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RESEARCH ARTICLE

Physiological, biochemical and hormonal response of wheat cultivars to foliar application of growth stimulants and zinc nano-chelate under water deficit stress

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Abstract

To investigate the effect of different foliar application treatments to improve drought tolerance in wheat genotypes, a factorial split-plot experiment was conducted based on the randomized complete block design with 3 replications in 2 locations. The main factor was irrigation (normal and water deficit) and the secondary factors were 4 levels of foliar application (control: without foliar application, jasmonic acid, zinc nano-chelate and succinate) and 3 genotypes of barley. Water deficit stress reduced the content of chlorophyll a (9.03 %), chlorophyll b (6.66 %), total chlorophyll (7.32 %) and auxin (4.21 %) and increased the catalase (18.18 %), superoxide dismutase activity (23.35 %), malondialdehyde (7.17 %), glucose (5.35 %), fructose (4.85 %) and sucrose (14.99 %) versus normal irrigation conditions. Foliar application of zinc nano-chelate increased chlorophyll a, chlorophyll b, total chlorophyll and fructose content by 15.45 %, 15.76 %, 14.70 % and 41.35 % respectively. The highest content of chlorophyll a, chlorophyll b and total chlorophyll was assigned to the Mihan cultivar. Foliar application with zinc nano-chelate in both environments resulted in the highest biological yield and grain yield and the lowest content of abscisic acid. Foliar application line 9 genotypes with zinc nano-chelate had the highest auxin, cytokinin, catalase and superoxide dismutase activity. While foliar application of nano-zinc chelate in the Mihan cultivar led to the highest biological yield, grain yield, glucose content and the lowest amount of malondialdehyde. Therefore, foliar application of zinc nano-chelate along with the appropriate variety can improve grain yield under different environmental conditions.

Keywords

Jasmonic acid; micronutrient; succinate; water deficit

Introduction

Wheat (*Triticum aestivum* L.) is a major cereal cultivated worldwide; the area under cultivation and the amount of wheat production in 2020-21 were estimated at 2015 million ha and 761.5 million tons respectively (1).

In Iran, only one-third of all wheat fields are irrigated and the rest are rainfed, where crop production depends on the amount of annual rainfall. In arid and semi-arid regions, winter wheat is often faced with water shortage stress during reproductive stages. In research conducted in Eastern Chi-

na on winter wheat, found that drought stress in the flowering and grain-filling stages significantly decreased grain yield (2). The reduction of grain yield in wheat due to water deficit stress has also been reported in other studies (3, 4). It is predicted that by 2050, wheat production should increase by 60 % to meet the food needs of the growing global population (5). In more than 50 % of the regions of the world where wheat is cultivated, this plant experiences intermittent periods of water shortage stress (6). Forecasts indicate that due to climate change, the frequency and severity of the water deficit will rise (7). Therefore, it is essential to understand the adverse effect of water shortages on wheat growth and find a solution to increase the resistance and production of this crop under water shortage stress (8).

Zinc is an essential microelement. This element has many roles in the plant, including participation in physiological and biochemical processes, e.g., the activities of various enzymes and plant growth and development (9-11). In various studies, foliar application with the mineral Zn resulted in improved resistance to drought stress in various crops. It has been found that zinc plays a significant role in improving wheat's resistance to drought stress (12). In drought-stressed wheat, photoprotection mechanisms were enhanced by applying ZnSO4 heptahydrate foliar at 0.1 % (13). It was observed that nano-ZnO (100 mg/ L) alleviated the adverse effect of water shortage stress through better stomatal movement, a higher net photosynthetic rate and improved water use efficiency on maize. Also, applying nano-ZnO (100 mg/L) increased drought stress tolerance in corn by activating antioxidant enzymes (14). Plants treated with nano-Zn had a more effective reactive oxygen species (ROS) scavenging system than untreated plants. The increased activity of antioxidant enzymes in zinc application treatments is due to the increased transcription of genes responsible for synthesizing ROS-scavenging enzymes under water stress (15). Improving the antioxidant defense mechanism under the foliar application of zinc elements in wheat is reported (16).

In addition, it was reported that the foliar application of zinc increased grain yield and the quality of wheat under drought stress conditions (17, 18).

Jasmonic acid (JA) is a lipid-derived plant hormone that mediates diverse biological phenomena and is a critical regulator of plant responses to stress (19). Jasmonic acid is a plant growth regulator that plays a key role in the regulation of many cell development processes, including seed germination, root growth and fertility (20). It has been reported that the external application of jasmonic acid increases and regulates the activity of antioxidant enzymes in various plants (21). Foliar application of jasmonic acid also increases antioxidant activity in tobacco (*Nicotiana tabacum* L.) (22).

Improvement of resistance to environmental stresses was reported in a previous study after succinate foliar treatment on soybean (23), corn (24) and alfalfa (25). The present research was designed and implemented to evaluate the effect of growth stimulant treatments and zinc nano-chelate foliar application on different wheat genotypes under normal and water deficit stress conditions.

Materials and Methods

Field experimental design

This experiment was conducted in Miandoab Agricultural Research Station (at 36°58 ⊠N and 46° 6⊠ and altitude of

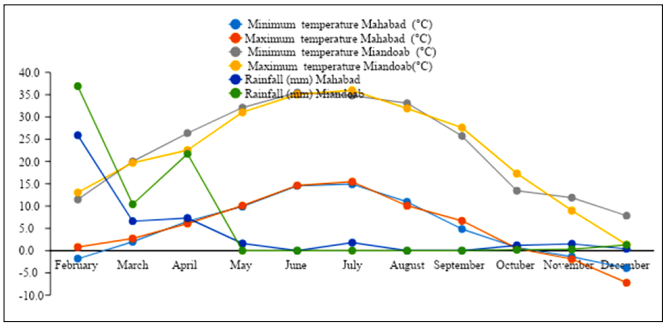

Fig. 1. Rainfall (mm), Minimum and maximum temperature (°C) records of experimental site during 2020.

1314 m above sea level) and Mahabad (at 36°10 ⊠N and 45° 43 ⊠ and altitude of 1320 m above sea level), North West-Iran, in the 2020 wheat growing seasons (Fig. 1).

Experimental design

The experiment was carried out in 2 locations in a split-plot factorial design based on a randomized complete block with 3 replications.

The main factor was irrigation (normal and water-deficit conditions). The first subfactor was 4 levels of foliar application (control: without foliar application, jasmonic acid: 1 millimolar, zinc nano-chelate: 1/1000 and succinate: 0.5 mM) and the second subfactor was wheat genotypes (line 9 and line 13 and Mihan cultivar) (Table 1).

Table 1. Pedigree of studied genotypes.

Genotypes	Pedigree
C-97-1	Mihan
C-97-9	Charger//CMH80A.768/3*Cno79
C-97-13	Boh4/7/Wa476/3/391//Num/5/W22/5/Ana/6/Tam200/Kasyan

Each repetition consisted of 12 experimental units and each experimental unit was considered 4 m^2, where 10 wheat lines were cultivated with a row spacing of 20 cm and a plant spacing of 5 cm. The distance between the experimental units was 50 cm.

Seeds were sown with a density of 350 plants per square meter (based on the customs of the region) on October 28 (Miandoab) and 29 (Mahabad), 2020. The plants were harvested after the maturity stage on July 10 (Miandoab) and 12 (Mahabad), 2020.

Nitrogen fertilizer (350 kg/ha of urea), phosphorus (50 kg/ha of triple superphosphate) and potassium (50 kg/ha of potassium sulfate (50 % K_2O)) were applied equally in the treatments based on the fertilizer recommendations.

All phosphorus and potassium fertilizers were applied before seeding, but nitrogen fertilizer was applied in three stages after planting to prevent nitrogen leaching. All

agricultural operations and conservation practices, including pest, disease and weed control, were carried out equally for all the experimental units during the crop-growing season.

Application of treatments

All the plants were irrigated with 90 mm of evaporation from the class A pan until the heading stage. After that, irrigation was stopped in water deficit treatments and continued as usual (90 mm of evaporation from class A) under normal conditions until the physiological treatment stage (stages of 89 Zadoks).

To estimate the % of moisture in the soil in the experimental units, several samples were randomly collected before irrigation and sent to the laboratory.

The formula to estimate the water needed for subsequent irrigations was based on bringing the soil moisture to the field capacity (FC):

$$In = (Fc-ai). D. b$$

Where, In: the depth of irrigation water (mm), Fc: field capacity (% by weight), ai: soil moisture before irrigation (weight percent), D: depth of root (mm) (1000 mm was considered for wheat), b: apparent specific mass (g/cm^3). Field soil moisture was considered constant during the experiment. After determining the depth of irrigation water, the amount of water required for irrigation was estimated based on the following formula; this amount was controlled by the meter located at the beginning of the irrigation pipes.

$$V = (In/1000). A$$

Where, In: the depth of irrigation water (mm), V: the amount of irrigation water (m^3) and A: the plot area (m^2).

The total water consumed under normal irrigation and water deficit stress was measured by a meter.

The foliar fertilization was performed first in the tillering growth stage and then, at 15-day intervals,

Traits measurement

In the mature physiological stage (stages of 94 Zadoks), to measure the traits, 10 plants were randomly selected from each experimental unit by removing the effect of margins.

To measure chlorophyll a, b and total chlorophyll, samples were taken from completely developed mature leaves; after digestion with 80 % acetone and centrifugation of the samples, the absorbance of each sample was measured using a spectrophotometer (26).

Catalase and superoxide dismutase activity were measured using Stewart and Bewley (27) method. The results were presented in terms of units per milligram of protein

To investigate the harmful effects of lipid peroxidation on plant cells, the content of malondialdehyde (MDA) was also measured. This evaluation was carried out based on

Haraguchi *et al.*'s (28) method and using a spectrophotometer. The concentration of malondialdehyde was calculated using the light absorption coefficient with an intensity of 155 × 10-6.

In this experiment, the content of abscisic acid (ABA), gibberellin and auxin (IAA) was measured (29-31) respectively.

Moreover, the method of Hendrix, (32) was followed to measure glucose, fructose and sucrose.

To measure the biological yield, whole experimental plots were harvested and after drying for 4 days, they were weighed and recorded as the biological yield of each experimental unit. Then, the grains in all harvested spikes were weighed and recorded as grain yields.

Statistical analysis

Before performing the analysis of variance, its assumptions were checked. The data were analyzed using SAS 9.4 and mean comparisons were performed with Duncan's method (LSR); in addition, Microsoft Excel software was used to plot the graphs.

Results

The combined analysis of variance (ANOVA) indicated significant differences among irrigation levels ($P < 0.01$) on all the investigated traits. The effect of foliar application treatment on all the investigated traits was significant ($P < 0.01$), except for malondialdehyde content. The difference between the genotypes was also significant in terms of all traits except for malondialdehyde, glucose, fructose and sucrose content ($P < 0.01$). The location x irrigation interaction effect was significant on the abscisic acid ($P < 0.01$), auxin ($P < 0.05$) and sucrose content ($P < 0.01$). The location x foliar application interaction was significant for superoxide dismutase activity and malondialdehyde, glucose and sucrose content ($P < 0.01$). The irrigation x foliar application interaction effect on abscisic acid, biological yield and grain yield was significant ($P < 0.01$). There was a significant difference between location x genotype interaction treatments on malondialdehyde, abscisic acid, fructose and sucrose ($P < 0.01$). The foliar application x genotype interaction effect was significant on abscisic acid, auxin, gibberellin, cytokinin, catalase activity, superoxide dismutase activity, malondialdehyde content, sucrose, biological yield and grain yield ($P < 0.01$) (Table 2).

Photosynthetic pigments

Water deficit stress reduced the content of chlorophyll a, chlorophyll b and total chlorophyll by 8.98 %, 7.14 % and 7.89 % respectively, compared to normal conditions.

Plants foliated with zinc nano-chelate showed higher content of chlorophyll a, chlorophyll b and total chlorophyll by 15.45, 15.63 and 14.86 % respectively, compared to the control treatment.

Among the cultivars, Mihan had the highest chlorophyll a, chlorophyll b and total chlorophyll content (Table 3).

Plant hormonal properties

Abscisic acid

Table 2. Combined variance analysis of the characteristics in wheat genotypes under foliar application treatments and irrigation conditions.

SOV	DF	MS						
		Chlorophyll a	Chlorophyll b	Total chlorophyll	Abscisic acid	Auxin	Gibberellin	Cytokinin
Location (L)	1	0.04^{ns}	0.07^{ns}	0.22^{ns}	95.57^{ns}	7.66ns	33.91^{ns}	97.29^{ns}
Repeat (Location)	4	0.09	0.03	0.28	93.46	55.13	551.29	181.40
Irrigation (I)	1	5.22**	0.75**	10.29**	125.58**	84.04**	12911.0**	127.64**
L×I	1	0.04^{ns}	0.009^{ns}	0.08^{ns}	3.55**	4.14*	68.86^{ns}	0.51^{ns}
Ea	4	0.005	0.0007	0.007	0.05	0.10	12.48	0.05^{ns}
Foliar application (F)	3	2.38**	0.71**	5.78**	627.13**	1056.59**	8606.74**	826.7**
L×F	3	0.005^{ns}	0.004ns	0.011^{ns}	0.004^{ns}	1.47ns	0.13^{ns}	2.25^{ns}
I×F	3	0.0002^{ns}	0.00005^{ns}	0.00005^{ns}	90.10**	0.0007ns	0.18^{ns}	0.0008^{ns}
L×I×F	3	0.0001^{ns}	0.00001^{ns}	0.00003^{ns}	0.0009^{ns}	0.0005ns	0.16	6.05^{ns}
Genotype (G)	2	0.22**	0.097**	0.73**	102.93**	0.37**	105.51**	100.20**
L×G	2	0.04^{ns}	0.011^{ns}	0.10^{ns}	9.57**	0.03ns	0.33^{ns}	0.13^{ns}
I×G	2	0.009^{ns}	0.001^{ns}	0.01^{ns}	0.08^{ns}	0.19ns	0.24*	6.73$_{ns}$
F×G	6	0.002^{ns}	0.00001^{ns}	0.08^{ns}	19.25**	7.74**	274.97**	16.08**
I×F×G	6	0.001^{ns}	0.04^{ns}	0.04^{ns}	0.39^{ns}	0.05ns	2.59^{ns}	0.0008^{ns}
L×I×G	2	0.002^{ns}	0.001^{ns}	0.0001^{ns}	0.0004^{ns}	0.0002ns	0.19^{ns}	0.16^{ns}
L×F×G	6	0.009^{ns}	0.005^{ns}	0.04^{ns}	0.37^{ns}	0.07ns	3.99^{ns}	0.0004^{ns}
P×I×F×G	6	0.0006^{ns}	0.0001^{ns}	0.001^{ns}	0.006^{ns}	0.01ns	1.43^{ns}	0.0005^{ns}
Eb	88	0.014	0.005	0.51	0.62	0.87	6.64	3.71
CV %	-	2.70	3.51	3.45	2.57	2.63	5.58	3.91

ns, *, **: Non-significant Significant at 5 % and 1 % probability levels respectively

Table 2.Continues

SOV	DF	MS							
		Catalase activity	Superoxide dismutase activity	Malondialde-hyde content	Glucose	Fructose	Sucrose	Biological yield	Grain yield
Location (L)	1	0.68^{ns}	0.39^{ns}	0.14^{ns}	83.56^{ns}	6.25^{ns}	1.26^{ns}	16.48^{ns}	3.85^{ns}
Repeat (Location)	4	0.21	0.22	0.29	8.22	5.49	0.23	10.75	4.21
Irrigation (I)	1	0.64**	0.45**	6.75**	153.69**	49.09**	22.62**	1405.94**	11.46**
L×I	1	0.007^{ns}	0.008^{ns}	0.02^{ns}	6.59**	1.71^{ns}	0.16*	1.67^{ns}	0.35^{ns}
Ea	4	0.0004	0.0004	0.006	0.11	0.046	0.02	51.66	0.03
Foliar application (F)	3	14.35**	74.15**	2.60^{ns}	1280.34**	1333.31**	0.47**	19.67**	0.74**
L×F	3	0.20^{ns}	0.21**	5.56**	61.05**	1.91^{ns}	1.44**	0.007^{ns}	0.007^{ns}
I×F	3	0.0009^{ns}	0.0001^{ns}	0.006^{ns}	0.001^{ns}	0.002^{ns}	0.001^{ns}	97.56**	0.81**
L×I×F	3	0.0001^{ns}	0.0002^{ns}	0.007^{ns}	0.0004^{ns}	0.0004^{ns}	0.002^{ns}	0.005^{ns}	0.07^{ns}
Genotype (G)	2	0.63**	0.78**	5.46^{ns}	0.41^{ns}	43.54^{ns}	0.39^{ns}	198.27**	2.90**
L×G	2	0.06^{ns}	0.10^{ns}	2.31**	0.25^{ns}	21.03**	0.50**	0.10^{ns}	0.04^{ns}
I×G	2	0.001^{ns}	0.0007^{ns}	0.01^{ns}	0.20^{ns}	0.06^{ns}	0.04^{ns}	145.21**	0.15^{ns}
F×G	6	0.22**	0.15**	3.08**	9.94^{ns}	0.007^{ns}	0.93**	45.86**	0.54**
I×F×G	6	0.001^{ns}	0.04^{ns}	0.003^{ns}	0.04^{ns}	0.021^{ns}	0.03^{ns}	0.55^{ns}	0.08^{ns}
L×I×G	2	0.001^{ns}	0.0007^{ns}	0.0001^{ns}	0.002^{ns}	0.95^{ns}	0.009^{ns}	0.04^{ns}	0.01^{ns}
L×F×G	6	0.003^{ns}	0.03^{ns}	0.002^{ns}	0.54^{ns}	0.005^{ns}	0.06^{ns}	0.004^{ns}	0.02^{ns}
P×I×F×G	6	0.009^{ns}	0.0003^{ns}	0.0007^{ns}	0.019^{ns}	0.005^{ns}	0.002^{ns}	9.49ns	0.01^{ns}
Eb	88	0.04	0.04	0.043	1.49	1.03	0.031	7.29	0.15
CV %	-	5.10	6.26	3.34	3.06	3.97	3.11	15.49	9.76

ns, *, **: Non-significant Significant at 5% and 1% probability levels, respectively.

Although the content of abscisic acid increased in response to water shortage stress, the foliar application of

Table 3. Mean comparison of the main effects of irrigation, foliar application and cultivars in terms of effects on the traits in wheat.

Treatments	Chlorophyll a (mg/g F W)	Chlorophyll b (mg/g F W)	Total chlorophyll (mg/g F W)	Abscisic acid (nano g/g F W)	Auxin (nano g/g F W)	Gibberellin (nano g/g F W)	Cytokinin (nano g/g F W)	Catalase activity (µmol / g FW)	Superoxide dismutase activity (µmol g/ FW)	Malondialdehyde content (µmol g/ FW)	Glucose (mg/g D W)	Fructose (mg/g D W)	Sucrose (mg/g D W)	Biological Yield (t/ha)	Grain yield (t/ha)
Irrigation															
normal	4.61a	2.25a	6.83a	29.80a	36.3a	153.93a	50.26a	3.52b	3.04b	6.02b	38.84b	33.38b	5.27b	20.55a	4.36a
stress	4.23b	2.10	6.33b	31.66a	34.77b	172.87a	48.38b	4.66a	3.75a	6.45a	40.91a	35.00a	6.06a	13.56b	3.79b
Foliar application															
Control	4.14d	2.03d	6.19d	26.04d	29.86d	151.30c	43.17c	3.34d	2.47d	6.55a	26.88d	26.88d	4.22c	15.185b	3.86c
Jasmonic acid	4.50b	2.22b	6.73b	32.40b	42.37a	184.19a	51.79ab	4.40b	3.95a	5.62d	41.20a	41.20a	6.23a	17.82a	4.05ab
Zinc nano-chelate	4.78a	2.35a	7.11a	35.63a	37.01b	166.15b	54.42a	4.47a	3.77b	6.15c	36.41b	36.41b	6.86a	18.28a	4.34a
Succinate	4.27c	2.11c	6.38c	28.86	32.88c	151.95c	47.92b	3.84c	3.37c	6.32b	32.27c	32.27c	5.34b	16.95ab	4.03bc
Genotypes															
Mihan	5.50a	2.23a	6.74a	29.37c	34.91b	162.64b	48.705b	4.00b	3.25c	6.18b	39.98a	32.08a	5.58a	18.84a	4.29a
Line 9	4.39b	2.16b	6.53b	32.28a	36.54a	165.11a	50.975a	4.22a	3.52a	6.60a	39.85a	34.13a	5.65a	18.37a	4.12b
Line 13	4.38b	2.15b	6.51b	30.55b	35.15b	162.45b	48.29b	4.05b	3.41b	5.93c	39.80a	31.01a	5.76a	15.11b	3.81c

Means in each column, followed by similar letter(s), are not significantly different at the 5 % probability level.

all 3 treatments of jasmonic acid, zinc nano-chelate and succinate in both environmental conditions reduced the content of this plant hormone compared to the control

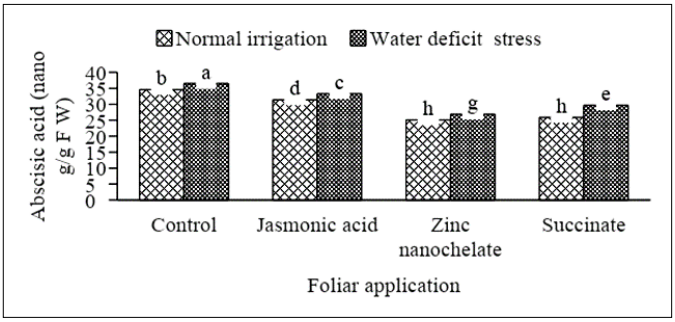

Fig. 2. Mean comparison of irrigation with foliar application interaction treatments in terms of the effect on the abscisic acid in wheat.

treatment. Non-foliar-applied plants under water shortage stress (36.51 ng/g FW) had the highest and the plants treated with zinc nano-chelate under normal irrigation conditions (25.1 ng/g FW) had the lowest concentration of this hormone (Fig. 2).

All 3 studied genotypes showed the highest concentration of ABA hormone in the non-foliar application treatment and foliar application of all 3 treatments significantly reduced the content of this hormone in all 3 genotypes. All 3 genotypes demonstrated the lowest content of this hormone in response to the foliar treatment of zinc nano-chelate (Table 4).

Gibberellin content

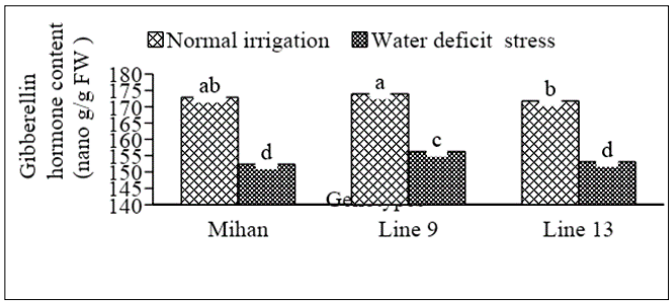

Fig. 3. Mean comparison of foliar application with genotypes interaction treatments in terms of the effect on the gibberellin hormone in wheat.

Water deficit stress significantly decreased gibberellin content in all 3 genotypes; the highest magnitude of decrease was assigned to the genotype of Mihan and line 13. Mihan cultivar and line 9 (172.89 and 173.94 ng/g FW respectively) had the highest and Mihan cultivar and line 13 under water deficit conditions had the lowest amount of gibberellin hormone (152.39 and 153.12 mg/g FW respectively) (Fig. 3).

The genotypes showed a positive reaction to jasmonic acid and zinc nano-chelate. Mihan cultivars, line 9 and line 13 had the highest gibberellin content of 186.25, 188.33 and 177.98 ng/g FW respectively in the Jasmonic acid foliar application. The lowest gibberellin content in

Table 4. Mean comparison of cultivar with foliar application interaction treatments in terms of the effect on the traits in wheat

Genotypes	foliar application	Abscisic acid (nano g/g F W)	Gibberellin (nano g/g F W)	Auxin (nano g/g F W)	Cytokinin (nano g/g F W)	Catalase activity (µmol / g FW)	Superoxide dismutase activity (µmol / g FW)	Malondialdehyde content (µmol g/ FW)	Glucose (mg/g D W)	Sucrose (mg/g D W)	Biological Yield (t/ha)	Grain yield (t/ha)
Mihan	Control	33.42d	151.34g	29.56i	43.65f	3.37g	2.45ef	6.73b	33.17g	4.2g	16.60cd	3.96def
	Jasmonic acid	29.76f	186.25a	40.65c	50.06d	4.25d	3.68b	5.67f	40.32d	5.86d	19.22ab	4.13bcd
	Zinc nano-chelate	26.08i	161.97e	36.73d	54.10b	4.73b	3.95a	6.00e	46.43b	6.73b	19.56ab	4.72a
	Succinate	28.21g	150.99g	32.69f	47.01e	3.65f	3.20d	6.30d	42.58c	5.53e	19.97ab	4.36b
Line 9	Control	37.44a	151.43g	30.85h	44.50f	3.47g	2.57e	7.08a	33.07g	4.24g	17.94bc	3.93def
	Jasmonic acid	34.94c	188.33a	43.65a	52.85bc	4.46c	4.08a	6.07e	42.02c	6.17c	19.00ab	4.23bcd
	Zinc nano-chelate	27.49h	164.13d	37.35d	56.75a	5.00a	4.10a	6.43cd	47.47a	6.76b	20.43a	4.29bc
	Succinate	29.24f	156.53f	34.3e	49.80d	3.95e	3.46c	6.81b	47.14ab	5.43e	16.09cde	4.02c-f
Line 13	Control	36.02b	151.12g	29.19i	41.35g	3.17h	2.39f	6.55c	32.38g	4.23g	13.70f	3.70f
	Jasmonic acid	32.49e	177.98b	42.8b	52.45c	4.49c	3.66b	6.00e	37.03f	6.67b	17.87bc	3.79ef
	Zinc nano-chelate	24.53j	172.36c	36.95d	52.41c	4.60bc	3.71b	5.15g	38.52e	7.08a	14.85def	4.03cde
	Succinate	29.14g	148.33h	31.65g	46.95e	3.93e	3.45c	6.03e	38.37e	5.06f	14.00ef	3.72ef

Means in each column, followed by similar letter(s), are not significantly different at the 5 % probability level.

the mentioned genotypes was allocated to the non-foliar application treatment (151.34, 151.43 and 151.12 mg/g FW respectively) (Table 4).

Auxin content

Water shortage stress reduced the concentration of auxin by 4.21 % compared to normal irrigation conditions (Table 3).

Spraying plants with jasmonic acid, zinc nano-chelate and succinate treatments significantly increased the content of auxin in all 3 genotypes compared to the corresponding control treatment; foliar application of line 9 with jasmonic acid (43.65 ng/g FW) gained the maximum and the Mihan cultivar and line 13 in the control treatment of foliar application (respectively 26.56 and 29.19 ng/g FW) gained the minimum auxin content (Table 4).

Cytokinin content

A deficit of supplied water reduced cytokinin content by 3.47 % compared to normal irrigation conditions (Table 3).

Although the foliar spraying of line 9 with zinc nan-chelate (56.75 ng/g FW) had the highest cytokinin content, the foliar application of all 3 treatments of jasmonic acid, zinc nano-chelate and succinate significantly increased the content of cytokinin in all 3 genotypes. The lowest cytokinin content was detected in line 13 under non-foliar treatments (46.95 ng/g FW) (Table 4).

Antioxidant properties

Catalase Activity

Plants subjected to a water deficit had higher catalase activity compared with the normal condition by 18.18 % (Table 3).

Foliar application of jasmonic acid, zinc nano-chelate and succinate significantly increased the activity of the catalase enzyme in all 3 studied genotypes and in the zinc nano-chelate foliar treatment, the activity of the catalase enzyme was maximized. Foliar application of zinc nano-chelate in line 9 and line 13 along with non-foliar applied treatment achieved the highest and lowest amount of catalase enzyme activity respectively (5.00 and 3.17 µmol /g FW) (Table 4).

Superoxide dismutase activity

The water deficit raised superoxide dismutase activity by 23.35 % compared to normal irrigation conditions (Table 3).

All 3 genotypes showed the highest and lowest amount of superoxide dismutase enzyme activity under foliar application of zinc nano-chelate and control treatment respectively. The maximum superoxide dismutase activity belonged to line 9 under the foliar application of zinc nano-chelate and jasmonic acid (4.10 and 4.08 µmol / g FW respectively). The difference between the mentioned treatments and the foliar application of nano-zinc chelate on the Mihan cultivar was not significant. The lowest superoxide dismutase activity was recorded in the Mihan cultivar under the control treatment of foliar application (2.45 µmol/ g FW) (Table 4).

Malondialdehyde

Water deficit stress increased the malondialdehyde content by 7.17 % compared to the normal irrigation treatment (Table 3).

The lowest content of malondialdehyde was recorded in genotypes grown under the foliar application of jasmonic acid, zinc nano-chelate and succinate. The maximum and minimum malondialdehyde contents were as-

signed to line 13 in the control treatment of foliar application (7.08 µmol /g FW) and line 13 in the zinc nano-chelate foliar application treatment (5.15 µmol / g FW) respectively (Table 4).

Soluble sugars

Glucose Content

The highest glucose content was assigned to the plants subjected to water deficit (Table 3).

In all 3 evaluated genotypes, the foliar treatments had a higher glucose content compared to the corresponding control. Furthermore, the foliar application of line 9 with zinc nano-chelate (47.47 mg/g DW) showed the highest, while the Mihan cultivar and lines 9 and 13 under the control treatment of foliar application showed the lowest glucose content (33.17, 33.07 and 32.38 mg/g DW respectively) (Table 4).

Fructose Content

Fructose content increased significantly under water stress conditions (35.00 mg/g DW) compared to normal irrigation conditions (33.38 mg/g DW) (Table 3).

Fructose content showed a positive reaction to foliar application treatments, such that foliar application of jasmonic acid, zinc nano-chelate and succinate increased fructose content by 53.27 %, 35.41 % and 20.05 % compared to the control treatment respectively (Table 3).

Sucrose Content

Under water stress conditions, the amount of sucrose increased by 14.99 % compared to normal conditions (Table 3).

The mean comparisons showed that all 3 genotypes had the highest and lowest sucrose content in zinc nano-chelate treatment and control respectively. Line 13 in the zinc nano chelate foliar application (7.08 mg/g DW) and Mihan cultivar and lines 9 and 13 in the control treatment of foliar application had the lowest sucrose content (4.20, 4.24 and 4.23 mg/g DW respectively) (Table 4).

Biological yield

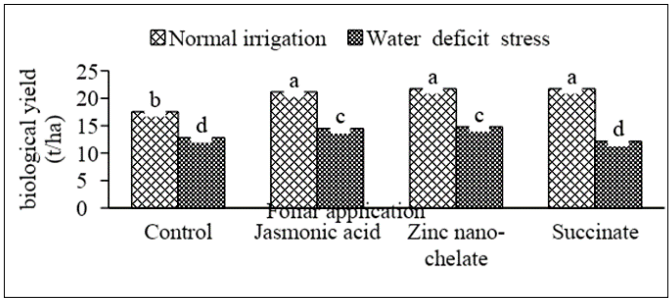

Fig. 4. Mean comparison of irrigation with foliar application interaction treatments in terms of the effect on the biological yield in wheat.

The foliar application of all 3 treatments of jasmonic acid, nano zinc chelate and succinate under normal irrigation conditions (21.16, 21.76 and 21.76 t/ha respectively) and the foliar application of jasmonic acid and nano zinc chelate under water stress conditions (14.48 and 14.80 t/ ha respectively) significantly increased the biological yield compared to the foliar application control treatments. The lowest biological yield was recorded in the control treatment of foliar application under water deficit stress conditions (12.82 t/ ha) (Fig. 4).

The biological yield of the examined genotypes showed a positive reaction to the foliar application of zinc nano-chelate and jasmonic acid. The biological yield in the treatment of zinc nano-chelate foliar application in line 9 had the maximum (20.43 t/ha), while the control treatment of foliar application in genotype 13 had the minimum biological yield (13.70 t/ha) (Table 4).

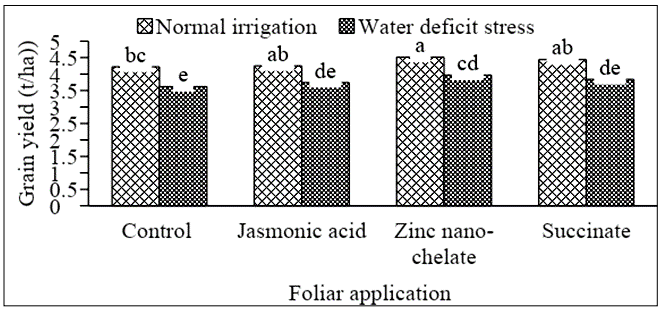

Fig. 5. Mean comparison of irrigation with foliar application interaction treatments in terms of the effect on the grain yield in wheat.

Grain Yield

The highest grain yield (4.51 t/ha) was produced under normal irrigation conditions and foliar application of zinc nano-chelate; the difference between this treatment, jasmonic acid and succinate foliar application under normal irrigation conditions was not statistically significant. The lowest grain yield was also recorded under water shortage stress and control of foliar application treatment (3.62 t/ha) (Fig. 5).

The highest grain yield was obtained in all 3 genotypes in response to the foliar application of nano zinc chelate. Foliar application of zinc nano-chelate raised the grain yield in Mihan, line 9 and line 13 genotypes compared to the corresponding control treatment by 19.19, 9.16 and 8.91 % respectively. Foliar application of zinc nano-chelate in the Mihan cultivar (4.72 t/ha) had the highest, whereas non-foliar application treatment in line 13 had the lowest grain yield (3.70 t/ha) (Table 4).

Discussion

Photosynthetic pigments

The content of photosynthetic pigments was reduced by the water shortage. The reduction of photosynthetic pigments under water stress conditions is related to the accumulation of oxygen free radicals such as H_2O_2 (33). Free radicals damage the thylakoid membrane and its peroxidation, which destroys the structure of chlorophyll (34). One of the reasons for the decrease in chlorophyll content under water shortage conditions is the increase in the activity of the chlorophyllase enzyme, which decomposes chlorophyll (35). It has been reported that water deficit stress in the post-pollination stage reduces the content of photosynthetic pigments in the leaves of wheat genotypes (36).

The highest photosynthetic pigments' content was

recorded in response to the foliar application of zinc nano-chelate. The Mihan cultivar had the highest photosynthetic pigments' content compared with other genotypes. One of the indicators of plants' resistance to drought stress is the maintenance of leaf chlorophyll content under stress conditions. It has been documented that the reduction of chlorophyll content in the sensitive variety (Beni-suef 5) was higher than in the resistant variety (Sids 1) of wheat (37). The reduction of leaf chlorophyll content in wheat under the water deficit treatment was also reported in previous studies (37-39).

The application of nano-zinc fertilizer can play a significant role in improving plant tolerance by increasing the synthesis of lipids, proteins, amino acids and photosynthetic pigments (40). The use of zinc nano-fertilizers increased growth, the content of photosynthetic pigments and gas exchange characteristics in wheat and reduced oxidative stress (41). The application of zinc nanoparticles in both soil and foliar applications enhanced the content of chlorophyll a, chlorophyll b and carotenoids in corn (41).

Plant hormonal properties

The foliar application of all three treatments of jasmonic acid, zinc nano-chelate and succinate in both environmental conditions and the 3 studied varieties reduced the content of ABA hormone. A major event in response to osmotic stress is the rapid and transient accumulation of abscisic acid, which facilitates stomatal closure and the expression of related genes that protect plants from damage caused by water stress (42). In wheat, the efficiency of photosynthesis and the content of photosynthetic pigments in the flag leaf are negatively affected by the content of abscisic acid (43).

Water deficit stress reduced gibberellin, auxin and cytokinin in all 3 genotypes. Cytokines have an antagonist relationship with abscisic acid; they stimulate the opening of leaf stomata and reduce the sensitivity of stomata to abscisic acid by delaying leaf senescence (44). Although cytokinin concentration often decreases under water deficit conditions, treatment with cytokinin increases biomass and grain yield under drought stress by improving nitrogen metabolism (45). It seems that auxin biosynthesis and signaling pathways were reduced by drought stress in wheat (46). Foliar application of zinc nano-chelate and jasmonic acid improves the amount of gibberellin, auxin and cytokinin of all 3 genotypes; the highest magnitude of this increase was detected in line 9 and Mihan cultivar.

Zinc is required for auxin (IAA) synthesis. Stopping growth and small leaves are among the most important signs of zinc deficiency, which may be due to low levels of IIA and degradation of IIA. There is considerable evidence that zinc is required for the synthesis of tryptophan (which is the precursor of IIA) (47). In research on wheat cultivars, with the intensification of salinity stress, the content of ABA increased and the auxin and cytokinin contents decreased. However, treatment with jasmonic acid increased the content of auxin and cytokinin and decreased ABA under salt stress conditions (48). The concentration of plant hormones in wheat leaves was significantly affected by using plant hormones such as indole acetic acid (IAA) (48).

Antioxidant properties

The shortage of water supply led to increased catalase and superoxide dismutase activity as well as malondialdehyde content, in our study. Catalase and superoxide dismutase activity peaked in all 3 genotypes under the foliar treatment of zinc nano-chelate; moreover, the foliar application of zinc nano-chelate in line 13 and line 9 induced the maximum activity of catalase and superoxide dismutase enzymes respectively. When plants are subjected to water shortages, ROS accumulation increases and plants stimulate their antioxidant defense systems, such as SOD and CAT, to clear the excess ROS (50). Under environmental stress, SOD is the first line of defense against ROS accumulation damage, accelerating the dismutation of O_2 to H_2O_2 and O_2. In the next step, CAT takes action and catalyzes H_2O_2 into H_2O and other non-toxic products (51). In research on wheat, water deficit stress increased the activity of catalase and superoxide dismutase and the peroxidation of cell membrane lipids in drought-resistant and sensitive varieties (37).

We found that the foliar application of all 3 treatments significantly reduced the content of malondialdehyde; the minimum malondialdehyde content belonged to line 13 treated with zinc nano-chelate.

The accumulation of ROS as a result of water deficit stress accelerates cell wall peroxidation; as a result of this process, malondialdehyde (MDA) is produced. The application of zinc nano-chelate under water stress conditions decreased H_2O_2 accumulation by increasing the activity of SOD, CAT and APX enzymes (52). Similarly, in another experiment, the adverse effect of oxidative stress under water stress conditions was minimized by the foliar application of nano-ZnO (53). It seems that Zn nanoparticles are involved in clearing ROS compounds by activating the antioxidant enzyme system (53).

In research on corn, water deficit stress increased malondialdehyde (MDA) content and superoxide dismutase (SOD) enzyme activity compared to normal irrigation conditions; moreover, the application of nano-ZnO elevated SOD and CAT activity in both water shortage and normal irrigation conditions (52). The enhanced activity of antioxidant enzymes under water stress treatment and zinc nano-foliar application has been reported in other studies (3). In another study on wheat, nanoparticles (ZnO) mitigated the adverse effects of drought stress by improving and increasing the activity of CAT and SOD enzymes and promoting resistance to drought stress (53, 54). In addition to zinc foliar application, jasmonic acid also improved the activity of antioxidant enzymes (55). Similarly, previous studies have shown that the application of JA effectively suppressed the toxic effects of oxidative burst by enhancing the ROS- scavenging potential of the antioxidant defense system in stressed plants (56-58). It has been reported that treatment of sugar beet with MeJA reduced the peroxidation of membrane lipids under normal irrigation and water deficit stress conditions (59). In another study, the foliar application of jasmonic acid reduced the content of MAD under water stress conditions in resistant

and sensitive wheat varieties (37).

Sucrose Content

The results showed that a reduction in water increased glucose, fructose and sucrose content. Fructose content demonstrated a positive reaction to all foliar application treatments. Furthermore, the foliar application of zinc nano-chelate increased the content of glucose and sucrose in all 3 genotypes. The highest glucose content in genotype 9 and the highest sucrose content in genotype 13 were recorded in response to the foliar application of zinc nano-chelate.

Another way to deal with drought stress in plants is to increase the accumulation of active solutes such as amino acids, free proteins and soluble sugars. This strategy can protect the plant against drought stress (60).

Soluble sugars can regulate the osmotic potential, maintain the turgor pressure of the cells, clean the free radicals, act as a source of energy and carbon and maintain and stabilize the cell membrane (61).

In wheat, soluble sugars make the largest contribution to OA when subjected to water shortage stress. When wheat was exposed to water stress for seven days, the content of soluble sugars in the plant increased by 80 % (62). The effect of Zn on total sugars can be due to its role in starch and nucleic acid metabolism and also to the activities of various enzymes involved in these biochemical reactions (63). Zn also plays an essential role in the formation and metabolism of carbohydrates (64). The positive effect of zinc foliar application on increasing the content of soluble sugars has been reported in other studies (65).

In this study, jasmonic acid treatment also raised the content of soluble sugars; a rise in the content of soluble sugars in plants treated with jasmonate treatments has been reported in other studies (37, 49, 66).

Yield

Water shortage stress has been reported to reduce the biological and grain yield. The foliar application of zinc nano-chelate and jasmonic under normal and water deficit stresses enhanced the biological and grain yield.

Moreover, the foliar application of zinc nano-chelate resulted in maximum biological and grain yield in the 3 studied genotypes, especially the Mihan cultivar.

The high grain and biological yield in this study in the treatment of nano-chelate foliar application and Mihan cultivar can be due to the positive effect of this treatment on improving the content of photosynthetic pigments, the activity of antioxidant enzymes, the content of soluble sugars and plant hormones. All of these processes increase the plant's resistance to water stress and normal irrigation conditions (the plant experiences some degree of water stress). Improving the plant's defense system mitigates the adverse effects of ROS and increases the stability of the cell membrane.

The reduction in yield caused by drought stress can be attributed to reduced cell division and growth, decreased photosynthesis, membrane degradation, incompatibility of water and nutrient uptake and their transmission (8). The reduction in grain yield in wheat under water shortage conditions has been illustrated in previous studies (3, 4).

As previously mentioned, the foliar application of zinc nano-chelate had a positive effect on the improvement of the economic yield of wheat under water deficit and normal irrigation conditions. In line with these results, the positive effect of zinc foliar application on improving grain yield in wheat has been proven in other studies (13, 16, 67). Zinc is involved in many biochemical processes such as nucleotide synthesis, cytochrome, plant hormone activity, metabolism and cell membrane maintenance and zinc deficiency decreases chlorophyll content, photosynthetic materials and grain yield (68). Numerous studies have documented that the application of zinc nano-chelate increases the plant's resistance to water deficit stress by improving the activation of antioxidant enzymes, facilitating the absorption process in plants, and increasing growth (69, 70). A study reported that the biological yield and grain yield increased in the foliar treatment of zinc nano-chelate under normal and deficit irrigation (3). Furthermore, activating some physiological processes such as stomatal regulation, enzymatic activation, chlorophyll formation and biochemical processes and increasing the production of dry matter were related to the application of zinc (71). It has been found that water shortage significantly decreased grain yield by over expressing cytokinin oxidase/dehydrogenase, but applying Zn increased yield and biological yield compared with the untreated sample (4).

Note that the foliar application of jasmonic acid had a positive effect on biological and grain yield. MeJA-induced tolerance against water deficits might also be attributed to decreased adverse effects of osmotic potential due to the accumulation of some solutes as a response to drought, which might help protect metabolic processes and contribute to plant growth through conserving cell turgor (15).

Conclusion

The most important economic trait in wheat is grain yield, which is the result of all quantitative and qualitative traits. In this study, foliar application of zinc nano-chelate under both normal environmental and water deficit stress conditions improved the grain yield in the studied cultivars over control. Furthermore, the highest grain yield was recorded for the Mihan cultivar in response to the foliar application of zinc nano-chelate. The treatment with zinc nano-chelate enhanced the plant's resistance to water deficit stress by improving the activity of antioxidant enzymes and compatible osmolytes such as soluble sugars. Improving the defense systems in plants stabilizes the cell membrane and reduces the peroxidation of lipids, as a result of which the destruction of chlorophyll (which is the site of photosynthesis) declines. The stability and increase in the

content of chlorophylls promote the production of photosynthetic substances and grain yield. Moreover, an improvement in plant hormones' content was observed under the treatment of nano-zinc chelate, which can have a positive effect on vegetative growth and grain yield.

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Authors' contributions

Habibi, Sharafi, Mohammadi and Mir Mahmoudi carried out the experiment. Habibi and Sharafi wrote the manuscript with support from Yazdanseta and Mir Mahmoudi.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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