



RESEARCH ARTICLE

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

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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 trials involved three irrigation levels: I₁ (100 mm evaporation from Class-A pan), I₂ (130 mm evaporation from Class-A pan) and I₃ (160 mm evaporation from Class-A pan), which were applied to the main plots. Two bread wheat genotypes, G₁ (Hydari) and G₂ (Zarrineh), were used in the trials. 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 I₁ 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 m³, 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 CaCl₂ 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: I₁ (100 mm evaporation from Class-A pan), I₂ (130 mm evaporation from Class-A pan) and I₃ (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 (C₆H₁₁O₄N)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 ZnSO₄·7H₂O, 80 kg ha⁻¹ KH₂PO₄ and 90 kg ha⁻¹ HPO₄(NH₄)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 measurements of CAT activity, ascorbate peroxidase (APOX)

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

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 m² 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 MgCl₂ and 1 mM EDTA) in a chilled mortar. The mixture was then centrifuged at 5000 rpm for 20 min at 4 °C. The resulting solution (extraction solution) was used to measure the activity of the enzymes APOX and CAT. APOX activity was calculated as micromoles of H₂O₂ per min per mg of protein at a wavelength of 290 nm. CAT activity was measured as micromoles of H₂O₂ 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 (I₃), 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 C₂: 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 x 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

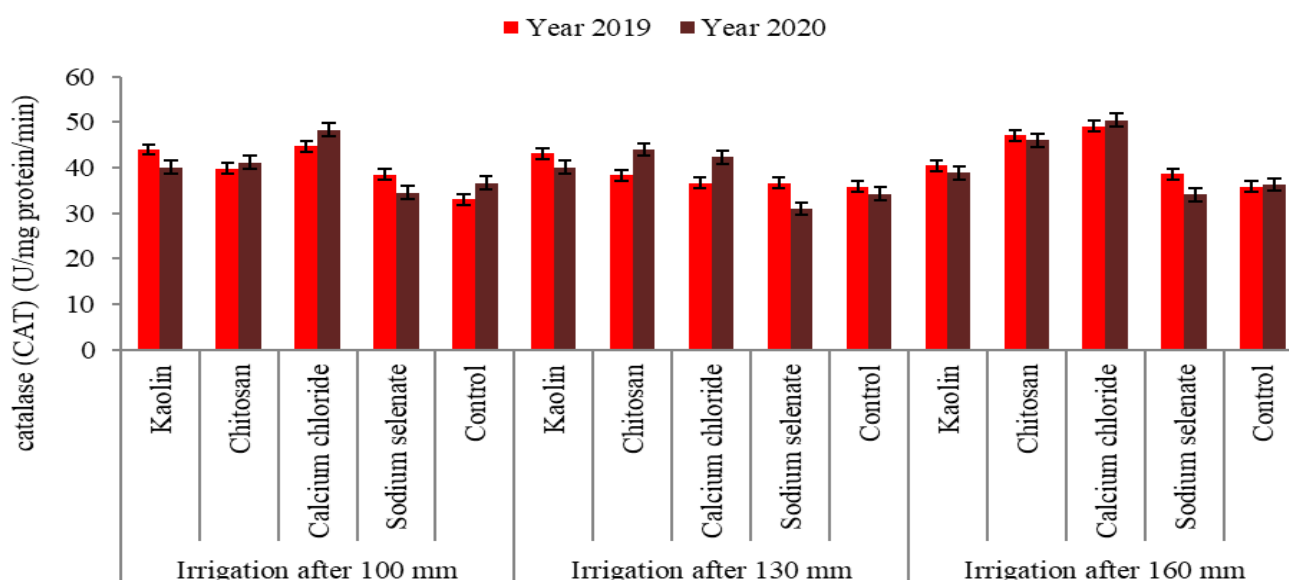


Fig. 1. Leaf catalase (CAT) content means within the interaction effect of anti-transpiration materials (F) x irrigation (I) x year (Y) in wheat cultivars (Tukey’s test; P≤0.05).

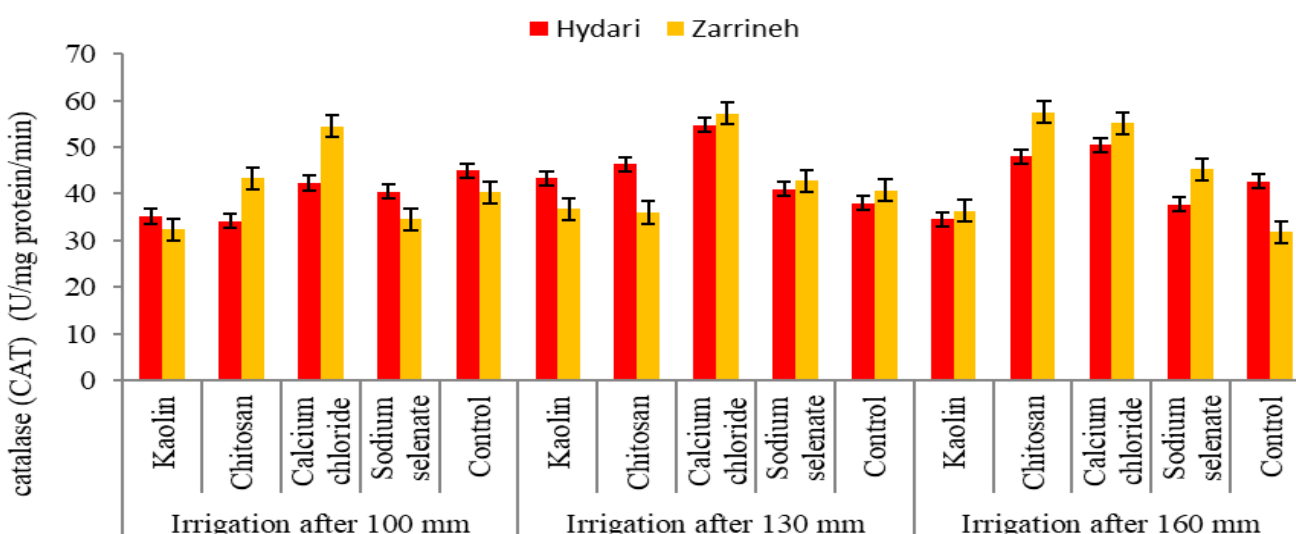


Fig. 2. Mean of leaf catalase (CAT) content of wheat cultivars in interaction effect of irrigation (I) x foliar application of anti-transpiration materials (F) x wheat cultivars (C) (Tukey’s test; P≤0.05).

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 ($3.16 \mu \text{mol H}_2\text{O. m}^{-2} \cdot \text{s}^{-1}$). In contrast, the highest transpiration rate occurred under

the 100 mm evaporation irrigation regime across both years, measuring at $6.55 \mu \text{mol H}_2\text{O. m}^{-2} \cdot \text{s}^{-1}$ (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 x irrigation x foliar application cultivar (Y x I x F x 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	

I₁, I₂ and I₃ irrigation after 100, 130 and 160 mm evaporation from class A pan, respectively.

C₁: Hydari cultivar and C₂: Zarrineh cultivar

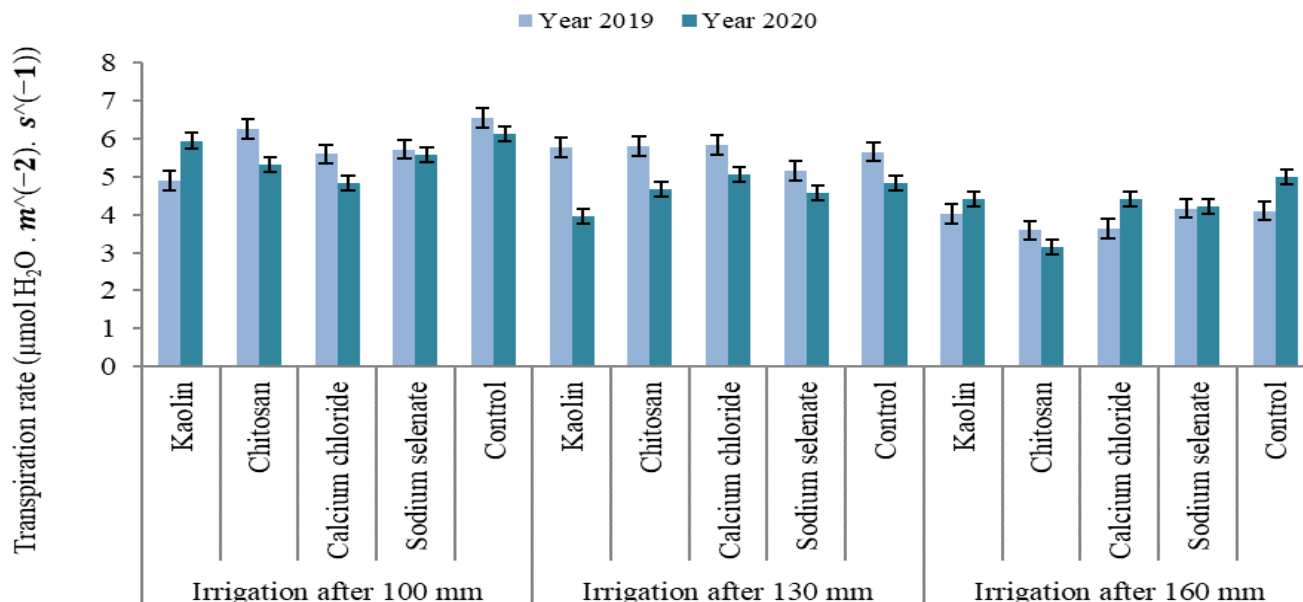


Fig. 3. Mean of leaf transpiration rate of wheat cultivars in interaction effect of irrigation (I) × foliar application (F) × wheat cultivars (C) (Tukey's test; P≤0.05).

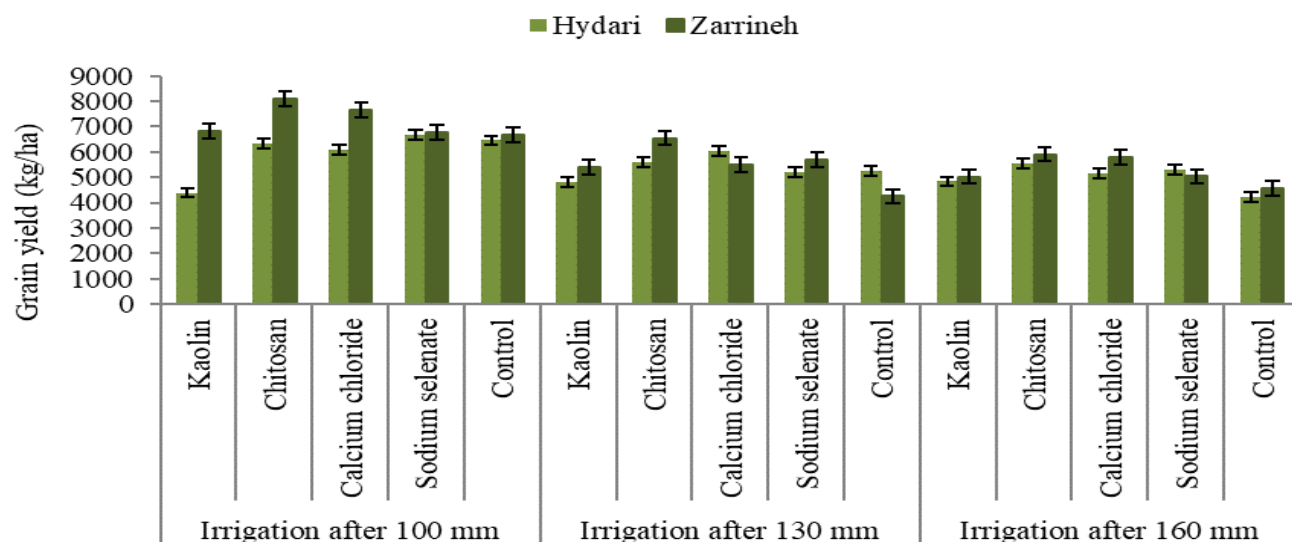


Fig. 4. Mean of grain yield of wheat cultivars in interaction effect of irrigation (I) × foliar application (F) × Year (Y) (Tukey's test; P≤0.05).

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 (I₃= 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.

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Compliance with ethical standards

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