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Original article

The Effects of Silicon Dioxide Priming on Some Germination and Vegetative Growth Parameters of Rocket Cultivars Under Salt Stress Conditions

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Abstract

Rocket (*Eruca vesicaria*), a plant from the cruciferous (*Brassicaceae*) family, whose leaves are eaten as a salad, is also considered a medicinal plant due to its different therapeutic properties. In this study, the effects of silicon dioxide priming (0.5, 1, 1.5 mM SiO2) on the germination and vegetative growth properties of two different rocket cultivars (Geniş Yaprak, Eda) were investigated under increased salt stress conditions (150, 200 mM NaCl). For this purpose, germination percentage (GP), germination index (GI), germination rate coefficient (CVG), mean germination time (MGT) germination rate index (GRI) were calculated as germination parameters. Shoot and root length (mm), leaf width and length (mm), plant fresh weight (g) were measured in seedlings developed from germinated seeds, and seedling vigour index (SVI) was calculated. The effects of SiO2 priming on germination and vegetative growth differed according to the cultivar. In Geniş Yaprak cv., 1 mM SiO2 application had a positive effect on germination parameters both in seeds not applied salt stress and under 150 mM salt stress, and increased the germination rate from 30% to 73%, especially in salty conditions. Under 200 mM salt stress, 1.5 mM SiO2 had a positive effect on germination parameters in same cultivar. Although SiO2 applications in Eda cv. were not effective on germination parameters under stress-free conditions, they had a positive effect on vegetative parameters. Especially, 1.5 mM application dose increased SVI, root and shoot length and plant fresh weight. In both salt concentrations, 1 mM application dose made a slight improvement in germination parameters of Eda cv. The positive effects of SiO2 applications on vegetative growth parameters such as leaves, roots and shoots were determined at different doses for both salt concentrations.

Keywords: Germination, Priming, Rocket, Salt Stress, Silicon Dioxide.

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INTRODUCTION

Eruca vesicaria is a leafy vegetable, also called rocket salad or aragula, belongs to the *Brassicaceae* (Cruciferae) family, which includes very important vegetables. It is widely used as culinary vegetable in salads and pizza due to its spicy sharp taste and being a health-beneficial herb (Essoh et al., 2020; Verkerk et al., 2010; Morales & Janick, 2002). Because of a great source of antioxidants due to the glucosinolates and various sulfur components it contains, its consumption increases as a protective vegetable against cancer day by day. In addition to having the highest ascorbic acid content among the leafy plants belonging to the *Brassicaceae* family, it also contains high carotenoids and minerals. Its rich nutritional content also provides protection against ulcer and liver diseases besides cancer (Kusvuran & Ellialtioglu, 2021; Hassan et al., 2017; Amiripour et al., 2016). This plant, which is easily grown in closed and controlled conditions, is a very suitable plant for vertical farming systems that will become more widespread in the future (Pensini et al., 2020).

Soil salinization in agricultural areas is a major problem on a global scale. Today, more than 20% of the irrigated areas and 40% of the agricultural products produced worldwide are affected by salinity. It is predicted that this situation will lead to a serious restriction in food production in the future, as more and more agricultural lands face the problem of salinity over the years (Smoleń et al., 2020; Kusvuran & Ellialtioglu, 2021). Considering the yield and quality losses caused by salinity stress in plants, it is necessary to develop some alternative applications in order to minimize the damage caused by this stress factor on plants. One of these methods, seed priming, is an effective method in both normal and stress conditions (Rhaman et al., 2020).

Silicon is the second most abundant element in the world after oxygen, which has been widely used in agricultural production in recent years to increase product yield and quality among harmless alternative materials for human health and the environment (Guntzer et al., 2012; Bassiony et al., 2016). Since it is a beneficial element especially for plants grown under stress conditions (Liang et al., 2006), its effect on plants under different stress conditions is being investigated. Silicon alleviated the negative effects of stress by significantly increasing antioxidant enzyme activities and reducing membrane lipid peroxidation under salt stress in barley (Liang et al., 2003) and cucumber (Zhu et al., 2004). Similarly, it has been reported that the use of silicon is effective in alleviating the effects of salt stress in two salt tolerant and salt sensitive rice cultivars by strengthening antioxidant defense mechanisms (Das et al., 2018). In okra, tolerance to salt stress was obtained by increasing the water content, nitrogen metabolism and enhancing antioxidant activity as in other studies (Abbas et al., 2017). It has been reported that silicon application is effective in eliminating the damages caused by calcium deficiency in arugula and increases the yield and quality (da Silva et al., 2021). Silicon dioxide application to arugula under drought-stress conditions made significant changes on vegetative growth (Hussain & Al-Taey, 2020). It has been determined that silicon priming has a reducing effect on the effects of stress under abiotic stress conditions in many cultivated plants. In rocket, there are few studies in which silicon is applied externally to seeds under abiotic stress conditions, including salt. Therefore the effects of silicon priming on the germination of the seeds and vegetative growth parameters of early seedling periods of arugula plant, which has very important effects on human health, were investigated under salt stress conditions.

MATERIALS and METHODS

Plant Material

The study was carried out in Akdeniz University Vocational School of Technical Sciences, Department of Plant and Animal Production. Two cultivars of rocket (Geniş Yaprak, Eda) used in the research were purchased from a commercial company.

Methods

Surface sterilization of the seeds and priming with silicon dioxide

Surface sterilization was applied to the seeds before priming. After the seeds were washed with detergent and rinsed in running tap water, they were kept for 10 minutes in 10% sodium hypochlorite solution with continuous stirring. Sterilization of the seeds was completed by keeping them in 70% alcohol solution for 2 minutes and rinsing with sterile distilled water 3 times for 5 minutes. Priming was done by keeping sterile seeds in solutions containing 0.5, 1 and 1.5 mM SiO_2 in sterile jars for 24 hours at room temperature (24±2ºC) in the dark. No pre-treatment was applied to control seeds after surface sterilization.

Germination and vegetative growth studies

Germination studies were carried out under controlled conditions in a climate room with a photoperiod of 16/8 hours at 24±1ºC.

The effect of SiO_2 on germination under saline conditions was investigated at two different salt concentrations (150, 200 mM NaCl). Control and SiO_2 primed seeds were placed in 90 x 15 mm petri plates with two layers of sterile blotting paper.

Germination experiments were carried out for 14 days in accordance with ISTA rules (ISTA 1985). During this period, the germinated seeds were checked daily and the control seeds were irrigated with distilled water. In the other two groups, drying was prevented by dropping equal amounts of 150 or 200 mM NaCl (2ml). The number of germinated seeds was determined from the first day the seeds were placed on petri plates and the emergence of the radicle from the testa was accepted as germination. Germinated seeds were counted every day and at the end of the 14th day, germination percentage (GP), mean germination time (MGT), germination rate coefficient (CVG), germination rate index (GRI), germination index (GI) were calculated as the germination parameters. Calculations were made for germination parameters using the following formulas.

GP (%) = Number of germinated seeds / Total number of seeds X 100 (Gosh et al., 2014)

MGT: Σ Dn / Σn D = days counted from the beginning of the test, n = number of seeds germinated on day D (Ellis & Roberts, 1981; Sivritepe, 2012).

CVG: N1+N2+….+Nx/100xN1T1+……+NxTx (Kotowski, 1926) T: number of days corresponding to N, N: number of seed germinated each day

GRI (%/day): G1/1+G2/2+……+Gx/x (Esechie, 1994)

G1: Germination percentage x100 at the first day after sowing, G2: Germination percentage x100 at the second day after sowing, GI: (14xn1) + (13xn2) +…..+ (1xn14) n1, n2,…, n14: number of germinated seeds on the first, second and subsequent days until the 14th; 14, 13…., and 1 are weights given to the number of germinted seeds on the first, second and subsequent days respectively (modified from Benech et al., 1991).

At the end of the 14th day as the vegetative growth parameters; shoot length (mm), root length (mm), leaf width (mm), leaf length (mm) were measured with a digital caliper, plant fresh weight (g) was weighed, and seed vigor index was calculated according to the formula below.

SVI = [Seedling length (cm) x GP (%)] (Baki & Anderson, 1973)

Statistical Analysis

In the experiments established with 4 replications, 25 seeds were used for each replication. Statistical analysis of the data was determined by analysis of variance using the MINITAB 17 package program and differences were determined and analyzed with the Tukey test.

RESULTS and DISCUSSION

The Effects of Exogenous Silicon Application on Germination Parameters of Rocket cv.

Early developmental stages such as seed germination and seedling formation in plants are very sensitive to salinity as well as to all other abiotic stress factors. Seed priming is a physiological method that increases germination rates by activating metabolic activities in the seed before germination, and by providing better water uptake of seeds, both under non-stress and stress conditions. Priming, which increases the durability of the seedlings after germination, can be applied before planting due to its low cost and effectiveness (Rhaman et al., 2020; Vishal & Kumar, 2018). Various priming applications have been made on different plants by many researchers, so in this study, SiO_2 priming was performed on arugula under salt conditions as an easy and effective method. The silicone priming has an encouraging effect on germination parameters, especially germination rate, under both normal and salt stress conditions, depending on the cultivar.

Priming with SiO_2 had a positive effect on germination parameters of Geniş Yaprak cv. both under non-stress and salt stress conditions. 1 mM SiO_2 application improved GP, GI, CVG and GRI values in both conditions, and the germination percentage, which was 30% especially at 150 mM salt concentration, increased it approximately 2.5 fold to 73% Although this application also shortened the mean germination time, the fastest germination was obtained with 0.5 mM SiO_2 priming in 150 mm salt stress conditions. At higher salt concentration (200mM), 1.5 mM SiO_2 pre-treatment increased the germination rate by 2 fold (Figure1).

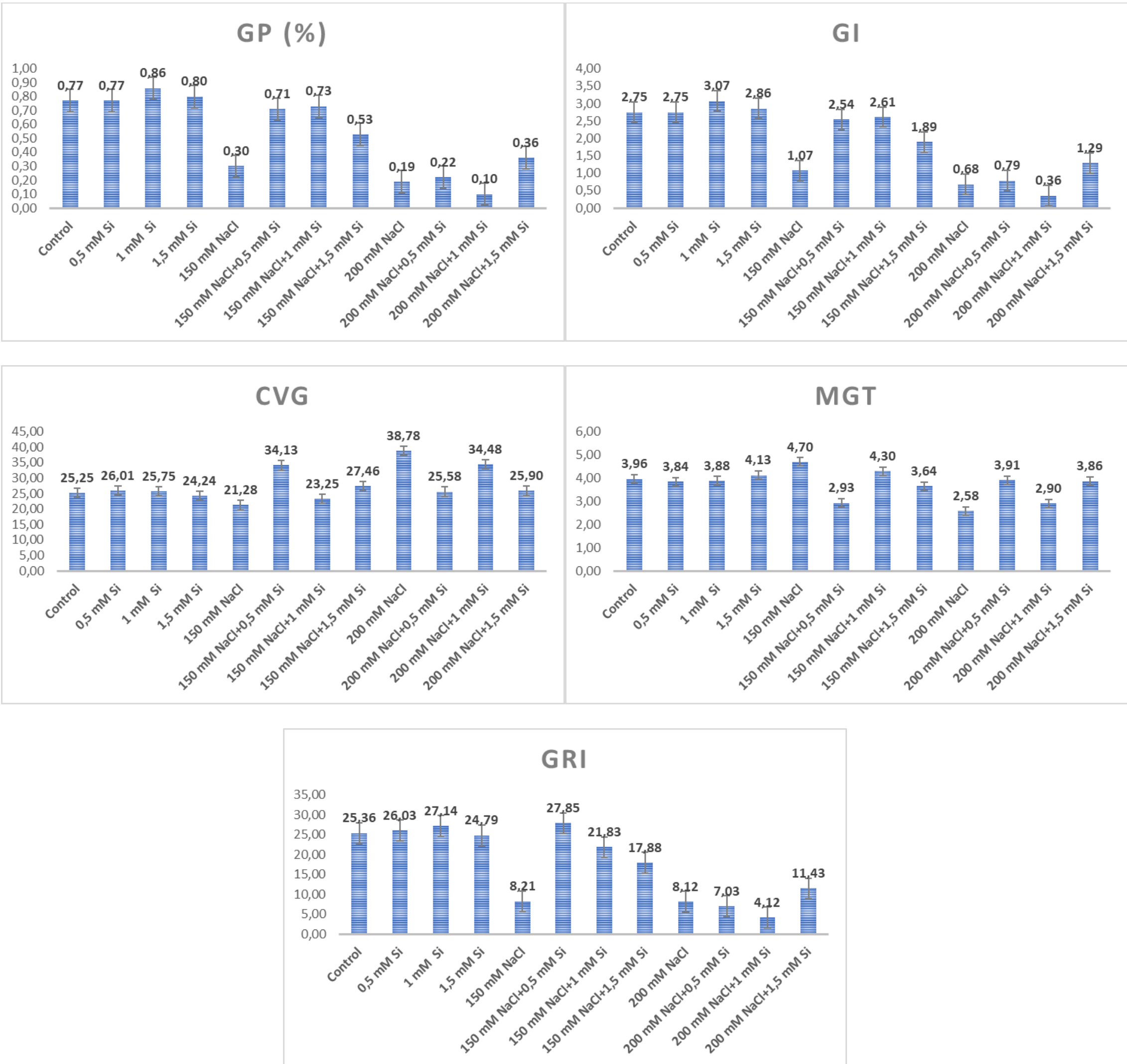

Figure 1. The Effects of Exogenous SiO_2 Priming on Germination Parameters of Geniş Yaprak cv.

SiO_2 priming was not as effective as Geniş Yaprak in both normal and salt stress conditions in Eda cv. Although the application of 1 mM SiO_2 at both salt concentrations increased the germination percentage, this increase was not as high as in Geniş Yaprak (Figure 2) .

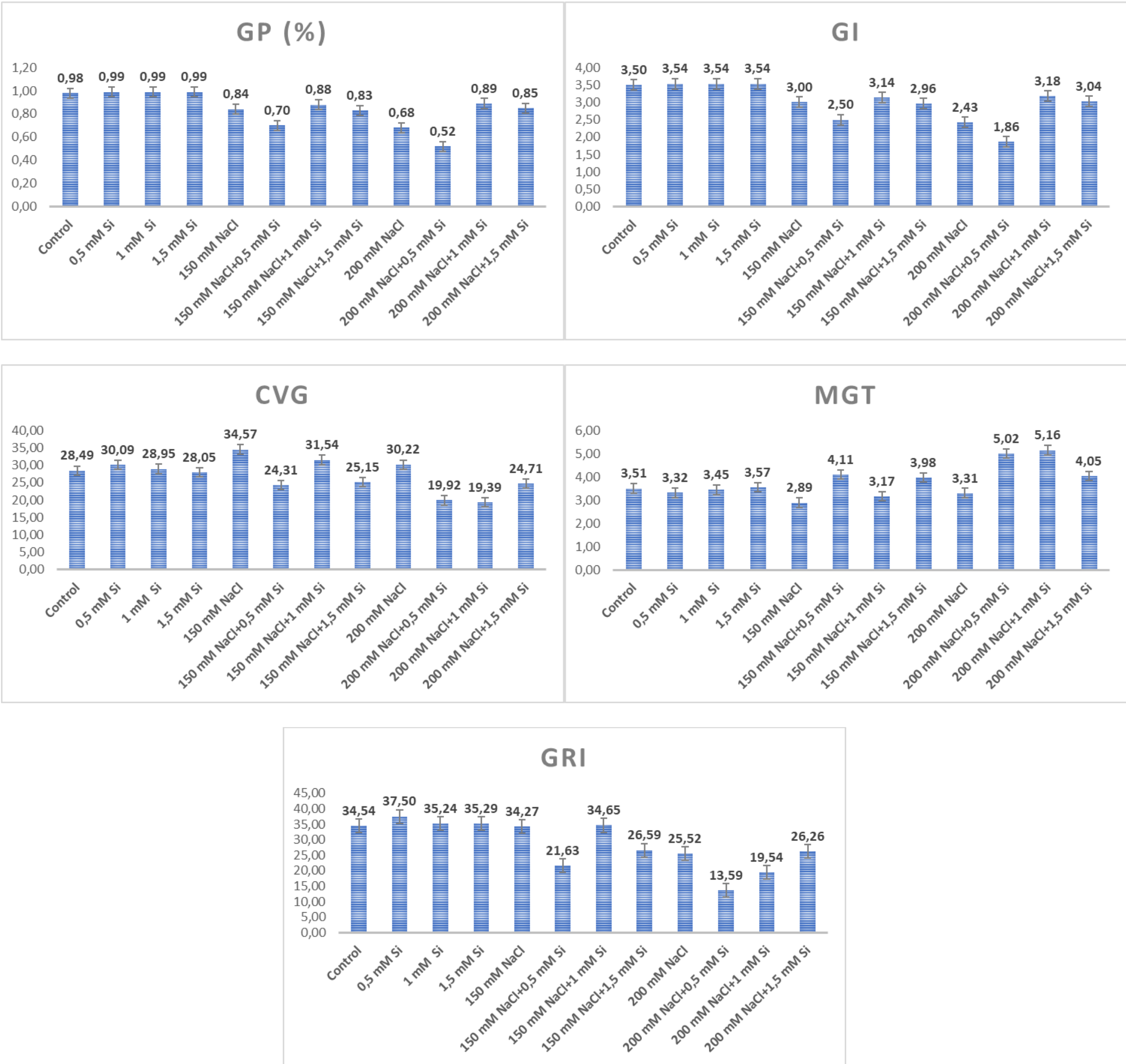

Figure 2. The Effects of Exogenous SiO_2 Application on Germination Parameters of Eda cv.

The Effects of Exogenous SiO_2 Application on Vegetative Growth Parameters of Rocket cv.

The negative effects of salt stress on plant growth and development are known. However, it can be a positive stimulus for an increase in the polyphenol content of some plants. Polyphenols are important in that they are associated with the antioxidant capacity of plants, which makes them more protective against many disease (Wong et al., 2006). Salt stress provides an increase in the content of polyphenols related to the antioxidant capacity of the plant in some members of cruciferous vegetables (Ksouri et al., 2007). There was an increase in the amount of total phenolics in lettuce grown in salty conditions (Kim et al., 2008). Higher antioxidant content was determined in rocket grown in a region with characteristic agricultural factors such as water salinity and high solar radiation in arid conditions, compared to commercial varieties. (Hamilton, 2010). For this reason, increasing the tolerance of plants to saline conditions is important both for the sustainability of agricultural production under increasing abiotic stress conditions and for enriching the nutritional content such as total phenolic ascorbic acid and antioxidant content of many leafy plants such as arugula (Hamilton & Fonseca, 2010). SiO_2 priming had a stimulating effect on germination and vegetative growth parameters in both normal and saline conditions in two rocket cultivars used in this study. This effect differed depending on the salt and the silicon dioxide concentration. Similarly, SiO_2 applications in carrots under salt stress conditions provided tolerance by reducing the effects of salt stress depending on the cultivar and application dose (Nasircilar et al., 2021).

The results of variance analysis showing the effects of cultivars, applications and their combinations on vegetative growth parameters were given in Table 1. It was determined that cultivars had no effect on root length and leaf width. On the other hand, it was determined that cultivar had a statistically significant effect on other parameters, and the interaction of application and cultivar X application had a statistically significant effect on all parameters (Table 1).

Table 1. Descriptives and variance analysis result with respect to the vegetative growth of rocket cv.

Variation source	df	SL (mm)	RL (mm)	LW (mm)	LL (mm)	PFW (g)	SVI
C	1	***	ns	ns	**	**	***
A	11	***	***	***	***	***	***
C*A	11	***	***	***	***	***	***
Mean		21.0	29.7	4.21	3.80	0.0202	37.5
Minimum		0.00	0.00	0.00	0.00	0.00	0.00
Maximum		0.00	99.1	7.19	6.08	0.0438	105
Standart deviation		7.56	19.9	1.67	1.21	0.00882	25.0

** p<0.005, ***p<0.001, ns: not significant SL: Shoot Length, RL: Root Length, LW: Leaf Width, LL: Leaf length, PFW: Plant Fresh Weight, SVI: Seedling Vigour Index

According to the correlation table (Table 2) between vegeatative parameters, there was a strong positive correlation between LL- LW (r=0.724) , PFW- RL (r=0.761), PFW-LL(r=0.710), SVI-RL (r=0.883), SVI-PFW(r=0.757)

Table 2. Correlation matrix of the vegetative growth parameters of the rocket cultivars

Correlation Matrix

	SL (mm)	RL (mm)	LW (mm)	LL (mm)	PFW (g)	SVI
SL (mm)	—					
	—					
RL (mm)	0.540	—				
	< .001	—				
LW (mm)	0.369	0.577	—			
	0.001	< .001	—			
LL (mm)	0.532	0.585	0.724	—		
	< .001	< .001	< .001	—		

Correlation Matrix

		SL (mm)	RL (mm)	LW (mm)	LL (mm)	PFW (g)	SVI
PFW (g)		0.670	0.761	0.698	0.710	—	
		< .001	< .001	< .001	< .001	—	
SVI		0.623	0.883	0.608	0.616	0.757	—
		< .001	< .001	< .001	< .001	< .001	—

SL: Shoot Length, RL: Root Length, LW: Leaf Width, LL: Leaf length, PFW: Plant Fresh Weight, SVI: Seedling Vigour Index

Exogenous SiO_2 application under salinity conditions in corn and soybean reduced the destructive effect of salt on germination and seedling growth. The curative effect of SiO_2 in saline conditions was through breaking seed dormancy and internal phytohormonal regulation (Sun et al., 2021; Lee et al., 2010). SiO_2 application in melon under saline conditions also alleviated the harmful effects of salinity by making an encouraging effect on vegetative growth parameters (Gomes et al., 2018). Silicon supplementation in calcium deficiency increased ascorbic acid, total phenol, carotenoid and photosystem II yield, and fresh and dry matter production in arugula plant. A similar effect was also detected in the presence of sufficient amount of calcium. This effect is thought to be by improving antioxidant enzyme systems, especially in calcium deficiency (da Silva et al., 2021). In addition, under drought stress conditions, silicon increases the resistance to stress conditions by increasing photosynthesis efficiency by causing an increase in leaf number, leaf area and chlorophyll content of rocket (Hussain & Al-Taey, 2020). Similar to drought stress, in this study, silicon application under salt stress conditions increased the leaf area by increasing the width and length of the leaf that is an organ in which photosynthesis takes place.

Under normal conditions, 0.5 mM SiO_2 is the most effective dose on the vegetative growth parameters in Geniş Yaprak cv. Priming with 0.5 SiO_2 had an inducing effect on all parameters and significantly increased root length and SVI value compared to control plants. The root length, which was 27.59 mm in the control plants, increased to 95.94 mm with this application, while the seed vigor index value from 42.99 increased to 100.79. 1.5 mM SiO_2 application increased shoot, root length and SVI value, while 1 mM SiO_2 application had an encouraging effect on leaf length, leaf width and plant fresh weight at 150 mM NaCl concentration. The effect of silicon dioxide was less effective at 200 mM salt concentration, and only shoot length and width increased with 1 mM priming application. However, seeds primed by 1.5 mM SiO_2 did not survive at 200 mM salt concentration (Table 3). Concentrates, in which the elements that increase the tolerance of plants to stress conditions, including silicon, are effective in plants, have an effect in a very narrow range. Taking more than the desired level of these elements, which have very important contributions to the plant at low levels, can have a lethal effect for the plant (Kaur et al., 2016). In this study, 1.5 mM $Si0_2$ priming performed at 200 mM salt concentration in Geniş Yaprak cv. had a similar lethal effect, and seedling growth did not occur from seeds germinating at this dose. For this reason, it is very important to determine the appropriate dose for different plants in priming studies carried out under stress conditions.

Table 3. The effect of SiO_2 applications on vegetative growth parameters of Geniş Yaprak cv. under normal and salt stress conditions.

Application	SL (mm)	RL (mm)	LW (mm)	LL (mm)	PFW (g)	SVI
Control	28.23 ^{ab}	27.59 ^{c}	5.36 ^{ab}	4.65 ^{ab}	0.0222 ^{bcd}	42.99 ^{c}
0.5 mM SiO_2	34.95 ^{a}	95.94 ^{a}	6.35 ^{a}	5.64 ^{a}	0.0420 ^{a}	100.79 ^{a}
1 mM SiO_2	12.83 ^{f}	18.87 ^{c}	4.35 ^{abc}	3.90 ^{bc}	0.0120 ^{e}	27.27 ^{d}
1.5 mM SiO_2	25.86 ^{bcd}	60.36 ^{b}	4.83 ^{abc}	4.67 ^{ab}	0.0269 ^{b}	68.98 ^{b}
150 mM NaCl	17.65 ^{def}	23.99 ^{c}	3.40 ^{bc}	2.81 ^{c}	0.0152 ^{de}	12.49 ^{ef}
150 mM NaCl+0.5 mM SiO_2	24.35 ^{bcde}	17.02 ^{c}	3.39 ^{bc}	2.52 ^{c}	0.0180 ^{bcde}	29.37 ^{d}
150 mM NaCl+1 mM SiO_2	15.03 ^{f}	18.68 ^{c}	5.51 ^{ab}	4.66 ^{ab}	0.0247 ^{bc}	24.61 ^{de}
150 mM NaCl+1,5 mM SiO_2	26.71 ^{abc}	23.23 ^{c}	2.97 ^{c}	3.24 ^{bc}	0.0200 ^{bcde}	26.47 ^{d}
200 mM NaCl	16.47 ^{ef}	22.13 ^{c}	3.70 ^{bc}	3.94 ^{bc}	0.0167 ^{cde}	7.33 ^{fg}
200 mM NaCl+0.5 mM SiO_2	12.33 ^{f}	16.33 ^{cd}	3.40 ^{bc}	3.18 ^{bc}	0.0120 ^{e}	6.30 ^{fg}
200 mM NaCl+1 mM SiO_2	18.97 ^{cdef}	19.08 ^{c}	5.22 ^{abc}	3.92 ^{bc}	0.0211 ^{bcde}	3.81 ^{fg}
200 mM NaCl+1.5 mM SiO_2	0.00	0.00	0.00	0.00	0.0000	0.00

SL: Shoot Length, RL: Root Length, LW: Leaf Width, LL: Leaf length, PFW: Plant Fresh Weight, SVI: Seedling Vigour Index

In Eda cv., 1.5 mM SiO_2 had an encouraging effect on all vegetative parameters except leaf length and width under normal conditions. At 150 mM salt concentration, 1.5 and 1 mM pre-application caused an increase shoot length and leaf width, leaf length and plant fresh weight values respectively. In this cultivar, 0.5 mM SiO_2 was very effective at 200 mM salt concentration and increased all parameters except leaf width. It is noteworthy that it has doubled the root length (Table 4).

Table 4. The effect of SiO_2 applications on vegetative growth parameters of Eda cv. under normal and salt stress conditions

Application	SL (mm)	RL (mm)	LW (mm)	LL (mm)	PFW (g)	SVI
Control	21.30 ^{bcde}	39.75 ^{abc}	6.60 ^{a}	4.40 ^{abc}	0.0245 ^{bc}	59.83 ^{bc}
0.5 mM SiO_2	14.84 ^{e}	49.05 ^{ab}	5.98 ^{ab}	3.86 ^{abc}	0.0212 ^{bc}	63.26 ^{ab}
1 mM SiO_2	18.99 ^{cde}	35.89 ^{abcd}	6.65 ^{a}	5.22 ^{a}	0.0269 ^{ab}	54.33 ^{bcd}
1.5 mM SiO_2	30.36 ^{a}	53.74 ^{a}	6.34 ^{ab}	5.04 ^{ab}	0.0378 ^{a}	83.26 ^{a}
150 mM NaCl	17.84 ^{de}	29.61 ^{bcd}	3.41 ^{c}	3.75 ^{abc}	0.0120 ^{c}	39.86 ^{cde}
150 mM NaCl+0.5 mM SiO_2	21.10 ^{bcde}	28.86 ^{bcd}	3.00 ^{c}	3.58 ^{abc}	0.0181 ^{bc}	34.97 ^{de}
150 mM NaCl+1 mM SiO_2	23.77 ^{abcd}	18.21 ^{cd}	3.50 ^{c}	4.78 ^{abc}	0.0217 ^{bc}	36.95 ^{de}
150 mM NaCl+1,5 mM SiO_2	28.66 ^{ab}	17.86 ^{cd}	4.29 ^{bc}	3.31 ^{c}	0.0190 ^{bc}	38.61 ^{de}
200 mM NaCl	23.45 ^{abcd}	16.53 ^{d}	3.22 ^{c}	3.22 ^{c}	0.0177 ^{bc}	27.19 ^{e}
200 mM NaCl+0.5 mM SiO_2	26.65 ^{abc}	33.47 ^{abcd}	2.86 ^{c}	3.34 ^{bc}	0.0212 ^{bc}	31.26 ^{e}
200 mM NaCl+1 mM SiO_2	20.41 ^{bcde}	25.24 ^{cd}	3.33 ^{c}	4.02 ^{abc}	0.0168 ^{bc}	40.63 ^{cde}
200 mM NaCl+1.5 mM SiO_2	23.48 ^{abcd}	21.88 ^{cd}	3.38 ^{c}	3.49 ^{bc}	0.0166 ^{bc}	38.56 ^{de}

SL: Shoot Length, RL: Root Length, LW: Leaf Width, LL: Leaf length, PFW: Plant Fresh Weight, SVI: Seedling Vigour Index

Conclusion

In recent years, when the effects of abiotic stress conditions have been felt strongly, besides breeding studies and biotechnological methods, some exogenous applications are being investigated more and more in order for plants to tolerate abiotic stress conditions.

Priming the seeds with different organic and inorganic components before sowing is applied in different plants as an easy, effective and inexpensive method. Silicon priming promotes germination and vegetative growth parameters in arugula, which is a very beneficial product for human health due to its components, both under normal and salt stress conditions. It can also be used as an alternative component for sustainable agriculture as an environmentally friendly.

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