



The effect of foliar application of plant growth regulators on functional and qualitative characteristics of wheat (*Triticum aestivum* L.) under salinity and drought stress conditions

Hamid Reza Tajdari¹ · Ali Soleymani² · Nosratolah Montajabi³ · Mohammad Reza Naderi Darbaghshahi¹ · Hamid Reza Javanmard¹

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Abstract

This study aimed to investigate the effect of salinity and water stress on the physiological and functional characteristics of winter wheat (*Triticum aestivum* L.) under the foliar application of plant growth regulators (PGRs). The experiment was carried out as a split plot based on a randomized complete block design with three replications in two environments. In each environment, water stress at two irrigation levels (after 90 and 120 mm of pan evaporation) and with two EC of 1.5 and 10 dS/m in the main plots and spraying of PGRs including salicylic acid (SA), gibberellic acid (GA₃), and cytokinins (CK) (purine) content with a concentration of 100 ppm and the control treatment (spraying solution with normal water) were placed in subplots. Results indicated that all treatments caused significant increases in functional and qualitative characteristics and yield of *Triticum aestivum* L. The saline environment and irrigation level after 120 mm of pan evaporation caused a reduction in grain yield in all traits except for seed proline, seed nitrogen content, and seed protein content. Also, the combined foliar application of GA₃ + CK + SA increased yield in most traits. The highest RWC of flag leaves was observed in the foliar application of GA₃ + SA (3.36 kg/ha) and then in the foliar application of GA₃ + SA + CK (57.87 kg/ha). GA₃ interacts with PGR spraying to balance another development under saline and non-saline conditions.

Keywords Cytokinin · Gibberellic acid · Salicylic acid · Water stress · Saline stress · 1000-grain weight

Introduction

Agricultural science requires the most research in the field of crop adaptation to climate change in the current situation. Winter wheat (*Triticum aestivum* L.) is one of the most important crops in terms of cultivated area and production rate in the world and plays an important role in providing the food needs of human societies (Kinga et al. 2020). Among

the environmental factors, saline stress has received serious attention from researchers in Iran for about 50 years. About eight million hectares of irrigated land are used to produce about 12% of Iran's produce. About 6.8 million hectares of these 8 million hectares have various restrictions, including salinity (Roozitalab et al. 2018). The salinization of water and soil resources has increased attention to research related to ways to improve the yield of agricultural plants in saline conditions (Pirasteh-Anosheh et al. 2015). Salinity causes the phenomenon of physiological dryness and directly damages the internal structure of the plant (El Karamany et al. 2019). In arid and semiarid regions, reducing the quantity and quality of water is one of the issues limiting production (Mosaffa and Sepaskhah 2019). Water stress is another important factor limiting the production of agricultural products, which strongly affects food security (Zhang et al. 2018). It also affects leaf water potential, photosynthetic pigments, stomatal conductance, and absorption of elements, especially phosphorus and nitrogen (Anli et al. 2020; Gowthami 2022; Samad et al. 2023).

✉ Ali Soleymani
a_soleymani@khuisf.ac.ir

¹ Department of Agronomy and Plant Breeding, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran

² Plant Improvement and Seed Production Research Center, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran

³ Soil and Water Research Department, Isfahan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Isfahan, Iran

Plant growth regulators (PGRs) are one of the solutions to increase the yield of agricultural plants under different stress conditions (Zhuang et al. 2019; Correia et al. 2020; Desta and Amare 2021). Its yield depends on the intensity, genotype, time of application, concentration used, and intensity of the stress level (Quamruzzaman et al. 2021). PGRs include salicylic acid (SA), gibberellic acid (GA_3), and cytokinins (CK). GA_3 has various effects on plants, inducing grain germination, and stimulating the production of important hydrolyzing enzymes in the germination of leguminous grains (Nagar et al. 2021). Chauhan et al. (2019), Al-Harathi et al. (2021), Ali et al. (2021), and Attia et al. (2022) certified the alleviating effect of GA_3 on plant tolerance to salt stress. SA, as one of the most important phytohormones, plays a crucial and emerging role in plants' response to salt stress and supports them against many water stresses. Ma et al. (2017), Xu et al. (2022), and Yang et al. (2023) revealed the protective roles of SA priming in plants' response to salt stress. CK reduces the adverse effects of non-living stresses and also causes cell division, removal of apical dominance, stem differentiation, and delaying aging (Veselov et al. 2017). Hai et al. (2020) acknowledged the role of CK in plant response to water stress.

The vast expanse of dry and salty lands in Isfahan province, especially in the northern plains of Golpayegan city, are harvested in a barren form or with a small area and a negligible yield. Water salinity in the region is one of the limiting factors for agricultural activities, which if reduced and adjusted with the help of growth regulators, will help to increase productivity per unit area, reduce unemployment and job creation, reverse migration, and ultimately increase economy growth at the level of the region and the country. Until now, there has been no solution to the problem mentioned, which was a research gap and the main objective of this study. Modifying the structure of the soil in a large area is practically impossible. In the current situation, it is vital to adopt agricultural management methods and the use of PGRs. The combined use of several PGRs on wheat plants in different environments and with different types and levels of stress, which have not yet experienced any type of PGRs, is an innovative method. In general, investigating the impact of biological stresses on the productivity and quality of crops and also determining the appropriate solution to reduce the negative effects of stresses and improve the quality of the produced product can be considered an effective step in the direction of improving and developing agricultural plants, including *Triticum aestivum L.* The climate of the Golpayegan region (relatively cold winters ($T_{\min} = -21$ °C) and hot and dry summers ($T_{\max} = +37$ °C) and a mean rainfall of about 300 mm) is completely suitable for wheat cultivation. Therefore, the results of this research can be generalized to other places where this product is cultivated. The hypotheses of this research can be stated as follows: a) Foliar spraying

with PGRs can cause resistance to environmental stresses (salinity and drought); b) the interaction of PGRs can reduce the effects of environmental stresses (salinity and drought); and c) environmental stresses (salinity and drought) can have a negative effect on the yield and yield components of the wheat plant. This experiment aimed to determine how PGRs affect the functional and qualitative characteristics of winter wheat (*Triticum aestivum L.*) under conditions of saline and water stress. Also, obtaining the best application rate of these regulators in creating resistance in wheat plants by spraying is another goal of this study.

Materials and methods

Experimental environment

Experiments were carried out in the crop year of 2022–2023 in two non-saline environments (irrigation with EC of 1.5 dS/m) at the agricultural and animal science research station with latitude ($33^{\circ} 28'$ north) and longitude ($50^{\circ} 17'$ east) and saline environment (irrigation with EC of 10 dS/m) in the fields of the northern plain of Golpayegan city with latitude ($33^{\circ} 30'$ north) and longitude ($50^{\circ} 23'$ east). The study area represents a semiarid climate based on the Köppen (1936) method. Its annual average temperature and rainfall are 18 °C and 300 mm, respectively (Table 1).

Agricultural operations

Soil samples were taken from the plot of land located in the agricultural research station and farms before planting. To conduct the soil test, 10 samples were taken randomly, one kilogram each, and then the soil samples were completely mixed, and a 1 kg sample was selected as a soil sample and sent to the soil science laboratory of Isfahan Agricultural Research Center. Table 2 presents the physicochemical characteristics of the soil at a depth of 0–30 cm. To strengthen the soil and provide the elements required by the plant and according to the result of the soil fertilizer test, before planting, all the required phosphorus fertilizers were added to the soil from the triple superphosphate source at the rate of (non-saline field: 170 kg/ha; saline field: 190 kg/ha), potash fertilizer in the form of potassium sulfate at the rate (non-saline field: 150 kg/ha; saline field: 170 kg/ha), and one-third of nitrogen fertilizer in the form of urea at the rate of (non-saline field: 120 kg/ha; saline field: 150 kg/ha). The planting operation was done with a density of 400 plants per hectare in the non-saline field on October 23, 2021, and in the salt field on December 6, 2021.

Table 1 Climate characteristics of the region in the cropping seasons 2021–2022

Month	Absolute maximum temperature (°C)	Absolute minimum temperature (°C)	Mean monthly evaporation (mm)	Mean monthly rainfall (mm)
November	15.5	3.9	0	52.1
December	12.9	0.8	0	41.9
January	8.9	−2.5	0	55.7
February	9.7	−2.0	0	21.5
March	3.5	3.5	0	40.5
April	21.3	6.8	205.3	2.3
May	24.7	10.9	275.6	7
Jun	31.9	16.6	368.5	0
July	35.9	19.5	421.5	0

Table 2 Physicochemical characteristics of the soil of non-saline and saline fields in Golpayegan in the season's year 2021–2022

Field	Soil depth (cm)	pH	Organic carbon (%)	Nitrogen (%)	Phosphorus (mg/kg)	Potassium (mg/kg)	Clay (%)	Sand (%)	Silt (%)	Soil texture
Non-saline	0–30	7.19	16.36	175	0.62	0.06	19	27	54	Loami silt
Salinity	0–30	0.04	11	216	7.62	0.55	20	29	51	Loami silt

Experimental design and treatments

The experiment was carried out as a split plot based on a randomized complete block design (RCBD) with three replications in two environments. Drought stress was executed at three irrigation levels after evaporation of 90 and 120 mm from Class A evaporation pan as non-stress and severe stress, respectively. In each environment, water stress at two irrigation levels (after 90 and 120 mm of pan evaporation) in the main plots and spraying of PGRs SA, GA₃, and CK (purine) with a concentration of 100 ppm, and the control treatment (spraying solution with normal water) were placed in subplots. PGR spraying was done in the pollination stage (to reduce the effects of heat stress on the results of flowering and grain formation of this stage). Foliar application was done with PGRs including GA₃, SA, and CK by spraying in two stages: 1—double ridge, 2—terminal spikelet stage in the amount of 25^{cc} of PGRs solution in 5 L of water per plot at the beginning of the morning. The measured traits include the relative water content (RWC) of flag leaves (Soltys-Kalina et al. 2016), proline content in flag leaves, the no. of spikes/m², the no. of spikelet and grains per spike, the thousand seed weight (PMG), biological yield (Yb), and seed content (nitrogen, protein, and gluten-free) (Fariñas et al. 2019).

Sampling and measurement

Sampling was done in five steps. The first stage of sampling was done in the non-salinity field 135 days after germination

and in the saline field 75 days after germination. The second stage and then every 15 days, and in the final harvesting stage, a sampling stage was done. Sampling was done by a 1 × 1 square meter, and the samples were placed in paper envelopes with a code written for each one. After measuring the traits, the envelopes were placed in an oven at 75 °C for 72 h and the results were recorded by a digital scale with a sensitivity of one hundredth. Also, in the final harvest, 1m² from the middle rows of each plot was removed by removing the marginal effect from the soil surface and the desired traits were measured.

Statistical analysis

All the obtained data were analyzed using statistical software SAS version 9.2 and the MSTAT-C Program version 7.0.1. To compare the means, Duncan's multiple range tests (DMRT) were used at the 5% level. STATGRAPHICS software version 18.1.001 was used to obtain correlation coefficients. Excel software was used to draw graphs. Bartlett's test was used for the combined analysis of traits and the possibility of combining the two environments (Table 3).

Results

The relative water content of flag leaves

The interaction effect of environment × irrigation levels × PGRs on the RWC of the flag leaf was significant at the

Table 3 Results of Bartlett's test for the studied traits

Traits	Number of spikes	Number of spikelet per spike	Number of seeds per spike	1000-grain weight (g)	Biological yield (kg/ha)
F	1.15 ^{ns}	1.32 ^{ns}	1.51 ^{ns}	1.36 ^{ns}	1.09 ^{ns}
Traits	Relative leaf water content (%)	Flag leaf proline content (mg/g)	Seed protein percentage (%)	Seed nitrogen percentage (%)	Grain gluten percentage (%)
F	1.58 ^{ns}	1.40 ^{ns}	1.08 ^{ns}	1.07 ^{ns}	1.17 ^{ns}

5% probability level (Table 4). In the current research, the highest RWC of flag leaves was obtained in the condition of non-saline, irrigation level after 90 mm of pan evaporation, and PGRs including GA₃ + CK + SA at the rate of 74.77%. Its difference with other foliar applications was significant except for CK + SA and GA₃ + SA (Fig. 1). The interaction effect of environmental levels × PGRs caused a 29% increase in the RWC of the flag leaf.

Proline content in flag leaves

The interaction effect of environmental levels × PGRs on the proline content of flag leaves was significant at the 5% level (Table 4). The highest content of proline in flag leaf was obtained in the conditions of a saline environment, irrigation level after 120 mm of pan evaporation, and PGRs including foliar application of GA₃ + CK + SA at the rate of 18.59 mg/g. The difference between this treatment with a non-saline environment, irrigation level after 90 mm of pan evaporation, and other PGRs and no PGRs was significant except for foliar application of GA₃ + SA (Fig. 2). The interaction effect of environment × irrigation levels × PGRs caused a 69% increase.

The number of spikes per square meter

The interaction effect of environment × irrigation levels on the no. of spikes/m² was significant at the 5% probability level (Table 4). The highest no. of spikes/m² was obtained in non-salinity, and the irrigation level after 90 mm of pan evaporation was equal to 436.26. It was significantly different from other treatments (Table 7). The interaction effect of environment × irrigation levels caused a 30% increase in the no. of spikes/m². The interaction effect of environment × PGRs on the no. of spikes/m² was significant at the 5% probability level (Table 4). The highest no. of spikes/m² was obtained in non-salinity and PGRs including GA₃ + CK + SA at the rate of 452.19. It was significantly different from other treatments (Table 8). The interaction of environment × PGRs caused a 43% increase in the no. of spikes/m².

No. of spikelet per spike

The effect of the environment on the no. of spikelet per spike was significant at the probability level of one percent (Table 4). The maximum no. of spikelet in the spike was obtained in the condition of non-salinity equal to 20.21. It was significantly different from the salinity environment (Table 5). Saline stress caused a 5% decrease in the no. of spikelet per spike. The effect of PGRs on the no. of spikelet per spike was significant at the 5% probability level (Table 4). The highest no. of spikelet per spike was obtained by PGRs including GA₃ + CK + SA at the rate of 20.63. It showed a significant difference with other PGRs and non-foliar spraying treatments (Table 5). PGRs including GA₃ + CK + SA increased the no. of spikelet per spike by 14% compared to other foliar application treatments and the control treatment.

The number of grains per spikelet

The effect of PGRs on the number of grains per spikelet was significant at the 5% probability level (Table 4). The highest number of grains per spikelet was obtained by PGRs including GA₃ + CK and GA₃ + CK + SA, equal to 55.19 and 55.05, respectively. This treatment, except for GA application, showed a significant difference from other PGRs and non-foliar spraying treatments (Table 5). PGRs including GA₃ + CK and GA₃ + CK + SA increased the no. of grains per spike by 25% compared to the control treatment. The interaction effect of environment × irrigation levels on the no. of grains per spike was significant at the 5% probability level (Table 4). The highest no. of grains per spike in the condition of no salinity and irrigation level after 90 mm of pan evaporation was obtained at the rate of 59.00, and the difference with other treatments was significant (Table 6). The interaction of environment × irrigation levels increased the no. of grains per spike by 28%.

The thousand seed weight (PMG)

The interaction effect of environment × irrigation levels on PMG was significant at the 5% probability level (Table 4).

Table 4 Composite analysis of physiological traits in two saline and non-saline environments under water stress and foliar application of PGRs

S. O. V	df	Relative leaf water content (%)	Flag leaf proline content (mg/g)	No. of spikes	No. of spikelets per spike	No. of seeds per spike	PMG (g)	BY (kg/ha)	Seed nitrogen percentage (%)	Seed protein percentage (%)	Grain gluten percentage (%)
Environment	1	3751.38**	729.36**	225,857.71**	28.90**	4335.88**	287.21**	581,829,690.04**	14.55**	475.96**	1165.73**
Rep in Environment	4	36.40	10.23	1836.27	1.03	114.95	24.44	3,326,688.08	0.17	5.49	3.77
Irrigation	1	739.20ns	63.16ns	27,594.27ns	1.27ns	230.86ns	620.52ns	247,645,201.50*	17.03ns	557.09ns	50.13ns
Environment × Irrigation	1	58.73*	20.30**	136.64ns	0.26ns	25.35ns	12.32ns	52,547.04**	0.29ns	9.32ns	9.52*
Error a	4	3.17	0.75	487.02	0.13	3.91	2.53	2,193,971.58	0.19	6.19	0.61
Regulator	7	51.69ns	54.31**	13,853.16**	13.92**	275.25**	14.78**	35,145,358.59**	6.61**	216.40**	71.76**
Environment × Regulator	7	14.84**	2.53**	714.14*	0.08ns	0.90ns	1.76ns	1,492,469.59ns	0.03ns	0.95ns	7.33*
Irrigation × Regulator	7	11.09ns	1.53ns	322.67ns	0.03	1.57	0.65*	372,557.29**	0.04*	1.32*	9.58ns
Error b	56	3.02	0.68	274.53	0.22	22.26	3.24	938,725.71	0.23	7.61	3.02
(%) C.V		2.66	6.53	4.48	2.40	9.21	4.85	5.21	13.02	13.03	7.01

RWC Relative leaf water content, BY Biological yield, PMG 1000-grain weight, S.O.V Source of variations, C.V coefficient of variations
 *, **, and ns represent significance at 5% and 1% probability levels and are not significant, respectively

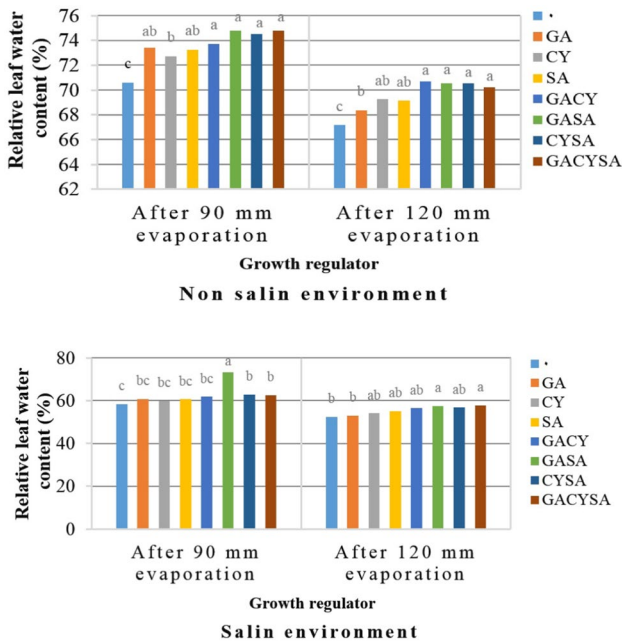


Fig. 1 Interaction effect of environment×irrigation levels×PGRs on relative leaf water content in both saline and non-saline environments

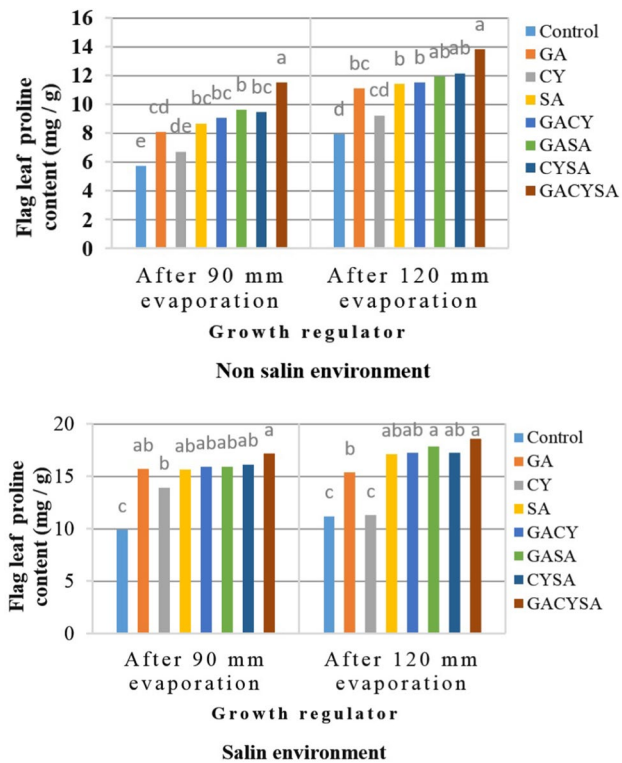


Fig. 2 Interaction effect of environment×irrigation levels×PGRs on flag leaf proline content in both saline and non-saline environments

The maximum PMG in the condition of no salinity and irrigation level after 90 mm of pan evaporation was obtained at the rate of 41.71 gr (Table 6). The interaction of environment×irrigation levels increased the RWC of flag leaves by 20%. The interaction effect of irrigation levels×PGRs on PMG was significant at the 5% probability level (Table 4). The highest weight of PMG was obtained at the irrigation level after 90 mm of pan evaporation and foliar application with PGRs including GA₃+CK at the rate of 40.90 gr (Table 7). The interaction between irrigation levels and PGRs caused an 18% increase in PMG.

Biological yield

The interaction effect of environment×irrigation levels on Yb was significant at the 5% probability level (Table 4). The highest Yb was achieved in the absence of salinity and the irrigation level after 90 mm of pan evaporation at the rate of 22,625.33 kg/ha, which was significantly different from the saline and irrigation environment at the irrigation level after 120 mm of pan evaporation (Table 5). The interaction of environment×irrigation levels increased the Yb by 35%. The interaction effect of irrigation levels×PGRs on Yb was significant at the probability level of 1% (Table 4). The highest Yb was achieved under irrigation conditions at the irrigation level after 90 mm of pan evaporation and PGRs including GA₃+CK+SA at the rate of 22,114.5 kg/ha. The difference between this treatment with the irrigation level after 120 mm of pan evaporation and other treatments of foliar spraying and no foliar spraying was significant (Table 8). The irrigation levels×PGRs increased the Yb by 38%. The foliar application of SA and GA (100 ppm) priming led to an increase in Yb under full irrigation conditions.

Grain nitrogen percentage

The effect of the environment on grain nitrogen percentage was significant at the probability level of 1% (Table 4). The highest percentage of grain nitrogen in saline conditions was 4.09%, which was significantly different from the non-saline environment (Table 5). Saline stress caused an increase of 18% in grain nitrogen. The interaction effect of irrigation levels×PGRs was significant at the 5% probability level (Table 4). The highest percentage of grain nitrogen at the irrigation level after 120 mm of pan evaporation was achieved by evaporation and PGRs including GA₃+CK+SA at the rate of 5.04%. This treatment showed a significant difference between evaporation and other foliar spraying and non-foliar spraying treatments with irrigation level after 90 mm of pan evaporation (Table 8). The irrigation level after 120 mm of pan evaporation and PGRs including GA₃+CK+SA increased grain nitrogen by 63%.

Table 5 Results of mean comparisons of physiological traits in two saline and non-saline environments, water stress, and foliar application of PGRs

Treatment	Relative leaf water content (%)	Flag leaf proline content (mg/g)	No. of spikes	No. of spikelets per spike	No. of seeds per spike	PMG (g)	BY (kg/ha)	Seed nitrogen percentage (%)	Seed protein percentage (%)	Grain gluten percentage (%)
Environment										
non-saline	71.47a	9.88b	418.12a	20.21a	57.97a	38.81a	21,042.6a	3.32b	18/97b	28.27a
Saline	58.97b	15.39a	321.11b	19.11b	44.53b	35.35b	16,118.9b	4.09a	23/42a	21.30b
Irrigation levels (mm)										
90	68.00a	11.82a	386.57a	19.77a	52.80a	39.62a	20,186.88a	3.28a	18/79a	25.51a
120	62.45a	13.44a	352.66a	19.54a	49.70a	34.54a	16,974.63b	4.13a	23/61a	24.07a
Regulator										
Control	62.14c	8.69d	303.60d	17.56f	41.01f	35.49d	15,005.92e	2.16f	12/36h	20.91d
GA	63.87bc	12.57b	357.04c	19.75c	54.87a	38.58a	17,548.00d	3.65cd	20/9e	23.76bc
CK	64.00bc	10.30c	358.00c	19.46d	51.75c	36.44cd	18,283.42cd	3.53de	20/17f	23.10cd
SA	64.54bc	13.20b	358.86c	18.67e	48.67e	35.74d	19,319.83abc	3.46e	19/82g	25.44bc
GACK	65.69abc	13.43b	397.65ab	20.68a	55.19a	38.19ab	18,852.17c	4.28b	24/49b	26.01b
GASA	69.04a	13.83ab	381.77bc	20.35b	53.33ab	37.20bc	19,124.67bc	4.12b	23/56c	25.63bc
CKSA	66.14abc	13.76ab	383.64bc	20.15b	50.10d	37.33abc	20,117.50ab	3.81c	21/77d	24.20cd
GACKSA	66.35ab	15.29a	416.34a	20.63a	55.05a	37.66abc	20,394.50a	4.63a	26/51a	29.25a

In each column, there is no significant difference between treatments with common letters according to the Duncan test
SA salicylic acid, GA Gibberellic acid, and CK Cytokinins

Table 6 Results of mean comparisons interaction effect of environment \times irrigation levels on physiological traits

Treatment	RWC (%)	Flag leaf proline content (mg/g)	No. of spikes	No. of spike-let per spike	No. of seeds per spike	PMG (g)	BY (kg/ha)	Seed nitrogen (%)	Seed protein (%)	Grain gluten (%)
Non-saline	90 mm	73.46a	436.26a	20.27a	59.00a	41.71a	8481.75a	2.84c	16/25c	29.31a
	120 mm	69.48b	399.97b	20.14a	56.93b	35.91c	7080.42b	3.79b	21/69b	27.24b
Saline	90 mm	62.53c	336.87c	19.28b	46.59c	37.53b	6105.17c	3.73b	21/33b	
	120 mm	55.41d	305.35d	18.94c	42.46d	33.16d	4753.92d	4.46a	25/52a	21.71c

In each column, there is no significant difference between treatments with common letters according to the Duncan test
 RWC Relative leaf water content, BY Biological yield, PMG 1000-grain weight

Grain protein percentage

The effect of the environment on grain protein percentage was significant at the probability level of one percent (Table 4). The highest percentage of grain protein was obtained in saline conditions at the rate of 23.42%, which was significantly different from the non-saline environment (Table 5). The results show that saline stress increased grain protein by 19%. The interaction effect of irrigation levels + PGRs was significant at the 5% probability level (Table 4). The highest percentage of grain protein was obtained at the irrigation level after 120 mm of pan evaporation, and foliar spraying of PGRs including GA₃ + CK + SA at the rate of 28.83%. This showed a significant difference between evaporation and other foliar spraying and non-foliar spraying treatments with an irrigation level after 90 mm of pan evaporation (Table 8). The irrigation level after 120 mm of pan evaporation and PGRs including GA₃ + CK + SA caused a 63% increase in grain protein.

Grain gluten

The interaction effect of environment \times irrigation levels \times PGRs on grain gluten content was significant at the 5% probability level (Table 4). The highest amount of grain gluten was obtained in non-saline conditions, the irrigation level after 90 mm of pan evaporation, and PGRs including GA₃ + CK + SA at the rate of 33.50%. Its difference with the non-saline environment level, the irrigation level after 120 mm of pan evaporation, and other foliar spraying and no foliar spraying treatments was significant (Fig. 3). The interaction effect of environment \times irrigation levels \times PGRs caused a 54% increase in grain gluten.

Discussion

The effect of the environmental stresses on the studied traits

The reduction of wheat grain yield in a saline environment is due to the reduction of three components: the no. of spikes, the number of grains per spikelet, and PMG (Zorb et al. 2018). In saline conditions, the acceleration of terminal spike growth reduces the no. of spikelets per spike. Also, the decrease in PMG in saline conditions can be justified by reducing the length of the grain-filling period. Water salinity, as a growth-inhibiting factor, with its negative oxidation effect, destroys the cell structure and reduces the osmotic potential of the plant tissue, affecting yield. RWC is the most appropriate method for measuring the amount of water in plant tissues. For this reason, its importance is greater than other methods. There is a positive and significant correlation

Table 7 Results of mean comparisons interaction effect of environment×PGRs on physiological traits

Treatment	RWC (%)	Flag leaf proline content (mg/g)	No. of spikes	No. of spike-let per spike	No. of seeds per spike	PMG(g)	BY (kg/ha)	Seed nitrogen (%)	Seed protein (%)	Grain gluten (%)
Non-saline										
Control	68.89b	6.83 h	352.55f	18.18f	47.85c	36.76bcde	18,077.00e	1.83j	10/46j	24.07 fg
GA	70.88ab	9.59f	410.56 cd	20.23bc	61.15ab	40.25a	19,510.83d	3.3fgh	18/85fgh	26.57de
CK	70.98ab	7.98 g	400.08d	19.89 cd	58.42ab	38.32abc	20,640.33c	3.14gh	17/96gh	26.82cde
SA	71.19a	10.05ef	405.32d	19.21e	55.58b	37.96abcd	22,096.50ab	3.08 h	17/63 h	28.33bcd
GACK	72.19a	10.3ef	455.4a	21.27a	61.92a	40.00a	21,079.67bc	3.78def	21/64def	29.59b
GASA	72.63a	10.78e	439.79ab	20.83a	60.42ab	39.1ab	21,325.00bc	3.72defg	21/26defg	28.77bc
CKSA	72.51a	10.82e	429.04bc	20.74ab	56.47ab	38.41abc	22,695.00a	3.42efgh	19/54efgh	29.46b
GACKSA	72.49a	12.68d	452.19a	21.30a	61.93a	39.69a	22,916.50a	4.27bcd	24/42bcd	32.57a
Saline										
Control	55.39 g	10.55ef	254.64j	16.95 g	34.17e	34.22 fg	11,934.83i	2.49i	14/25i	17.75j
GA	56.86 fg	15.54c	303.52 h	19.27e	48.58c	36.91bcde	15,585.17 h	4.01cde	22/95cde	20.94hi
CK	57.02 fg	12.63d	315.92 h	19.03e	45.07 cd	34.56efg	15,926.50gh	3.91cdef	22/38cdef	19.38ij
SA	57.89ef	16.36bc	312.4 h	18.13f	41.77d	33.52 g	16,543.17fgh	3.85def	22/00def	22.56gh
GACK	59.18de	16.57b	339.9 fg	20.09 cd	48.46c	36.38cdef	16,624.67fgh	4.78ab	27/34ab	22.43gh
GASA	65.44c	16.88b	323.76gh	19.86 cd	46.24 cd	35.31efg	16,924.33efg	4.52abc	25/85abc	22.49gh
CKSA	59.77de	16.70b	338.24 fg	19.56de	43.74 cd	36.26cdef	17,540.00ef	4.20bcd	24/00bcd	18.94ij
GACKSA	60.20d	17.90a	380.49e	19.97 cd	48.17c	35.64defg	17,872.50e	5.00a	28/60a	25.93ef

In each column, there is no significant difference between treatments with common letters according to Duncan RWC Relative leaf water content, BY Biological yield, PMG 1000-grain weight, SA salicylic acid, GA Gibberellic acid, and CK Cytokimins

Table 8 Results of mean comparisons interaction effect of irrigation levels \times PGRs on physiological traits

Treatment	RWC (%)	Flag leaf proline content (mg/g)	No. of spikes	No. of spike-lets per spike	No. of seeds per spike	BY (kg/ha)	PMG (g)	Seed nitrogen (%)	Seed protein (%)	Grain gluten percentage (%)	
90 mm	Control	64.47bcde	7.81h	318.77h	17.73i	42.92j	37.58e	16,379.67j	1.83l	10/44l	22.22ef
	GA	67.07bc	11.80ef	380.73cdef	19.87e	56.40a	41.25a	19,492.00f	3.24i	18/53i	24.71bcde
	CK	66.32bcd	10.33fg	378.56cdef	19.59f	53.2cde	39.21c	19,961.17e	3.12ij	17/87ij	23.70cde
	SA	66.94bc	12.14ef	374.09ef	18.74h	49.96gh	38.34d	20,921.33c	3.06j	17/50j	26.33bcde
	GACK	67.79bc	12.47de	414.38ab	20.87a	56.63a	40.90a	20,333.83d	3.74g	21/38g	26.96abc
	GASA	74.05a	12.78cde	403.51bc	20.36bcd	54.99abc	39.71bc	20,684.83c	3.68g	21/05g	26.91abcd
	CKSA	68.67b	12.80cde	390.65bcde	20.28d	52.26def	40.01b	21,607.67b	3.38h	19/33h	22.79ef
	GACKSA	68.65b	14.35abcd	431.85a	20.73a	56.03ab	39.96b	22,114.5a	4.23d	24/2d	30.46a
120 mm	Control	59.81f	9.57gh	288.42i	17.40j	39.10k	33.4h	13,632.17l	2.49k	14/27k	19.60f
	GA	60.67ef	13.25bcde	333.35h	19.64f	53.34cde	35.9f	15,604.00k	4.07e	23/26e	22.81def
	CK	61.67ef	10.28fg	337.44gh	19.34g	50.29fg	33.67h	16,605.67j	3.93f	22/48f	22.50ef
	SA	62.14def	14.26abcd	343.64gh	18.59h	47.39i	33.14h	17,718.33h	3.87f	22/13f	24.56bcde
	GACK	63.59cdef	14.39abcd	380.93cdef	20.49bc	53.76cde	35.47f	17,370.50i	4.83b	27/60b	25.07bcde
	GASA	64.02cdef	14.88ab	360.04fg	20.33cd	51.68efg	34.69g	17,564.50hi	4.56c	26/06c	24.35bcde
	CKSA	63.62cdef	14.71abc	376.62def	20.01e	47.95hi	34.66g	18,627.33g	4.23d	24/21d	25.61bcde
	GACKSA	64.04cdef	16.22a	400.83bcd	20.54b	54.07bcd	35.36f	18,674.50g	5.04a	28/83a	28.04ab

In each column, the common denominators do not differ significantly from the Duncan multiplier test at 5%

RWC Relative leaf water content, BY Biological yield, PMG 1000-grain weight, SA salicylic acid, GA Gibberellic acid, CK Cytokinins

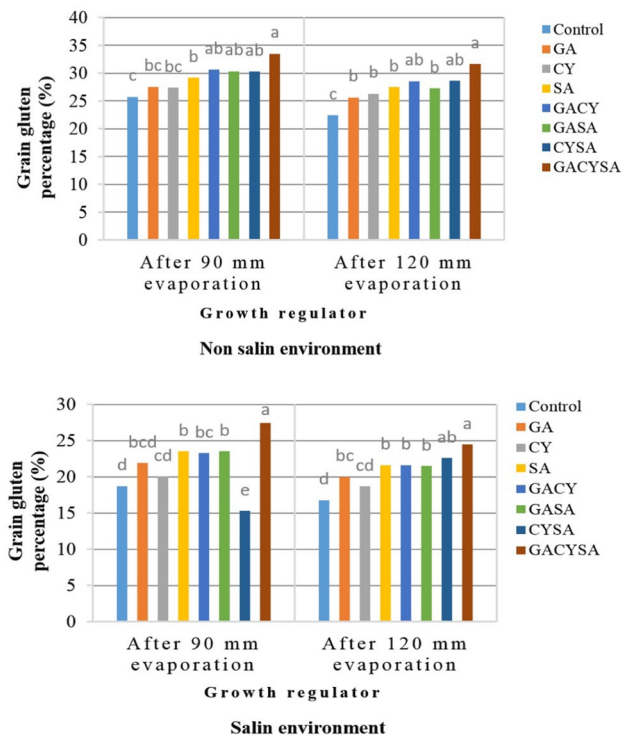


Fig. 3 Interaction effect of environment x irrigation levels x PGRs on grain gluten percentage in both saline and non-saline environments

between the RWC and the no. of spikes ($r=0.846^{**}$), the no. of spikes ($r=0.595^{**}$), the no. of grains ($r=0.755^{**}$), 1000-grain weight ($r=0.398^{**}$), Yb ($r=0.807^{**}$), gluten index ($r=0.783^{**}$), and flag leaf proline content ($r=0.520^{**}$) (Table 9). The no. of spikelets is dependent on suitable water and soil conditions, and the salinity condition reduces the no. of spikelets in the spike. In the yellow stage, the living flowers that will produce grains are mature and close to pollination. The fifth and higher flowers are aborted. At this stage, the no. of spikelets was determined to be fertile and will produce grains. A decrease in the irrigation level leads to an increase in water consumption efficiency (Mosaffa and Sepaskhah 2019). Also, by conducting another experiment, it was shown that the RWC of the flag leaves was 76% in the control treatment and 5.47% in the saline treatment (10 dS). In the present experiment, there was a significant correlation between the no. of spikes and the no. of spikelets ($r=0.745^{**}$), the no. of grains ($r=0.818^{**}$), and PMG ($r=0.412^{**}$), which indicates the trend between these traits (Table 10). The application of different irrigation levels and salinity treatments has caused spikelets to be infertile and also reduced the transfer of photosynthetic materials to grains. Salinity affects grain yield components depending on when the stress is applied to the plant (Mashi et al. 2018). The no. of grains is directly related to the environmental conditions, duration, quality, and time of the stress peak,

which in this experiment is the transition from the vegetative phase to the reproductive phase and also the flowering time from the stress-sensitive stages of the plant in terms of grain formation. Therefore, salinity stress hurts the vegetative growth stages and thereby decreases the grain yield and its components.

The effect of irrigation levels

Triticum aestivum L. grain yield components include the no. of spikes/m², the number of grains per spikelet, and PMG, which develop in a time sequence. Considering that PMG is the last component of grain yield development, if other grain yield components (no. of grains per spike and no. of spikes/m²) decrease due to water stress, increasing PMG can compensate for this deficiency. The no. of spikes/m² had a direct positive effect (0.38) and an indirect effect (0.27) through PMG on plant grain yield under drought stress conditions (Torabian and Maghsoudi 2012). In water stress conditions, absorption and stabilization of CO₂ are reduced due to the partial closing of the stomata or the reduction of their degree of opening, so the total amount of cultivated material for grain filling is reduced. However, water stress does not reduce the transfer of nitrogen from the leaves to the grain, and this causes an increase in grain protein (Sehgal et al. 2018). Desta and Amare (2021) showed that the spraying of PGRs increased the mentioned traits in both normal irrigation conditions and water stress conditions. Water stress lowers all the physiological activities of *Triticum aestivum* L. This is while it seems that the use of SA priming with a concentration (of 100 ppm) in the conditions of water stress has improved the condition of the plant by increasing the plant's resistance to stress. As a result, it has led to higher grain yield. With increasing stress intensity, the traits of the no. of grains per spike, PMG, leaf area index, Yb, and photosynthetic pigments decreased, while proline content, soluble protein content, and soluble carbohydrates increased (Gowthami 2022). In an experiment, the highest and lowest biological yield of winter wheat were observed in the treatments of 125% of winter wheat water requirement x 1.6 dS/m salinity and 50% of winter wheat water requirement x salinity 7.8 S/m, respectively. Poor treatment compared to superior treatment showed a decrease of about 65% in this trait.

The effect of PGRs

In the present experiment, the effect of PGRs separately and in combination caused an increase in the values of each of the studied traits. PGRs including GA₃, CK, and SA priming according to the type of their influence on traits (especially in grains that need cold or light), improving the biochemical characteristics of the plant such as the content of soluble

Table 9 The results of the mean comparisons interaction effect of environment × irrigation levels × PGRs on physiological traits

Treatment	No. of spikes	No. of spike- let per spike	No. of seeds per spike	PMG (g)	BY (kg/ha)	Seed nitro- gen percent- age (%)	Seed protein percentage (%)			
Non-saline	90 mm	Control	372.59ghi	18.33jkl	49.97bcdef	39.42bcde	19302ghi	1.40l	8/01m	
		GA	432.94cd	20.28cdef	62.62a	43.29a	21,467/33de	2.82ijk	16/15ijk	
		CK	427.77cd	19.99cdefgh	58.99ab	41.48abc	22,344/67cd	2.70jk	15/42jkl	
		SA	420.3de	19.27ghi	56.3abc	40.83abcd	23,651/67abc	2.65jk	15/14kl	
		GACK	475.12a	21.36a	62.78a	43.17a	22,552/67bcd	3.14hijk	17/96hijkl	
		GASA	452.67abc	20.84abc	61.77a	41.96abc	22,899/33abcd	3.24ghij	18/51ghijk	
		CKSA	441.53bcd	20.75abcd	57.62ab	41.32abc	24,166/33ab	2.95hijk	16/87hijkl	
		GACKSA	467.21ab	21.34a	62.00a	42.20ab	24,618/67a	3.83defgh	21/93defgh	
	120 mm	Control	332.52kl	18.03kl	45.73efg	34.10jklmnop	16852jkl	2.26k	12/91l	
		GA	388.18fgh	20.18cdefg	59.69a	37.20efghijk	17,554/33ijk	3.77defgh	21/55defgh	
		CK	372.40ghi	19.8fgh	57.86ab	35.16hijklmno	18936ghi	3.58fghij	20/50fghij	
		SA	390.35fgh	19.15hij	54.85abcde	35.09ijklmno	20,541/33efg	3.52fghij	20/12fghijk	
		GACK	435.69cd	21.18ab	61.07a	36.82efghijkl	19,606/67fgh	4.43bcdef	25/32bcdef	
		GASA	426.91cd	20.82abc	59.08ab	36.23efghijklm	19,750/67efgh	4.20cdef	24/00cdef	
		CKSA	416.54def	20.72abcde	55.32abcd	35.50ghi- jklmno	21,223/67def	3.88defgh	22/21defgh	
		GACKSA	437.17cd	21.27a	61.86a	37.17efghijk	21,214/33def	4.71abcd	26/92abcd	
	Saline	90 mm	Control	264.96no	17.12m	35.86hi	35.74fghijklmn	13,457/33p	2.25k	12/87l
			GA	328.51kl	19.46fghi	50.18bcdef	39.20bcdef	17,516/67ijk	3.66efghi	20/92efghi
			CK	329.35kl	19.19hi	47.42cdefg	36.95efghijkl	17,577/67ijk	3.55fghij	20/31fghijk
			SA	327.89kl	18.22kl	43.62fgh	35.85fghijklmn	18191hij	3.47fghij	19/87fghijk
			GACK	353.64ijk	20.38bcdef	50.47bcdef	38.64cdefgh	18115hij	4.34bcdef	24/81bcdef
GASA			354.34ijk	19.89defgh	48.22cdefg	37.46efghij	18,470/33hij	4.12cdefg	23/59cdefg	
CKSA			339.77jk	19.82fgh	46.89defg	38.69cdefg	19049ghi	3.81defgh	21/79defgh	
GACKSA			396.5efg	20.13cdefg	50.07bcdef	37.73defghi	19,610/33fgh	4.63abcd	26/46abcd	
120 mm		Control	244.32o	16.78m	32.47i	32.69nop	10,412/33q	2.73ijk	15/63ijkl	
		GA	278.52mn	19.09hij	46.99defg	34.61ijklmnop	13,653/67op	4.37bcdef	24/98bcdef	
		CK	302.48lm	18.88ijk	42.73fgh	32.18op	14,275/33nop	4.28cdef	24/46cdef	
		SA	296.92m	18.03l	39.92ghi	31.18p	14,895/33mnop	4.22cdef	24/14cdef	
		GACK	326.17kl	19.81fgh	46.45defg	34.12jklmnop	15,134/33lmnop	5.22ab	29/88ab	
		GASA	293.17mn	19.83efgh	44.27fgh	33.16mnop	15,378/33lmno	4.92abc	28/12abc	
		CKSA	336.7jk	19.30ghi	40.58ghi	33.83klmnop	16031klmn	4.58abcde	26/22abcde	
GACKSA	364.49hij	19.81efgh	46.28defg	33.54lmnop	16,134/67klm	5.37a	30/74a			

In each column, there is no significant difference between treatments with common letters according to Duncan test

RWC Relative leaf water content, BY Biological yield, PMG 1000-grain weight, SA salicylic acid, GA Gibberellic acid, and CK Cytokinins

proteins, free proline, photosynthetic pigments, and the no. of plant hormones (Samad et al. 2023). These increments are similar to those reported by Kumar et al. (2018) and El Karamany et al. (2019), on different plant species. Also, as a result of increasing grain yield in saline stress conditions, it causes cell division, stimulates morphogenesis, stimulates the growth of lateral buds, and stimulates the growth of leaves in plants (Forghani et al. 2018; Kumar et al. 2018). These results agree that the foliar application of PGRs had an enhancement effect on growth, as reported in some of the findings of Forghani et al. (2018) and Kumar et al. (2018). PGRs can reduce the damages to a minimum by

creating resistance or modulating adverse effects in stressful environments (El Karamany et al. 2019). It delays the leaf senescence in plants. Also, it includes organic compounds that in small amounts can modify the growth (Chaurasiya et al. 2014). Also, it increases the active period of grain growth (grain germination times), controls the vegetative growth, flowering, fruiting, and seed production in plants, and increases PMG and the no. of grains per plant. Finally, PGRs increase the crop grain yield (Dwivedi et al., 2014; Safdari et al. 2014). The increase in *Triticum aestivum* L. grains PMG with the use of CK has been reported in various studies (Zheng et al. 2016). Cell division is one of the most

Table 10 Correlation between measured traits

Traits	1	2	3	4	5	6	7	8	9	10
1 No. of spikes	1									
2 No. of spikelet	0.745**	1								
3 No. of seeds per spike	0.818**	0.714**	1							
4 PMG	0.412**	0.488**	0.501**	1						
5 BY	0.857**	0.704**	0.767**	0.421**	1					
6 RWC	0.846**	0.595**	0.755**	0.398**	0.807**	1				
7 Seed nitrogen (%)	0.11	0.17	0.164	0.443**	0.112	0.117	1			
8 Seed protein (%)	0.11	0.17	0.164	0.443**	0.112	0.117	1.000**	1		
9 Grain gluten (%)	0.858**	0.647**	0.732**	0.351**	0.800**	0.783**	0.06	0.06	1	
10 Flag leaf proline content (mg/g)	-0.378**	0.036	-0.314**	0.058	-0.291**	-0.520**	0.292**	0.292**	-0.34**	1

ns non-significant, RWC Relative leaf water content, BY Biological yield, PMG 1000-grain weight

** $P < 0.01$, * $P < 0.05$

important roles of CK, and it seems that foliar application of CK has increased biological grain yield by increasing the cell division of vegetative organs, increasing photosynthesis, and increasing the durability of leaf surface index and grain weight. Increasing the Yb of *Triticum aestivum L.* with the use of CK has also been reported in other studies (Zaheer et al. 2019). The effect of SA priming in the treatment without saline had a lesser effect on the RWC of leaves (4%). In the case of 10dS salinity treatment, the effect of SA priming increased the RWC of leaves by 20%. The physiological efficiency of nitrogen increased with increasing consumption of SA priming. Also, the consumption of 6 mM SA priming with ratios of 124.6 and 132.1 g of total dry matter per gram of absorbed nitrogen had the highest physiological efficiency of nitrogen in both irrigation and non-irrigation conditions, respectively. Irrigation interruption conditions have caused the re-transfer of nitrogen to increase, and the physiological efficiency of nitrogen has increased to 113.95 g of total dry matter per gram of absorbed nitrogen. On the other hand, SA priming moderated the water stress by increasing the physiological efficiency and re-transfer of nitrogen, which improved the yield of barley grains under water stress conditions compared to full irrigation (Abhari and Radman 2020).

Conclusion

The results of this research indicated the negative effect of salinity and water stress on the physiological and functional traits of the *Triticum aestivum L.* plant. Increasing the irrigation water salinity to 10 dS/m and irrigation levels after evaporation of 120 mm led to a significant decrease in the RWC of the flag leaf, the no. of spikes/m², the no. of spikelets per spike, the number of grains per spikelet, PMG, the biological yield, and the amount of grain gluten. It also decreased the proline content of flag leaves and the

percentage of nitrogen and grain protein. Foliar application of different PGRs has a significant effect on the growth, photosynthetic pigments, yield, and yield attributing characters significantly. Based on our study, the spraying of PGRs in combination (GA₃ × SA × CK, GA₃ × CK, and GA₃ × SA) with a recommended 100-ppm concentration was the most effective foliar application for increasing growth and yield of *Triticum aestivum L.* Due to the specialized effects of each sprayed in two stages: 1—double ridge and 2—terminal spikelet, all traits modify the effects of environmental stress and increase yield in traits. Interaction of environment × irrigation level × PGRs showed the most RWC of the flag leaf in the non-stress of salinity under the influence of the spraying of PGRs at the irrigation level after 90 mm of pan evaporation in GA₃ + CK + SA was observed at 74.77 kg/ha. At the irrigation level after 120 mm of pan evaporation in GA₃ + CK, GA₃ + SA, and CK + SA, the amount of 70.51 kg/ha was observed. The highest RWC was influenced by the spraying of PGRs at the irrigation level after 90 mm of pan evaporation in the foliar application of GA₃ + SA at 73.36 kg/ha. It was also observed at the irrigation level after 120 mm of pan evaporation in the foliar application of GA₃ + SA + CK the amount of 57.87 kg/ha. Our results reveal the novel finding that GA₃ interacts with the spraying of PGRs to balance another development under saline and non-saline conditions in *Triticum aestivum L.* According to the results, salicylic acid priming and PGR spraying can partially reduce the negative effects of deficient irrigation and soil salinity stress. In general, it seems that by using these compounds, it is possible to reduce the number of times of irrigation and, in addition to managing water resources, reduce the adverse effects of drought stress in plants. Since our results are based on the data from a single year, at least an additional year hassle of study is recommended. The results will be useful for the local farmers as well as future research initiatives. In the area where this experiment is

carried out, salinity and drought stress are the two dominant limitations compared to other cases in the climatic conditions of this region. Therefore, carrying out drought stress in the region in the range of 90 and 120 mm of evaporation allows us to investigate the plant's reaction to these environmental stresses in saline and non-saline environments (control) under the influence of foliar spraying of PGRs. In general, the results showed that the spraying of PGRs can be effective in increasing the quantitative and qualitative yield of wheat by improving the production components and reducing the salinity effect of irrigation water, and it can be suggested to researchers and farmers.

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Availability of data and materials All data and materials as well as a software application or custom code support our published claims and comply with field standards.

Declarations

Conflict of interest There is no conflict of interest among authors.

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