

Research Article

Effect of silicon on antioxidant responses of two grape genotypes (*Vitis vinifera* L.) under salinity stress conditionsShabnam Heidarpour¹, Nasser Abbaspour^{1*}, Nayer Mohammadkhani², Fatemeh Rahmani¹¹ Department of Biology, Faculty of Science, Urmia University, Urmia, Iran² Shahid Bakeri High Education Center of Miandoab, Urmia University, Urmia, Iran**Abstract**

Silicon (Si) mitigates the impact of salinity stress in plants and enhances salt tolerance in grapevines. This study investigated the Si (3 mM Na₂SiO₃) impacts on reducing salinity stress (50–100 mM NaCl) and its effects on the levels of toxic ions, enzymatic and non-enzymatic antioxidant activity, and modifications in the composition of phenolic compounds in two hydroponically cultured grape (*Vitis vinifera* L.) genotypes: Chawga and AghUzum. The presence of high salt levels resulted in a notable reduction in the height of the plants as well as in the fresh and dry weights of both the leaves and roots. Furthermore, in response to NaCl-induced stress, there was an increase in Cl⁻ and Na⁺ levels, accompanied by a decrease in K⁺ and NO₃⁻ concentrations. Exposure to NaCl stress led to a high accumulation of H₂O₂, MDA, proline, total soluble sugar, and glycine betaine in AghUzum compared to Chawga. Si regulated osmolyte levels, reduced membrane damage, and increased antioxidant activity. The results demonstrated that Si significantly reduced the toxicity caused by salinity; however, its impact is genotype-dependent. In Chawga, Si induced more tolerance to salt stress than AghUzum. Si made salt tolerance in Chawga by maintaining ionic balance, while in AghUzum, it was through the antioxidant enzyme activation. These results can help develop new strategies to protect grapevines from saline stress and explain how silicon makes different grape genotypes less sensitive to salt.

Keywords: Salt stress, *Vitis vinifera*, Physiological changes, Si, Salt tolerance**Introduction**

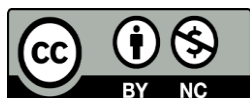
Salinity is one of the significant environmental challenges to plant growth and crop production because of an imbalance of soil solutes (Kashif *et al.*, 2020; Munns *et al.*, 2020). Reports indicate that salt impacts approximately 1125 million hectares of agricultural land globally, accounting for nearly one-third of global productivity (Liu *et al.*, 2020; Li-ping *et al.*, 2015). Salinity causes functional and metabolic changes in plants due to the toxicity of sodium (Na⁺) and chloride (Cl⁻) ions, as well as osmotic stress (Munns and Tester, 2008; Rahnesan *et al.*, 2018; Kumar *et al.*, 2021). The osmotic impact leads to inefficient water uptake, reduced cell expansion, and impaired growth and development of plant leaves. Moreover, the excessive accumulation of Na⁺ and Cl⁻ in plant organs inhibits protein biosynthesis, numerous enzymatic reactions, and photosynthetic processes, resulting in oxidative stress

due to the generation of reactive oxygen species (ROS) (Zhou-Tsang *et al.*, 2021). The overproduction of ROS, such as hydrogen peroxide (H₂O₂), superoxide (O₂⁻), and hydroxyl radicals (OH[•]), can cause oxidative damage to proteins, lipids, nucleic acids, and the plasma membrane of cells, resulting in cell death (Ahanger *et al.*, 2017; De Rossi *et al.*, 2021).

Plants use many defense mechanisms to manage salt stress, such as morphological, physiological, and molecular techniques. These strategies are crucial for enhancing plant tolerance to salinity (Shahid *et al.*, 2020). These systems involve the synthesis of osmolytes, such as soluble sugars, proline, and proteins, which protect plant cells against the adverse effects of salt (Farouk *et al.*, 2020). To respond to the harmful effects of over-production of ROS and lipid peroxidation on cellular membranes, plants increase the efficiency of both enzymatic (such as catalase,

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peroxidase, superoxide dismutase, and ascorbate peroxidase) and non-enzymatic antioxidant systems (Farouk *et al.*, 2020). Non-enzymatic antioxidants such as phenolic and flavonoid compounds scavenge and detoxify ROS, thereby mitigating the damage oxidative stress causes to cellular structures (Jaleel *et al.*, 2009; Ashraf *et al.*, 2019).

The grapevine (*Vitis vinifera* L.), due to its high economic value and beneficial impact on human health, is considered one of the most important fruit crops in the world. It is cultivated under varied agro-ecological conditions, from tropical to temperate; however, some cultivars have cultivation potential in arid and semi-arid areas, where soil salinity is a serious concern due to high evaporation (Golla, 2021). Grapevines are moderately sensitive to salinity, with chloride ions primarily responsible for the damage (Zhou-Tsang *et al.*, 2021). Salinity induces necrosis of adult leaves in grapes, and the subsequent increase in Na⁺ influx and K⁺ leakage results in a higher Na⁺/K⁺ ratio in tissues. This disruption affects grapevine growth, yield, and physiological processes (Zhou-Tsang *et al.*, 2021). In grapevines, salt tolerance is closely associated with the ability of genotypes to regulate the transport of both Cl⁻ and Na⁺ ions, thereby avoiding toxicity. Some genotypes demonstrate a higher efficiency in controlling the transport of Na⁺, Cl⁻, or both, which has led to their classification as salt-tolerant genotypes. This tolerance arises from various mechanisms of maintaining ion balance (Teakle and Tyerman, 2010). However, even salt-tolerant genotypes exhibit a reduction in yield when exposed to electrical conductivity (EC) levels higher than 3.3 dS/m (Zhang *et al.*, 2002).

Various strategies have been employed to solve the salinity problem, i.e., washing and leaching to eliminate excess salt from the plant's rhizosphere (Inoue, 2012), adopting diverse irrigation practices (Belkheiri and Mulas, 2013), and enhancing plant salt tolerance (Shams *et al.*, 2019). However, these methods may be ineffective in mitigating the salinization threats due to their high cost. Therefore, developing new strategies for regulating plants' physiological and metabolic defense mechanisms against the damaging effects of salinity may be essential for grapes cultivated in salt-affected soil. In this regard, beneficial elements such as silicon (Si) have emerged as an eco-friendly approach to improving plants' ability to tolerate salt stress. Proposed Si-mediated mechanisms to alleviate salinity stress in various plant species include Si deposition in the root cortex obstructing the apoplastic movement of Na⁺ and Cl⁻ ions, converting them into stable complexes, and thereby reducing their transport from roots to shoots (Zhao *et al.*, 2013). Si also protects cytoplasmic functions under salt stress by promoting vacuolar sequestration of Na⁺ through increased H⁺-ATPase activity and influencing H⁺-dependent sodium flux (Coskun *et al.*, 2018; Bhardwaj *et al.*, 2023). Additionally, Si supplementation mitigates the oxidative effects of salinity by reducing the excessive

accumulation of ROS, decreasing damage to cell membrane fatty acids, and regulating enzymatic and non-enzymatic antioxidants in plants (Zhu *et al.*, 2019). The effects of Si are indirect and depend on the dosage of Si, plant species, and the intensity and duration of salinity stress (Coskun *et al.*, 2018; Bhardwaj and Kapoor, 2021).

Climate change is the main factor contributing to the salinization of agricultural soil worldwide, so it is critical to develop strategies that increase plant tolerance to salinity. Grapevine (*Vitis vinifera* L.) is susceptible to soil salinization. However, the mechanisms by which Si develops salinity tolerance in grapevines and the influence of Si application on different grape genotypes under salt stress are not fully understood. Therefore, this study aims to investigate the effectiveness of Si on non-enzymatic antioxidants and ion balance in two grape genotypes differing in salt tolerance under salinity stress. The findings will serve as a foundation for developing strategies to mitigate the risks associated with salinity toxicity and maintain sustainable plant production.

Materials and methods

Plant material, treatment combinations, and design:

Based on previous screening results in West Azerbaijan Province (Mohammadkhani *et al.*, 2013), two native grape genotypes, Chawga (salt tolerant) and AghUzum (salt sensitive), were selected. Chawga is known for its ability to maintain high levels of potassium (K⁺), while AghUzum is sensitive to salt stress. This study aimed to evaluate the biochemical impact of Si on mitigating salt stress-induced damage in grapevine plants. Hardwood cuttings were obtained from the Agricultural Research Center (Kahriz, Urmia, Iran). The cuttings were disinfected with 1.5% (w/v) benomyl for 15 min and soaked in 0.1% (w/v) IBA (indole-3-butyric acid) from the basal parts for 5–10 s. Subsequently, all cuttings were placed in a mist chamber set at a relative humidity of 80% with a heat-bed temperature ranging from 25 to 30°C. After one month, the rooted cuttings were transferred into the hydroponic culture in 3L pots containing aerated modified Hoagland's solution (pH of 6.30-6.35) [containing 1M KNO₃, 2M Ca(NO₃)₂, 2M MgSO₄·7H₂O, 1M KH₂PO₄, 0.028 g/L Fe-EDTA, 28.5 g/L H₃BO₃, 18.1 g/L MnCl₂·4H₂O, 2.2 g/L ZnSO₄·7H₂O, 0.8 g/L CuSO₄·5H₂O, and 0.5 g/L Na₂MoO₄·2H₂O] (Walker *et al.*, 2004). The nutrient solution was exchanged every four days to ensure consistent nutrition levels. Si treatment (3 mM Na₂SiO₃) and salinity treatments (50 mM and 100 mM NaCl) were applied at the 6-leaf stage and continued for two weeks. Salinity was gradually introduced to prevent osmotic shock. After the two-week treatment period (Abbaspour *et al.*, 2014), the plants were harvested, immediately frozen in liquid nitrogen, and stored at -80°C for subsequent analysis. Plant tissues, including leaves, petioles, stems, and roots, were weighed separately and then dried at 70°C for 48 hours.

Parameters for plant growth: After harvesting, the roots and shoots were separated to assess root length, shoot length, root fresh weight, and shoot fresh weight. The plant material was dried in an oven at a temperature of 70°C for 3 days, and the weight of the dried material was then recorded.

Determination of some mineral contents, Cl, Na, K: Ground samples (0.1 g) of leaves were weighed in 15 mL plastic centrifuge tubes. Then, 10 mL of deionized H₂O was added to each tube, following the method described by Abbaspour *et al.* in 2014. The tubes were then placed in a boiling water bath for approximately 1 hour. Subsequently, the sample tubes were centrifuged at 5,000 rpm. The supernatant was carefully transferred into new tubes, and the quantity was modified to 10 mL using deionized H₂O. The quantification of chloride was conducted using the silver ion titration technique using a chloride meter (Corning 926 Model). The amounts of sodium and potassium were measured using a flame photometer (Fater Electronics 405, Iran).

NO₃: The concentration of nitrate (NO₃⁻) was determined using the salicylic sulfuric acid method, as described by Cataldo *et al.* (1975). At first, 0.5 mL aliquots of the material were mixed with 0.8 mL of 5% (w/v) salicylic acid in concentrated sulfuric acid (H₂SO₄). After being left at room temperature for 20 minutes, 19 mL of a 2N NaOH solution was slowly added to increase the pH level above 12. Subsequently, the samples were allowed to reach room temperature, following which the absorbance at a wavelength of 410 nm was determined.

Si: A quantity of 0.2 g of powdered dry leaf tissue materials was measured and then subjected to a temperature of 650±50°C in an oven for a duration of 2 hours. Afterwards, the samples underwent digestion in a mixture of 3 mL of nitric acid (HNO₃) and 2 mL of hydrogen peroxide (H₂O₂) for 12 hours. After digestion, the leaf samples were moved to 25-mL plastic flasks and mixed with around 15 mL of deionized H₂O. Ultimately, 1 mL of hydrofluoric acid (HF) was added and left overnight. After the samples were diluted to a ratio of 1:50 (v/v), the Si concentration was measured using inductively coupled plasma optical emission spectrometry (ICP-OES; SPECTRO, ARCOS, Germany).

Quantification of total soluble sugar, proline, and glycine-betaine contents: The quantification of total soluble sugars was conducted using the phenol-sulfuric acid method, as described by Dubois *et al.* (1956). The leaves were crushed and mixed with 70% ethanol to create a uniform mixture. The mixture was then centrifuged at 2,000 rpm for 20 minutes. Next, 1 mL of supernatant was mixed with 1 mL of phenol solution (5%) and 5 mL of sulfuric acid (98%). The absorbance of the mixes was measured at a wavelength of 485 nm after 1 hour. The quantity of soluble sugar present was determined by employing a glucose calibration curve ranging from 10 to 40 mg/mL, and the results were

represented as mg g⁻¹ DW.

The Bates method, with a little modification, was utilized to determine the concentration of free proline (Bates *et al.*, 1973). The plant leaves were homogenized in 10 mL of a solution containing 3% sulfosalicylic acid, and the homogenates were centrifuged at 4,000 rpm for 10 minutes. Next, 2 mL of the supernatant was mixed with 2 mL of ninhydrin solution (3% v/v) and 2 mL of glacial acetic acid. The solution was subjected to incubation at a temperature of 100°C for 1 hour, followed by cooling in an ice bath for a period of 15 minutes. Subsequently, 4 mL of toluene was added to the mixture as a reaction reagent, and the absorbance was quantified at a wavelength of 520 nm. The proline concentration was quantified using the standard curve and reported as mg g⁻¹ DW.

The glycine-betaine concentration in leaf samples was quantified using spectrophotometry on dried powder. This was achieved by reacting the samples with KI-I₂ and measuring the absorbance at 520 nm. The experimental procedure followed the method outlined by Grieve and Grattan in 1983.

ROS determination (H₂O₂ contents) and lipid peroxidation (MDA contents): The concentration of hydrogen peroxide (H₂O₂) was determined using the method described by Velikova *et al.* (2000). Leaf tissues (0.2 g) were homogenized in 5 mL of a solution containing 0.1% trichloroacetic acid (TCA) and then subjected to centrifugation at a speed of 8,000 rpm for 15 minutes. The absorbance of the reaction solution, including 0.5 mL of phosphate buffer with a concentration of 10 mM, 1 mL of potassium iodide (KI) solution with a concentration of 1 M, and 0.5 mL of supernatant, was determined at a wavelength of 390 nm.

The determination of malondialdehyde (MDA) content was conducted using the method described by Heath and Packer (1968). Leaf samples (0.5 g) were ground in 5 mL of trichloroacetic acid (0.1%) and thereafter subjected to centrifugation at 10,000 rpm for 10 minutes. Afterward, 1 mL of supernatant was mixed with 4 mL of trichloroacetic acid (20%) and 2-thiobarbituric acid (0.5%). The mixture was then heated at a temperature of 100°C for 1 hour. Following another round of centrifugation at 10,000 rpm for 10 minutes, the absorbance values of the samples were measured three times at wavelengths of 532, 600, and 450 nm. The MDA content was determined using the following equation:

$$\text{MDA } (\mu\text{mol g}^{-1} \text{FW}) = 6.45 (A_{532} - A_{600}) - (0.56 A_{450})$$

Antioxidant enzyme assay: The extraction processes for antioxidant enzymes followed the approach described by Garratt *et al.* (2002).

The assessment of peroxidase (POD) activity was conducted by measuring the oxidation of guaiacol (with an extinction coefficient of 26.6 mmol L⁻¹ cm⁻¹) at a wavelength of 470 nm, as described by Plewa *et al.* (1991). The reaction mixture consists of 2 mL of phosphate buffer with a concentration of 50 mM and a pH of 7.0, 0.5 μL of guaiacol, 200 μL of H₂O₂, and 100

μM of enzyme extract.

The activity of catalase (CAT) was assessed by measuring the reduction in absorbance at 240 nm for 1 minute using an extinction coefficient of $40 \text{ mM}^{-1} \text{ cm}^{-1}$. This reduction in absorbance is a result of the analysis of hydrogen peroxide (H_2O_2) (Cakmak *et al.*, 1993). The reaction mixture included 2.5 mL of phosphate buffer with a concentration of 100 mM and a pH of 7.0, 100 μL of enzyme extract, and 200 μL of H_2O_2 solution.

The enzymatic activity of ascorbate peroxidase (APX) was assessed by measuring the decrease in ascorbate level and the corresponding change in absorbance at 290 nm for 1 minute using an extinction coefficient of $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ (Asada and Chen, 1989). The reaction mixture includes 2 mL of phosphate buffer with a concentration of 50 mM and a pH of 7.0, 150 μL of ascorbic acid, 200 μL of H_2O_2 , and 150 μL of enzyme extract.

The activity of the enzyme superoxide dismutase (SOD) was assessed using the photochemical inhibition of nitroblue tetrazolium (NBT) technique, with certain alterations made to the original protocol by Giannopolitis and Ries in 1977. The reaction mixture includes 4 mL of 50 mM potassium phosphate buffer with a pH of 7.8, 33 μM NBT, 10 mM L-methionine, 66 mM EDTA, 33 μM riboflavin, and 1 mL of extract. The measurement of absorbance was conducted at a wavelength of 560 nm.

Statistical analysis: The data experienced a one-way analysis of variance (ANOVA) and Tukey's multiple comparisons test ($P < 0.05$) using SPSS version 16. The GLM (General Linear Model) analysis was employed to determine differences across genotypes interactions between genotypes, salinity, and Si treatments. The results are displayed as average values, with the standard error (SE) indicated. The graphical display was executed using the GraphPad Prism 8 program.

Results

Parameters for plant growth: The impact of NaCl on grapevine growth was evaluated by quantifying plant height and analyzing the fresh and dry weights of both leaves and roots. The results indicated a significant reduction in plant growth due to salinity stress, as illustrated in Table 1. Plants treated with 50 and 100 mM NaCl significantly reduced their height compared to the control (Table 1). In Chawga, the drops were 14.3% and 32.7%, and in AghUzum, they were 24.7% and 45.58%. At NaCl concentrations of 50 and 100 mM, the shoot fresh weight decreased by 20.7% and 37.9% in Chawga and by 28.9% and 42.1% in AghUzum, respectively. The stress factor also changed the leaves' dry weight. In Chawga and AghUzum, it decreased by 36.2% and 39.1% at 50 mM and by 50.9% and 65.6% at 100 mM. However, the external application of Si to the salt-stressed grape genotypes resulted in a significant increase ($P < 0.05$) in various growth parameters. The fresh weight of the shoots increased by 14.4% and

13.8%, the fresh weight of the roots increased by 15.5% and 12.3%, the dry weight of the shoots increased by 18.1% and 14.6%, and the dry weight of the roots increased by 13.0% and 11.2% in Chawga and AghUzum, respectively. This was compared to saline alone. Also, adding Si to plants that were stressed by 100 mM NaCl made the shoots and roots longer by 23.5% and 17.1%, respectively. It also increased the fresh weight by 15.7% and 10.9%, the root fresh weight by 26.9% and 14.1%, the shoot dry weight by 24.9% and 23.4%, and the root dry weight by 23.5% and 17.1% in Chawga and AghUzum. These changes were made compared to plants that were stressed by 100 mM NaCl stress alone.

Leaf mineral contents: The mineral concentration in grapevine leaves was significantly affected by salinity stress, as shown in Figure 1. The Cl^- concentration significantly increased at NaCl concentrations of 50 mM and 100 mM, showing a 4.53-fold and 6.15-fold increase in Chawga and a 4.81-fold and 8.09-fold increase in AghUzum, respectively, compared to the control. The NO_3^- levels in vine leaves showed a notable reduction ($P < 0.05$) as the level of sodium chloride (NaCl) increased in the nutrition solution (Fig. 1B). When exposed to a concentration of less than 100 mM NaCl, Chawga showed a lower reduction in NO_3^- leaf contents (18.7% of the control), whereas AghUzum had a higher reduction (63.8% of the control) compared to the plants in the control group. In addition, the levels of Na^+ increased as the salinity became more concentrated. In the Chawga genotype, the concentrations reached 5.69 and 9.12 folds higher at medium and high saline concentrations, respectively, compared to the control. In the AghUzum genotype, the concentrations reached 11.23 and 13.68 folds higher at medium and high saline concentrations, respectively, compared to the control. In AghUzum, exposure to salinity stress at concentrations of 50 and 100 mM NaCl resulted in a reduction in K^+ levels to 53.6% and 60.02%, respectively, compared to the control. Nevertheless, the K^+ level exhibited a significant rise in Chawga when exposed to 50 and 100 mM NaCl. The inclusion of Si in the base medium resulted in an elevated Si concentration in the leaves of both genotypes as compared to the control groups. Notably, Chawga exhibited substantially higher levels of Si in leaves compared to AghUzum, as shown in Figure 1E.

The addition of Si resulted in a considerable decrease in the levels of Cl^- and Na^+ in the leaf tissues of both genotypes when exposed to NaCl treatment. The leaf tissues of plants treated with 100 mM NaCl and Si showed a decrease in Cl^- and Na^+ content. Specifically, Chawga had reductions of 19.5% and 25.3% in Cl^- and Na^+ content, while AghUzum experienced reductions of 11% and 11.9% in Cl^- and Na^+ content, respectively. These reductions were compared to plants treated with 100 mM NaCl alone. The decrease was more significant in Chawga compared to AghUzum. Nevertheless, the addition of Si treatment (100 mM NaCl + Si) resulted in

Table 1. The fresh weight (FW), dry weight (DW), and length (cm) of two grapevine genotypes (*Vitis vinifera* L.), Chawga and AghUzum, under different salt stress conditions (0, 50, and 100 mM NaCl), with and without Si treatment

Genotypes and Salinity (mM NaCl)	Sodium silicate (mM)	Fresh Weight (gr)		Dry Weight (gr)		Length (cm)	
		Shoot	Root	Shoot	Root	Shoot	Root
Chawga 0	0	16.395±0.23 ^a	10.292±0.39 ^{ab}	3.188±0.20 ^a	1.135±0.02 ^b	19.33±0.88 ^{ab}	17.77±0.8 ^{ab}
	3	17.375±0.64 ^a	11.265±0.31 ^a	3.237±0.02 ^a	1.333±0.05 ^a	21.03±0.73 ^a	18.33±0.44 ^a
50	0	12.994±0.31 ^c	7.878±0.42 ^c	2.032±0.08 ^c	0.970±0.01 ^c	16.55±0.72 ^b	13.83±0.72 ^{cd}
	3	15.178±0.49 ^b	9.320±0.34 ^b	2.483±0.01 ^b	1.115±0.05 ^b	19.16±1.01 ^{ab}	15.77±0.48 ^{bc}
100	0	10.180±0.35 ^d	5.444±0.33 ^d	1.565±0.09 ^d	0.663±0.03 ^d	12.99±1.45 ^c	12.66±1.20 ^d
	3	12.085±0.40 ^c	7.450±0.47 ^c	2.090±0.07 ^c	0.918±0.03 ^c	16.99±0.88 ^b	14.13±0.51 ^{cd}
AghUzum 0	0	15.164±0.18 ^a	8.870±0.13 ^b	2.677±0.12 ^a	1.086±0.03 ^b	19.04±0.92 ^a	16.16±0.44 ^a
	3	16.088±0.49 ^a	9.758±0.07 ^a	2.867±0.10 ^a	1.224±0.06 ^a	19.83±1.09 ^a	16.50±0.76 ^a
50	0	10.779±0.21 ^c	6.391±0.20 ^d	1.630±0.05 ^c	0.933±0.01 ^c	14.33±0.68 ^{bc}	12.16±0.72 ^{bc}
	3	12.505±0.67 ^b	7.299±0.21 ^c	1.909±0.07 ^b	1.051±0.01 ^b	15.91±1.34 ^b	14.00±0.57 ^b
100	0	8.969±0.69 ^d	4.855±0.10 ^f	0.920±0.02 ^e	0.562±0.02 ^e	10.36±0.75 ^d	10.06±0.72 ^c
	3	10.072±0.29 ^c	5.695±0.35 ^e	1.202±0.01 ^d	0.729±0.02 ^d	12.50±0.28 ^{cd}	11.20±0.43 ^c

Data shows the average ± standard error (SE) (replications = 3). Different letters in each column indicate significant differences (one-way ANOVA, Tukey, P<0.05).

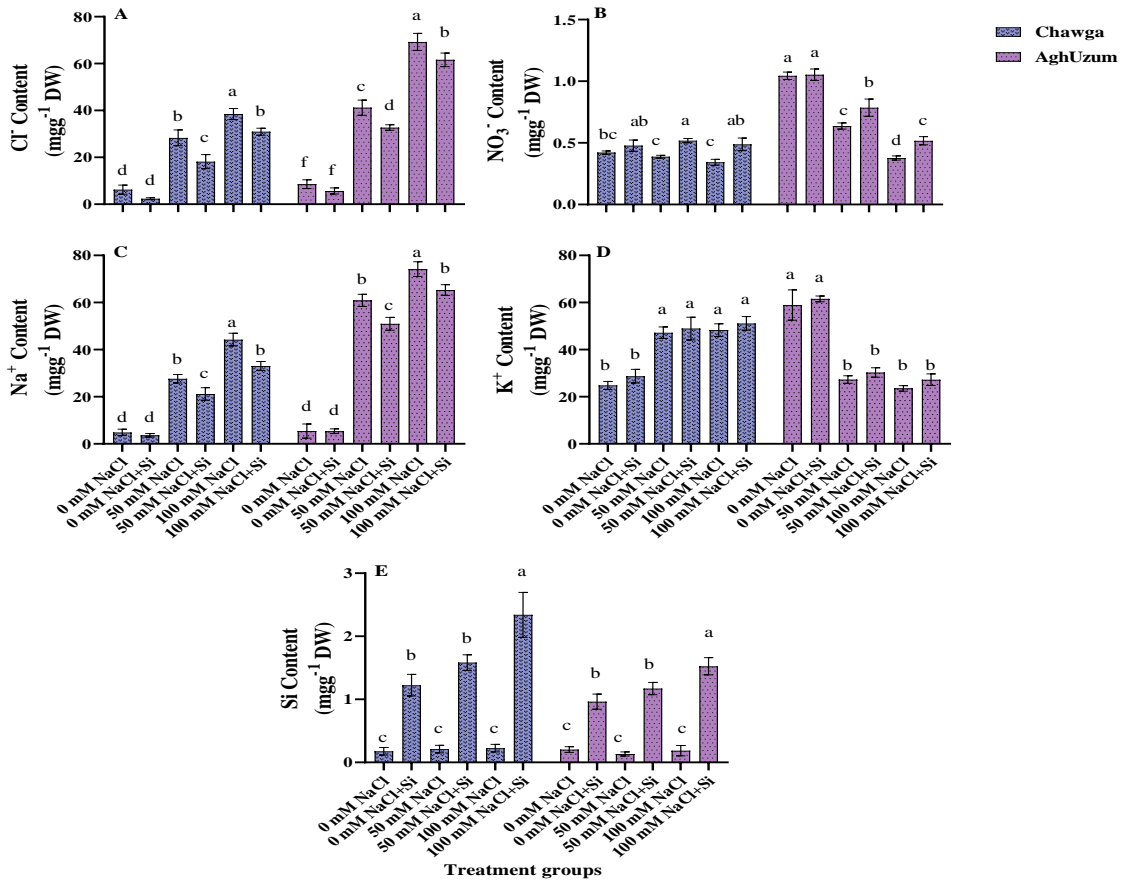


Figure 1. Cl⁻ (A), NO₃⁻ (B), Na⁺ (C), K⁺ (D), and Si (E) contents of leaf tissue in two grapevine (*Vitis vinifera* L.) genotypes, Chawga and AghUzum, in response to NaCl (0, 50, and 100 mM) and Si (0 and 3 mM) treatments. The bars show the average ± standard error (SE) (replications = 3). Different letters in each genotype indicate significant differences (one-way ANOVA, Tukey, P<0.05).

a significant increase in the accumulation of NO₃⁻ in leaves. Specifically, there was a 29.9% increase in the Chawga genotype and a 27.0% increase in AghUzum,

compared to the same salinity level without Si amendment. In salt-stressed grape plants provided with Si, the K⁺ content in the leaf did not show a significant

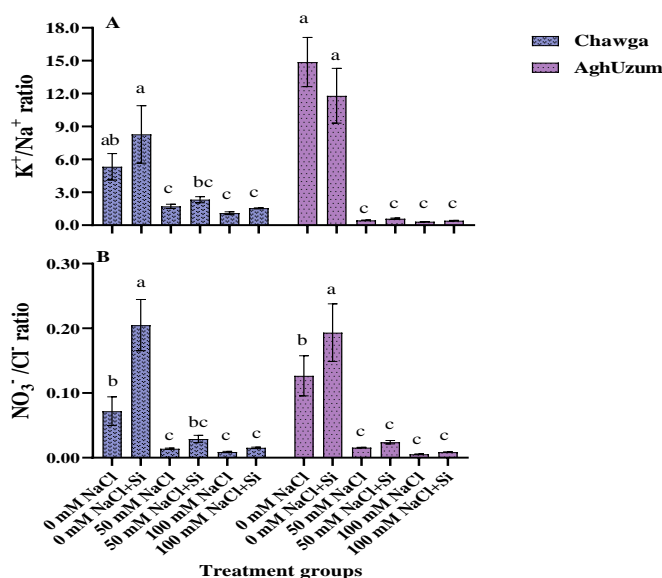


Figure 2. The effect of exogenous Si (Si) supply on Na⁺/K⁺ (A) and NO₃⁻/Cl⁻ (B) ratios of two grapevines (*Vitis vinifera* L.) genotypes, Chawga and AghUzum, grown in a saline medium. The bars show the average ± standard error (SE) (replications = 3). Different letters in each genotype indicate significant differences (one-way ANOVA, Tukey, P<0.05).

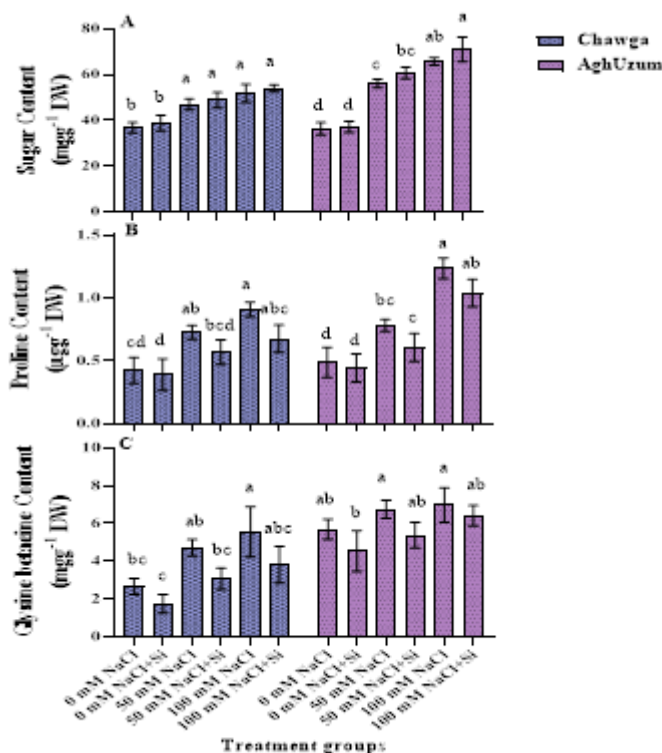


Figure 3. Sugar (A), proline (B), and glycine betaine (C) contents of leaf tissue in two grapevine (*Vitis vinifera* L.) genotypes, Chawga and AghUzum, in response to NaCl (0, 50, and 100 mM) and Si (0 and 3 mM) treatments. The bars show the average ± standard error (SE) (replications = 3). Different letters in each genotype indicate significant differences (one-way ANOVA, Tukey, P<0.05).

change compared to the same salt level without Si amendment (Fig. 1C).

NO₃⁻/Cl⁻ and K⁺/Na⁺ ratios: The data shown in Figure 2 demonstrates the changes in the ratios of

K⁺/Na⁺ and NO₃⁻/Cl⁻ in grape genotypes when subjected to salt stress and treated with Si. Exposure to 50 and 100 mM NaCl resulted in a decrease of the K⁺/Na⁺ and NO₃⁻/Cl⁻ ratios by 4.8 and 8.9 folds in the Chawga and by

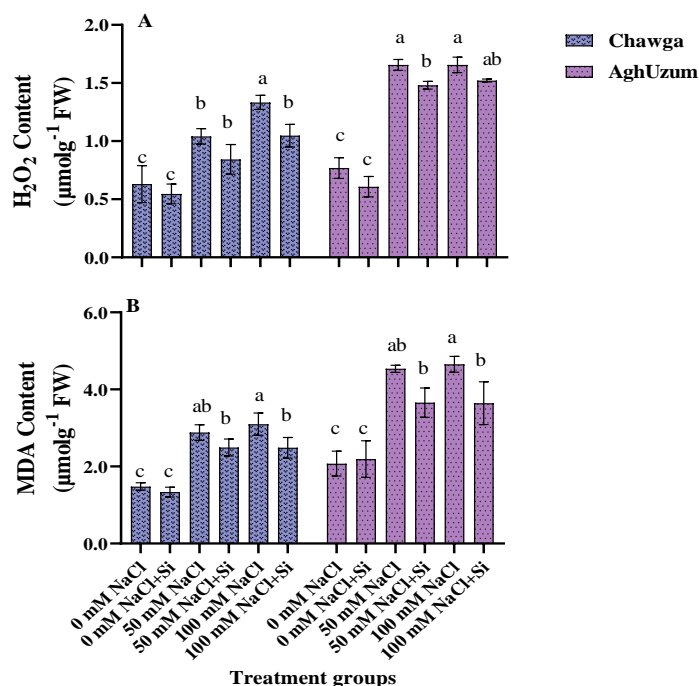


Figure 4. H₂O₂ (A) and MDA (B) contents of leaf tissue in two grapevine (*Vitis vinifera* L.) genotypes, Chawga and AghUzum, in response to NaCl (0, 50, and 100 mM) and Si (0 and 3 mM) treatments. The bars show the average ± standard error (SE) (replications = 3). Different letters in each genotype indicate significant differences (one-way ANOVA, Tukey, P<0.05).

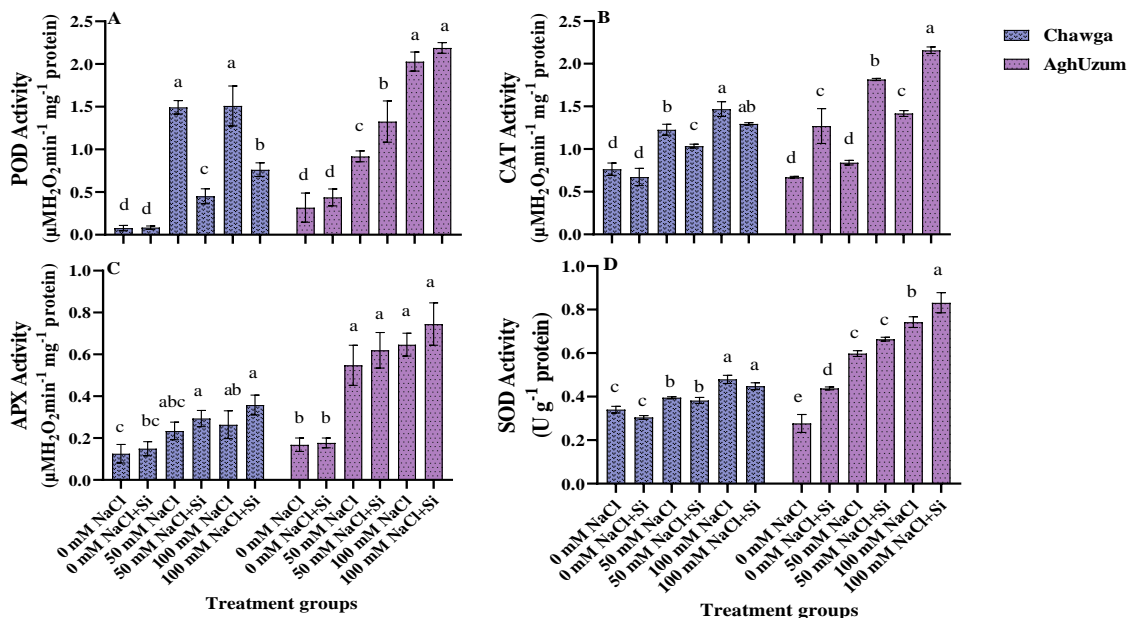


Figure 5. POD (A), CAT (B), APX (C), and SOD (D) activity of leaf tissue in two grapevines (*Vitis vinifera* L.) genotypes, Chawga and AghUzum, in response to NaCl (0, 50 and 100 mM) and Si (0 and 3 mM) treatments. The bars show the average ± standard error (SE) (replications = 3). Different letters in each genotype indicate significant differences (one-way ANOVA, Tukey, P<0.05, n = 3).

40.4 and 25.2 folds in the AghUzum, respectively, compared to the controls. When a high salt concentration (100 mM NaCl + Si) was added, the ratio

of K⁺/Na⁺ selectivity increased by 29.1%, and the ratio of NO₃⁻/Cl⁻ selectivity increased by 46.6% in Chawga. Also, in AghUzum, adding Si under the same conditions

made the ratio of K^+/Na^+ selectivity rise by 23.7% and the ratio of NO_3^-/Cl^- selectivity rise by 44.4% compared to plants that were only treated with 100 mM NaCl.

Total soluble sugar, proline, and glycine-betaine contents: In this experiment, when the leaves of both genotypes of grapes were exposed to salt, the amounts of sugars, free proline, and glycine betaine significantly increased ($P < 0.05$) (Fig. 3). The highest concentrations of these compounds were observed at the 100 mM salt level, whereas the lowest concentrations were found in the control group. Chawga showed a lower increase in the leaf's sugar, proline, and glycine betaine contents compared to control plants (28.8%, 53.4%, and 52.0% of control, respectively). In contrast, AghUzum showed higher leaf increments (44.6%, 60.5%, and 18.36% of control, respectively). Adding Si at high NaCl levels reduced the amounts of proline (25.4% and 15.8%) and glycine betaine (31.1% and 8.0%) in the Chawga and AghUzum genotypes. However, the sugar content did not change significantly in salt-stressed grape plants supplied with Si.

H_2O_2 and MDA contents: Plants that received a treatment of 3 mM Si under conditions without salt showed the least amount of H_2O_2 concentration, as shown in Figure 4A. The application of Si resulted in a decrease of hydrogen peroxide levels by 21.2% in the Chawga genotype and 8.1% in the AghUzum genotype when exposed to salt stress. However, Si had a limited effect on H_2O_2 levels in AghUzum leaves compared to Chawga.

A high level of Na^+ ions at a concentration of 100 mM NaCl resulted in a significant increase in the MDA content by 52.2% and 55.4% in the leaves of the Chawga and AghUzum genotypes, respectively, when compared to control plants (Fig. 4B). The findings suggest that the addition of Si (3 mM) decreased salt-induced lipid peroxidation by reducing the MDA concentration in the tissues of both grape genotypes. When Si was added to plants grown under salt stress, there was a reduction in MDA levels by 19.7% in the Chawga genotype and 21.7% in the AghUzum genotype, compared to plants cultivated under salt stress without Si addition.

Antioxidant enzyme: The activity of antioxidant enzymes in the leaves of grape genotypes showed significant modifications in response to both NaCl toxicity and Si supplementation, as shown in Figure 5. The activity of POD, CAT, and APX enzymes was enhanced in response to both levels of salinity stress. Significant increases have been observed under 100 mM NaCl, with POD increasing by 94.8%, CAT by 47.9%, and APX by 52.6% in Chawga plants. In AghUzum plants, the increases were 84.3%, 52.7%, and 73.9% for POD, CAT, and APX, respectively, compared to the control group. In contrast, the Si application increased the activity of POD by 7.2%, CAT by 34.3%, and APX by 13.2% in AghUzum when exposed to NaCl stress (100 mM), compared to the treatment with the same concentration of salt stress. Unexpectedly, Si did not

have any impact on the activity of POD, CAT, and APX enzymes in Chawga plants when they were exposed to salinity treatment. The Chawga genotype exhibited a decrease in the antioxidant activity of superoxide dismutase (SOD) with Si supplementation, resulting in a 6.6% reduction under salt stress (100 mM). Conversely, the AghUzum genotype showed an increase in the antioxidant activity of SOD by 10.7% under the same conditions.

Discussion

Salt stress inhibited the growth of both grape genotypes, which was evident in the reduced plant height and fresh and dry weights of the leaves (Table 1). The growth parameters of AghUzum decreased more significantly than those of Chawga under NaCl stress. In this study, Si application significantly enhanced the growth parameters of grape genotypes, particularly noticeable in Chawga, across all applied concentrations of NaCl (Table 1). Numerous studies have highlighted the inhibitory effect of salinity stress on plant growth across various plant species (Oueslati *et al.*, 2010; Bettaieb Rebey *et al.*, 2017; Bistgani *et al.*, 2019). In saline environments, plant growth is primarily inhibited by osmotic stress, which prevents the uptake of crucial macro- and micronutrients (Oueslati *et al.*, 2010). In this study, salt stress led to decreased concentrations of NO_3^- in both genotypes. However, the response to potassium was different, with the K^+ content decreasing in AghUzum and increasing in Chawga with rising salinity levels. This variation in mineral concentrations may be directly related to increased uptake of Na^+ and Cl^- ions by the grape roots, as observed in similar studies (Mohammadkhani *et al.*, 2013). The Na^+ and Cl^- were accumulated in the leaf tissue of two genotypes under salinity (Fig. 2). The accumulation of Na^+ and Cl^- in leaves is a crucial display for assessing root control capability. The ability to regulate the transport and exclusion of toxic ions from shoots is closely connected to salt tolerance. In our study, AghUzum showed higher Cl^- and Na^+ accumulation in the leaves compared to the Chawga genotype, indicating a lack or ineffective exclusion mechanism in AghUzum and an absence of control over the transport of toxic ions to the leaves. Maintaining ionic homeostasis, especially K^+ , is crucial for essential cellular processes, including efficient photosynthetic system functioning and regulation of stomatal opening (Bettaieb Rebey *et al.*, 2017). Potassium (K^+) plays a significant role in plant resistance to salinity. Therefore, maintaining high K^+ concentrations is necessary to alleviate osmotic stress in a saline environment (Benito *et al.*, 2014). The data indicate that Si application did not significantly affect the K^+ concentration of grape plants under salt stress, different from some previous reports claiming an induction effect of Si on K^+ absorption. Among the two studied genotypes, Chawga had considerably more K^+ than Na^+ in its leaves under 50 and 100 mM NaCl concentrations. Toxic ion accumulation was reduced in

leaves by Si application under salt stress. The effect of Si on reducing the Na^+ and Cl^- contents of leaves in the Chawga genotype was more than in the AghUzum genotype (Fig. 2). Si reduces the apoplastic transport and transpiration bypass flow of Na^+ and Cl^- ions from roots to shoots by blocking the apoplast. This mechanism leads to the return of salt concentration to a tolerable level in shoots (Shi *et al.*, 2013). Si increases the ratio of K^+/Na^+ in the grapevines (Fig. 2) by reducing the influx and increasing the efflux of Na^+ . The increase of Na^+ efflux in grape genotypes by Si can be attributed to the enhancement of H^+ -ATPase and/or Na^+/H^+ -ATPase activity under salinity stress induced by Si. Similarly, under salinity stress, the elevated level of Cl^- in plants can cause toxicity. Under salt stress, the $\text{NO}_3^-/\text{Cl}^-$ ratio is an important factor in the grapevine, and Si-treated plants maintain higher NO_3^- content under high salinity conditions. The increase in NO_3^- absorption under salinity stress in Si-treated plants is probably related to the reduction of Cl^- absorption. Therefore, the effect of Si in reducing Cl^- accumulation in grape leaves is of great importance for improved performance under salt stress. To our knowledge, there is limited information in the literature about the specific effect of Si on the Cl^- absorption mechanism of plants under salinity stress. It seems that the beneficial effects of Si in reducing salinity in grapes may be related to alleviating the absorption and exclusion of toxic ions (Na^+ and Cl^-).

In response to salt stress, osmolytes such as organic and inorganic solutes play a crucial role in regulating the cellular osmotic potential of plants. Higher concentrations of proline and soluble sugars facilitate stress-tolerant cultivar selection (Shabala and Pottosin, 2014; Mahouachi, 2018), maintain osmotic potential and ionic balance, improve water and mineral absorption, enhance cell membrane stability (Sarker and Oba, 2020), and protect enzyme structures against ROS (Rahneshan *et al.*, 2018; Kumar *et al.*, 2021). In this study, the soluble sugar, free proline, and glycine betaine contents significantly increased under salt stress (Fig. 4). Moreover, these compounds' contents were notably higher in the AghUzum genotype compared to Chawga. Si application decreased proline and glycine betaine levels in both genotypes under salinity treatments. However, Si did not affect the soluble sugar content in either genotype. Reduced Na^+ uptake and the balance of ROS production due to Si under salinity may be the causes of the drop in proline and glycine betaine content. Higher enzymatic activities, facilitating the regulation of cellular structures and functions through interactions with macromolecules, may explain the increase in total soluble sugars (Gupta and Huang, 2014; Al Hassan *et al.*, 2016).

Salt stress can disrupt the delicate balance between the production and scavenging of ROS, leading to oxidative stress. Raised levels of radical species like H_2O_2 , $\text{O}_2^{\cdot-}$, and OH^\cdot can cause damage to various plant cell components, ultimately resulting in cell death

(Alam *et al.*, 2019; El-Badri *et al.*, 2021). In this study, we measured H_2O_2 levels to study the redox state of two grape genotypes. Our findings showed a significant increase in hydrogen peroxide levels with rising NaCl concentrations (Fig. 5). Interestingly, H_2O_2 contents were higher in the AghUzum genotype compared to Chawga. Furthermore, Si application appeared to reduce the content of H_2O_2 more significantly in Chawga than in AghUzum, probably due to the better effect of Si on maintaining ion balance and consequently reducing ROS production under salinity stress in this genotype. Increased ROS generation due to abiotic stress often leads to the peroxidation of cellular and organelle membrane lipids, resulting in membrane integrity loss (Gill and Tuteja, 2010). MDA is a widely used marker for detecting lipid peroxidation. Salinity stress induces higher oxidative damage, as indicated by elevated MDA levels under stress (Hong *et al.*, 2000; Shabala *et al.*, 2014; Ali *et al.*, 2020). In our study, MDA content was significantly higher under stress than in the control in both genotypes (Fig. 4). We observed a higher MDA content in AghUzum under NaCl treatments, indicating more damage and reduced membrane stability in the sensitive genotype. However, Si application decreased the MDA content in stressed plants (Fig. 4). Si's role in regulating the level of osmolytes and reducing ROS production under saline conditions induced this improvement in membrane stability (Singh *et al.*, 2022).

The main antioxidant enzymes involved in scavenging and detoxifying ROS in plant cells include CAT, POD, APX, and SOD (Mushtaq *et al.*, 2020). In our study, the activities of these enzymes increased in both genotypes under salt stress levels (Fig. 5). However, enzyme activity was higher in AghUzum than in Chawga under saline conditions. Plants protect against oxidative stress by increasing antioxidant enzymes and scavenging ROS production to deal with the harmful effects of salinity stress (Molassiotis *et al.*, 2006; Akram *et al.*, 2017; Hasanuzzaman *et al.*, 2020). Under saline imposition, SOD converts superoxide radicals into H_2O_2 , while POD and CAT detoxify H_2O_2 into H_2O and O_2 , thus regulating the formation of H_2O_2 (Hossain *et al.*, 2015). The lower enzyme activity in the Chawga genotype under salt stress is probably due to lower oxidative toxicity and a lower need for antioxidant enzyme activity. The impact of Si on enzyme activity under salt stress changed between the two grape genotypes. The Si application enhanced the activity of CAT, POD, APX, and SOD in AghUzum. Still, it did not impact enzyme activities in Chawga under salinity treatment, likely due to the better performance of the tolerant genotype in reducing oxidative stress and ROS production (Fig. 5).

In this study, the two genotypes under investigation may exhibit differences in their ability to isolate salt within leaf cells. One strategy for salt resistance observed in AghUzum involves the activation of antioxidant enzymes and proline biosynthesis. In contrast, Chawga employs a dissimilar strategy

characterized by the uptake and maintenance of essential ions, such as K^+ and NO_3^- , which may require less energy compared to the activation of antioxidant enzymes. These genotype-specific strategies highlight the diverse mechanisms active in plants to mitigate the effects of salt stress and underline the importance of understanding intraspecific variation in response to environmental challenges.

Conclusion

Our findings show the potential of Si to mitigate salinity stress in grape plants by improving growth characteristics, maintaining nutrient balances, reducing cellular oxidation, and regulating osmolyte production. The impact of Si in alleviating salt stress varies depending on the genetic traits of the grape genotypes studied. Under saline conditions, Si treatment resulted in decreased accumulation of Na^+ and Cl^- in the leaves, along with improved growth parameters, compared to untreated plants. Salinity stress influenced the

concentrations of osmolyte compounds differently in grape genotypes; However, Si application exhibited a more evident effect on most osmotic compounds in AghUzum than Chawga, depending on the salt stress levels. Our observations suggest that AghUzum employs a salt resistance strategy involving the activation of antioxidant enzymes and proline biosynthesis, and Chawga utilizes a different strategy characterized by the uptake and maintenance of essential ions, such as K^+ and NO_3^- , which may require less energy compared to the activation of antioxidant enzymes. The alteration of the salinity stress response in grape plants influenced by Si highlights its potential to enhance crop production and agricultural sustainability in regions affected by soil salinity. Such studies could provide a valuable understanding of the greater applicability of Si as a mitigation strategy for salinity stress in grapevine cultivation.

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