treatments on photosynthetic pigments of wheat.

Treatments	NaCl	Chlorophyll a		Chlorophyll b		carotenoids		Total pigments	
		mg/g	%	mg/g	%	mg/g	%	mg/g	%
NaCl	0.0	4.3 ab	100	2.04 cd	100	1.52 b	100	7.86 d	100
	100	3.4 abcde	79.0	1.63 e	79.9	1.13 cd	74.3	6.16 g	78.3
	200	2.6 bcde	60.4	1.20 gh	58.8	0.78 f	51.3	4.58 k	58.2
	300	1.5 e	34.8	0.95 j	46.5	0.50 hi	32.8	2.95 n	37.5
IBA	0.0	4.5 ab	104.6	2.32 b	113.7	1.62 b	106.5	8.44 b	107.3
	100	3.9 abcd	90.6	1.88 d	92.0	1.25 c	82.2	7.03 f	89.4
	200	3.0 bcde	69.7	1.35 fg	66.0	0.96 e	63.1	5.31 i	67.55
	300	2.0 de	46.5	1.00 j	49.0	0.70 fg	46.0	3.7 m	47.0
Crude powder	0.0	4.2 abc	97.6	2.05 c	100.4	1.50 b	98.6	7.75 e	98.6
	100	3.0 bcde	69.7	1.51 ef	74.0	1.17 cd	76.9	5.68 h	72.2
	200	2.2 cde	51.0	1.18 hi	57.8	0.60 gh	39.4	3.98 l	50.6
	300	1.6 e	37.2	1.02 ij	50.0	0.42 i	27.6	3.04 n	38.5
Mixed	0.0	5.2 a	120.9	2.53 a	124.0	1.80 a	118.4	9.53 a	121.2
	100	4.5 ab	104.6	2.00 cd	98.0	1.51 b	99.3	8.01 c	101.9
	200	3.7 abcd	86.0	1.42 f	69.6	1.09 de	71.7	6.21 g	79.0
	300	2.9 bcde	67.4	1.21 gh	59.3	0.81 f	53.2	4.92 j	62.5

3.2 An Effect of Spraying Application of (IBA) on Photosynthetic Pigments of Salt-Stressed of Wheat.

The results also in table (1) showed that spraying of Indole3- Butyric acid of wheat under salinity levels significantly increased the content of photosynthetic pigments, which increased by (11.6%, 12.1%, 7.9% and 11.1%) of treatment (IBA + 100 mM NaCl), (9.3%, 7.2%, 11.8% and 9.35%) of treatment (IBA + 200 mM NaCl), and (11.7%, 2.5%, 13.2% and 9.5%) treatment (IBA + 300 mM NaCl) of chlorophyll contents (a, b), carotenoids, and total pigments respectively, compared to the untreated plant. Our results are consistent with those (Iqbal *et al*., 2022; Yilmaz *et al*., 2022), which found Foliar application of phytohormones of (GA3, and SA) improved the growth traits of salinized wheat. Maybe because they enhance essential inorganic nutrients as well as maintain membrane permeability, balance, and osmotic capacity in plants and Increase activity of antioxidant enzymes that regulate ROS levels (kaya *et al*., 2010; Piotrowska-Niczyporuk *et al*., 2018), which Improves efficiency of photosystem II (Aroca *et al*.,2013).

3.3 An Effect of Adding a Crude Powder of P. oceanica on Photosynthetic Pigments of Salt-Stressed of Wheat.

Results showed that adding a crude powder of *P.oceanic* (25 g /pot) had no significant effect compared with different salinity levels, on chlorophyll (a, b), carotenoids, and total pigments, the achieved results were in accordance with (Latique *et al*., 2013) who found that high concentrations of *Ulva rigida* extract had no effect in enhancing chlorophyll content compared to control, this result differed with a study (Castaldi and Melis, 2002) which confirmed that can be used *P.oceanic* as an agricultural fertilizer because it contains a good proportion of carbon, nitrogen, and phosphorus. The reason for ineffectiveness of *P.oceanica* may be the high levels of growth-promoting substances such as indole-3-acetic acid (IAA), gibberellins A and B, cytokinins (Kalaivanan and Venkatesalu, 2012), or chromosome abnormalities caused by effect of seaweed (Hamouda *et al*., 2022).

3.4 An Effect of Mixed (crude powder of P.oceanica and IBA) Photosynthetic Pigments of Salt-Stressed of Wheat.

The application of mixed(crude powder of *Posidonia oceanica* + IBA) was the most effective treatment, significantly highest increased the content of the photosynthetic pigments, by (25.6%, 18.1%, 25.0%, and 23.6%)of content chlorophyll (a, b), carotenoids, and total chlorophyll respectively, as compared with 100 mM NaCl, and with an increase in the concentration of NaCl to 200 and 300mM, treatment Mixed recorded an increase by (25.6%, 10.8%, 20.4%, 20.8%) and (32.6%, 12.8%, 20.4%, 25.0%) of content chlorophyll (a, b), carotenoids, and total pigments for two concentrations, respectively.

4 Conclusion

Salt stress leads to disruption of chlorophyll synthesis, This was clear from this study's results on photosynthetic pigments content of wheat under different salinity levels. Treatment of the (mixture) of adding crude powder of *Posidonia oceanica* 25 g/pot, and spraying with 100 mg/L of IBA was the most effective treatment in alleviating, salt stress and increasing improving the photosynthetic pigment content, followed by the spraying of IBA, while the treatment of adding 25g/pot crude powder of *P.oceanica* was not successful in reducing salt stress. So this study recommends a combination of treatments as a way to overcome salinity damage in wheat (Marjawi).

Conflict of Interest: The authors declare that there are no conflicts of interest**.**

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