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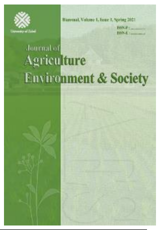

Physiological, antioxidant and yield responses of barley cultivars in nutrients-mediated alleviation of salinity stress

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ABSTRACT

Salinity is one of the most important abiotic stresses and variables restricting the successful production of plant products around the world, with negative consequences for plant development and other metabolic processes. The effect of nutrient management (control, 0.5 percent K_2SO_4, 0.5 percent $ZnSO_4$, and 1.5 Mm salicylic acid) on physiological parameters, antioxidant activities, and grain yield responses of three barley (*Hordeum vulgare* L.) cultivars (Armaghan, Goharan, and Mehr) were examined under salinity stress (1 and 12 dS/m of salinty). Salinity stress considerably lowers growth, yield components, and grain yield, according to the findings. The number of grains per spike and 1000-grain weight of all cultivars tested increased after foliar application of salicylic acid. ZnSO4, K2SO4, and salicylic acid influenced grain and biological yields. At a salinity of 12 dS/m, foliar treatment of $ZnSO_4$, K_2SO_4, and salicylic acid boosted peroxidase, superoxide dismutase, ascorbate peroxidase, and catalase while decreasing hydrogen peroxidase and malondialdehyde. Under the influence of foliar application, the relative water content increased by 12 percent, while the leaf water potential dropped by 8 percent. Salicylic acid treatment had a stronger impact on Mehr cultivar yield and physiological parameters than $ZnSO_4$ or K2SO$_4$. These findings revealed that under the impact of salicylic acid, the Mehr cultivar was more appropriate than other cultivars.

1. Introduction

Barley is a major staple food crop in the world. Increasing grain yield and improving quality are of great importance to the increasing human population (Curtis and Halford, 2014). Although all abiotic stresses adversely affect barley growth and production, water scarcity imposes the most severe effects on this crop (Gonzalez et al., 2010). Researchers have stated that plants are very sensitive to salinity stress at pollination and grain-filling stages. Salinity stress leads to overproduction of Reactive Oxygen Species (ROSs), which is known as a defense mechanism of plants (Thapa et al., 2018). Under salinity stress conditions, tolerant cells activate their antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), which contribute to ROs accumulation (Sinclair, 2011).

Salicylic acid is a plant growth regulator that plays a significant role in abiotic stress. It was revealed that salicylic acid increased the abscisic acid content, leading to the accumulation of proline, and that soaking grains in acetyl-salicylic acid improved the salinity tolerance in wheat (Farooq et al., 2009).

Low crop productivity leads to the problem of the world's food security being under salinity stress, particularly in those areas that receive less rainfall annually (Beheshti and Behboodifard, 2010). Adequate moisture supply is vital for successful germination and crop productivity (Manivannan et al., 2008). During the spring season, high temperatures and higher rates of transpiration cause severe barely yield losses due to low moisture supply. Higher yield losses are reported when the spells of salinity stress reach their limit, especially during the critical growth

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periods (reproductive and grain filling stages) of the crop (De Jonge et al., 2015). Barely, due to its higher nutritional quality with respect to grain and fodder, it ranks 3rd among the cereal crops around the world. It is grown under a wide range of physiographic, soil, and climatic conditions. Salinity stress suppresses the crop yield by reducing the yield attributes (grain weight, grain number) (De Jonge et al., 2015).

Potassium creates resistance against salinity stress in the plant body by activating the antioxidant enzymes (Asada, 2000; Martineau et al., *20*17; Hussain et al., 2018a, b). The current scenario for the causes of the low yield of barely is the decreasing availability of potassium (0.8 kg ha^{-1} $year^{-1}$) in Pakistan and other developing countries as compared to the world's potassium use (15.1 kg ha^{-1} $year^{-1}$). For sustainable and more yielding growth barely under salinity stress, the presence of potassium in ample quantity in the plant tissues is very vital (Bly et al., 2002; Valadabadi and Farahani, 2009). Barely crop is also very sensitive to micronutrient deficiencies, particularly zinc deficiency. Indeed, under zinc-deficient conditions, the activities of antioxidant enzymes become limited, resulting in oxidative damage to the cell membrane of barely plants (Aref, 2010). The major yield limiting factors among the nutrients under abiotic stress include the low supply of zinc in semi-arid regions (Cakmak, 2008; Balakrishnan et al., 2017).

The goal of this study was to explore the influence of nutrient management on physiological parameters, antioxidant activities, and grain production responses of three barley cultivars under salt stress.

2. Materials and methods

Three barley cultivars, including (Armaghan, Goharan, and Mehr), were selected. Seeds of uniform size of three cultivars were sown in a field at the research farm of the Kabotarabad station in Isfahan, Iran, during the 2018–19 growing season. The crop was irrigated with good-quality irrigation water and salinity water quality (12 dS/m).

The soil texture is loam, with an acidity of 6.8 and an electrical conductivity of 7.62 dS/m. The experiment was set up in a split-split-plot complete randomized block design with three replicates. The main plots were salinity treatments (1 and 12 dS/m of NaCl), foliar application (control, 0.5 % K_2So_4, 0.5 % $ZnSo_4$, and 1.5 Mm salicylic acid), and the three barley cultivars as sub-subplots.

Measurements included the relative water content of the flag leaf (RWC) (Castillo, 1996). soluble sugars (Zhang et al., 2017) and soluble proteins (Bradford, 1976); activities of peroxidase (POD) (Cakmak et al., 1993), ascorbate peroxidase (APX) (Nakano and Asada, 1981), catalase (CAT) (Aebi, 1984), and superoxide dismutase (SOD) (Dhindsa and Matow, 1981); levels of hydrogen peroxide (H_2O_2) (Veljovic-Jovanovic et al., 2002) and malondialdehyde (MDA) (Hodges et al., 1999); concentrations of calcium, potassium and magnesium by flame photometer (model 410; Corning Inc., Corning, NY, USA); and leaf water potential (Ψ_w) (PMS Instrument Company, Albany, OR, USA). At maturity, grain yield, number of grains per spike, 1000 grain weight, and harvest index were measured.

Analysis of variance was performed on the data for each parameter by using SAS version 9.2 software (SAS Institute, Cary, NC, USA). Significant differences among mean values were compared using Duncan's multiple range test (at $P \leq 0.05$).

3. Results and discussion

Salinity stress 12 ds/m significantly reduced the grain number per spike by 25.33% in Mehr and 34.21% in Armaghan. The negative impact of salinity stress on the number of grains per spike was alleviated by the application of potassium sulfate and zinc sulfate (Table 1). The 1000-grain weight of barley cultivars decreased significantly under salinity stress. The cultivar Mehr had a higher 1000-grain weight under salinity stress (Table 1).

Table 1. Influence of separate or application of 0.5 % Potassium sulfate, 0.5 % Zinc sulfate, and 1.5 Mm Salicylic acid on yield, yield components, biological yield and harvest index of three barley cultivars (Armaghan, Goharan and Mehr) under field salinity stress and non-stress conditions.

Salinity stress	Chemical treatment	No. of grains per spike			1000-grain weight (g)			Grain yield ($g\ m^{-2}$)		
		Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan
1	0	30.01b	30.56b	29.36b	52.00ab	52.30b	51.10a	455.00b	570.87b	455.87c
(dS/m)	Potassium	30.61b	33.12a	31.12a	52.32a	52.42b	50.22b	452.65bc	570.32b	480.32a
	Zinc	30.54b	31.32b	30.52b	52.81a	52.01b	50.81a	490.23a	482.23c	445.32d
	Salicylic acid	33.52a	33.63a	31.23a	53.15a	53.15a	51.15a	476.47d	578.32a	472.23b
12	0	25.11e	20.56e	21.56e	32.52e	31.02d	31.12e	310.27g	467.77d	257.87g
(dS/m)	Potassium	26.13d	24.02d	22.42d	35.05cd	34.15cd	32.15d	344.25e	435.53e	315.23ef
	Zinc	27.15cd	24.12d	22.12d	35.00cd	34.80cd	32.00d	340.42f	430.30e	320.40e
	Salicylic acid	28.32c	25.12c	23.12c	36.01c	36.11c	33.01c	340.13f	405.45f	285.45f

For each parameter, means followed by the same letter are not significantly different at P=*0.05*

The decline in 1000-grain weight was considerably less in plants supplied with potassium sulfate and zinc sulfate than when these treatments were not applied. Therefore, foliar application of these treatments can significantly improve 1000-grain weight under field salinity conditions; The maximum weight of 1000 grains under the influence of salicylic acid was obtained by the Mehr cultivar (Table 1). However, foliar application of zinc sulfate, potassium sulfate, and salicylic acid caused a significant increase in grain yield under saline conditions. The effect of salicylic acid was greater than that of potassium sulfate or zinc sulfate applied (Table 1).

In three cultivars, the biological yield decreased significantly under salinity stress conditions; however, potassium sulfate and zinc sulfate -treated plants had higher biological yields than untreated plants under salinity stress alone. The effect of salicylic acid application on biological yield was greater than that of potassium sulfate or zinc sulfate applied (Table 1). Salinity stress decreased the harvest index for Goharan only. Foliar application of salicylic acid significantly promoted the harvest index of three barley varieties under saline conditions (Table 1).

Table 1 Continued.

Salinity stress	Chemical treatment	Biological yield (g m^{-2})			Harvest index		
		Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan
1	0	1376.62b	1300.52b	1310.85b	33.18b	33.83b	31.03d
(dS/m)	Potassium	1357.10c	1310.23ab	1170.23c	34.45a	33.71b	39.1a
	Zinc	1389.23a	1315.02ab	1545.02a	33.50b	33.38b	33.08c
	Salicylic acid	1350.23d	1364.02a	1164.02c	32.38bc	32.80b	34.10b
12	0	700.68g	735.32e	715.82e	25.00d	33.69b	33.09c
(dS/m)	Potassium	750.10f	770.50d	670.50f	25.84d	34.03ab	33.83c
	Zinc	753.14ef	770.25d	678.35f	28.02c	35.92ab	32.32cd
	Salicylic acid	768.12e	900.88c	890.88d	28.45c	38.02a	33.02c

For each parameter, means followed by the same letter are not significantly different at P=*0.05*

Salinity affects water relations and photosynthetic pigments of barely crops and leads to a reduction in grain and biological yield. Osmotic adjustment is a mechanism for maintaining transpiration under drought, but its role in maintaining growth and yield is not so simple (Turner, 2018). Values of LWP, OS, and TP declined with salinity stress due to insufficient water available to fulfill the needs of transpiration (Waraich et al., 2012; Wang et al., 2013). Potassium and zinc improve water relations and chlorophyll content as these nutrients are part of many biochemical and physiological processes (Kambe et al., 2015) and under stress, the concentration of potassium in the cell maintains these processes and resists the production of oxygen reactive species (Morshedi and Farahbakhsh, 2010; Osakabe et al., 2014), which leads to tolerance. As a result, because the potassium concentration in the soil was moderate, applying potassium at a lower rate than recommended did not improve the processes, even when supplemented with zinc (Table 2). Although zinc is an important plant micronutrient, its role under salinity stress is not well defined. Plants treated with Potassium and zinc had significantly higher soluble sugar content than untreated plants under salinity stress alone.

The influence of salicylic acid on soluble sugars in plants under salinity stress tended to be greater than that of potassium or zinc applied. The responses of cultivars to potassium and zinc varied significantly, with Mehr being more responsive. in potassium, zinc, and salicylic acid treatments and under salinity stress, soluble sugar content was 19.75%, 15.20%, and 27.22% higher, respectively, in Goharan, and 12.08%, 15.88%, and 21.34% higher in Armaghan than with no foliar application (Table 2). In three cultivars, the levels of soluble proteins decreased markedly under saline conditions.

Table 2. Influence of separate or application of 0.5 % Potassium sulfate, 0.5 % Zinc sulfate, and 1.5 mM Salicylic acid of soluble sugars, soluble proteins and mineral nutrients in the leaves of three barley cultivars (Armaghan, Goharan and Mehr) under field salinity stress and non-stress conditions Measures of sugars and minerals by dry weight, protein by fresh weight.

Salinity stress	Chemical treatment	Soluble sugars			Soluble proteins			Potassium		
		Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan
1	0	33.80e	33.12f	32.12f	16.50b	15.50bc	14.50bc	58.63f	47.08ef	46.08e
(dS/m)	Potassium	36.12d	37.32e	36.32e	14.85c	15.32bc	13.32c	58.52f	48.10e	46.10e
	Zinc	37.10cd	37.86e	36.86e	14.00cd	15.69bc	14.69bc	62.00e	52.01d	51.01d
	Salicylic acid	37.18cd	40.00d	59.00c	15.65c	18.23a	17.23a	63.00d	52.11d	51.11d
12	0	41.11c	52.02c	56.02d	10.52e	12.36d	12.36d	66.23c	65.00c	65.00c
dS/m)	Potassium	48.23b	65.51ab	64.51ab	13.02d	14.36c	13.36c	71.85b	73.10b	72.10b
	Zinc	49.26b	61.45b	61.45b	13.00d	14.32c	13.32c	73.15a	78.00ab	78.00a
	Salicylic acid	33.80e	33.12f	32.12f	16.50b	15.50bc	14.50bc	58.63f	47.08ef	46.08e

For each parameter, means followed by the same letter are not significantly different at P=*0.05*

Table 2 Continued.

Salinity stress	Chemical treatment	Magnesium				Calcium	
		Armaghan	Mehr	Armaghan	Mehr	Armaghan	Mehr
1	0	2.30b	2.31e	2.30b	2.31e	2.30b	2.31e
(dS/m)	Potassium	2.31b	2.28e	2.31b	2.28e	2.31b	2.28e
	Zinc	2.30b	2.29e	2.30b	2.29e	2.30b	2.29e
	Salicylic acid	2.29b	2.31e	2.29b	2.31e	2.29b	2.31e
12	0	2.88ab	5.07b	2.88ab	5.07b	2.88ab	5.07b
(dS/m)	Potassium	2.85ab	5.10b	2.85ab	5.10b	2.85ab	5.10b
	Zinc	3.02a	5.65a	3.02a	5.65a	3.02a	5.65a
	Salicylic acid	3.10a	5.71a	3.10a	5.71a	3.10a	5.71a

For each parameter, means followed by the same letter are not significantly different at P=*0.05*

Application of potassium and zinc improved the soluble protein levels of salinity -stressed plants of three cultivars compared with plants exposed to salinity stress without potassium and zinc application, and the effect of salicylic acid on soluble protein content was greater than that of potassium or salicylic acid applied. Foliar application of salicylic acid also significantly increased soluble protein content by 6.80% and 15.32%, respectively, in Armaghan and Mehr under non-stress conditions (Table 2). Salinity-stressed plants fed with potassium and zinc accumulated a greater concentration of potassium than control plants. Supplementation with zinc and salicylic acid caused a marked increase in magnesium concentration in salinity - stressed plants compared with those receiving no foliar treatment (Table 2). Calcium concentration increased significantly in three cultivars under salinity stress; foliar application of potassium, zinc, and magnesium caused a further increase in this nutrient only in Mehr. The concentrations of the three mineral nutrients (potassium, magnesium, and Calcium) were greater in Mehr than in Armaghan under salinity stress conditions (Table 2).

In three cultivars, application of potassium, zinc, and salicylic acid significantly increased POD activity in salinity stressed plants; the influence of salicylic acid was greater than that of potassium or zinc. POD was much higher in Mehr than in Armaghan under salinity stress conditions, especially with foliar-applied salicylic acid (Table 3).

Table 3. Influence of separate or application of 0.5 % Potassium sulfate, 0.5 % Zinc sulfate, and 1.5 Mm Salicylic acid on activities of peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT), as well as levels of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) of three barley cultivars (Armaghan, Goharan and Mehr) under field salinity stress and non-stress conditions.

Salinity stress	Chemical treatment	POD ($U\ mg^{-1}$ protein)			SOD ($U\ mg^{-1}$ protein)			APX ($U\ mg^{-1}$ protein)		
		Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan
1	0	23.18d	37.02f	37.12e	3.20d	4.32d	5.14c	2.85c	2.18d	2.18d
(dS/m)	Potassium	23.46d	39.32e	37.12e	3.18d	4.35d	4.24d	2.04cd	2.22d	2.32d
	Zinc	23.62d	36.25f	34.15f	4.00c	4.07d	3.13e	2.75c	2.86c	2.86c
	Salicylic acid	24.88c	39.98e	35.98f	4.50b	5.02c	3.14e	2.77c	3.83b	2.73c
12	0	28.19b	65.56d	63.56d	5.00b	7.02bc	10.16a	3.14b	3.87b	3.77b
(dS/m)	Potassium	29.26b	74.32b	75.32b	5.50ab	8.02b	9.13a	4.85a	4.62ab	4.62ab
	Zinc	28.09b	70.65c	69.65c	5.50ab	8.32b	6.32b	4.92a	4.88a	4.78a
	Salicylic acid	32.87a	80.36a	79.36a	6.01a	9.54a	6.42b	4.87a	4.86a	4.96a

For each parameter, means followed by the same letter are not significantly different at P=*0.05*

Table 3 Continued.

Salinity stress	Chemical treatment	CAT ($U\ mg^{-1}$ protein)			H_2O_2 ($U\ mg^{-1}$ protein)			MDA ($U\ mg^{-1}$ protein)		
		Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan
1	0	2.15e	2.12d	3.12cd	8.71a	5.23c	5.23bc	5.42e	5.54d	3.14e
(dS/m)	Potassium	2.16e	2.10d	3.10cd	8.02b	8.00b	5.00c	4.00f	4.14e	4.14d
	Zinc	2.15e	2.13d	3.23cd	8.00b	8.12bc	5.12c	3.47g	3.23f	3.23e
	Salicylic acid	2.57d	2.89c	3.89c	7.81b	8.00b	5.00c	3.20h	3.14f	3.14e
12	0	4.11c	3.21b	4.31b	8.62a	8.71a	5.71a	15.20a	10.36a	9.56a
(dS/m)	Potassium	4.41b	3.84a	4.84a	8.01b	8.23b	5.23bc	14.35b	9.23b	8.15b
	Zinc	4.55b	3.02b	4.02bc	8.58a	8.34b	5.34b	11.63c	6.32c	5.42c
	Salicylic acid	4.84a	3.87a	4.87a	8.78a	8.34b	5.34b	11.23d	6.42c	5.52c

For each parameter, means followed by the same letter are not significantly different at P=*0.05*

Plants treated with potassium or zinc had greater SOD activity than those grown solely under water limitation. The effect of salicylic acid was greater than that of zinc applied. Varietal response to potassium and zinc varied significantly for SOD activity; Mehr was more responsive. In addition, under normal salinity conditions, application of salicylic acid significantly promoted SOD activity relative to no foliar application in both barley varieties (Table 3).

Optimal potassium-zinc nutrient supply resulted in increased dry matter production due to increased activation of enzymes and many physio-chemical processes (Kambe et al., 2015). Soil application of zinc and potassium synergistically enhances chlorophyll production, which results in more photosynthetic assimilates (Tariq et al., 2014) and ultimately produces higher yields (Abid et al., 2016) even under salinity stress. Irrigation water use efficiency (IWUE) is a very important attribute as it describes the efficiency of total applied water.

Table 4- Influence of separate or application of .5 % Potassium sulfate, 0.5 % Zinc sulfate, and 1.5 Mm Salicylic acid on relative water content and leaf water potential of three barley cultivars (Armaghan, Goharan and Mehr) under field salinity stress and non-stress conditions.

Salinity stress	Chemical treatment	Relative water content (%)			Leaf water potential (–MPa)		
		Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan
1	0	72.12a	73.11b	70.01b	1.73e	1.80c	1.60c
(dS/m)	Potassium	72.31a	74.19a	73.09a	1.98d	1.78c	1.61c
	Zinc	72.00a	74.20a	73.10a	1.35fg	1.78c	1.63c
	Salicylic acid	72.10a	74.10a	73.00a	1.53f	1.68d	1.78bc
12	0	60.23d	67.02d	65.42e	2.69a	2.97a	1.97a
(dS/m)	Potassium	65.55c	68.32c	67.32d	2.40b	1.96b	1.87b
	Zinc	65.20c	69.23c	66.33e	2.31b	1.43d	1.63c
	Salicylic acid	69.60b	68.20c	66.25e	2.11c	1.75cd	1.85b

For each parameter, means followed by the same letter are not significantly different at P=*0.05*

The IWUE in the current study was higher under SD than under MD and WW, which indicates barely used water more efficiently when applied in limited amounts. Under WW conditions, IWUE was low due to more wastage of irrigation water. Ul-Allah et al. (2014,15) reported higher IWUEs of fodder crops, including barely under water stress conditions in a semi-arid climate and suggested reevaluating the irrigation scheduling for optimum water use efficiency. As application of zinc combined with higher levels of potassium produces maximum biological yield, therefore, IWUE was also maximum for this treatment (Table 4). Higher IWUEs with the application of potassium-zinc nutrition were due to the role of these nutrients in osmotic adjustment and regulation of biochemical processes under water stress conditions (Osakabe et al., 2014; Kambe et al., 2015).

The Activity of APX also increased in both barley varieties under salinity stress, and this increase was more pronounced in Goharan. Application of potassium sulfate and zinc sulfate had no significant effect on APX activity in Armaghan under either salinity regime, whereas in Goharan, APX significantly increased with the application of zinc and salicylic acid under normal salinity conditions and with the application of potassium, zinc and salicylic acid under salinity stress (Table 3).

Levels of H_2O_2 increased markedly under saline conditions. Plants treated with potassium and zinc had lower H_2O_2 levels than plants under salinity stress alone. Furthermore, the influence of salicylic acid application on H_2O_2 content was greater than with either potassium or zinc applied. With application of potassium, zinc, and salicylic acid, and under salinity stress, H_2O_2 content was lower than with no foliar application in both cultivars (Table 3).

For any food and feed crop, nutritional quality has the same importance as that of yield. Grain starch, oil and protein content are important for human consumption. The Reduction in gain nutritional content under salinity stress has been attributed to the downregulation of enzymatic activities (Halford et al., 2014; Ignjatovic-Micic et al., 2015; Kambe et al., 2015), which results in lower conversion to assimilate into grain starch, oil, and proteins. Plant researchers believe that because of the effect of water stress on nutritional quality in a climate change scenario. potassium-zinc improves regulation of enzymatic activities under salinity stress, which results in improvement of these attributes (Valadabadi and Farahani, 2009).

Conclusion

Salinity stress significantly reduces growth, yield components, and grain yield in barley. Foliar salicylic acid reduces the negative effects of salinity stress and, consequently, improves. The results of the study highlight the role of Potassium sulfate and zinc sulfate applications in regulating the salinity stress response of barley, suggesting that salicylic acid is involved in physiological activities.

These results showed positive effects of potassium and salicylic acid in terms of increased antioxidant activity as well as relative water content and leaf water potential. In addition, potassium sulfate and zinc sulfate stimulated the active accumulation of some The effects of salicylic acid application on the Mehr cultivar on yield and physiological parameters were greater than those of potassium sulfate or zinc sulfate. Therefore, the Mehr cultivar was more suitable than other cultivars under the influence of salicylic acid.

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