PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(3): 758-767
https://doi.org/10.14719/pst.3266

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Physiological responses of wheat cultivars to anti-transpiration compounds and drought stress ameliorators under water-deficient conditions

Somayyeh Razzaghi[*]

[*]Soil Science and plant Nutrition, Erciyes University, Kayseri 38030, Turkey

*Email: srazzaghi@erciyes.edu.tr

ARTICLE HISTORY
Received: 09 January 2024
Accepted: 18 June 2024

Available online
Version 1.0 : 25 August 2024
Version 2.0 : 29 August 2024

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE

Razzaghi S . Physiological responses of wheat cultivars to anti-transpiration compounds and drought stress ameliorators under water-deficient conditions. Plant Plant Science Today. 2024; 11(3): 758-767. . https://doi.org/10.14719/pst.3266

Abstract

Anti-transpiration compounds are essential for mitigating the impact of water scarcity by reducing plant water uptake and enhancing water use efficiency. To evaluate the effects of these compounds on the physiological traits of wheat cultivars under water deficit conditions, 2 field trials were conducted during the 2019 and 2020 cultivation periods. The trails involved three irrigation levels: I_1 (100 mm evaporation from Class-A pan), I_2 (130 mm evaporation from Class-A pan) and I_3 (160 mm evaporation from Class-A pan), which were applied to the main plots. Two bread wheat genotypes, G_1 (Hydari) and G_2(Zarrineh), were used in the trails. Various anti-transpiration and stress amelioration compounds were tested, including kaolin (5 %), chitosan (200 mg L^{-1}), calcium chloride (50 mM), sodium selenate (40 mg L^{-1}) and a control treatment, which were randomized in subplots. In both years, the most significant reduction in transpiration was observed with the foliar application of chitosan following irrigation after 160 mm evaporation. Catalase (CAT) activity increased in both cultivars when subjected to foliar application of calcium chloride. Additionally, the chitosan treatment exhibited the highest peroxidase activity and the lowest malondialdehyde (MDA) activity. Notably, the chitosan treatment, particularly under irrigation withholding at the I_1 level, resulted in the highest grain yield, especially with the Zarrineh cultivar. The application of anti-transpiration compounds demonstrated the ability to elevate levels of antioxidant enzymes and enhance the physiological traits of wheat under stress conditions. However, it is important to note that the extent of these improvements varies based on the timing of stress application and the type of cultivar involved.

Keywords

Enzyme; anti-transpiration; drought stress; wheat cultivar

Introduction

To mitigate environmental damage, plants possess an antioxidant protection mechanism comprising both non-enzymatic compounds and enzymatic substances (1). Among the enzymatic components are catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APOX), peroxidase (POX), glutathione reductase (GR) and polyphenol oxidase (PPO) (2-4). Under stressful conditions, researchers have observed an increase in the concentration of these antioxidant enzymes, resulting in enhanced resistance to oxidative stress (5).

Indeed, transpiration is essential for photosynthesis; however, under certain conditions, it can be detrimental. Consequently, the use of anti-transpirants is one of the most effective methods for reducing moisture loss through transpiration (6, 7). Finding practical methods to mitigate transpiration can significantly decrease water requirements, especially in arid regions. Various efforts have focused on utilizing anti-transpiration or transpiration-reducing compounds to minimize water loss (8-10). Typically, anti-transpirant coatings, composed of emulsions derived from substances such as wax, latex or plastic are applied by spraying onto the foliage. The primary function of these coatings is to reduce stomatal conductance, thereby minimizing water loss from the plant (11). To achieve this goal, it is imperative to employ innovative approaches that effectively reduce agricultural water consumption while simultaneously preserving product quality and stability.

Anti-transpiration materials play a pivotal role in reducing water absorption by plants, thereby enhancing water use efficiency (WUE) by curbing plant transpiration (12). Chitosan, derived from chitins, is an agricultural biostimulant and elicitor known for its non-toxicity and broad applicability. It enhanced physiological responses and mitigates the effects of abiotic stress through stress transduction pathways (13).

Kaolin, with the chemical formula $Al_4Si_4O_{10}(OH)_8$, is an example of a reflective anti-transpirant (14). Kaolin spray helps lower leaf temperature by increasing leaf reflectance, which reduced transpiration rates more than it affects photosynthesis in plants exposed to intense sunlight (15). Similar effects have been observed in tomatoes and potatoes treated with a foliar application of kaolin suspension (14). Additionally, this mineral has demonstrated efficacy across a broad spectrum of crops, with particular relevance to nut-bearing trees such as walnuts (16). The application of kaolin as an anti-transpiration material has also led to an increase in the relative water content of olive leaves, thereby enhancing its effectiveness (17). In chickpeas, the application of the anti-transpirant chitosan resulted in increased leaf foliage thickness and leaf mesophyll tissue (18). Chitosan is a natural, low-toxic and inexpensive compound derived from deacetylated chitin. Its biological activity, which helps improve plant resistance, depends on its molecular weight and degree of deacetylation (19). The foliar application of chitosan (750 ppm) on fava bean crops, especially under an irrigation regime of 4800 m^3, significantly improved the physiological attributes of the plants, notably enhancing seed yields compared to the control group (20). Additionally, the external application of calcium mitigates the negative impacts of abiotic stresses by regulating osmotic adjustment, photosynthetic efficiency and antioxidant activities (21). The application of $CaCl_2$ increased water deficit tolerance in *Viola cornuta* by inducing stomatal closure and maintaining high relative water content (22). However, the current understanding of the influence of anti-transpiration materials on wheat cultivars under limited irrigation conditions is incomplete, particularly regarding their effects on stomatal aperture, transpiration rate, antioxidant activity and water retention. Given the critical role of these factors in determining plant resilience and yield, a comprehensive investigation is imperative. This study aims to address this gap by systematically evaluating the effects of anti-transpiration materials on key physiological parameters in 2 wheat cultivars under imitated irrigation conditions. The insights gained from this research are expected to significantly contribute to our knowledge of sustainable water management practices in agriculture and enhance the development of resilient crop varieties.

Materials and Methods

Cultivation practices and associated procedures

The study was conducted at the Agricultural Research Station of Khoy/ Iran, located at 38° 33' northern latitude and 44° 55' east longitude, with an altitude of 1103 m above sea level. Two field experiments were carried out during the 2019 and 2020 growing seasons. The experiment followed a split-plot factorial design based on a randomized complete block design (RCBD) with 3 replications. The main plots were assigned different irrigation levels: I_1 (100 mm evaporation from Class-A pan), I_2 (130 mm evaporation from Class-A pan) and I_3 (160 mm evaporation from Class-A pan). Two varieties of bread wheat, G1 (Hydari) and G2 (Zarrineh), along with various anti-transpiration and stress amelioration compounds, including kaolin (5 %), chitosan (200 mg L^{-1}), calcium chloride (50 mM), sodium selenate (40 mg L^{-1}), and a non-spray control treatment were randomized in subplots. Each plot comprised of 6 rows, each 6 m long and spaced 20 cm apart. The kaolin used in this study is a non-toxic aluminosilicate manufactured by Bayer in Germany, marketed under the brand name Surround WP. It is known for its light-reflecting properties and was applied at a concentration of 40 g/L (14, 23). Chitosan, produced by Sigma Aldrich and with the chemical formula $(C_6H_{11}O_4N)_8$, was dissolved at a concentration of 5 g/L using 1 % molar acetic acid. The pH of the solution was adjusted to 5.6 using NaOH (24). Calcium chloride, another stress-reducing substance, plays a crucial role in maintaining and improving the turgor of leaf cells through the symplastic pathway, facilitated by aquaporins in leaf mesophyll cells. Additionally, calcium ions enhance water absorption by roots through the apoplastic pathway, thereby promoting overall plant growth and development (25). Sodium selenite, naturally present in mineral form in the soil, is another stress-mitigating substance that plays a significant biological role in plants (26). In the experiment, sodium selenite and calcium chloride were applied at concentration of 40 mg L^{-1} and 50 mM respectively (27). Antiperspirant foliar spraying was conducted 3 days before the initial irrigation treatment in spring. All plots received fertilizer applications, including 30 kg ha^{-1} urea (46 % N), 18 kg ha^{-1} $ZnSO_4.7H_2O$, 80 kg ha^{-1} KH_2PO_4 and 90 kg ha^{-1} $HPO_4(NH_4)_2$ before planting. Additionally, 160 kg ha^{-1} of urea was applied at the tillering and pre-anthesis stages. The primary physicochemical characteristics of the soil are outlined in Table 1.

Wheat seeds were planted on October 6, 2019 and 2020, at a seed density of 450 seeds per square meter (approximately 180 kg ha^{-1}). All plots received equal irrigation to maintain soil moisture at field capacity, with irrigation intervals adjusted according to treatment protocols. In mid-April, favourable environmental conditions allowed for the application of chemical weed control using 2,4-D herbicides. Nitrogen fertilizers was uniformly applied during the tillering and stem elongation stages to ensure even distribution. The study focused on various physiological traits, including

Table 1. The soil properties of the experimental site.

Soil texture	**Clay** (%)	**Silt** (%)	**Sand** (%)	**Carbonate Calcium** (%)	**Saturation Humidity** (%)	**pH**
Silt	30	48	22	10.5	31	8
EC (ds/m)	**Zn** (mg/kg)	**Fe** (mg/kg)	**K** (mg/kg)	**P** (mg/kg)	**T N** (%)	**O. C** (%)
0.79	0.60	5.87	24.10	12.04	0.14	0.89

measurements of CAT activity, ascorbate peroxidase (APOX) activity, leaf MDA content, transpiration rate and yield. To determine grain yield, 2 side rows (out of the 6 rows per plot) located half a meter from the beginning and end of each plots were excluded. Grain yield after harvesting was calculated from a standardized area of 4.2 m^2 within each plot and then converted to kg ha^{-1}. Protein content was analysed using a Kjeldahl machine (model V40) (28). Leaf transpiration rate was measured one week after foliar application using a Leaf Porometer (model AP4). The MDA rate was determined as follows: A 1 g plant tissue sample was weighed and immersed in 2.5 mL of 10 % trichloroacetic acid solution. The resulting solution was then centrifuged at 15000 rpm for 20 min. After centrifugation, an equal volume of extraction solution containing 5 % phenol sulfuric acid and 20 % trichloroacetic acid solution was added to the test tube, which was then placed in an incubator at 96 °C for 30 min. Finally, the tubes were cooled in cold water for 5 min and then centrifuged at 10000 rpm for 5 min. The absorption of the resultant solution was measured using the Dynamic-halo xb-10 UV-Vis spectrophotometer at wavelengths of 532 nm and 600 nm (29). For determination of APOX and CAT activity, 0.5 g of leaf tissue was ground with 3 mL of extraction buffer (Tris-HCl buffer, pH 7.0, containing 50 millimolar HCl, 3 mM $MgCl_2$ and 1 mM EDTA) in a chilled mortar. The mixture was then centrifuged at 5000 rpm for 20 min at 4 ºC. The resulting solution (extraction solution) was used to measure the activity of the enzymes APOX and CAT. APOX activity was calculated as micromoles of H_2O_2 per min per mg of protein at a wavelength of 290 nm. CAT activity was measured as micromoles of H_2O_2 per min per mg of protein at a wavelength of 240 nm (30, 31). Statistical analysis of the data was performed using SAS and SPSS software. Excel software was used for creating figures.

Statistical analyses

After conducting the required tests and confirming the normality of the data distribution, a combined analysis of variance was performed using SAS and SPSS software. One-way ANOVA followed by Tukey's test was employed to assess mean comparability. Figures were created using Excel software.

Results

Combined analysis of variance for the data

The combined analysis of variance indicated that the effects of year, irrigation, foliar spraying, cultivars and their interactions on the examined traits were statistically significant (Table 2).

The mean comparison of the interaction of data

The leaf catalase (CAT) activity

The comparison of the mean interaction effect resulting from foliar application with anti-transpiration materials (F) x irrigation (I) x year (Y) on leaf CAT activity showed that the highest CAT rates were observed with foliar application of calcium chloride in both years (49.1 and 50.5 U/mg). CAT activity exhibited varying patterns over the 2 years, with the highest enzyme activity consistently associated with foliar application. This peak enzyme activity was consistently observed under conditions of 160 mm evaporation rate (I_3), as depicted in Fig. 1. The significant CAT rates observed with

Table 2. Combined analysis of variance for traits under varying irrigation regimes and genotypes.

S.O.V	**d.f**	**CAT**	**APOX**	**MDA**	**Transpiration**	**Yield**
Y (Year)	1	93.889^{ns}	0.074^{*}	1.420^{**}	4.346^{ns}	32413126.05^{ns}
R (Year)	4	165.056	0.004	0.209	0.313	16430660.38
I (Irrigation)	2	211.072^{**}	0.119^{**}	4.012^{**}	40.254^{**}	11734306.55^{**}
I x Y	2	43.706^{ns}	0.038^{*}	0.007^{ns}	6.971^{*}	63556.01^{ns}
Error	8	23.064	0.008	0.060	1.306	575866.41
F (Foliar application)	4	7981.661^{**}	0.295^{**}	11.398^{**}	1.999^{*}	1161121.31^{ns}
Y x F	4	33.361^{ns}	0.016^{*}	0.154^{ns}	0.803^{ns}	6264.89^{ns}
I x F	8	575.107^{**}	0.296^{**}	4.777^{**}	1.629^{*}	3043753.89**
Y x I x F	8	79.449^{**}	0.010^{ns}	0.142^{ns}	1.338^{*}	16462.30^{ns}
C (Cultivar)	1	121.689^{**}	0.109^{**}	2.378^{**}	0.114^{ns}	26749074.06^{**}
Y x C	1	2.689	0.001^{ns}	0.152^{ns}	0.030^{ns}	144556.67
I x C	2	282.170^{**}	0.207^{**}	1.361^{**}	0.609^{ns}	90338.10^{**}
Y x I x F	2	0.072^{ns}	0.016^{ns}	0.175^{ns}	0.755^{ns}	485.57^{ns}
I x F	4	190.606^{**}	0.458^{**}	4.682^{**}	1.373^{ns}	3732329.35^{**}
Y x F x C	4	5.050^{ns}	0.007^{ns}	0.158^{ns}	1.167^{ns}	20109.35^{ns}
I*F*F	8	274.568^{**}	0.385^{**}	6.236^{**}	0.922^{ns}	1313568.97^{*}
Y*I*F*C	8	12.454^{ns}	0.017^{**}	0.321^{**}	0.726^{ns}	7098.77^{ns}
Error	108	15.080	0.006	0.048	0.621	604673.02
C.V (%)	**-**	8.93	4.83	3.41	15.89	13.47

ns: Not significant * and **: Significant at 5 % and 1 % probability levels respectively

C1: Hydari cultivar and $C_{2:}$ Zarrineh cultivar calcium chloride in both years indicate a notable impact of this anti-transpiration material on enhancing enzymatic activity.

As illustrated in Fig. 2, the combined effect of irrigation withholding (I) x foliar application of anti-transpiration materials (F) x wheat cultivars (C) influenced leaf CAT activity in both cultivars. The highest CAT activity was associated with foliar application of chitosan and calcium chloride at various irrigation stages, with the Zarrineh cultivar exhibiting the highest levels at 160 mm evaporation, recording 57.5 and 55.2 U/mg protein. Min^{-1} respectively.

The ascorbate peroxidase (APOX) and malondialdehyde (MDA)activity

Based on combined analysis of variance, the interaction effects of irrigation × foliar application (I x F), irrigation x cultivar (I x C), foliar application x cultivar (F x C)) and year x irrigation x foliar application cultivar (Y x I x F x C) were found to be statistically significant for APOX activity (Table 1). The peak APOX activity was consistently observed in both years of the experiment under conditions of irrigation withholding at a 160 mm evaporation rate in the Chitosan-treated Zarrineh cultivar, reaching 2.283 U/mg protein/min (Table 3). In contrast, the lowest APOX activity was associated with the control treatment under a 100 mm evaporation irrigation regime in the Hydari cultivar, registering at 1.443 U/mg protein/min (Table 3). APOX activity showed higher levels with foliar application of the anti-transpiration material chitosan compared to other treatments. The enhanced growth of plants under stressful conditions may be attributed to the pivotal role of antioxidant enzymes in mitigating the harmful effects of oxidative stress, supported by previous research on wheat. This assertion finds support in previous research conducted on wheat (32). The peak MDA activity was consistently observed in both experimental years under conditions of irrigation withholding at a 160 mm evaporation rate. Specifically, the highest MDA activity was recorded in the Calcium chloride-treated Zarrineh cultivar, reaching 9.897 U/mg protein/min in the first year. In

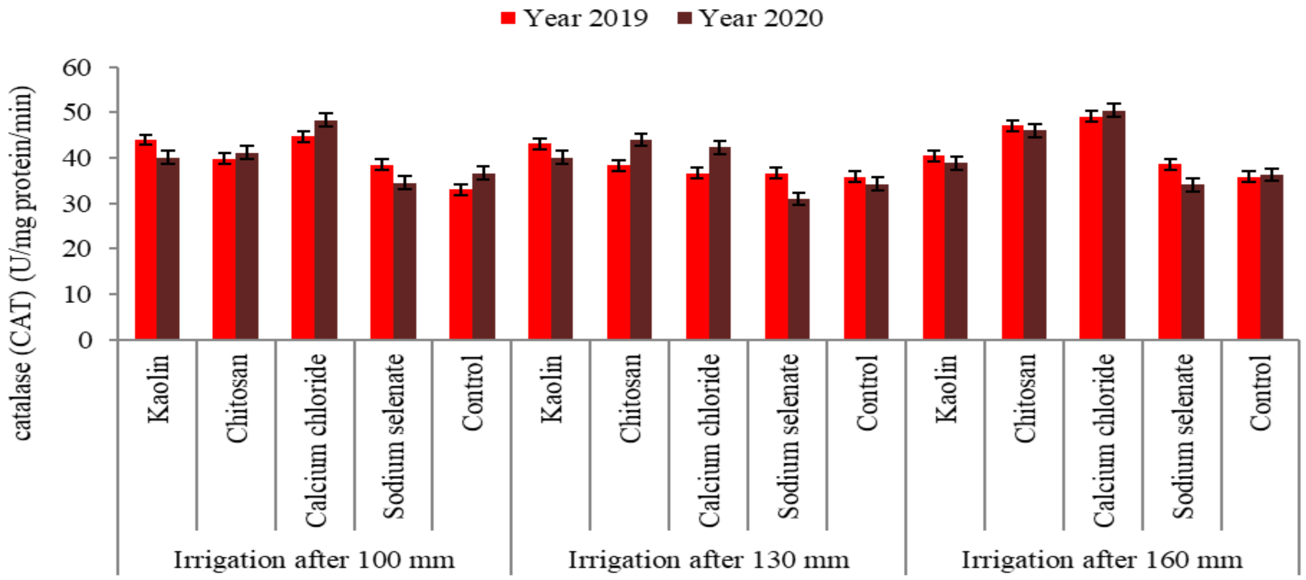

Fig. 1. Leaf catalase (CAT) content means within the interaction effect of anti-transpiration materials (F) × irrigation (I) × year (Y) in wheat cultivars (Tukey's test; P≤0.05).

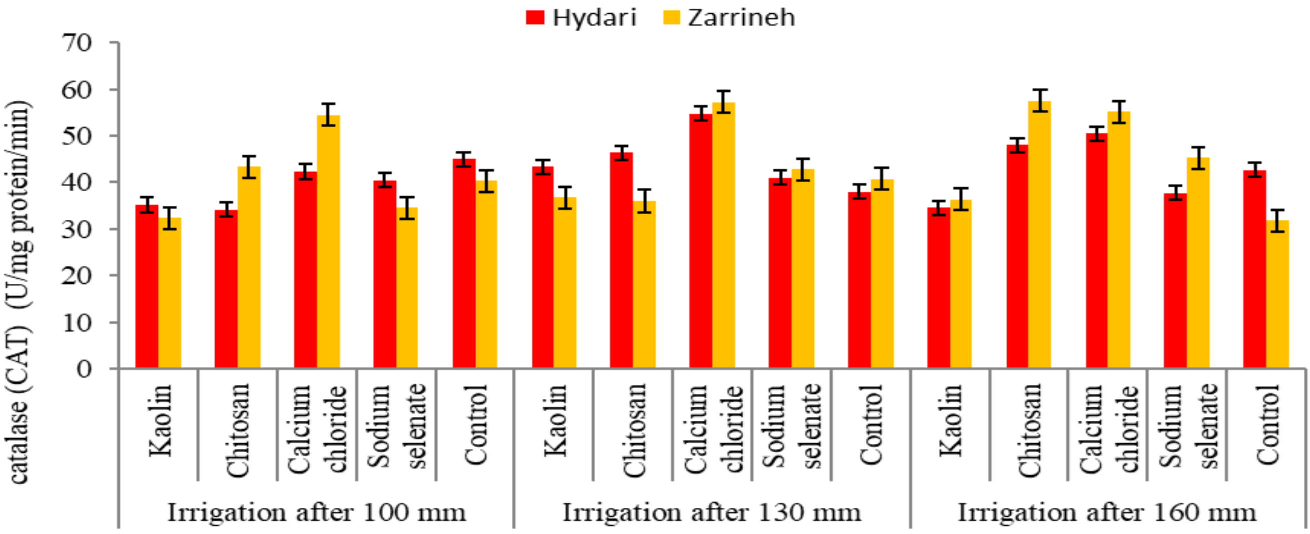

Fig. 2. Mean of leaf catalase (CAT) content of wheat cultivars in interaction effect of irrigation (I) × foliar application of anti-transpiration materials (F) × wheat cultivars (C) (Tukey's test; P≤0.05).

contrast, the lowest MDA activity was associated with the Sodium selenite treatment under irrigation withholding at a 130 mm evaporation rate in the Hydari cultivar during the second year, measuring at 6.413 mM/g FW.

The leaf transpiration rate

The interaction effect of irrigation withhold x foliar application x year (I x F x Y) on leaf transpiration rate revealed significant mean differences. Specifically, the lowest transpiration rate was observed with foliar application of Chitosan under the 160 mm evaporation irrigation level in both years, with a further reduction noted in the second year (3.16 μ mol H_2O. m^ (-2). s^ (-1). In contrast, the highest transpiration rate occurred under the 100 mm evaporation irrigation regime across both years, measuring at 6.55 μ mol H_2O. m^ (-2). s^ (-1) (Fig. 3).

The grain yield

The analysis of the interaction effect involving irrigation withhold (I) x foliar application (F) x year (Y) on grain yield revealed significant variations (Fig. 4). Specifically, the highest grain yield was observed in the chitosan treatment under irrigation withholding at the 100 mm evaporation regime, particularly in the Zarrineh cultivar, reaching 7783 kg/ha. In contrast, the lowest grain yield was associated with the Kaolin treatment under irrigation withholding at the 160 mm evaporation regime, specifically in the Hydari cultivar, with a recorded yield of 6390 kg/ha (Fig. 4).

Table 3. The means comparison of the interaction effect of year × irrigation × foliar application cultivar (Y × I × F × C) on ascorbate peroxidase (APOX) and malondialdehyde (MDA) activity.

Irrigation	Foliar application	Cultivar	APOX (U/mg protein/min)		MDA (U/mg protein/min)	
			2019	2020	2019	2020
I_1	Kaolin	C_1	1.33	1.29	8.58	8.68
		C_2	1.543	1.543	7.867	7.69
	Chitosan	C_1	1.267	1.34	7.747	7.51
		C_2	1.373	1.28	9.29	8.75
	Calcium chloride	C_1	1.38	1.302	6.82	6.47
		C_2	1.873	1.76	7.82	7.75
	Sodium selenate	C_1	1.797	1.817	7.933	7.28
		C_2	1.88	1.95	7.417	7.65
	Control	C_1	1.573	1.55	8.273	8.437
		C_2	1.443	1.56	8.127	8.047
I_2	Kaolin	C_1	2.047	2.03	8.847	8.12
		C_2	1.477	1.453	8.1	8.07
	Chitosan	C_1	1.76	1.75	8.88	8.41
		C_2	1.49	1.433	8.653	7.04
	Calcium chloride	C_1	1.57	1.583	9.123	9.063
		C_2	1.767	1.78	8.3	9.06
	Sodium selenate	C_1	1.46	1.423	6.86	6.413
		C_2	1.567	1.653	8.877	8.453
	Control	C_1	1.76	1.723	6.3	9.853
		C_2	1.68	1.853	7.61	7.8
I_3	Kaolin	C_1	1.52	1.747	7.927	7.55
		C_2	1.507	1.613	8.59	8.19
	Chitosan	C_1	1.573	1.693	8.723	8.95
		C_2	2.173	2.283	8.37	7.41
	Calcium chloride	C_1	1.517	1.5	7.86	7.563
		C_2	1.603	1.697	9.897	9.523
	Sodium selenate	C_1	2.073	1.8	6.693	6.9
		C_2	1.887	1.66	6.427	6.86
	Control	C_1	1.19	1.33	8.07	8.093
		C_2	1.69	1.677	7.07	7.02
LSD at 5 %			0.22		1.29	

I_1, I_2 and I_3 irrigation after 100, 130 and 160 mm evaporation from class A pan, respectively.

C_1: Hydari cultivar and C_2: Zarrineh cultivar

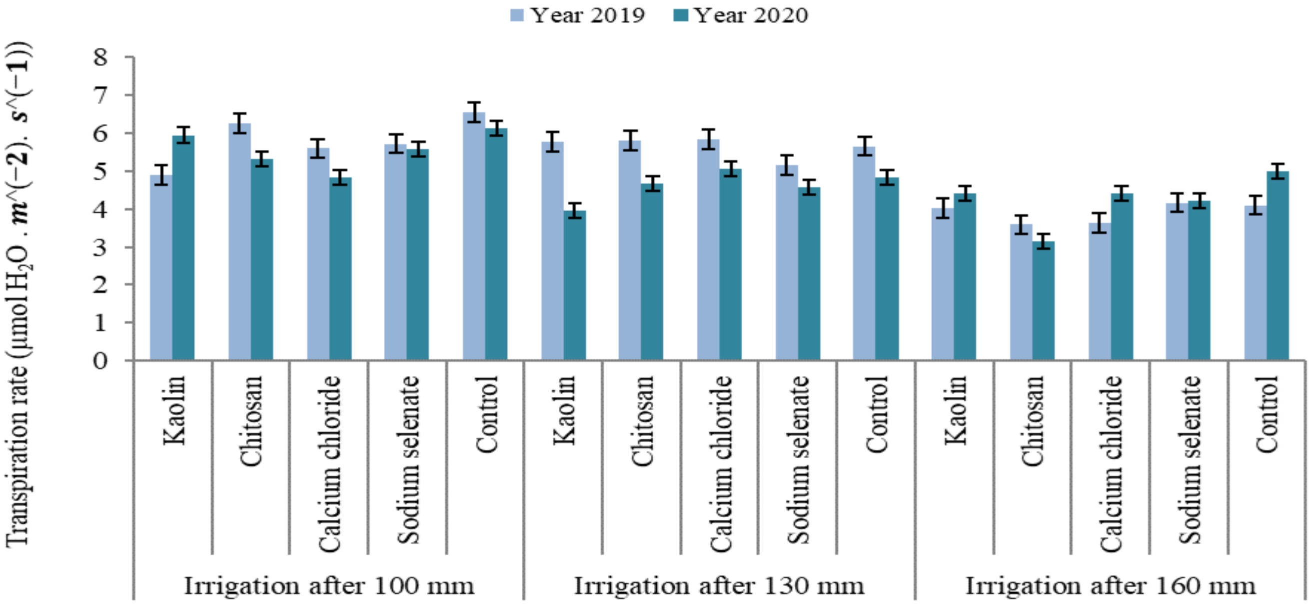

Fig. 3. Mean of leaf transpiration rate of wheat cultivars in interaction effect of irrigation (I) × foliar application (F) × wheat cultivars (C) (Tukey's test;

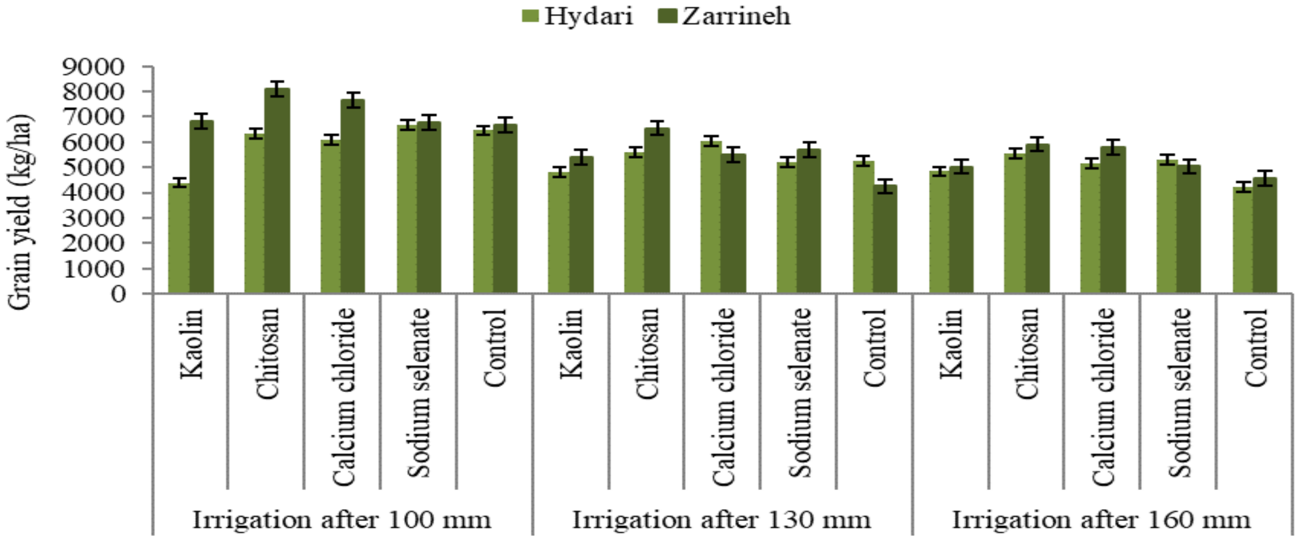

Fig. 4. Mean of grain yield of wheat cultivars in interaction effect of irrigation (I) × foliar application (F) × Year (Y) (Tukey's test; P≤0.05).

Discussion

The results of the combined analysis of variance conducted in this study highlights the significance of several factors, including year (Y), irrigation (I), foliar spraying (F), cultivars (C) and their interactions, in influencing the studied traits. The following discussion contextualizes these findings within the existing literature, emphasizing the effect of drought stress, antioxidant enzyme activities and the effectiveness of anti-transpiration materials in improving wheat plant responses to stress.

The Leaf Catalase (CAT) Activity

The increased CAT activity observed, especially with calcium chloride foliar application, underscores the crucial role of anti-transpiration materials in enhancing antioxidant defences. This finding is consistent with previous research (33), which highlights the beneficial effects of calcium chloride on the physiological, biochemical and yield aspects of sunflower plants under water deficit stress conditions. The sustained impact of foliar application, reflected in consistent peaks of CAT activity over 2 years, underscores its potential in mitigating drought stress. Cultivar-specific responses to foliar application of Chitosan and calcium chloride further highlights the nuanced influence of anti-transpiration materials on CAT activity. The highest levels of CAT activity in Zarrineh cultivar under specific irrigation conditions (I_3= Drought stress) emphasize the potential for customized applications of anti-transpiration materials based on cultivar characteristics. The documented effect of drought on antioxidant enzyme activities, particularly catalase (CAT), aligns with previous studies across various plant species, including wheat (34, 35), barley (36), sesame (37), soybeans (38) and canola (39). These studies provide a solid foundation for understanding the impact of drought on CAT, highlighting its universality across diverse plant species. As mentioned earlier, in our study, the increased CAT activity observed in foliar applications with anti-transpiration materials appears to mitigate damage caused by free oxygen radicals and lipid oxidation. Consequently, this phenomenon contributes to an increase in the membrane stability index within cultivars, emphasizing the effectiveness of these materials in enhancing tolerance to drought stress conditions. It has been observed that the heightened activity of antioxidant enzymes plays a crucial role in mitigating molecular disruptions responsible for physiological damage in plants under drought stress (40). This damage is often linked to increased production of reactive oxygen species. All plant species inherently possess both enzymatic and non-enzymatic antioxidant mechanisms, which serve as intrinsic defense against the harmful effects of active oxygen species (41). In general, imposition of drought stress triggers an increase in plant antioxidant activity, particularly catalase (CAT) activity.

The Ascorbate Peroxidase (APOX) and Malondialdehyde (MDA) Activity

The Chitosan-treated Zarrineh cultivar exhibited peak APOX activity and the lowest MDA levels, underscoring the efficacy of chitosan in maintaining redox balance under drought-induced stress (41). Our findings align with previous research (42) which demonstrated that foliar application of anti-transpiration materials, particularly chitosan, enhances APOX and CAT activity. The application of chitosan foliarly resulted in the lowest MDA levels, a critical indicator of lipid peroxidation induced by reactive oxygen species (ROS). Increased MDA concentrations indicate heightened lipid peroxidation and oxidation of membrane fatty acid (43). Previous research has linked elevated lipid peroxidation to reduce cell membrane stability across various plant species, including wheat (44), bean (45) and grass (46). Consistent with an earlier study (47), on the aquatic plant *Hydrilla verticillata*, chitosan application led to lower MDA content compared to the control group, suggesting a role for chitosan in reducing lipid peroxidation and enhancing membrane stability. Other investigations have similarly observed increased enzyme activity under drought conditions (48), further supporting chitosan's potential in mitigating lipid peroxidation among diverse plant species.

As previously discussed, Chitosan has demonstrated the ability to enhance APOX and CAT activity, thereby aiding in the efficient scavenging of active oxygen species and subsequently reducing plasma membrane damage. This mechanism results in decreased MDA accumulation, indicating that chitosan can mitigate lipid oxidation through the direct removal of free radicals and/or prevention of MDA increase facilitated by antioxidant enzymes (49). The neutralization of free radicals by chitosan is likely attributed to its distinctive structure, characterized by a significant presence of amine and hydroxyl groups. These structural components facilitate reactions with free oxygen radicals (ROS), forming the basis for chitosan's observed capacity to neutralize and mitigate oxidative stress (50, 51). Our results indicate a consistent peak in MDA activity observed in the calcium chloride-treated Zarrineh cultivar under conditions of irrigation withholding at a 160 mm evaporation rate (I_3), suggesting the potential of this material in mitigating lipid oxidation.

The Leaf Transpiration Rate

The lowest transpiration rate observed with Chitosan under a 160 mm evaporation level (I_3), coupled with a reduction in the second year, underscores the role of anti-transpiration materials in regulating water loss. In contrast, the highest transpiration rate associated with the 100 mm evaporation irrigation regime (I_1) and the control treatment (no anti-transpiration materials) emphasizes the complex interplay of factors influencing transpiration dynamics. These findings align with previous studies that have documented reduced stomatal conductance and transpiration rates with the use of anti-transpiration materials (13, 42). Notably, as highlighted in previous study (13), the application of anti-transpiration materials not only enhances photosynthesis but also improves drought resistance in plants like sweet pepper by regulating stomatal activity, enhancing water storage, promoting plant growth and increasing leaf water potential, thereby reducing water stress during critical growth stages. Another study has similarly shown that leaves treated with anti-transpiration materials exhibit increased water potential, underscoring their significance in alleviating water stress and enhancing overall plant vigor (52).

The Grain Yield

The examination of grain yield provides further evidence for the complex interactions involving irrigation (I), foliar application (F) and year (Y). Notably, the application of Chitosan emerges as a significant factor, resulting in the highest grain yield in the Zarrineh cultivar under irrigation withholding at the 100 mm evaporation regime (I_1). This underscores the practical importance of anti-transpiration materials in optimizing crop productivity, demonstrating their effectiveness even under varied environmental conditions. Moreover, previous studies have highlighted the benefits of chitosan in enhancing wheat's ability to cope with drought stress. For instance, research using chitosan nanoparticles at a concentration of 90 ppm showed improvements in wheat physiology and yield, enhancing resilience during drought periods (53). Additionally, spraying chitosan on wheat leaves, particularly at a concentration of 0.1 % during the vegetative stage, positively impacted growth, development and economic yield under drought stress conditions (54). These findings suggest that chitosan could serve as a valuable tool for enhancing crop resilience to water scarcity (54). It has been observed that the use of chitosan nanoparticles, especially at concentrations of 60 and 90 ppm, significantly enhances barley plant characteristics during late-season drought stress, suggesting a potential approach to mitigate the adverse effects of water scarcity on growth and yield (55). Furthermore, the application of zinc-chitosan-salicylic acid (ZCS) nanoparticles at a concentration of 100 mg L^{-1} has been found to enhance water content, reduce oxidative stress and increase wheat grain yield, highlighting their potential in alleviating drought effects and promoting sustainable agriculture (56). The detrimental impact of drought stress or deficit irrigation on agricultural production is evident from the production of reactive oxygen species, which lead to membrane lipid peroxidation and interactions with macromolecules, ultimately resulting in reduced plant growth and yield (57). These findings underscore the complex interplay between environmental stressors, protective measures like anti-transpiration materials and the overall productivity of crops, underscoring the necessity for targeted strategies in agricultural practices. Moreover, focusing on grain yield underscores the significant role of chitosan treatment, which notably enhanced grain production in the Zarrineh cultivar under specific irrigation conditions. This emphasizes the practical importance of anti-transpiration materials, such as chitosan, in improving crop productivity, particularly in mitigating the detrimental effects of drought stress on yield.

Conclusion

The study demonstrates that the activity of antioxidant enzymes in wheat varies significantly with different anti-transpiration materials under conditions of water deficit. Specifically, foliar application of chitosan during irrigation withholding enhances CAT and APOX activity, underscoring its potential to enhance wheat's antioxidant defenses and crop productivity. The response of enzymatic activity varies across cultivars and is influenced by the timing of stress imposition. Calcium chloride consistently induces the highest CAT activity levels. Moreover, chitosan application reduces MDA levels, highlighting its role in mitigating oxidative stress. The highest grain yield observed, particularly in the Zarrineh cultivar, underscores the potential of chitosan to enhance crop performance under water-deficient conditions. These findings provide valuable insights into sustainable water management, emphasizing the efficacy of anti-transpiration compounds, particularly chitosan, in improving wheat's physiological responses and resilience to water deficit, addressing the global need for water-efficient agricultural strategies amidst changing climates.

Acknowledgements

The author would like to extend special thanks to Associate Professor Mohammad Rezaei for invaluable assistance, insightful discussions and technical support in the research field throughout this work.

Compliance with ethical standards

Conflict of interest: **:** The author does not have any conflict of interest to declare

Ethical issues: None

References

1. Kıran S, Baysal Furtana G. Responses of eggplant seedlings to combined effects of drought and salinity stress: Effects on photosynthetic pigments and enzymatic and non-enzymatic antioxidants. Gesunde Pflanzen. 2023;75:2579-90. https://doi.org/10.1007/s10343-023-00901-9.
2. Zhou Y, Hu L, Ye S, Jiang L, Liu S. Genome-wide identification of glutathione peroxidase (GPX) gene family and their response to abiotic stress in cucumber. 3 Biotech. 2018;8:1-11. https://doi.org/10.1007/s13205-018-1185-3
3. Zhou Y, Hu L, Wu H, Jiang L, Liu S. Genome-wide identification and transcriptional expression analysis of cucumber superoxide dismutase (SOD) family in response to various abiotic stresses. International Journal of Genomics. 2017;2017. https://doi.org/10.1155/2017/7243973
4. Rajput VD, Harish, Singh RK, Verma KK, Sharma L, Quiroz-Figueroa FR, et al. Recent developments in enzymatic antioxidant defence mechanism in plants with special reference to abiotic stress. Biology. 2021;10(4):267. https://doi.org/10.3390/biology10040267
5. Hasanuzzaman M, Nahar K, Rahman A, Anee TI, Alam MU, Bhuiyan TF, et al. Approaches to enhance salt stress tolerance in wheat. In: Wanyera R, Owuache J, editors. Wheat improvement, management and utilization. InTech; 2017.151-87. https://doi.org/10.5772/67247
6. Abdallah MMS, El-Bassiouny HMS, AbouSeeda MA. Potential role of kaolin or potassium sulfate as anti-transpirant on improving physiological, biochemical aspects and yield of wheat plants under different watering regimes. Bulletin of the National Research Centre. 2019;43:1-12. https://doi.org/10.1186/s42269-019-0177-8
7. Rahim RWA, Manea AI, Al-Anbagi RAA. Techniques of arbuscular mycorrhiza as a biofertilizer and an anti-transpiration for promoting plant growth and fruit chemical features of pepper. IOP Conf. Series: Earth and Environmental Science: 4th International Agricultural Conference (IAC-2023); IOP Publishing; 2023.1213. https://doi.org/10.1088/1755-1315/1213/1/012065
8. Zeboon NH, Baqir HAA. The role of anti-transpiration in plant growth. Iraqi Journal of Soil Science. 2022;22(2). Available from: https://iasj.net/iasj/article/247817
9. Al-Saidy NJH, AL-Mayahi MZS. Effect of adding polymer (sap) levels, spraying kaolin anti-transpiration and bread yeast suspension on some qualitative traits in fruits of *Carica papaya* L. Journal of Pharmaceutical Negative Results. 2023;6650-57. https://doi.org/10.47750/pnr.2022.13.S07.806
10. MM Elmasry H, HA Al-maracy S. Efficacy of anti-transpiration on yield and quality of sugar beet subjected to water stress. Journal of Central European Agriculture. 2023;24(1):268-81. https://doi.org/10.5513/JCEA01/24.1.3634
11. AbdAllah AM, Burkey KO, Mashaheet AM. Reduction of plant water consumption through anti-transpirants foliar application in tomato plants (*Solanum lycopersicum* L). Scientia Horticulturae. 2018;235:373-81. https://doi.org/10.1016/j.scienta.2018.03.005
12. Del Amor FM, Cuadra-Crespo P, Walker DJ, Cámara JM, Madrid R. Effect of foliar application of antitranspirant on photosynthesis and water relations of pepper plants under different levels of CO_2 and water stress. Journal of Plant Physiology. 2010;167(15):1232-38. https://doi.org/10.1016/j.jplph.2010.04.010
13. Hidangmayum A, Dwivedi P, Katiyar D, Hemantaranjan A. Application of chitosan on plant responses with special reference to abiotic stress. Physiology and Molecular Biology of Plants. 2019;25:313-26. https://doi.org/10.1007/s12298-018-0633-1

14. Cantore V, Pace B, Albrizio R. Kaolin-based particle film technology affects tomato physiology, yield and quality. Environmental and Experimental Botany. 2009;66(2):279-88. https://doi.org/10.1016/j.envexpbot.2009.03.008

15. Nakano A, Uehara Y, editors. The effects of kaolin clay on cuticle transpiration in tomato. International Symposium on Plant Production in Closed Ecosystems; 1996. 440:233-38. https://doi.org/10.17660/ActaHortic.1996.440.41

16. Gharaghani A, Javarzari AM, Vahdati K. Kaolin particle film alleviates adverse effects of light and heat stresses and improves nut and kernel quality in Persian walnut. Scientia Horticulturae. 2018;239:35-40. https://doi.org/10.1016/j.scienta.2018.05.024

17. Burme L, Moallemi N, Mortazavi S. Anti-transpiration effect of kaolin on some physiological traits of four olive cultivars. Isfahan University of Technology-Journal of Crop Production and Processing. 2011;1(1):11-23. Available from: http://jcpp.iut.ac.ir/article-1-1347-en.html

18. Farouk S, Amany AR. Improving growth and yield of cowpea by foliar application of chitosan under water stress. Egyptian Journal of Biology. 2012;14:14-16. https://doi.org/10.4314/ejb.v14i1.2

19. Falcón AB, Cabrera JC, Costales D, Ramírez MA, Cabrera G, Toledo V, et al. The effect of size and acetylation degree of chitosan derivatives on tobacco plant protection against *Phytophthora parasitica* nicotianae. World Journal of Microbiology and Biotechnology. 2008;24:103-12. https://doi.org/10.1007/s11274-007-9445-0

20. Fouda SE, El-Saadony FM, Saad AM, Sayed SM, El-Sharnouby M, El-Tahan AM, et al. Improving growth and productivity of faba bean (*Vicia faba* L.) using chitosan, tryptophan, and potassium silicate anti-transpirants under different irrigation regimes. Saudi Journal of Biological Sciences. 2022;29(2):955-62. https://doi.org/10.1016/j.sjbs.2021.10.007

21. Shoresh M, Spivak M, Bernstein N. Involvement of calcium-mediated effects on ROS metabolism in the regulation of growth improvement under salinity. Free Radical Biology and Medicine. 2011;51(6):1221-34. https://doi.org/10.1016/j.freeradbiomed.2011.03.036

22. Park S, Moon Y, Waterland NL. Treatment with calcium chloride enhances water deficit stress tolerance in Viola (*Viola cornuta*). HortScience. 2020;55(6):882-87. https://doi.org/10.21273/HORTSCI14835-20

23. Ranjita B, Janawade A, Palled Y. Effect of irrigation schedule, mulch and anti transpirant on growth, yield and economy of wheat. Karnataka Journal of Agricultural Science. 2007;20(1):6-9.

24. Iriti M, Picchi V, Rossoni M, Gomarasca S, Ludwig N, Gargano M, et al. Chitosan antitranspirant activity is due to abscisic acid-dependent stomatal closure. Environmental and Experimental Botany. 2009;66(3):493-500. https://doi.org/10.1016/j.envexpbot.2009.01.004

25. Gilliham M, Dayod M, Hocking BJ, Xu B, Conn SJ, Kaiser BN, et al. Calcium delivery and storage in plant leaves: exploring the link with water flow. Journal of Experimental Botany. 2011;62(7):2233-50. https://doi.org/10.1093/jxb/err111

26. Germ M, Stibilj V. Selenium and plants. Acta Agriculturae Slovenica. 2007;89(1):65-71. https://doi.org/10.14720/aas.2007.89.1.14980

27. Mohammadi H, Sepehri A, Sabaghpour H. Effect of antitranspiration substances and drought stress ameliorators on leaf area duration, water use efficiency and grain yield of chickpea (*Cicer arietinum* L.) under different irrigation regimes. Applied Research in Field Crops Journal. 2018;31(2):92-118. Available from: https://sid.ir/paper/367225/fa

28. Fischer R, Rees D, Sayre K, Lu ZM, Condon A, Saavedra AL. Wheat yield progress associated with higher stomatal conductance and photosynthetic rate and cooler canopies. Crop Science. 1998;38(6):1467-75. https://doi.org/10.2135/cropsci1998.0011183X003800060011x

29. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics. 1968;125(1):189-98. https://doi.org/10.1016/0003-9861(68)90654-1

30. Kar M, Mishra D. Catalase, peroxidase and polyphenoloxidase activities during rice leaf senescence. Plant Physiology. 1976;57(2):315-19. https://doi.org/10.1104/pp.57.2.315

31. Aebi H. Catalase *in vitro*. Methods in Enzymology. Elsevier. 1984;105:121-26. https://doi.org/10.1016/S0076-6879(84)05016-3

32. Mandhania S, Madan S, Sawhney V. Antioxidant defense mechanism under salt stress in wheat seedlings. Biologia Plantarum. 2006;50:227-31. https://doi.org/10.1007/s10535-006-0011-7

33. Ibrahim M, Faisal A, Shehata S. Calcium chloride alleviates water stress in sunflower plants through modifying some physio-biochemical parameters. American-Eurasian J Agric Environ Sci. 2016;16(4):677-93. https://doi.org/10.5829/idosi.aejaes.2016.16.4.12907

34. Zhang J, Kirkham M. Drought-stress-induced changes in activities of superoxide dismutase, catalase and peroxidase in wheat species. Plant and Cell Physiology. 1994;35(5):785-91. https://doi.org/10.1093/oxfordjournals.pcp.a078658

35. Yang S, Deng X. Effects of drought stress on antioxidant enzymes in seedlings of different wheat genotypes. Pak J Bot. 2015;47(1):49-56. Available from: https://www.pakbs.org/pjbot/paper_details.php?id=4115

36. Zhanassova K, Kurmanbayeva A, Gadilgereyeva B, Yermukhambetova R, Iksat N, Amanbayeva U, et al. ROS status and antioxidant enzyme activities in response to combined temperature and drought stresses in barley. Acta Physiologiae Plantarum. 2021;43:1-12. https://doi.org/10.1007/s11738-021-03281-7

37. Fazeli F, Ghorbanli M, Niknam V. Effect of drought on biomass, protein content, lipid peroxidation and antioxidant enzymes in two sesame cultivars. Biologia Plantarum. 2007;51:98-103. https://doi.org/10.1007/s10535-007-0020-1

38. Vasconcelos ACFd, Zhang X, Ervin EH, Kiehl JdC. Enzymatic antioxidant responses to biostimulants in maize and soybean subjected to drought. Scientia Agricola. 2009;66:395-402. https://doi.org/10.1590/S0103-90162009000300015

39. Mirzai M, Moeini A, Ghanati F. Effects of drought stress on the lipid peroxidation and antioxidant enzyme activities in two canola (*Brassica napus* L.) cultivars. Journal of Agricultural Science And Technology (JAST) [Internet]. 2013;15(3):593-602. Available from: https://sid.ir/paper/606996/en

40. Hasanuzzaman M, Nahar K, Gill SS, Fujita M. Drought stress responses in plants, oxidative stress and antioxidant defense. Climate Change and Plant Abiotic Stress Tolerance. 2013:209-50. https://doi.org/10.1002/9783527675265.ch09

41. Ahmad P, Prasad MNV. Abiotic stress responses in plants: metabolism, productivity and sustainability: Springer Science and Business Media; 2011. https://doi.org/10.1007/978-1-4614-0634-1

42. Pourghasemian N, Moradi R, Iriti M. Assessing anti-transpiration potential of beeswax waste on *Calendula officinalis* under drought stress conditions. Scientia Horticulturae. 2023;315:111987. https://doi.org/10.1016/j.scienta.2023.111987

43. Hu R, Fan Z, Chen S, Huang Y, Lv X, editors. Effects of different buckle shed time on catalase activity and malondialdehyde content in grape leaves. IOP Conference Series: Earth and

Environmental Science; 2019: IOP Publishing.2019:358(3). https://doi.org/10.1088/1755-1315/358/3/032066

44. Sairam RK, Rao KV, Srivastava G. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Science. 2002;163(5):1037-46. https://doi.org/10.1016/S0168-9452(02)00278-9
45. Türkan I, Bor M, Özdemir F, Koca H. Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. Plant Science. 2005;168(1):223-31. https://doi.org/10.1016/j.plantsci.2004.07.032
46. Jiang Y, Huang B. Drought and heat stress injury to two cool-season turfgrasses in relation to antioxidant metabolism and lipid peroxidation. Crop Science. 2001;41(2):436-42. https://doi.org/10.2135/cropsci2001.412436x
47. Xu Q-j, Nian Y-g, Jin X-c, Yan C-z, Jin L, Jiang G-m. Effects of chitosan on growth of an aquatic plant (*Hydrilla verticillata*) in polluted waters with different chemical oxygen demands. Journal of Environmental Sciences. 2007;19(2):217-21. https://doi.org/10.1016/S1001-0742(07)60035-7
48. Zhang G, Tanakamaru K, Abe J, Morita S. Influence of waterlogging on some anti-oxidative enzymatic activities of two barley genotypes differing in anoxia tolerance. Acta Physiologiae Plantarum. 2007;29:171-76. https://doi.org/10.1007/s11738-006-0022-1
49. Zeng D, Luo X. Physiological effects of chitosan coating on wheat growth and activities of protective enzyme with drought tolerance. Open Journal of Soil Science. 2012;2(03):282. https://doi.org/10.4236/ojss.2012.23034
50. Sun XX, Hodge JJ, Zhou Y, Nguyen M, Griffith LC. The eag potassium channel binds and locally activates calcium/calmodulin-dependent protein kinase II. Journal of Biological Chemistry. 2004;279(11):10206-14. https://doi.org/10.1074/jbc.M310728200
51. Picchi V, Gobbi S, Fattizzo M, Zefelippo M, Faoro F. Chitosan nanoparticles loaded with N-acetyl cysteine to mitigate ozone and other possible oxidative stresses in durum wheat. Plants. 2021;10(4):691. https://doi.org/10.3390/plants10040691
52. Fathi A, Jiriaieb M. Interaction of PGPR and water deficit stress on yield and protein percent in wheat. Advances in Crop Science and Technology. 2014;4:82-90.
53. Aali MRM, Eivazi A, Mohammadi S, Shir-Alizadeh S. Effect of drought stress on dry matter remobilization and grain yield of winter bread wheat genotypes. Iranian Journal of Crop Sciences. 2013;15(3). Available from: https://www.cabidigitallibrary.org/doi/full/10.5555/20143192609
54. Masjedi MH, Roozbahani A, Baghi M. Assessment effect of chitosan foliar application on Total chlorophyll and seed yield of wheat (*Triticum aestivum* L.) under water stress conditions. Journal of Crop Nutrition Science. 2017;3(4):14-26. Available from: https://sanad.iau.ir/journal/jcns/Article/543135?jid=543135
55. Behboudi F, Tahmasebi Sarvestani Z, Kassaee MZ, Modares Sanavi SAM, Sorooshzadeh A, Ahmadi SB. Evaluation of chitosan nanoparticles effects on yield and yield components of barley (*Hordeum vulgare* L.) under late season drought stress. Journal of Water and Environmental Nanotechnology. 2018;3(1):22-39. https://doi.org/10.22090/jwent.2018.01.003
56. Das D, Bisht K, Chauhan A, Gautam S, Jaiswal JP, Salvi P, et al. Morpho-physiological and Biochemical responses in wheat foliar sprayed with zinc-chitosan-salicylic acid nanoparticles during drought stress. Plant Nano Biology. 2023;100034. https://doi.org/10.1016/j.plana.2023.100034
57. Bistgani ZE, Siadat SA, Bakhshandeh A, Pirbalouti AG, Hashemi M. Interactive effects of drought stress and chitosan application on physiological characteristics and essential oil yield of *Thymus daenensis* Celak. The Crop Journal. 2017;5(5):407-15. https://doi.org/10.1016/j.cj.2017.04.003