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INFLUENCE OF THE TIMING OF THE APPLICATION OF SALICYLIC ACID ON THE QUANTITATIVE YIELD AND SOME BIOCHEMICAL CHARACTERISTICS OF BARLEY (*Hordeum vulgare* L.) UNDER DEFICIT IRRIGATION

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ABSTRACT

Using salicylic acid (SA) to feed drought-stressed plants plays a vital role in reducing the adverse effects of water stress and improving plant performance. This study explores the role of salicylic acid and different barley cultivars in mitigating the effects of drought stress on barley.The study examined three irrigation levels—one-time irrigation (severe stress), two-time irrigation (moderate stress), and four-time irrigation (control)—along with foliar and non-foliar applications of salicylic acid (SA) at three key stages of the Zadoks Growth Scale (ZGS): ZGS 29 (end of tillering), ZGS 34 (50% stem elongation), and ZGS 39 (completion of flag leaf emergence). These treatments were applied to three barley cultivars—Khatam, Reyhan, and Nosrat—which are considered semi-tolerant to drought stress. The findings showed that the interaction of reduced irrigation and SA increased chlora (8.8%) and b (7.12%) in the ZGS34 treatment under control conditions compared to the treatment without SA. The proline content increased with increasing drought stress, with the highest proline content obtained at the end of the tillering stage in the control condition. Compared to the control, which had no foliar spraying, the specific leaf area increased by 3.8, 1.8, and 0.4%, respectively. Relative water content in Khatam (35.6%), Reyhan (33.3%) and Nosrat (30.5%) decreased with increasing stress in the control treatment compared to the minimum stress. The most sensitive cultivar to lack of irrigation was Khatam. The rate of yield increase by SA compared to the control was (10.33%) among the barley cultivars cultivated, the cultivar Reyhan had a comparative advantage in more measures, mainly when applied at ZGS29. In conclusion, SA improved the drought tolerance of the barley and increased the yield by improving the biochemical characteristics.

Keywords: Antioxidant capacity, Barley, Cultivars, Drought stress, Phytohormone

INTRODUCTION

Barley grain remains found at archaeological sites in the Fertile Crescent indicate that it was domesticated there from its wild relative *Hordeum Vulgare* L. about 10,000 years ago (Badr et al., 2000). It is a perennial plant of the wheat family and one of the oldest cultivated plants with a wide range of distribution and climatic compatibility. This plant is usually grown for grain production and has many uses in human and animal nutrition (Langridge, 2018). Barley is a globally important crop with multiple uses (feed, food and beverage) (Rehman et al., 2021). It is the fourth most important crop in the world in terms of dry matter production after maize, wheat and rice, and in 2021 world barley production reached 145 million tones (FAO, 2022).

Rainfall and temperature are the most important abiotic factors affecting crop yield, especially in arid areas (Kheiri et al., 2021), and warming and climate change are expected to aggravate drought (Rousta et al., 2023). Drought stress is the most important factor limiting agricultural production (Meza et al., 2021). At any stage of plant growth, water deficit stress alters agronomic and physiochemical traits (Nouri et al., 2020; Matinizadeh et al., 2024). The lack of water reduces the uptake and production of substances in the leaf by closing the stomata and increasing respiration. This leads to a reduction in leaf weight. Finally, it reduces leaf area, increases tissue senescence, and a negative effect on assimilate metabolism (Rozentsvet et al., 2022). Changes in the physiological and morphological characteristics of leaves, such as changes in weight and leaf area, are responses of plants to drought (Moshki et al., 2024). These changes affect the rate of photosynthesis and ultimately the plant's yield. The change in leaf thickness and its degree of fleshiness is one of the leaf responses to drought

(Papkyadeh et al., 2023). It is assessed by measuring two indices of specific leaf area to estimate leaf thickness (Yan et al., 2019). Based on previous reports, there is a weak correlation between leaf thickness and yield (Khoshouei et al., 2024). Under drought stress, the leaf area decreases due to a reduction in cell size, which causes a reduction in its specific area. Based on researchers, the lower leaf area compared to its dry weight is one of the adaptive aspects of plants under dry conditions (Liu et al., 2022).

Drought tolerance is a major trait for increasing and stabilizing barley productivity in arid areas worldwide (Sallam et al., 2019). It appears that cultivars that produce the same yield under favorable and low irrigation conditions have a relatively higher tolerance to drought. Identifying and studying plant growth indicators can be very helpful in analyzing factors affecting yield and yield components. In addition, by measuring the dry matter produced during the growth period, we can better understand the distribution of photosynthetic substances in different organs and their accumulation (Ghanem and Al-Farouk, 2024). Maintaining optimal crop performance under drought stress conditions is an important goal shared by researchers and breeders working in semi-arid areas around the world (Williams et al., 2022).

It has been shown that salicylic acid (SA) to confer tolerance in plants to various abiotic stresses such as heat, salinity, heavy metal toxicity and drought (Singh and Usha, 2003). Plant responses to drought are modulated by plant growth regulators such as SA, auxins, gibberellins, cytokinins and abscisic acid (Singh, 2023). The use of chemicals such as SA is easier and cheaper than breeding methods that are time-consuming and costly (El-Tayeb, 2005). SA is a beta-hydroxyphenolic acid widely produced by prokaryotes and plants (Ding and Ding, 2020). In this regard, the role of SA as a growth regulator to induce tolerance to numerous biotic and abiotic stresses such as drought stress has been considered (Pirnajmedin et al., 2020; Kaur et al., 2022). In general, it appears that SA can improve nutrient uptake under low irrigation and salinity conditions and increase growth (external traits such as plant height, length and number of internodes) (Hafez and Farig, 2019; Pirnajmedin et al., 2020; Singh, 2023). SA is involved in germination, seedling establishment, cell growth, stomatal closure, senescence, increased enzymatic activity and photosynthesis under stress conditions (Shakirova et al., 2003; Kaur et al., 2002; Singh, 2002). The foliar application of SA on the leaves is a suitable method to mitigate the negative effects of drought stress (Safar-Noori et al., 2018). Studies have demonstrated the role of SA in improving plant biochemical characteristics such as soluble protein content, free proline, antioxidant activity, photosynthetic pigments and phytohormone levels, thereby increasing yield under stress conditions in many plants such as barley (El-Teyeb, 2005; Abdelaal et al., 2020; Kaur et al., 2022), wheat (Hafez and Farig, 2019; Shakirova et al., 2003) and rice (Asma et al., 2023).

Water is one of the most important inputs; however, water for irrigation is a scarce resource (Ledesma-Ramírez et al., 2023), while 73% of the land in Iran is under arid and semi-arid conditions (Maghsoudi et al., 2019). Water scarcity is the main constraint for barley production in the semi-arid Mediterranean, especially in Iran, and it is expected to become more severe under the current climate change. Therefore, a correct decision on irrigation regime should be based on a thorough understanding of the factors influencing plant growth, development, yield and quality. Indeed, a better understanding of the effects of irrigation levels on barley will promote better management of deficit irrigation. The present study evaluates the changes caused by foliar application of salicylic acid on some important agronomic and biochemical indices of barley cultivars during the growth period. In addition, the effect of deficit irrigation on these indices and on the trend of the total accumulation of dry matter will be evaluated. Therefore, the main objective of this study is to evaluate the positive effect of SA and cultivars in reducing the adverse effects of drought stress in barley.

MATERIALS AND METHODS

Experiment setup and treatment application

A factorial split-plot experiment based on a randomized complete block design with three replications was conducted in two cropping seasons (2020-2021 and 2021-2022) from December to May. The field experiment was conducted in Neyriz under the supervision of the College of Agriculture, Islamic Azad University in Fasa, Iran, at 29º 12' N, 54º 20' E and 1595 m above sea level. Figure 1 shows the monthly averages of minimum/maximum temperature and rainfall during the two barley cropping seasons in this area and Table 1 shows the different physicochemical properties of the soil collected from the study site.

Table 1. The physical and chemical properties of the soil of the experimental site (0-30 cm).

Year	EC	pH	N	P	K	Fe	Zn	Mn	Cu	OC	Sand	Silt	Clay
	dS m^{-1}					PPM						(%)	
2020	3.2	7.6	0.01	11.4	238	7.1	1.4	19.8	1.50	0.46	33	43.2	23.8
2021	3.5	7.1	0.02	11.9	222	7.1	1.5	18.8	1.55	0.45	35	42.2	22.8

The studied treatments consisted of irrigation treatments as the main factor, including 1-off irrigation from the tillering stage to the end of the growing season (severe stress), 2-off irrigation from the stem elongation stage to the end of the growing season (moderate stress), and 3-off irrigation after the milky stage (control). The second factor was foliar application of salicylic acid (SA) at a concentration of 1 mM applied at different growth stages as none (control), sprayed at the end of tillering stage (Zadoks, 29), fifty percent of spike emergence (ZGS34) and flag leaf full emergence (ZGS39) and the third factor included three barley cultivars (Khatam, Reyhan and Nosrat). Table 2 shows the characteristics of the cultivars studied.

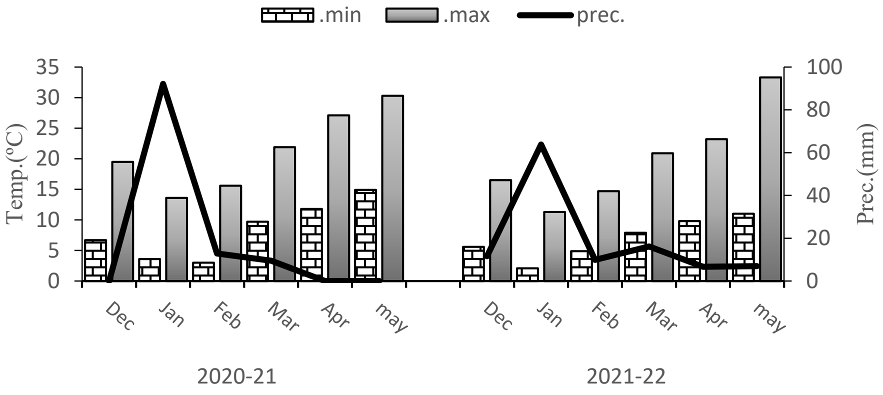

Figure 1. Average temperature (°C) and amount of precipitation (mm) in 2020, 2021 and 2022

Table 2. Some characteristics of studied barley cultivars

Cultivar name	Pedigree	Spike type	Maturity type	Growth type	Origin
Nosrat	Karoon/Kavir	six-rowed	Winter	Moderate maturity	Iran
Khatam	LB.Iran/Una 8271//Gloria"S"/Come"s"/3/Kavir	six-rowed	Spring	Moderate maturity	Iran
Reyhan	Rihane	six-rowed	Spring	Early maturity	ICARDA

ICARDA, International Center for Agricultural Research in the Dry Areas

The sowing date was the beginning of December and the harvest date was the beginning of June.

Rainfall during the growing season was 90 mm in the first year and 114 mm in the second year of the experiment.

Each plot was composed of six lines of 6m in length, sown at a distance of 20 cm between lines to achieve a density of 400 grains per m^2. Broadleaf weeds were controlled with 2,4-D herbicide at a rate of five litres per hectare prior to emergence. Grains were collected from the Agricultural Research Centre and Natural Resources of Fars Province. The SA was purchased from Merck Co. (Darmstadt, Germany).

Measurement of traits

Five plants were taken from the third and fourth rows by removing the two lateral edges and immediately taken to the laboratory to be measured. In this study, chlorophyll a and chlorophyll b contents (Lichtenthaler and Wellburn, 1983) and proline contents (Bates et al., 1973) were measured using a spectrophotometer (PG Instrument Ltd., UK). Total chlorophyll was measured using a SPAD-CCM-200 plus (Opti-Science). Samples were taken mainly from the apex of the plant, as these are the youngest fully developed leaves, to reduce variation due to leaf age.

Extract preparation for antioxidant enzyme assay, enzymatic antioxidants. Flag leaves of three barley cultivars (0.25 g) were extracted in 5 ml of potassium phosphate buffer (pH 7.8) at 4°C and centrifuged at 12,000 rpm for 15 minutes. The supernatant was used to determine the activities of enzymatic antioxidants.

The catalase activity of the extract was expressed as catalase activity units: min-1 mg-1 protein (Cakmak and Horst, 1991). The peroxidase activity was measured specifically with guaiacol. The increase in absorbance at 470 nm was recorded in a mixture of 0.1 ml of enzyme extract with 3 ml phosphate buffer (50 mM; pH=7) containing 0.05 ml guaiacol and 0.03 ml H2O2 (Nakano et al., 1980). Enzyme activity was expressed as units of enzyme activity per mg of protein.

To measure the relative water content (RWC) of the leaves, samples from the most recently developed leaf were collected from all experimental treatments. Fresh leaves were weighed, then submerged in distilled water for 24 hours, after which they were gently dried with tissue paper and reweighed to determine their turgid weight. Leaves were dried in a drying oven (at 70 °C for 48 h) to constant weight. The RWC was calculated using equation 1 (Ritchie and Nguyen, 1990):

$$RWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100 \quad (1)$$

In both years, agronomic yield was determined by measuring the plant height (PH; cm) of the main above-ground shoot at maturity in each plot after the plants had matured. Five spikelets were separated to determine the spike length (SL; cm) and weight (g) and the number of grains per spikelet (NG). Total aboveground biomass (i.e. straw and grain) was manually harvested on a surface of 1 m2 near the soil surface, sun-dried and then calculated as biological yield (BY; kg ha-1) weight, while grain yield (GY; kg ha-1) was determined after threshing the grain from the biomass. Specific leaf area (SLA) was calculated using equation 1 (Beadle 1993):

$$SLA=\frac{LA}{LDW} \quad (2)$$

In this formula, LA is the leaf area per unit area and LDW is the leaf dry weight.

Statistical analysis

After the physiological and biochemical evaluation, statistical analyses were carried out with the use of MSTAT-C and the SAS system software version 9.4 (Delwiche and Slaughter, 2019).

Data were analyzed using a combined analysis of variance and means compared with Duncan's multiple range tests. Finally, tables and calculations were carried out using Excel software. The results of the Bartlett's test to examine the homogeneity of the data in two crop years did not show a significant difference. They indicated the homogeneity of the data in the two years of the study. Two-year combined analysis of variance was performed on the data for this purpose.

RESULTS AND DISCUSSION

SLA and RWC

Analysis of variance showed that the mean squares due to drought stress (DS), cultivar (C), salicylic acid (SA) and their interactions were significant ($p \leq 0.05$ or $p \leq 0.01$) for SLA and RWC (Table 3). Year and often its interaction effects did not significantly affect SLA and RWC. Foliar application of salicylic acid increased SLA and RWC at all growth stages (Figure 2).

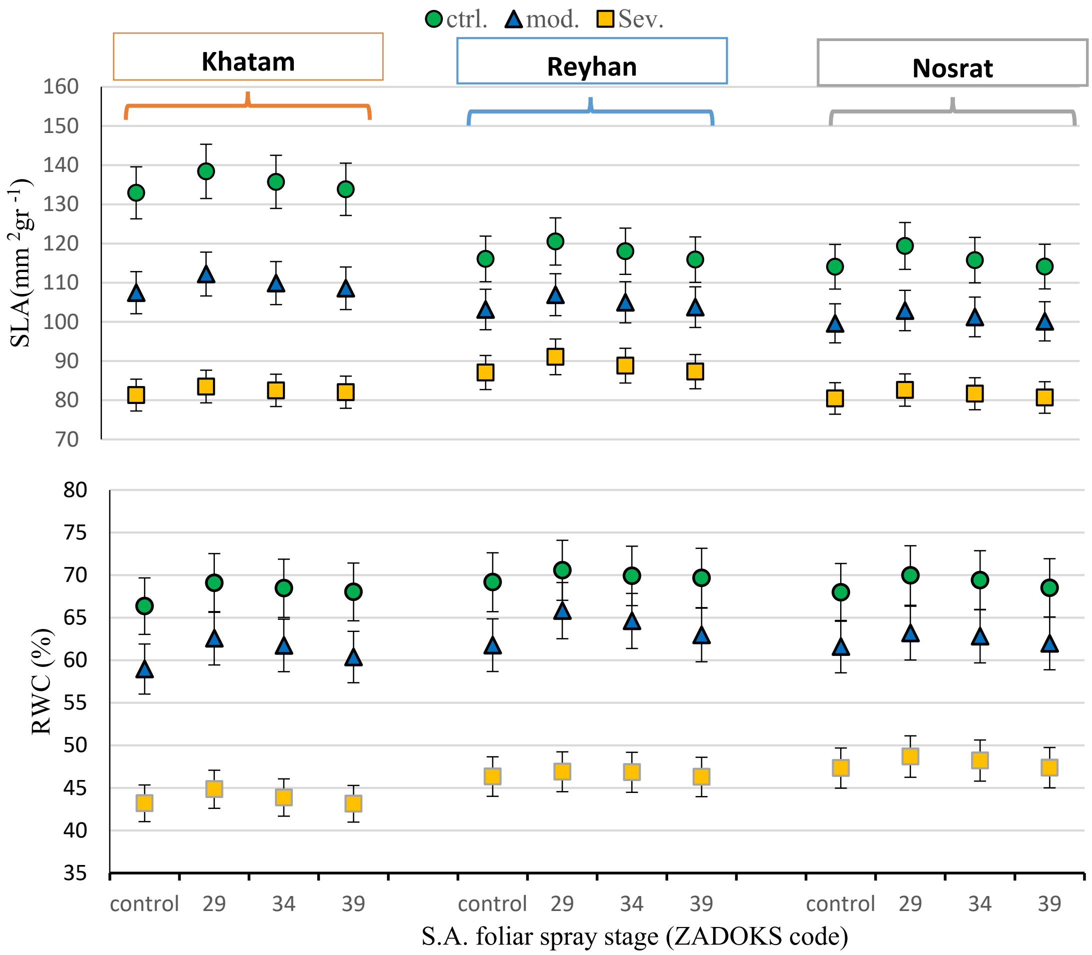

Figure 2. Triple interaction of investigated factors on the concentration of Specific leaf area (SLA), Relative water content (RWC), Columns with at least one letter in common do not have a statistically significant difference (Duncan 5%).

Based on the findings, the highest SLA of the flag leaf was with the SA leaf application at ZGS 29. A delay in foliar application reduced the plant's ability to use SA and its effect on SLA (Figure 2). The highest SLA was obtained from cultivar Reyhan in the high stress treatment (88.56 $mm^2.g^{-1}$). As the stress level decreased, Khatam cultivar's SLA increased compared with other cultivars. In the moderate and control treatments, the SLA of this cultivar was 109.54 and 135.23 mm2/g, respectively. In the severe stress treatment, the SLA of Khatam (39.1%), Reyhan (24.7%) and Nosrat (29.8%) cultivars decreased compared to the control, indicating the greater tolerance of Reyhan cultivar to the reduction in SLA due to low irrigation (Figure 2).

Table 3. Combined analysis of variance of the effect of levels irrigation, cultivar and time of salicylic acid foliar application on Specific leaf area (SLA), Relative water content (RWC), Plant Height (PH), Spike Length (SL), Number of Grains $Spike^{-1}$ (NG), Biological Yield (BY), Grain Yield (GY) during the two growing seasons

SOV	df	SLA	RWC	PH	SL	NG	BY	GY
Year (Y)	1	476.15^{ns}	1219.18^{ns}	2028.91^{ns}	1.0556^{ns}	91.00^{ns}	171760.56^{ns}	8957260.2^{ns}
Replications (R)	4	0.04	0.21	26.26	0.04861	0.21	45.16	197.3
Irrigation (I)	2	27180.97**	9960.59**	40830.72**	133.09**	3796.53**	10985868.29**	452554229**
Y × I	2	39.46^{ns}	468.01^{ns}	242.13^{ns}	0.05296^{ns}	9.57^{ns}	28797.23^{ns}	715622.3^{ns}
Yr × I	8	0.20	0.19	55.54	0.08627	0.21	43.64	699.4
Cultivar (C)	2	1684.14**	135.53**	4652.18**	17.1119**	161.28**	122386.79**	3180166.5**
Yr × C	2	30.70^{ns}	1.48^{ns}	106.70^{ns}	0.26601^{ns}	4.71^{ns}	2496.84^{ns}	401415.3^{ns}
I × C	4	942.80**	21.20*	517.32**	1.77359**	29.67**	30454.54**	1068303**
Yr × I × C	4	2.54^{ns}	2.69^{ns}	65.13^{ns}	0.27246*	6.25^{ns}	2097**	151209.3**
Salicylic acid (SA)	3	168.02**	38.64**	299.38**	5.33418**	13.24**	17474.17**	1239667.1**
Yr × SA	3	1.88**	0.39**	7.72^{ns}	0.09156^{ns}	0.81^{ns}	212.98^{ns}	7236.5^{ns}
I × SA	6	5.87**	3.91**	14.64**	0.33371**	0.42**	2726.29**	241932.8**
Yr × I × SA	6	1.16^{ns}	3.30^{ns}	17.69^{ns}	0.28531*	0.41^{ns}	267.59**	5536.1**
C× SA	6	0.81**	1.69**	2.58**	0.42666**	1.34**	168.16**	20706.3**
Yr × C × SA	6	0.86**	3.20**	9.29^{ns}	0.07411^{ns}	0.38^{ns}	314.91^{ns}	2463.6^{ns}
I × V× SA	12	1.19**	2.43**	3.42**	0.30924**	0.14**	138.25**	5224.2**
Yr× I × C × SA	12	1.30^{ns}	0.52^{ns}	4.80^{ns}	0.10736^{ns}	0.20^{ns}	354.49^{ns}	2635^{ns}
Error	132	0.11	0.09	6.38	0.10837	0.16	57.14	307.7
CV%		12.8	11.9	14.2	14.5	12.2	29.8	18.9

^{ns}, * and **: no significant, significant at the 5% and 1% probability levels, respectively

Under stress conditions, severe and moderate leaf RWC belonged to cultivar Khatam (63.06 and 68.57%). However, with increasing irrigation cycles under control conditions, the maximum RWC of flag leaves belonged to Reyhan and Nosrat cultivars. The RWC of each cultivar decreased in the severe stress treatment compared to the control. This reduction belonged to Khatam, Rehan and Nosrat cultivars at severe stress treatment with 7.7, 11.3 and 34.1%, respectively, compared to the control. Compared to the other cultivars studied; Khatam showed the lowest sensitivity to low irrigation (Figure 2). In the interaction of SA foliar application and stress treatments, it was observed that the RWC of the flag leaf with foliar application at ZGS29 was the highest among the foliar application treatments in each stress treatment. Flag leaf RWCs in this treatment were 2.6, 1.5 and 2% higher than control in heavy, medium and control treatments, respectively. Siosemardeh et al. (2004) stated that wheat tolerant cultivars have higher relative water content than drought-sensitive cultivars under stress conditions. Colem et al. (2003) considered the reason for the decrease in the relative moisture content of the leaf to be the decrease in water absorption from the roots in dry conditions. Colom and Vazzana (2003) considered the reason for the decrease in the relative moisture content of the leaf to be the decrease in water absorption from the roots in dry conditions.

Under drought stress, photosynthetic machinery can be damaged by stomatal and non-stomatal limitations (Reddy et al. 2004). Drought reduces the level of photosynthesis and the amount of photosynthesis, which reduces dry matter accumulation (Lawlor, 1981). Based on results from Ghotbi-Ravandi et al. (2014), stomatal conductance is the main factor limiting photosynthesis in Barley under mild drought stress. Low irrigation of wheat plants during the reproductive stage caused a reduction in most of the measured traits compared to the control. The greatest reduction was observed in the low irrigation treatment during the flowering stage. The decrease in photosynthesis and transfer of nutrients to the spike reduces the dry matter of each plant, which ultimately reduces yield (Barati et al., 2020).

Singh et al. (2023) have reported Moisture stress induced a reduction of relative water content. Moreover, a decrease in the RWC in plants under stresses such as drought and salinity may be due to the loss of turgor pressure, resulting from the cell's limited access to water (Soltys-Kalina et al., 2016). Cornic (2000) reported a decrease in the RWC has been known to induce stomata closure and thus a parallel reduction in photosynthetic efficiency. Barley plants treated with salicylic acid have more stem length and weight than non-treated plants. These plants have higher sugar and proline content, indicating that they apply higher osmotic regulation to increase the RWC in the plant (Abdelaal et al., 2020). Singh et al. (2023) have shown that Application of SA and GB enhanced photosynthesis, improved stomatal conductance, and maintained higher RWC. Wheat treatment with salicylic acid increases the leaf's RWC (Hafez and Farig, 2019). In other words, the leaf's RWC is a key indicator of the degree of cell and tissue dehydration (Silva et al., 2007). Thus, measuring the RWC of the flag leaf is used as a significant index in determining the water status of the plant and identifying cultivars resistant to drought stress (El-Seidy et al., 2013).

Plant Height, Spike Length, Number of Grains Spike−1, Biological Yield and Grain Yield

Analysis of variance showed that the mean squares due to drought stress (DS), cultivar (C), salicylic acid (SA) and their interactions were significant ($p \leq 0.05$ or $p \leq 0.01$) for plant height, spike length, number of grains spike-1, biological yield and grain yield (Table 3).

The highest plant height in the control conditions belonged to Reyhan and Nosrat cultivars. As the stress level increased, the plant height and two cultivars' height decreased by 48.7% and 60.6%, respectively. The lowest plant height with an average of 28.25 cm belonged to cultivar Khatam under severe stress conditions (Table 4). SA applied on the leaves on ZGS29 had the highest plant height in all stress conditions. Foliar application at this growth stage increased plant height by 6.6, 5.7 and 21.5% under control, moderate and severe stress conditions, respectively, compared to the non-foliar treatment. Under control conditions, the maximum spike length was obtained from Reyhan and Nosrat with an average of 6.53 and 6.4 cm, respectively.

Table 4. Means comparison of interaction effects of levels irrigation, cultivar and time of salicylic acid foliar application on Plant Height (PH), Spike Length (SL), Number of Grains Spike^{-1} (NG), Biological Yield (BY), Grain Yield (GY)

Drought stress	Cultivar	PH (cm)	SL (cm)	NG	BY (kg ha^{-1})	GY (kg ha^{-1})
Control	Khatam	75.21 c	5.29 b	38.61 b	11460.67 c	5069.75 c
	Reyhan	84.29 a	6.53 a	39.64 a	13020.42 a	5934.17 a
	Nosrat	83.50 a	6.4 a	36.33 c	12230.58 b	5649.5 b
Moderate	Khatam	57.92 e	4.33 c	36.5 c	9900.96 f	4140.5 f
	Reyhan	81.75 b	5.49 b	37 c	10630.38 d	4339.54 e
	Nosrat	64.17 d	5.18 b	33.98 d	10500.58 e	4434.58 d
Severe	Khatam	28.25 h	3.17 d	24.58 f	4630.71 i	644.54 h
	Reyhan	43.25 f	3.52 d	26.34 e	4810.29 g	739 g
	Nosrat	32.92 g	3.43 d	22.08 g	4740.54 h	782.08 g
Drought stress	**S.A. foliar spray stage (ZGS)**					
Control	control	78.83 c	5.85 abc	37.49 c	11930.22 d	5269.06 d
	29	84.06 a	6.4 a	38.77 a	12590.83 a	5800.33 a
	34	81.72 b	6.26 a	38.57 ab	12320.78 b	5666.39 b
	39	79.39 c	5.95 ab	37.94 bc	12110.06 c	5468.78 c
Moderate	control	66.33 f	4.88 d	34.34 e	10150.39 h	4041.17 h
	29	70.11 d	5.46 a-d	35.57 d	10610 e	4502.67 e
	34	67.78 e	5.22 bcd	35.42 d	10410.39 f	4419.89 f
	39	67.56 e	4.45 cd	34.75 e	10220.11 g	4255.78 g
Severe	control	32.06 i	3.2 e	24 f	4690.89 k	702 j
	29	38.94 g	3.66 e	24.68 f	4800.61 i	746.11 i
	34	35.61 h	3.43 e	24.54 f	4720.39 j	730.33 ij
	39	32.61 i	3.2 e	24.11 f	4690.83 k	709.06 j
Cultivar	**S.A. foliar spray stage (ZGS)**					
Khatam	control	51.94 i	4.3 f	32.71 c	8506.1 k	3100.28 l
	29	56.61 g	4.53 de	33.5 b	8862.2 h	3394.33 i
	34	54.22 h	4.32 ef	33.41 b	8742.2 i	3363.44 j
	39	52.39 i	3.9 g	33.29 b	8573.9 j	3281.67 k
Reyhan	control	67.28 c	4.89 c	32.86 c	9325 d	3487.11 g
	29	73 a	5.7 a	34.23 a	9716.7 a	3859.83 a
	34	70.67 b	5.38 b	34.1 a	9531.7 b	3741.5 c
	39	68.11 c	4.75 cd	32.9 c	9387.8 c	3595.17 e
Nosrat	control	58 fg	4.75 cd	30.27 g	8953.9 g	3424.83 h
	29	63.5 d	5.28 b	31.28 d	9435.6 c	3794.94 b
	34	60.22 e	5.22 b	31.02 e	9191.7 e	3711.67 d
	39	59.06 ef	4.77 c	30.61 f	9068.3 f	3556.78 f

Averages with at least one common letter in each part of the column do not have a statistically significant difference (Duncan 5%)

Plant height and flag leaf are two important agronomic traits for crop yield (Cheng et al., 2023). For their part, Ledesma-Ramírez et al. (2023) evaluated three irrigation schedules, 2, 3 and 5 irrigations, where the phenological characters and especially the plant height were affected by the limited irrigation. These results are in agreement with the results of the present study. Drought stress causes a decrease in turgor pressure and thus a decrease in cell growth and development, especially in the spike and leaves. Drought-stressed plants had shorter duration of grain filling than well-watered plants. The reduction in cell enlargement and cell division reduces leaf area, photosynthesis rate, and ultimately plant height. The reduction in growth and development limits cell growth. In other words, a reduction in photosynthetic material due to a decrease in leaf area and a reduction in the transfer of photosynthetic products to the reproductive organs due to low irrigation leads to a decrease in yield (Lv et al., 2023). Samarah (2005) showed that drought stress treatments reduced grain yield of barley by reducing the number of tillers, spikes and grains per plant and individual grain weight. Salicylic acid will likely improve nutrient uptake under drought and salt stress conditions, increasing growth and plant height (Moharekar et al., 2003). Plant height is influenced by several factors that are closely related to yield and quality, broadly divided into endogenous hormones and the external environment (Cheng et al., 2023). Researchers reported a 13% increase in wheat yield under salinity stress conditions due to the application of salicylic acid (Arfan et al., 2006).

Under the severe stress conditions, the spike length of the investigated cultivars did not show any statistically significant difference. Spike length decreased by 40.1, 46.1 and 46.4% in Khatam, Reyhan and Nosrat, respectively, under severe stress compared to the control. SA foliar application caused a significant increase in spike length in control and moderate stress treatments. However, foliar application of SA under severe stress did not significantly affect spike length in any of the treatments studied. Each cultivar's highest spike length was associated with foliar application of SA at the end of tillering (ZGS29). The highest spike length was associated with cultivar Reyhan with a mean of 5.7 cm and foliar application at ZGS29 (Table 4).

Reyhan cultivar had the highest number of grains per spike in each stress level. 36.3, 33.6 and 39.2% in Khatam, Reyhan and Nosrat cultivars reduced the number of grains per spike in the severe stress conditions compared to the control treatment. In the control and SA, the highest number of grains per spike was found in stages 29 and 34 of Zadoks, without significant statistical difference. The foliar application of SA did not significantly affect the number of grains per spike under severe stress conditions. In each of the cultivars studied, foliar application of SA at the end of tillering had the most excellent effect on the number of grains per spike. Reyhan had the highest spike number in the experiment under the effect of leaf spray at the end of tillering stages 29 and 34 of Zadoks, with an increase of 4.2% and 3.8%, respectively, compared to the non-applied control (Table 4). The spike is the grain-bearing organ in cereals that is an essential proxy for grain yield and quality (Ling et al. 2023), and spike length is a trait that plays a key role in grain yield. Low irrigation can reduce spike length in wheat plants by shortening the growing season (Sallam et al., 2019) and increasing the growth rate. Drought can also reduce spike length in wheat plants through a direct negative effect on the terminal meristem that forms the spike (Gooding et al., 2003). Research on wheat plants reported a reduction in the number of grains per spike due to low irrigation (Cattivelli et al., 2008). The reduction in the number of grains per spike can be due to the role of drought in slowing down the spike formation or the division of meiosis in the gametes, and the fertility of the eggs and the earlier development of the grains. The present study results revealed that salicylic acid foliar application under drought stress significantly increased the number of grains per spike. It is in line with the results of a study conducted by Hafez and Farig (2019), who stated the Phytohormone Salicylic Acid positively affect the number of grains per wheat spike in low-irrigation conditions. The biological yield includes the dry weight of all the aerial parts of the plant, which is affected by genotype and growing environment conditions (El-Seidy et al., 2013).

The highest biological yield in each stress condition belonged to the Reyhan cultivar and the lowest in all stress conditions was obtained from the Khatam cultivar. After foliar application of SA in each growth stage, the biological yield increased compared to the control without foliar application (Table 4). In each stress condition, the biological yield increased after foliar application at ZGS29, which was 5.5%, 4.5% and 2.3% in minimum, moderate and severe stress conditions, respectively, compared to the control without foliar application. The foliar application in ZGS29 had the highest biological yield in each of the cultivars studied. The increase in yield due to foliar application at this growth stage compared to the control was 1.4%, 4.2% and 5.4% in Khatam, Reyhan and Nosrat, respectively.

Under control conditions, the highest grain yield belonged to the cultivar Reyhan with an average of 5934.17 kg per hectare (Table 4). The highest grain yield was obtained from Nosrat cultivar in moderate and severe stress conditions. Grain yield of Khatam cultivar was lower than other cultivars in all stress conditions. Applying SA in all stress conditions increased grain yield compared to the control without foliar application. This rate of increase due to foliar application in ZGS29 was greater than other foliar treatments. The foliar application of SA at this growth stage increased grain yield by 10.1%, 11.4% and 6.3%, respectively, in minimum, moderate and severe stress conditions compared to the control. The results showed that the highest increase in grain yield due to foliar application occurred under control conditions. In all the cultivars studied, the highest yield increase after foliar application was obtained in ZGS29. This increase in Khatam, Reyhan and Nosrat cultivars compared to the control was 9.5%, 10.7 and 10.8%, respectively, indicating the greater effectiveness of SA foliar application on grain yield of Nosrat cultivar from Table 4. The delay in SA foliar application in the cultivars caused a reduction in grain yield (Table 4).

SPAD index Chlorophyll a and b

Analysis of variance showed that the mean squares due to drought stress (DS), cultivar (V), salicylic acid (SA) and their interactions were significant ($p \leq 0.05$ or $p \leq 0.01$) for the SPAD index chlorophyll a and b (Table 5). The highest level of this reduction in the severe stress treatment compared to the control belonged to cultivar Khatam (26.6%). The reduction in leaf chlorophyll index under severe stress compared to the control was 25% and 25.8% in Reyhan and Nosrat cultivars, respectively. Leaf chlorophyll index increased with increasing irrigation cycles in each SA foliar treatment (Figure 3). Despite the positive effect of SA foliar application, in the stress treatments, the control treatment (no foliar application) was not significantly different in terms of leaf chlorophyll index due to foliar application at the end of ZGS39 in each of the stress treatments. In each stress treatment, the foliar application of SA at ZGS29 had the highest leaf chlorophyll index. However, the foliar application of SA could not compensate for the reduction in leaf chlorophyll index caused by the decrease in the number of irrigations. The highest leaf chlorophyll index was obtained from the foliar application at ZGS29 with control irrigations (55.12 units).

Table 5. combined analysis of variance of the effect of irrigation, cultivar and time of salicylic acid foliar application on Leaf Chlorophyll Index (SPAD), Chlorophyll a (Chl a), Chlorophyll b (Chl b), peroxidase activity (POX), catalase activity (CAT), and Proline Concentration (PC) during the two growing seasons

SOV	DF	SPAD	Chl a	Chl b	POX	CAT	PC
Year (Y)	1	16.58^{ns}	0.130^{ns}	0.037^{ns}	544427.6^{ns}	35.46^{ns}	581.91^{ns}
Replications (R)	4	0.66	0.001	0.000	14413.75	23.33	4.41
Irrigation (I)	2	3445.23**	3.748**	1.855**	7148.26**	661.69**	397.07**
Y × I	2	2.49^{ns}	0.204^{ns}	0.009^{ns}	1518.15^{ns}	6.03 ns	15.88^{ns}
Yr × I	8	0.39	0.001	0.000	10345.12	46.69	10.65
Cultivar (C)	2	1103.20**	0.453**	0.023**	9708.38**	63.16**	50.75**
Yr × C	2	9.07^{ns}	0.034^{ns}	0.004^{ns}	5856.32	0.46	2.03^{ns}
I × C	4	11.43**	0.101**	0.110**	1506.18**	23.29**	17.74**
Yr × I × C	4	13.21^{ns}	0.015^{ns}	0.003^{ns}	730.81^{ns}	0.40^{ns}	0.71^{ns}
Salicylic acid (SA)	3	47.21**	0.018**	0.007**	5705.21**	12.62**	11.31**
Yr × SA	3	1.66**	0.005**	0.000**	19.57**	0.52**	0.45**
I × SA	6	0.39**	0.007*	0.001*	399.10**	4.02**	1.63**
Yr × I × SA	6	0.72^{ns}	0.001^{ns}	0.001^{ns}	335.21^{ns}	0.43 *	0.77^{ns}
C × SA	6	1.07*	0.003*	0.001**	1857.44*	27.53*	0.07**
Yr × C × SA	6	1.26**	0.004**	0.001**	468.04**	0.37**	0.03**
I × C× SA	12	1.28**	0.002**	0.001**	527.52*	4.77*	0.11**
Yr× I × C × SA	12	0.47^{ns}	0.002^{ns}	0.001^{ns}	428.21**	0.30**	0.00^{ns}
Error	132	0.36	0.001	0.000	575.37	5.36	0.01
CV%		12.1	9.8	11.3	22.5	13.80	12.27

^{ns}, * and **: no significant, significant at the 5% and 1% probability levels, respectively

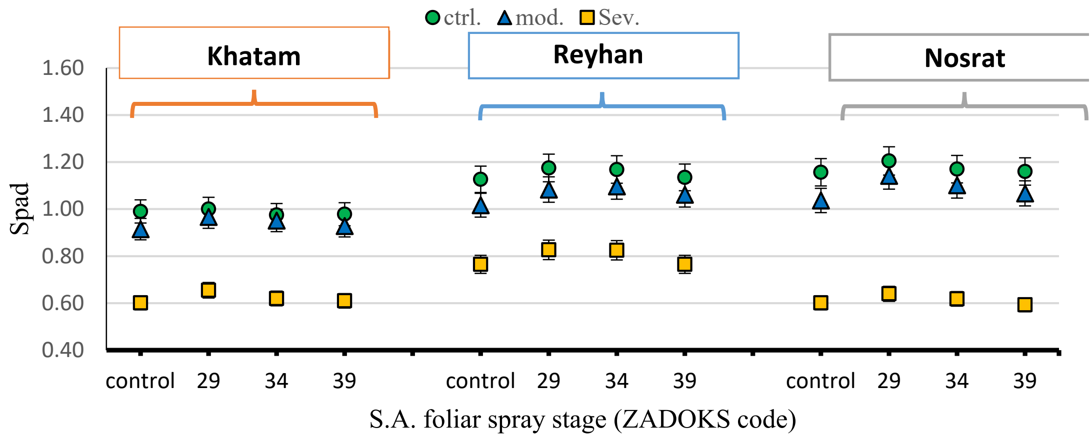

Figure 3. Triple interaction of investigated factors on the concentration of Spad. Columns with at least one letter in common do not have a statistically significant difference (Duncan 5%)

The lowest reduction of chlorophyll a and b in moderate stress conditions belonged to cultivar Khatam with an average of 4.5%. The lowest level of chlorophyll a reduction under severe stress conditions belonged to cultivar Reyhan with an average of 24.8%. Also, the highest chlorophyll b content with an average of 0.58 mg. g-1 fresh weight of leaves was obtained from cultivar Khatam under control conditions. The chlorophyll b content of this cultivar was the highest among the cultivars studied under moderate stress conditions. Under severe stress conditions, the highest amount of chlorophyll b belonged to cultivar Reyhan. Under severe stress conditions, the highest chlorophyll b content was obtained with SA foliar application at ZGS29 stage. However, it was not significantly different from the flag leaf emergence stage in the severe stress conditions (Figure 4). The highest chlorophyll content in each stress condition belonged to the SA foliar application at ZGS29 stage. In addition, the highest chlorophyll b content was obtained from the foliar application at the stage of ZGS29 in cultivar Nosrat with an average concentration of 0.42 Mg. g-1 fresh weights of leaves (Figure 4).

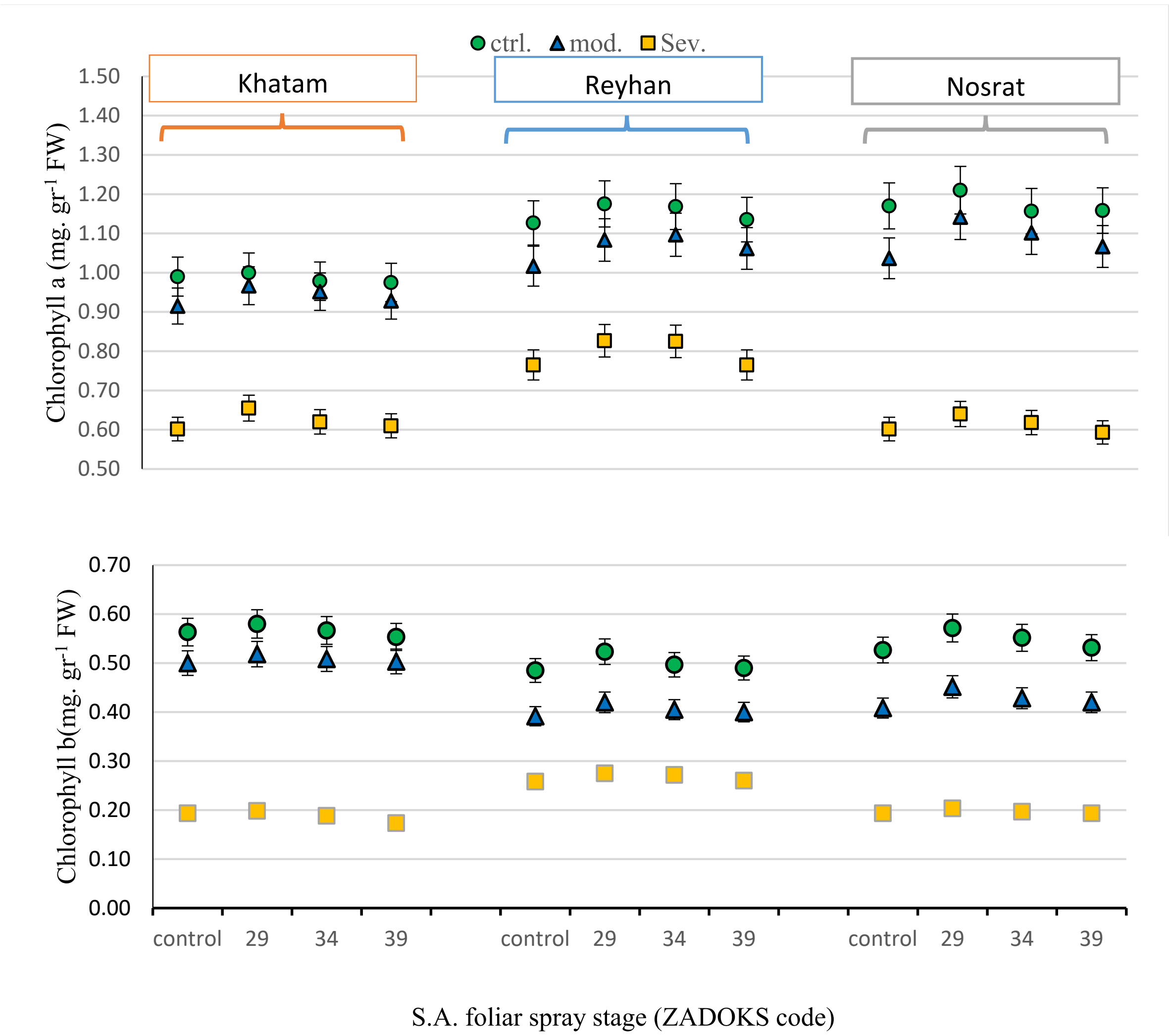

Figure 4. Triple interaction of investigated factors on the concentration of chlorophyll a and chlorophyll b Columns with at least one letter in common do not have a statistically significant difference (Duncan 5%)

The reduction in chlorophyll content in low irrigation conditions may be due to the reduction in chlorophyll synthesis and its destruction (Nouri et al., 2020). Indeed, with drought stress, the amount of photosynthesis decreases and the chlorophyll index decreases (Stone et al., 2003). The researchers examined the effect of drought stress on the activity of antioxidant enzymes in barley plants. The chlorophyll content decreased with the aging of the plant and under the impact of drought stress. Drought stress reduced plant yield, leaf area, chlorophyll content, and overall plant growth (Zhu et al. 2004). Also, Pirnajmedin et al. (2020) have shown that Mild and intense drought stress conditions led to depression in photosynthetic pigments including chlorophyll a and b, total chlorophyll contents and carotenoids. Salicylic acid acts as an antioxidant in drought stress conditions and prevents pigment damage, especially chlorophyll. Based on the reports, salicylic acid improves photosynthesis in drought stress by preventing damage to chlorophyll (Khan et al., 2003; Pirnajmedin et al., 2020).

Catalase activity, Peroxidase activity and Proline content

ANOVA revealed that the means of drought stress (DS), cultivar (C) and salicylic acid (SA) were significant (p≤0,05 or p≤0,01) in catalase activity, peroxidase activity and proline content (Table 5). Catalase activity increased significantly under stress conditions in the cultivars that were able to tolerate stress (Figure 5). The highest catalase activity was obtained in cultivar Reyhan with an average of 9.80 U mg-1 protein.The highest catalase activity was obtained from the interaction of cultivar Reyhan with foliar application in ZGS39. Foliar application in ZGS39 increased catalase activity by 40.3, 27.2 and 17.4% under normal, moderate and severe stress conditions compared to non-foliar treatment (Figure 5).

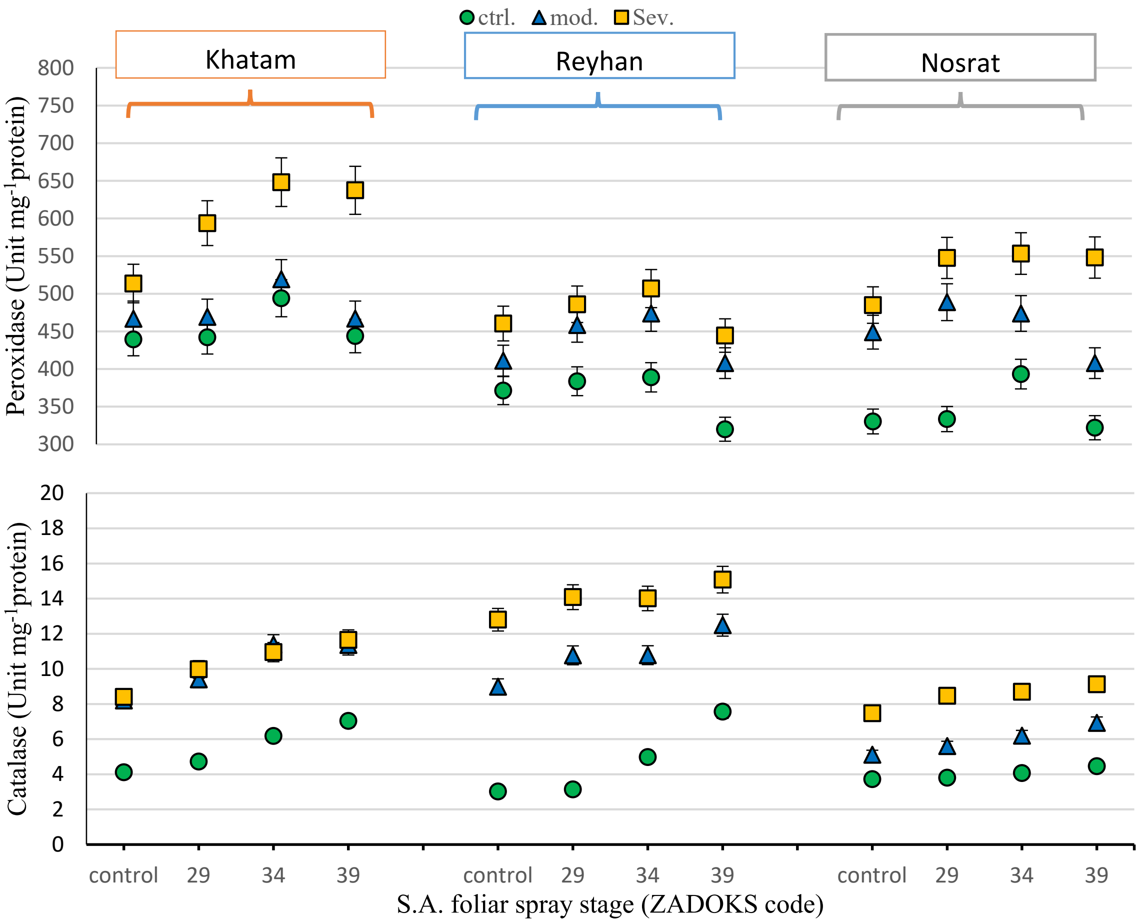

Figure 5. Triple interaction of investigated factors on the concentration of Peroxidase and Catalase Columns with at least one letter in common do not have a statistically significant difference (Duncan 5%)

There was an increase in peroxidase activity in the cultivars under stress conditions (Figure 1). The highest peroxidase activity was obtained in cultivar Khatam with an average of 511.2 U mg^{-1} proteins. Peroxidase activity increased in the severe stress treatment compared to the control in cultivars Khatam (31.5%), Reyhan (29.6%) and Nosrat (54.7%).The highest peroxidase activity was obtained from the interaction of cultivar Khatam with foliar application at ZGS34 (Figure 5).

The highest proline levels were found in the Reyhan cultivar, averaging 13.99 mmol g^{-1}. The application of regular irrigation in Khatam, Reyhan and Nosrat cultivars reduced the proline content by 46.2, 66.6 and 52.4% respectively compared to the severe stress treatment. This index's lowest and highest values were obtained for all SA levels in control, moderate and severe stress, respectively. The proline content of Reyhan cultivar decreased with the delay in foliar application. However, this trend increased in Khatam and Nosrat. The three-way interaction of the studied factors showed that the highest proline content in the severe stress treatment was obtained in the Reyhan cultivar and no leaf application with a mean of 15.08 mmol/g fresh weight of the plant (Figure 6). However, in the normal irrigation treatment, the highest proline content was obtained from the foliar application treatment at ZGS29, in all the cultivars studied.

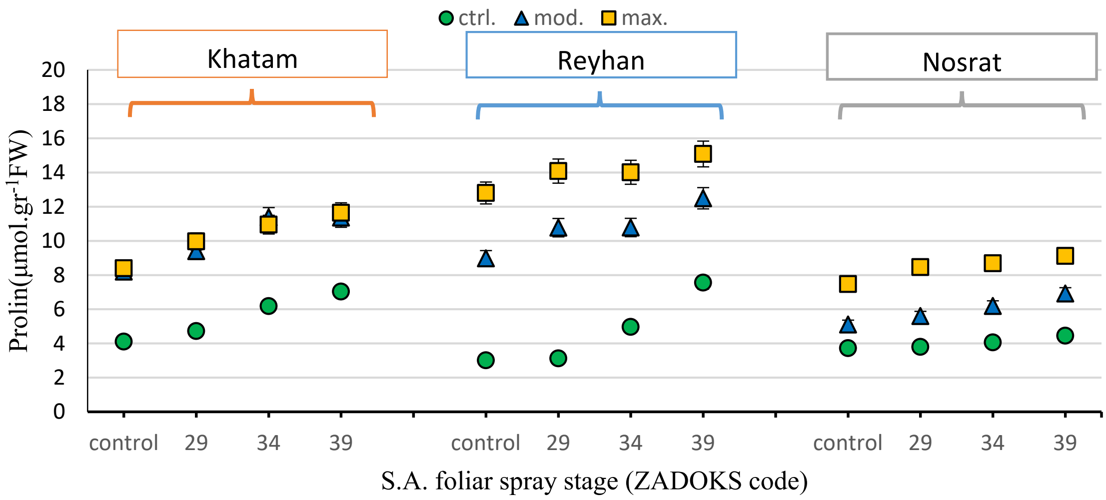

Figure 6. Triple interactions of investigated factors on proline concentration Columns with at least one letter in common do not have a statistically significant difference (Duncan 5%)

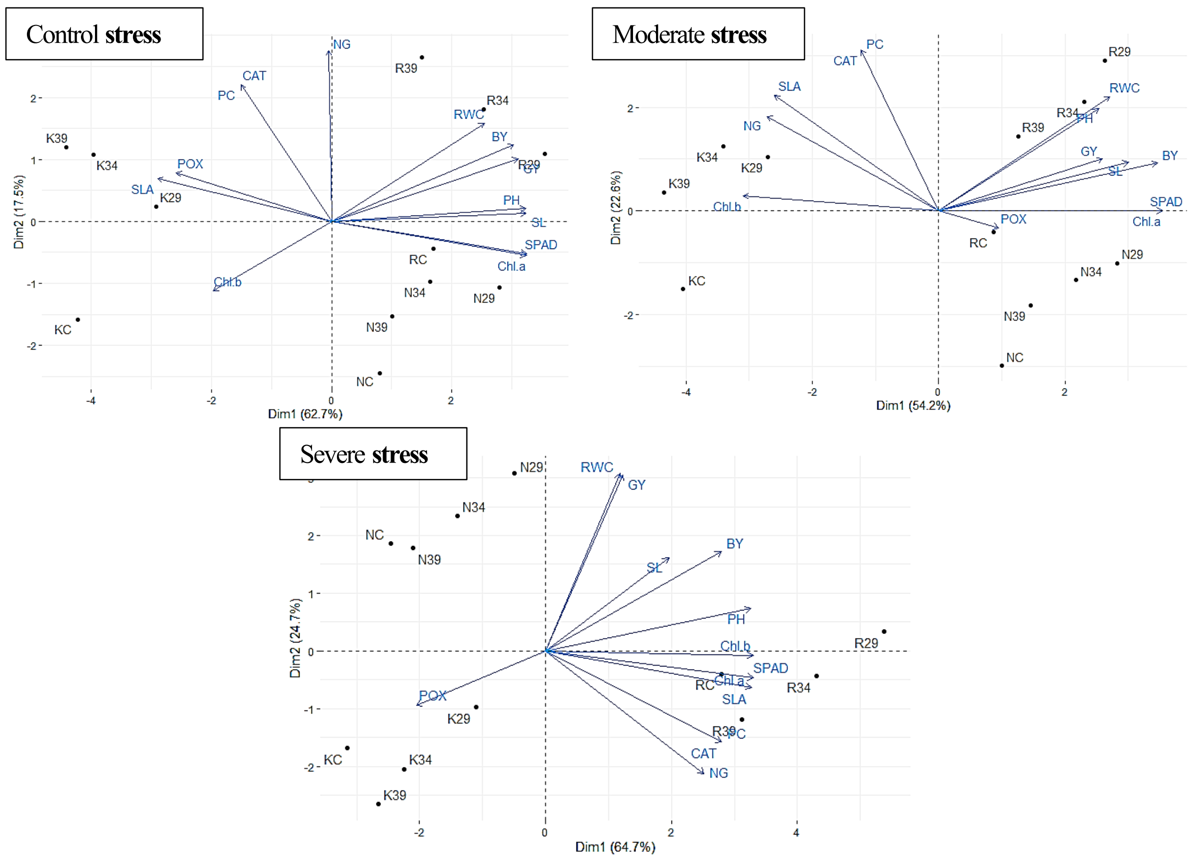

Figure 7. Principal Component Analysis (PCA) of data for all characteristics of plants under the different Irrigation. Plant Height (PH), Spike Length (SL), Number of Grains Spike^{-1} (NG), Biological Yield (BY), Grain Yield (GY), Leaf Chlorophyll Index (SPAD), Chlorophyll a (Chl a), Chlorophyll b (Chl b), peroxidase activity (POX), catalase activity (CAT), and Proline Concentration (PC).

A series of secondary metabolites, especially amino acids, accumulate in plants when they are exposed to stress conditions. Amino acids as precursors of protein compounds play a significant role in plant growth and development metabolism (Trovato et al., 2021). Based on the results of the present experiment, Application of SA decreased the adverse effects of drought stress by elevation of photosynthetic pigments and could enhance barley yield and drought tolerance in Case Study genotypes.

Proline as an amino acid plays a significant role in modulating drought stress. This role is due to maintaining osmotic potential, inhibiting reactive oxygen species, and finally preventing oxidative stress in plants (Verbruggen and Hermans, 2008). The increase of proline content along with the increase of environmental stresses such as drought is considered a strategy to protect the plant. Plants reduce the water potential by increasing organic compounds such as sugars and proline in the cell and through osmotic regulation. They provide the possibility of absorbing more water from environments under low water conditions (Ashraf and Foolad, 2007), so proline content in the tolerant line became more sensitive. Proline content was identified as the best physiological index for indirect selection of drought-tolerant genotypes in this present study these results are in agreement with the results reported by Barati et al. (2020).

A principal components analysis was conducted to evaluate the relationships between traits under drought stress treatments (Figure 7). As illustrated in the figure, the first and second components in control, moderat and serve stress accounted for approximately 62.7% and17.5%, 54.2% and 22.5%, and 64.7% and 24.7%, respectively. Approximately, all associations between characters have been affected by irrigation levels. Furthermore, most parameters were integrally occupied with high correlation with Reyhan cultivar in all drought stress specially in serve stress level, POX was associated with the Khatam (Figure 7).

CONCLUSIONS

The bush height decreased with the severity of drought stress. Drought stress also reduced grain yield and other yield-related components. Drought stress increased leaf proline. This increase in its concentration in the barley plant under drought conditions is a kind of defense mechanism for mitigating the effects of stress on plant cells. Spraying the leaves with salicylic acid increased the proline in the leaves and reduced the destructive effects of the stress. The chlorophyll a and b concentration also increased at the end of flag leaf emergence and 50% of stem emergence. The highest level of chlorophyll a was measured in the cultivar Reyhan and the highest level of chlorophyll b was measured in the cultivar Khatam. The application of salicylic acid compensated the effects of deficit irrigation on photosynthetic pigment traits, relative leaf water content, spike length, plant height, and number of grains per spike, biological yield, and grain yield. Salicylic acid generally increased grain yield, this positive effect was strongly correlated with foliar application time. Salicylic acid foliar application at the end of tillering stage had the highest yield among other foliar treatments. Generally, based on the results obtained, salicylic acid foliar application can improve plant tolerance against drought stress and reduce its complications by increasing the growth indices and affecting the biochemical characteristics of the studied barley cultivars. Among the investigated cultivars, grain yield of Reyhan cultivar was higher than other cultivars. It seems that this cultivar's superior yield is due to its superiority of the number of grains and spike length among the studied cultivars according to the studied yield components. The basil cultivar with the highest percentage of relative moisture in the flag leaves had the highest drought tolerance among the cultivars studied. The highest proline concentration was observed in the Reyhan cultivar, indicating greater drought stress tolerance than other cultivars. This result opens new perspectives in economics, the application of plant hormones, and plant breeding for crop production in deficit-irrigation conditions. Furthermore, it seems that more investigations are required in order to elucidate the physiological and biochemical mechanisms of drought tolerance in barley and in fact Our results demonstrate the potential of Timely use of salicylic acid for the protection and optimization of cereal crop yields under Deficit-Irrigation Condition.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest or personal relationships.

STATEMENTS AND DECLARATIONS

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