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Improving wheat (*Triticum aestivum* L.) antioxidative defense mechanisms against salinity stress by exogenous application of potassium silicate

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ABSTRACT

The primary objective of this study was to investigate the beneficial effects of seed priming and foliar spray of potassium silicate on antioxidant activities under different salinity levels, thereby potentially improving wheat growth. Seeds were soaked into solutions containing potassium silicate (K_2SiO_3, 1.5 mM) for 6 h, while foliar spray with K_2SiO_3 (4 mM) was applied at the early and the late stages of tillering. Lake Urmia water was used to prepare salinity levels of 0, 3, 5, 8, 10, 12, and 14 dS m^{-1}. For such traits as anthocyanin, catalase, ascorbate peroxidase, guaiacol peroxidase, and superoxide dismutase activity, an initial increase was observed at lower salinity levels; higher salinities subsequently decreased these traits or they remained mostly constant. Salinity also increased phenol, malondialdehyde, hydrogen peroxide, and polyphenol oxidase, but decreased flavonoid, nitrate content, and nitrate reductase activity. Seed priming and foliar spray provided effective approaches to reduce reactive oxygen species (ROS) manifestation in wheat grown under saline conditions. The improved antioxidant defense abilities by seed priming and foliar spray alleviated the oxidative damage of proteins and lipids and improved nitrate content and nitrate reductase activity.

Abbreviations: CAT: catalase; APX: ascorbate peroxidase; FW: Fresh weight; GPX: guaiacol peroxidase; SOD: superoxide dismutase; PPO: polyphenol oxidase and; PAL: phenylalanine ammonia-lyase; POD: peroxidase; MDA: malondialdehyde content; NR: nitrate reductase; ROS: reactive oxygen species.

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Antioxidants; foliar spray; radical scavenging; salinity stress; seed priming

Introduction

High salt concentrations are toxic to plants at all growth stages by causing oxidative damage such as oxidation of certain macromolecules such as DNA, proteins, and lipids, due to the production of reactive oxygen species (ROS), ultimately diminishing the growth, economic yield and/or quality of crops (Arora et al. 2008; Bela et al. 2015). To keep ROS levels tightly regulated and to minimize ROS-derived damages, different antioxidant systems have been evolved in aerobic organisms, including the diverse super-family of peroxidase enzymes (Munns and Gilliham 2015; Riasat et al. 2018). The primary scavenger in the detoxification of ROS in plants is superoxide dismutase (SOD), which converts superoxide to hydrogen peroxide (H_2O_2) and molecular oxygen.

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Ascorbate peroxidase (APX), the most important peroxidase in detoxifying H_2O_2, and catalase (CAT) can reduce H_2O_2 to water (Bhanuprakash and Yogeesha 2016). Phenylalanine ammonia-lyase (PAL) is the main enzyme responsible for phenolic synthesis under a variety of stresses. Moreover, polyphenol oxidase (PPO) shares a role in phenolic and lignin synthesis: PPO may remove excess ROS caused by stress, thus serving a detoxifying role during stress (Ali et al. 2006).

Other antioxidant compounds such as phenolic compounds, flavonoids, and anthocyanin, in concentration and structure-dependent ways, may contribute to antioxidant activity and the scavenging of free radicals, lipid peroxidation inhibitors, and membrane stabilizers (Saleh and Madany 2015). Previous reports on the antioxidative response of plants to salt stress indicate an increase in the activities of non-enzymatic and enzymatic antioxidant systems and lipid peroxidation (Tarchoune et al. 2010; Ouhibi et al. 2014; Panuccio et al. 2014). In contrast, Noreen, Ashraf, and Akram (2010) reported that the imposition of varying levels of salinity could substantially decrease the activities of SOD, peroxidase (POD), and CAT in turnip cultivars. Some wheat cultivars subjected to long-time exposure to salinity showed an increase in certain antioxidant enzymes such as POD and SOD (Pessarakli, Haghighi, and Sheibanirad 2015). Moreover, Shahzad et al. (2019) reported that salinity stress caused reductions in plant growth due to increased lipid peroxidation and decreased total phenols in cowpea. Ultimately, the salt tolerance of plants may be enhanced if ROS could be scavenged by an improved antioxidative defense system. It is therefore important to study antioxidative response during the growth and development of plants under salinity stress (Turan and Tripathy 2013) and to identify the most effective and easiest techniques that improve the antioxidative defense system.

Seed priming techniques have been widely used to improve plant growth under different abiotic stresses. Past studies show that seed priming may increase SOD, POD, and CAT activities in barley (Ali et al. 2013), CAT activity in sunflower (Kibinza et al. 2011), and CAT, POD, and APX activities in wheat (Islam et al. 2015), while also enhancing anthocyanin and PPO; however, seed priming lowered phenol and the reducing power in rice (Paul et al. 2017). Additionally, seed priming can activate signaling pathways in the early stages of growth, resulting in faster plant defense responses. The biological origin of enhancing salinity tolerance in plants by seed priming is associated with increased antioxidant enzyme activities, protection of cell membranes as a result of decreased lipid peroxidation, and improved leaf photosynthetic rates. However, the exact molecular mechanisms behind priming are not completely understood (Bhanuprakash and Yogeesha 2016). Moreover, foliar spray, as another beneficial technique, could enhance the activity of antioxidant enzymes (SOD, APX, CAT, and GSH-Px) and reduce the contents of H_2O_2 and malondialdehyde (MDA) in plants under salinity stress (Subramanyam, Laing, and Van Damme 2019). Bybordi (2012) also reported that foliar spray of K_2SiO_3 increased APX and nitrate reductase (NR) activities in canola.

Although the beneficial effects of seed priming and foliar spray against abiotic stresses are evident from previous studies, not much is known about molecular regulation and the physiological/biochemical mechanisms of seed priming and foliar spray-mediated stress tolerance. Therefore, the hypothesis of our study is that K_2SiO_3 may enforce the antioxidant system of wheat plants, which ultimately could scavenge ROS accumulations and improve plant growth, thus inducing a defense against salinity stress. The main objectives of this study hence are to clarify the involvement of exogenous K_2SiO_3, as a seed priming and foliar spray agent, in non-enzymatic and enzymatic antioxidant systems and lipid peroxidation processes in wheat subject to varying salinity levels, as well as elucidating the possible mechanisms of K_2SiO_3-enhanced salt tolerance in wheat.

Materials and methods

Seed materials

Healthy and uniform-sized seeds of wheat (*Triticum aestivum* L. Var. Chamran) were prepared for our study by the Cereal Laboratory of the Agricultural Research Center of West Azerbaijan

Province, Urmia, Iran. The mean 100-seed weight and seed moisture content were 3.57 g and 9-10%, respectively.

Seed priming, foliar spray, and salinity treatments

Prior to the experiments, seeds exhibited a 100% germination rate at 25°C, as assessed using Petri dishes. For priming, the seeds were soaked for 6 h in a solution containing K_2SiO_3 (1.5 mM). This agent and its specific concentration were selected following Feghhenabi et al. (2020). Once primed, the seeds were thoroughly rinsed with tap water and then distilled water, after which they were dried back to their original moisture content at room conditions (about 25°C and 42% relative humidity), as determined by the changes in seed weight. All seeds were subsequently surface-sterilized in a 1% sodium hypochlorite solution for 10 min to be used in the main experiments.

By considering silicate at a concentration of 4 mM as the most effective in modulating the effects of salinity (Bybordi 2015; Alzahrani et al. 2018), foliar spray at this concentration was applied to wheat leaves in two consecutive rounds. Tween 20 at 0.01% (v/v) was used furthermore as a surfactant to increase foliar spray efficiency by increasing solution penetration. Half of the pots were randomly selected and sprayed separately so that they were not in contact with other pots. The remaining pots without any treatment were considered as no foliar sprayed pots. Foliar spray was applied at the early and the late stages of tillering (31 and 45 days after sowing). The first round was applied before sunset and the second round just before dawn.

The open greenhouse pot experiments

Greenhouse-based pot experiments were undertaken within an open environment. Two-year average maximum and minimum temperatures were 24.86 and 10.50°C, the number of sunny hours was 9.85 hours, and rainfall throughout the growing season was 18.8 mm. The growth medium was a field soil collected from a 30-cm deep layer at an alfalfa farm. The field soil was classified as a loam soil (sand, silt, and clay percentages were 46, 33, and 21%, respectively) that was relatively non saline (the electrical conductivity of the saturation paste was 1.1 dS m^{-1}), containing 18.5% equivalent $CaCO_3$, with a pH value of 7.7. The field-collected soil was air-dried, ground, and passed through a 2-mm sieve. In order to maintain the growth of the seedling, a basal dose of urea, di-ammonium phosphate and potassium sulfate was added and mixed well into the soil. A small amount of urea was also applied as a top dressing. A total of 16.4 kg of the field soil with a bulk density typical of the plow layer of a cultivated field was placed into each 18.5 L plastic pot. The amount of soil in each pot was measured on a dry weight basis. Pot cultivation was carried out manually at 3 cm depth in an open greenhouse. Watering was performed immediately after seed sowing using different levels of prepared salt water and continued until harvesting time. The amount of water required per pot at 30% of field capacity was 2.4 liters. For the experiments we used salinity levels of 0, 3, 5, 8, 10, 12, and 14 dS m^{-1}, obtained by diluting saline water from Lake Urmia, in the northwest of Iran.

Sampling materials

Fresh samples obtained from fully matured flag leaves of wheat at the early flowering stage were frozen in liquid nitrogen and stored at −80°C. Liquid nitrogen was also used to powder the frozen samples. The whole process of triturating was done in a cold condition.

Measurement of non-enzymatic antioxidants content

Phenol content

Phenol was extracted according to methods outlined by Schaller, Brackhage, and Dudel (2012), with some modifications. Samples (0.5 g each) were homogenized with 95% (v/v) ethanol. The extracts were well mixed with an equal volume of Folin-Ciocalteu-reagent for 3 min, after which 10% Na_2CO_3 was added at half of their volume. After 1 hour's standing, the absorbance of the reaction solution was read at 760 nm by spectrophotometry. The phenol content was expressed in mg g^{-1} fresh weight (FW).

Flavonoid content

Flavonoid content was measured employing the colorimetric method described by Zhishen, Mengcheng, and Jianming (1999). 0.5 ml aliquots of extracts were added to a 10 ml volumetric flask containing 4.5 ml distilled water. 0.3 ml 5% sodium nitrite was added to each aliquot after 5 min, and then also 0.6 ml of 10% aluminum chloride. After 6 min, 2 ml of 1 M sodium hydroxide was added to the mixture followed by the addition of 2.1 ml distilled water. Absorbance was recorded at 510 nm, and the flavonoid content was expressed as mg g^{-1} FW.

Anthocyanin content

Determination of anthocyanin contents was carried out using the method of Wagner (1979). To calculate the content of anthocyanin, an extinction coefficient of 33,000 mol cm was used, with the resulting anthocyanin content expressed as μg FW.

Measurement of enzymatic antioxidants activities

Fresh samples from fully mature flag leaves of the wheat crop were used for enzyme analysis. One-gram leaves were homogenized in a 3 ml of 0.05 M sodium phosphate buffer (pH 7.8), including 1 mM EDTA and 2% (w/v) Polyvinylpyrrolidone (PVP). The homogenates were centrifuged at 13,000 × g for 20 min at 4°**C**. Supernatants were used for enzyme activity and protein content assays. All steps in the preparation of the enzyme extracts were carried out at 4°**C**. The extracts were used for measuring the enzyme activity of CAT, APX, guaiacol peroxidase (GPX), and SOD.

Catalase, ascorbate peroxidase, guaiacol peroxidase, and superoxide dismutase activities

CAT, APX, and GPX activities were measured according to Cakmak and Marschner (1992), Nakano and Asada (1981), and Egley et al. (1983), respectively. Activities of the extracts were expressed as mM g^{-1} FWmin^{-1}. One unit of SOD activity was defined as the amount of enzyme which caused 50% inhibition of photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm according to the method of Giannopolitis and Ries (1977). SOD activities of the extracts were expressed as % inhibition g^{-1} FWmin^{-1}.

Polyphenol oxidase activity

PPO activity was assayed using the method of Singh et al. (1999). First, samples were homogenized using a sodium phosphate buffer (200 mM, pH 6.5), after which10mM pyrogallol was added to each test tube. The test tubes subsequently were incubated for 15 min in a water bath at 30°**C**. Enzyme extracts were then added to the test tubes. The absorbance of the resulting solutions were recorded at 334 nm for 2 min with a spectrophotometer. PPO activity of the extract was expressed as mM g^{-1} FWmin^{-1}.

Phenylalanine ammonia-lyase activity

Extractions and assays of PAL (EC 4.3.1.5) were carried out according to methods outlined by Beaudoin-Eagan and Thorpe (1985). A standard curve of t-cinnamic acid was used; PAL activities were expressed as nmol t-cinnamic acid mg^{-1} protein min^{-1}.

Measurement of signaling molecule content and lipid peroxidation

The H_2O_2 content, as a signaling molecule, of the samples was determined following Velikova, Yordanov, and Edreva (2000). Lipid peroxidation (MDA content) was determined using thiobarbituric acid reactions (Bailly et al. 1996), with slight modifications. The MDA content was calculated using:

$$\text{MDA content } \left(\mu\text{mol g}^{-1}\ \text{FW}\right) = \frac{[(OD_{532} - OD_{600})/155] \times 5}{0.1} \quad (1)$$

where OD_x is the absorbance of the extract at a given wavelength x.

Measurement of nitrate and nitrate reductase activity

The nitrate content was obtained using methods described by Cataldo et al. (1975), and the NR activity using the method of Klepper, Flesher, and Hageman (1971). Nitrate reductase activity was expressed as μmol h^{-1} FWg^{-1}.

Statistical analysis

A randomized complete block design was used with a factorial arrangement of treatments with three replications. The first factor covered alleviating treatments by K_2SiO_3 and the Control (no seed priming and foliar spray), and the second was salinity (0, 3, 5, 8, 10, 12, and 14 dS m^{-1}). The combined analysis of variance for two years (a four-way ANOVA, considering the year as a random effect) was performed using the SAS 9.4 software. Consequently, multiple comparisons were performed using the protected least significant difference (PLSD) test at the $P < 0.05$ level of significance. The Excel 2017 software was used to create the figures and regression analyses. Two-year data averages were used to draw the graphs.

Results and discussion

Results of the two-year data of the analysis of variance showed a significant effect of salinity on all measured traits (Table 1). Furthermore, treatments (seed priming and foliar spray) had a significant ($p < 0.05$ or 0.01) effect on all measured traits (Table 1). For some traits (phenol, flavonoid, H_2O_2, nitrate content, SOD, NR, and PAL), a significant modification was observed with the change of year. Furthermore, double interactions (treatment × salinity) were significant only for GPX, NR, and MDA.

Non-enzymatic antioxidants content

Phenol and flavonoid content

The phenol and flavonoid content curves were fitted with a linear regression (Figure 1a and b). The phenol content demonstrated an upward trend with salinity. Boudet (2007) documented that the increased expression and activity of the enzyme PAL, a key enzyme in producing phenylpropanoids, raised phenolic compounds. The phenolic compounds can be oxidized by PPO, leading

Table 1. Analysis of variance of the alleviating treatments' effects on wheat response to salinity stress during two consecutive years (2017-2018).

			Mean Squares											
SOV	df	phen	flav	antho ($\times 10^{-8}$)	CAT	APX	GPX	SOD	PPO	PAL	H_2O_2	MDA	Nit ($\times 10^{-8}$)	NR
Y	1	51.34**	0.02*	117^{ns}	0.14^{ns}	0.003^{ns}	1.62^{ns}	915.54**	2.29^{ns}	0.03*	20.68**	0.28^{ns}	157**	39.64**
B	4	4.63**	0.05**	3372^{ns}	1.63**	2.20**	1.87*	375.16**	11.64**	0.02**	0.90^{ns}	2.05^{ns}	168**	7.10**
T	1	12.67**	0.01*	4795*	7.08**	3.85**	25.83**	3092.10**	199.47**	0.22**	3.00**	30.91**	212**	25.97**
S	6	61.25**	0.31**	5478*	26.45**	55.78**	99.42**	14215.69**	18.09**	0.50**	60.06**	147.61**	3032**	116.88**
T × S	6	0.68^{ns}	0.003^{ns}	163^{ns}	0.61^{ns}	0.56^{ns}	1.55*	67.02^{ns}	3.26^{ns}	0.01^{ns}	0.43^{ns}	1.55**	13^{ns}	1.64**
Y × T	1	0.08^{ns}	0.007^{ns}	13^{ns}	0.04^{ns}	0.04^{ns}	0.58^{ns}	324.53*	1.95^{ns}	0.01^{ns}	0.07^{ns}	0.38^{ns}	1^{ns}	0.74^{ns}
Y × S	6	0.32^{ns}	0.0003^{ns}	26^{ns}	0.02^{ns}	0.01^{ns}	1.51^{ns}	3.74^{ns}	0.13^{ns}	0.0004^{ns}	0.52^{ns}	0.31^{ns}	12^{ns}	0.10^{ns}
Y × T × S	6	0.17^{ns}	0.0003^{ns}	25^{ns}	0.01^{ns}	0.01^{ns}	0.26^{ns}	3.03^{ns}	0.11^{ns}	0.0002^{ns}	0.01^{ns}	0.09^{ns}	2^{ns}	0.27^{ns}
Error	108	1.29	0.003	2014	0.44	0.43	0.75	106.75	2.95	0.006	0.46	0.32	11	0.66
CV (%)		12.9	14.0	17.9	14.9	17.5	7.5	6.2	10.6	16.49	18.4	11.5	19.2	7.11

Note: SOV, sources of variation; Y, year; B, block; T, treatments; S, salinity; phen, phenol; flav, flavonoid; antho, anthocyanin; CAT, catalase; APX, ascorbate peroxidase; GPX, guaiacol peroxidase; SOD, superoxide dismutase; PAL, phenylalanine ammonia-lyase; H_2O_2, hydrogen peroxide; MDA, malondialdehyde content; Nit, nitrate content. *, **and ^{ns}, denote significant at 5%, 1%, and not significant, respectively.

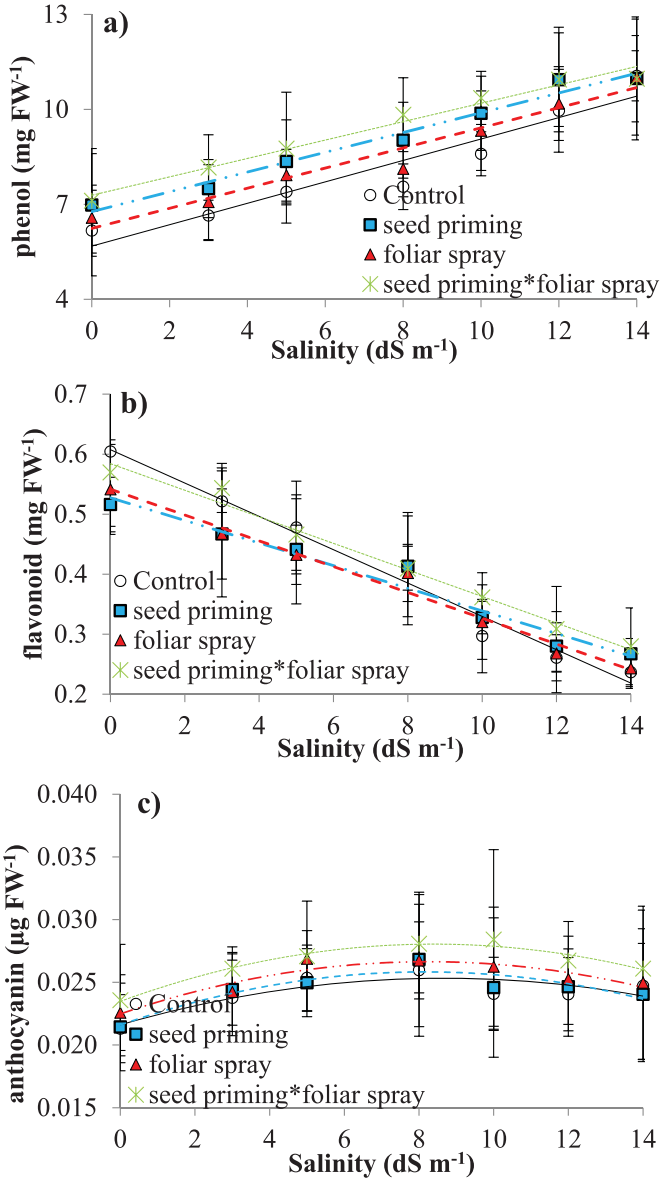

Figure 1. Effect of seed priming and foliar spray of potassium silicate on phenol (a), flavonoid (b) and anthocyanin (c) content at different salinity levels. Bars represent two-year mean values with standard deviations.

to the formation of quinones, which are involved in the defense system of a plant. Moreover, by donating electrons or hydrogen atoms, these compounds can break down the chain reactions that form free radicals and react with peroxide precursors, thereby preventing the formation of peroxide. Phenolic compounds hence could be regarded as excellent oxygen radical scavengers (Ouhibi et al. 2014). However, under salinity, the flavonoid content showed a trend opposite to that of the phenol content. The slope of the phenol content changes for control was such that the content of phenol became 1.8 times higher at $EC = 14$ dS m^{-1}. Instead, the flavonoid content for the control reduced by half at $EC = 14$ dS m^{-1}. Reduced flavonoid content could be due to channeling to cause anthocyanin overproduction, thus being unavailable for energy required for the

synthesis of flavonoid due to the lower rate of photosynthesis (Chaparzadeh and Hosseinzad-behboud 2015). However, Taïbi et al. (2016) concluded that the high-yielding genotypes may offer better protection against oxidative damage by increasing the amount of total flavonoids and the activity of antioxidant enzymes under high salinity. Furthermore, Ouhibi et al. (2014) concluded that salt stress induced the stimulation of the phenol content, flavonoids, and total antioxidant capacity.

Compared to using seed priming and foliar spray alone, our results showed that their combined use increased the content of flavonoids more effectively. Compared to the control, the application of seed priming and foliar spray had a positive effect on the regression slope and intercept of phenol content changes. Our results revealed the significant positive effect of seed priming and foliar spray on phenol content with 11 and 5% increases (Figure 1a and Table 2). The application of seed priming along with foliar spray increased phenol content by up to 15% (Table 2). These results are consistent with findings by Dallagnol et al. (2015) who observed significant higher contents of phenolic compounds in K_2SiO_3-treated plants. Induced accumulation of phenol content can control the production of H_2O_2, which consequently may play an important role in the oxidative stress tolerance of plants. Moreover, the regression slope of flavonoid content changes became milder as a result of seed priming and foliar spray. The effects of seed priming and foliar spray on the flavonoid content caused a decrease of almost 2%. Instead, seed priming along with foliar spray statistically increased the flavonoid content by up to 5% (Table 2).

Anthocyanin content

The anthocyanin biosynthetic pathway is controlled by both external and internal factors. Its synthesis hence is inducible under stress, as our results showed by the effect of salinity. All data related to the anthocyanin content were fitted based on quadratic regression (Figure 1c). The behavior of the anthocyanin for controlling salinity was such that it increased (22%) up to $EC = 8$ dS m^{-1}, and then declined (5%) with a further salinity increase. Moreover, seed priming along with foliar spray was most significant for anthocyanin, which increased by 13% (Table 2). On the other hand, seed priming alone had no considerable effect. Anthocyanin has antioxidative properties because of its ability as a hydrogen or electron donor, its ability of the polyphenol-derived radicals to stabilize and delocalize unpaired electrons, and its ability to chelate transition metal ions. Increasing anthocyanin hence will be useful in modulating the effects of salinity. However, overproduction of anthocyanin can be associated with decreasing chlorophyll content, biosynthesis of nitrogen-containing compounds, and the content of phenolic compounds such as flavonoids (Chaparzadeh and Hosseinzad-behboud 2015).

Enzymatic antioxidants activities

Catalase activity

According to the regression analysis, a quadratic function describing the CAT activity response to increasing salinity fitted its behavior best (data not shown). The CAT activity for the control intensified (2.92 times) up to $EC = 12$ dS m^{-1}, after which it showed a downward trend. Seed priming had no significant effect on CAT activity. However, CAT activity was enhanced approximately 18% by the combined treatments of seed priming and foliar spray, which statistically was equal to the application of foliar spray alone (Table 2). In contrast, Bibordy (2016) found that the CAT and SOD activities were suppressed by supplying silicon (2 or 4 g L^{-1}) to canola plants grown under salinity conditions. The high levels of CAT activity indicated efficient scavenging of H_2O_2 (Vaidyanathan et al. 2003). Indeed, foliar spray indirectly reduced H_2O_2.

Table 2. Influence of alleviating treatments on the antioxidative defense system of wheat.

					Mean of two-year data				
Treatments	phen	flav	antho	CAT	APX	GPX	SOD	PPO	PAL
control	8.20 ± 1.87c	0.40 ± 0.15ab	0.024 ± 0.004b	4.10 ± 1.26b	3.43 ± 1.45b	10.47 ± 1.97c	160.45 ± 27.29b	14.25 ± 1.34b	0.39 ± 0.16b
Seed priming (1)	9.09 ± 1.98ab	0.39 ± 0.11b	0.024 ± 0.004b	4.10 ± 1.26b	3.51 ± 1.40b	11.35 ± 2.24b	160.80 ± 24.01b	14.46 ± 1.81b	0.53 ± 0.16a
Foliar spray (2)	8.60 ± 1.87bc	0.38 ± 0.12b	0.025 ± 0.004ab	4.80 ± 1.02a	3.97 ± 1.72a	11.99 ± 1.92a	174.80 ± 24.64a	18.14 ± 1.82a	0.42 ± 0.16b
(1)*(2)	9.45 ± 1.89a	0.42 ± 0.12a	0.027 ± 0.004a	4.82 ± 1.12a	4.01 ± 1.65a	12.21 ± 2.28a	176.11 ± 23.39a	18.12 ± 2.22a	0.53 ± 0.14a

Data represent means ± SD of three replicates and two years. Difference in letters indicates significant difference according to LSD's test ($P < 0.05$). phen, phenol; flav, flavonoid; antho, anthocyanin; CAT, catalase; APX, ascorbate peroxidase; GPX, guaiacol peroxidase; SOD, superoxide dismutase; PAL, phenylalanine ammonia-lyase.

Ascorbate peroxidase activity

The response of the APX activity to salinity is best demonstrated by quadratic regression (Figure 2a). APX activity increased up to $EC = 6$ dS m^{-1}, and then reduced to its initial value at $EC = 0$ dS m^{-1}. Indeed, increasing the APX activity produced a more efficient antioxidant system. Although the application of K_2SiO_3 in the form of seed priming had no significant effect on APX activity, an increase in APX activity was observed with the application of K_2SiO_3 in the form of foliar spray, even in combination with seed priming, specifically at $EC < 10$ dS m^{-1} (Figure 2a and Table 2). The increased APX activity may be the result of stimulation to upregulate the expression levels of APX genes in salinity conditions (Sheteiwy et al. 2021).

Guaiacol peroxidase activity

The effect of salinity on the GPX activity was significant. According to the regression analysis, the relationship between GPX activity and salinity as affected by different treatments could be best described using quadratic regressions (Figure 2b). GPX activity for the control increased up to $EC = 8$ dS m^{-1}, followed by a slight decrease to reaching 11.47 mM $gFW^{-1}\,min^{-1}$ at $EC = 14$ dS m^{-1}. The positive effect of seed priming and foliar spray on GPX activity was significant at all salinity levels. Foliar spray was more efficient than priming at all levels of salinity. Moreover, the combination of seed priming and foliar spray had a synergistic effect at $EC > 7$ dS m^{-1} (Figure 2b and Table 2). Both forms of K_2SiO_3 had a positive effect on GPX activity at all salinity levels (Figure 2b and Table 2). Increasing GPX activity along with glutathione reductase (GR) will help maintain a balanced state of reduced glutathione/oxidized glutathione (GSH/GSSG). On the other hand, improving the ascorbic acid–glutathione cycle and the related activity of enzymes through K_2SiO_3 significantly increased the activity of antioxidant enzymes, such as APX and GPX (Pinedo-Guerrero et al. 2020).

Superoxide dismutase activity

The effects of salinity on SOD activity were statistically significant (Figure 2c and Table 2). The response of the SOD activity to salinity was best demonstrated by quadratic regression. SOD activity increased linearly (51%) up to $EC = 10$ dS m^{-1}, but then remained mostly constant until $EC = 14$ dS m^{-1}. SOD converts superoxide radicles ($O_2^{\cdot-}$) into H_2O_2. Superoxide radicals play a crucial role in signaling NaCl-induced up-regulation of antioxidative enzymes. Kováčik et al. (2009) showed that salinity exposure enhanced superoxide radical accumulation in chamomile plants and that the foliar spray strategy increased SOD activity; superoxide radicles consequently declined. Furthermore, since SOD is involved in the control of H_2O_2 and hydroxyl radicals, which are highly reactive causing major damage to protein membranes, part of its activity may be used to regulate H_2O_2 and reduce hydroxyl radicals (Bhanuprakash and Yogeesha 2016).

Our results show that application of K_2SiO_3 in the form of foliar spray alone as well as in combination with seed priming, enhanced SOD activity equally. SOD activity increased up to 9% using these two treatments (Figure 2c and Table 2). Furthermore, seed priming had no significant effect on SOD activity. In contrast, Soylemezoglu et al. (2009) reported that silicon lowered SOD and CAT activity, but increased APX in *Vitis vinifera* L. grown in saline conditions. However, the effects of silicon on antioxidant enzyme activities have been reported previously. Under salt stress, silicon significantly increased the activity of antioxidant enzymes such as SOD, GPX, CAT, and APX, suggesting that silicon may be involved in metabolic or physiological activities under salinity stress (e.g., Manivannan et al. 2016).

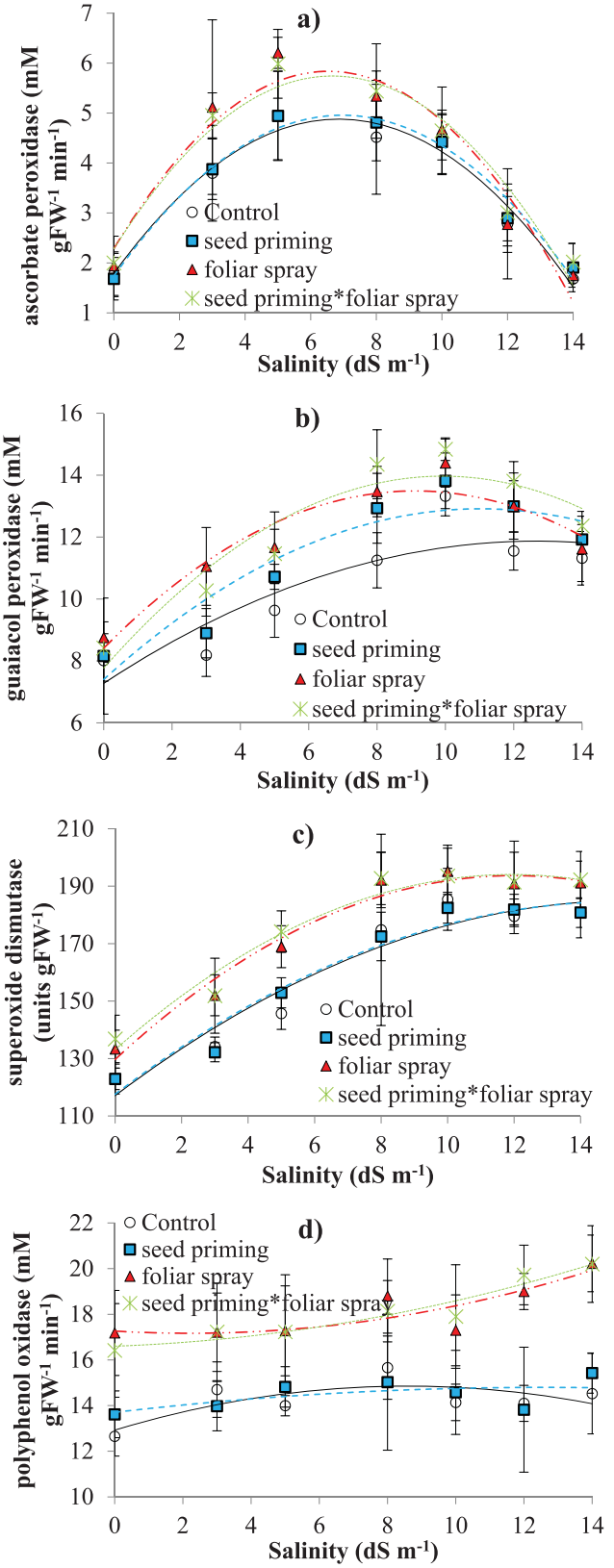

Figure 2. Effect of seed priming and foliar spray of potassium silicate on the activity of enzymatic antioxidants at different salinity levels. Bars represent two-year mean values with standard deviations.

Table 3. Influence of alleviating treatments on H_2O_2, MDA, Nit, and NR of wheat.

	Mean of two-year data			
Treatments	H_2O_2	MDA	Nit	NR
control	3.89 ± 1.81a	5.98 ± 2.86a	0.0014 ± 0.001c	10.37 ± 2.59c
Seed priming	3.96 ± 1.79a	4.36 ± 2.20c	0.0018 ± 0.001b	11.39 ± 2.17b
Foliar spray	3.48 ± 1.44b	5.23 ± 2.53b	0.0017 ± 0.001b	12.04 ± 2.31a
Seed priming*foliar spray	3.44 ± 1.51b	4.10 ± 2.01d	0.0020 ± 0.001a	12.03 ± 2.16a

Data represent means ± SD of three replicates and two years. Difference in letters indicates significant difference according to LSD's test ($P < 0.05$). H_2O_2, hydrogen peroxide; MDA, malondialdehyde content; Nit, nitrate content.

Polyphenol oxidase activity

PPO has been shown be involved in phenolics oxidation processes in plants, with a correlation existing between its activity and the phenolics level, while external factors may also affect its activity (Dallagnol et al. 2015). While PPO plays a role in defense responses to biotic stresses, PPO levels are known to vary in response to different forms of abiotic stresses. For instance, our results show that salinity has a significant influence on PPO activity; quadratic regression best described the trend of PPO activity over various salinity levels (Figure 2d). The trends of PPO activity for the control and seed priming were such that its activity at $EC = 14$ dS m^{-1} was on average 14% higher than its initial activity at $EC = 0$ dS m^{-1}, whereas for foliar spray and the combination of seed priming and foliar spray, this increase was on average 21%. The effect of seed priming on increasing PPO activity was not comparable to that of foliar spray nor the combination of foliar spray and seed priming (Figure 2d and Table 2). Enhanced PPO activity under the influence of salinity or K_2SiO_3 may not be a guarantee for increasing salinity tolerance. Taranto et al. (2017) claimed that the genotypes in which PPO activity was suppressed by water stress resulted in more tolerance to stress.

Phenylalanine ammonia-lyase activity

Salinity significantly influenced PAL activity. We found a linear relationship between the increase in PAL activity and salinity levels for both the control and foliar spray (data not shown). In contrast, the response of PAL activity to salinity under seed priming and the combination of seed priming and foliar spray was best described using quadratic regression. As salinity increased, PAL activity for the control increased to a maximum of 0.59 nmol cinamate gFW^{-1} at EC = 14 dS m^{-1} (data not shown). Despite the significant effects of K_2SiO_3 in the form of foliar spray on most traits, it had no effect on PAL activity (Table 2). In contrast, K_2SiO_3 in the form of seed priming alone or along with foliar spray had a significant effect on increasing PAL activity. Overall, these two treatments increased PAL activity by 36% (Table 2). Since phenylalanine is a substrate for PAL (Kováčik et al. 2009), regarding the increase of PAL, it seems that these two treatments both increased salinity-reduced phenylalanine. Conversion of phenylalanine into trans-cinamic acid is a step required to channel the carbon input from primary metabolism to secondary phenylpropanoid metabolism. Although carbon consumption in this way can eventually lead to increased salinity tolerance, it will also reduce yield (Pinedo-Guerrero et al. 2020).

Signaling molecule content (H_2O_2 content)

H_2O_2 was influenced by both salinity and foliar spray, but not by seed priming (Table 3). According to the regression analysis, H_2O_2 response to different salinity levels under various treatments could be described better using quadratic regression (Figure 3a). As salinity levels became more destructive, the H_2O_2 content increased for all treatments. However, foliar spray and the combination of seed priming and foliar spray lowered H_2O_2 on average 11% at all salinity levels, especially at $EC > 5$ dS m^{-1} (Figure 3a and Table 3). High levels of H_2O_2 may

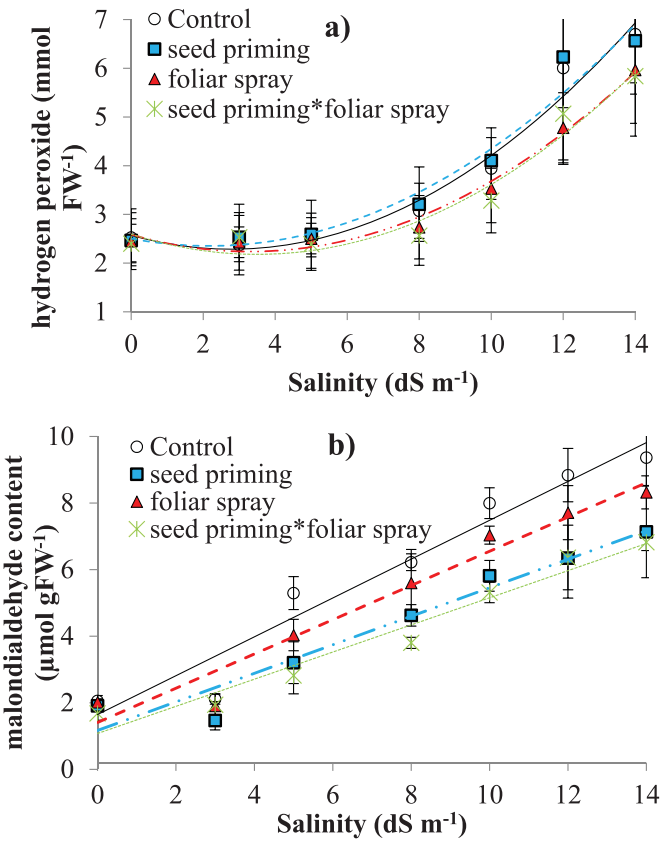

Figure 3. Effect of seed priming and foliar spray of potassium silicate on hydrogen peroxide (a) and malondialdehyde content (b) at different salinity levels. Bars represent two-year mean values with standard deviations.

accelerate processes like Haber–Weiss reaction, resulting in the formation of hydroxyl radicals that could cause lipid peroxidation and increase MDA (Khan et al. 2019). This is reflected by the greater extent of lipid peroxidation at the higher levels of salinity. These results hence suggest that at moderate and high levels of salinity stress, wheat suffers mainly from the toxic effects of H_2O_2 (due to a lack of efficient H_2O_2 detoxification mechanisms) as also reported by Vaidyanathan et al. (2003). The H_2O_2 increase was alleviated by foliar spray (Table 3). Likewise, Li et al. (2015) reported increased H_2O_2 concentrations in salt-stressed tomato seedlings grown in a sand culture; however, silicon application reversed this stress-induced change.

Lipid peroxidation content (MDA content)

Salinity had a significant effect on MDA content, which increased in all treatments over the entire salinity range (Figure 3b). The MDA content at all salinity levels was the highest for the control, with the differences becoming greater at the higher salinity levels. Compared to the control, the alleviating treatments not only reduced the content of MDA, but also moderated the slope of MDA content versus salinity. Furthermore, application of seed priming along with foliar spray showed the greatest effect (31%) on reducing the MDA content. Among the two treatments of seed priming and foliar spray, seed priming was more effective in decreasing MDA. The MDA contents obtained with seed priming and foliar spray were 73 and 87% of the control, respectively (Figure 3b and Table 3). Abdel Latef and Tran (2016) also found that seed priming with silicon

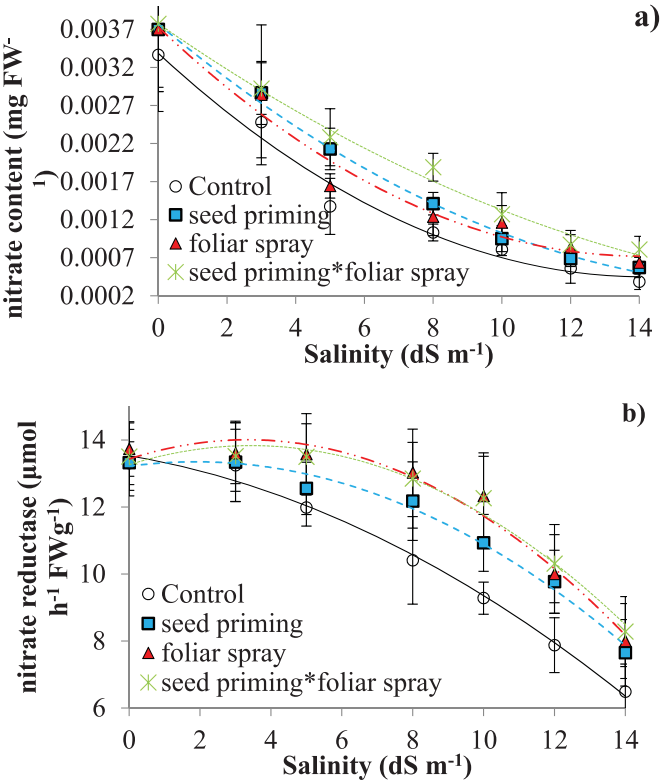

Figure 4. Changes in nitrate content (a) and nitrate reductase (b) activities affected by seed priming and foliar spray of potassium silicate at different salinity levels. Bars represent two-year mean values with standard deviations.

improved the growth of plants under stress, which was accompanied by elevated activities of SOD, CAT, and POD enzymes and lower contents of MDA in stressed plants, relative to plants subjected to only alkaline stress.

Nitrate content

The response curve of nitrate was fitted by quadratic regression (Figure 4a). The nitrate content of the control was at its maximum (0.003 mg FW^{-1}) at $EC = 0$ dS m^{-1}, and decreased with increasing salinity to 0.0004 mg FW^{-1}. However, seed priming and foliar spray positively influenced the nitrate content. The average increase in nitrate content at all salinity levels for these two treatments was 25% (Table 3). Furthermore, seed priming along with foliar spray had greatest effect on enhancing the nitrate content (43%).

Nitrate reductase activity

Increasing the salinity caused the NR activity to drop. The reduction in NR activity of leaves may be due to an imbalance of salts in cells, enzyme degradation/inactivation, and a reduction in gene expression and NR protein synthesis (Souza et al. 2016). There is well-documented information available concerning lower nitrate reductase activity depending on nitrate content. As Botella et al. (1993) stated, salinity significantly decreased NR activity, with the effects depending on nitrate content, alteration in nitrate uptake, and the NO_3^-/Cl^- ratio. In addition to NR, SOD and POD activities could be reduced by nitrogen deficiency (Pessarakli, Haghighi, and Sheibanirad

2015). Our results showed that the response of NR activity to salinity was best described using quadratic regression (Figure 4b). The NR activity changes for the control were affected even by the low salinity and decreased. The changes in NR activity against salinity were affected by seed priming and foliar spray. The changes of NR activity for the alleviating treatments were milder up to $EC < 5$ dS m^{-1}, after which they were declined by an average of 40% up to $EC = 14$ dS m^{-1}. The average NR activity of the Control was 10.4 μmol h^{-1} gFW^{-1}, being 91% of that of seed priming (Figure 4b and Table 3). The highest NR activity was obtained for foliar spray and the combined seed priming and foliar spray treatment. Overall, the rate of decrease, and the value and starting point of the reductions, were all affected by the alleviating treatments (Figure 4b and Table 3). Angrish, Kumar, and Datta (2001) reported that the positive effects on salinity stress were due to the enhanced N status and NR activity by seed priming of wheat seeds. Our results similarly showed the positive effect of seed priming and foliar spray on NR activity and nitrate content, which may ultimately help to improve conditions for maintaining wheat yield in saline environments.

Overall, changes in the activity of antioxidant enzymes and the content of antioxidants against salinity indicated a significant effect of salinity and increased oxidative stress. One may conclude that ROS production is enhanced in plants in response to salinity stress. Plants with high concentrations of antioxidant enzymes tolerate much better the oxidative damage caused by salinity stress (Pakar et al. 2016). The result of antioxidants changes was reflected in traits such as nitrate content, NR activity, and MDA content. Increases in scavenging of H_2O_2 occurred as a result of increased antioxidant enzymes activities (CAT, APX, GPX, and SOD). Indeed, the free radicals are inhibited by processes between these enzymes (as mentioned in the introduction). Phenolic compounds such as phenol and anthocyanin were enhanced by increasing the activity of the PAL enzyme and were converted to o-quinones by the PPO enzyme. These enzymes, along with the content of phenolic compounds, helped to tolerate salinity. All antioxidants triggered by seed priming and foliar spray were effective in controlling ROS, which is very important for increasing tolerance to salinity stress. As shown by our findings, silicon can reduce the oxidative damage by increasing antioxidant enzymes activities, maintaining optimal membrane fluidity, plasma membrane H^+-ATPase, and reducing the generation of ROS in many plant species under salinity conditions (Kim et al. 2014; Li et al. 2015; Muneer and Jeong 2015). Furthermore, Alzahrani et al. (2018) concluded that enzyme activity (e.g., of SOD, CAT, and POD) improved by applying silicon, which may have been related to elevated antioxidants and osmoprotectants (e.g., proline, soluble sugars, ascorbic acid, and glutathione) contents, probably by providing antioxidant defenses against abiotic stress in wheat. Maghsoudi et al. (2019) also suggested the active role of silicon in plant physiological activities, including of the oxidative defense system and the water status.

Conclusions

Our results show that seed priming and foliar spray with K_2SiO_3 are effective approaches to reduce reactive oxygen species (ROS) manifestation in wheat grown under saline conditions, particularly at relatively low salinity levels. Indeed, under different salinity levels, seed priming and foliar application showed varying responses to ROS scavenging by activation of the endogenous defense systems of plants. The improved antioxidant defense abilities by seed priming and foliar spray, which were linked to elevated non-enzymatic antioxidants (e.g., phenol and anthocyanin), and enzymatic antioxidants, alleviated the oxidative damage of proteins and lipids and increased nitrate content and nitrate reductase activities, mostly due to the more pronounced effect of combined application of both alleviators.

Disclosure statement

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Faride Feghhenabi, Hashem Hadi, and Habib Khodaverdiloo initiated, designed, and conducted the research. Faride Feghhenabi also prepared the manuscript. Hashem Hadi, Habib Khodaverdiloo, Martinus van Genuchten, and Mohammad Pessarakli contributed to manuscript preparation and its improvement.

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