



Silicon and Potassium-Induced Modulations in Leaf Carbohydrate Metabolism Confer Freezing Tolerance in Satsuma Mandarin

Shahid Iqbal¹ · Rashad Mukhtar Balal² · Mahmoud F. Seleiman³ · Matthew Mattia⁴ · John M. Chater⁵ · Muhammad Adnan Shahid¹

Received: 1 May 2024 / Accepted: 13 June 2024 / Published online: 25 June 2024

This is a U.S. Government work and not under copyright protection in the US; foreign copyright protection may apply 2024

Abstract

Freezing temperatures are a severe issue in North Florida, primarily due to occasional cold snaps and frost events in winter and early spring that cause damage to citrus groves, resulting in reduced fruit yield. The apoplasm is the primary cell component that interacts with environmental stress and is essential for plant tolerance to freezing temperatures. The present study was conducted to gain insight into how the application of silicon (Si) and potassium (K) are involved in the leaf apoplasm contributes to freezing stress tolerance, and regulates carbohydrate metabolism. We used Satsuma mandarin (*Citrus unshiu* Marc.), the most successful commercially grown citrus cultivar in North Florida, and treated trees with two concentrations of Si and K (50 and 100 ppm) both individually and combined as foliar spray to determine their effect as they relate to improving cold hardiness. Freezing stress (-6 °C) caused a severe reduction in photosynthesis, and modulations in leaf carbohydrate metabolism resulted in inhibited plant growth. The exogenous application of Si and K both improved the photosynthesis rate, soluble sugars, and activities of enzymes of carbohydrate metabolism such as fructokinase, phosphofructokinase, hexokinase, sucrose and phosphate synthase, and acid and neutral invertase. Applying Si (100 ppm) and combined treatment (Si + K-50) showed the best response by inducing the maximum tolerance to freeze stress. Our data demonstrated the ameliorative effect of Si and K under freezing stress in citrus is associated with modification in carbohydrate metabolism in the leaf apoplasm. This study provides direction for future research to investigate the effect of Si and K on the transcriptome and metabolome in citrus plants and their tissues under freezing stress.

Keywords Cold hardiness · Citrus · Low-temperature · Sugar metabolism

1 Introduction

Freezing temperature is one of the major factor affecting plant growth, limiting yield and having a negative effect on economic value [1]. Citrus is an important perennial

fruit crop and an excellent source of vitamin C, but its production is negatively affected due to several issues such as Huanglongbing (HLB) and other abiotic stresses such as salinity, heat, flooding, and freezing temperature. Freezing stress is one of the principal abiotic stress that leads to

✉ Muhammad Adnan Shahid
mshahid@ufl.edu

Shahid Iqbal
shahidiqbal@ufl.edu

Rashad Mukhtar Balal
uaf_rashad@yahoo.com

Mahmoud F. Seleiman
mseleiman@ksu.edu.sa

Matthew Mattia
matthew.mattia@usda.gov

John M. Chater
jchater@ufl.edu

¹ Horticultural Science Department, North Florida Research and Education Center, University of Florida/IFAS, Quincy, FL 32351, USA

² Department of Horticulture, College of Agriculture, University of Sargodha, Sargodha 40100, Pakistan

³ Department of Plant Production, College of Food and Agriculture Sciences, King Saud University, P.O.Box 2460, 11451 Riyadh, Saudi Arabia

⁴ United States Horticultural Research Laboratory, United States Department of Agriculture, Agricultural Research Service, 2001 South Rock Road, Ft. Pierce, FL 34945, USA

⁵ Horticultural Science Department, Citrus Research and Education Center, University of Florida/IFAS, Lake Alfred, FL 33850, USA

severe losses in citrus [2]. Some citrus species can tolerate temperatures as low as -7°C , but their growth gradually decreases due to several physiological and biochemical alterations inside the plants, such as carbohydrate metabolism, antioxidant enzyme activities, hormonal changes, etc. [3]. Freezing temperature affects plants with clear symptoms like surface lesions, discoloration, a water-soaked appearance, tissue breakdown, membrane damage, and quick decay of the plant by adversely affecting metabolic activities within plant tissues [4, 5]. Freezing is the most challenging factor [6], particularly for citrus production in areas with severe unpredicted freeze events in winter and early spring. Thus, considering the above limitations under freezing, cost-effective, eco-friendly, and sustainable mitigation strategies are highly demandable for perennial fruit production in tropical and subtropical regions.

Plants exposed to an environment with specific conditions reach an optimal state where their metabolism is improved. This optimization includes changes in enzymatic activity and metabolic capacity, allowing the plants to perform as efficiently as possible under these conditions [7]. This environmental change interferes with plants in three categories, i.e., molecular, physiological, and biochemical [8–10]. In citrus, a series of physiological changes occur when the plants are exposed to low temperatures, which includes an increase in unsaturated fatty acids, a decrease in enzymatic and photosynthetic activity, disruption in chloroplast structure, and ion leakage [8, 11]. During freezing stress, the fluidity of the cell membrane decreases due to a higher amount of unsaturated fatty acids in membrane lipids, which alters the ratio and composition of lipids to proteins [12]. The detrimental effect of freezing is the formation of ice, which first forms in the intercellular spaces of the plant, reduces the water potential, and causes dehydration, which further leads to electrolyte leakage [13]. The severity of these ice crystals ruptures the plant cell walls, leads to cytosol outflow, and eventually causes plant tissues and possibly the entire plant to die [14].

Plants exposed to freezing temperatures have a variety of metabolic changes that enable them to withstand stress conditions and exhibit changes in soluble carbohydrates, including soluble sugars (sucrose, glucose, and fructose) and sugar alcohols such as mannitol, sorbitol, xylitol, etc. [15], whereas the specific functions of these various carbohydrates remain largely unknown, they may protect plants from chilling stress in a variety of ways [16, 17]. The formation of soluble sugars [12, 18] and starch [19] are some of the main metabolic changes caused when the plants are exposed to freezing temperatures. Sugars that accumulate in freeze-adapted plants have numerous important functions, such as soluble sugars that can control gene expression in photosynthesis, sugar metabolism, and osmolyte production [20]. Sucrose and glucose are osmolytes that help to maintain cellular homeostasis [21] and play a role in cellular

signal transduction in stressful environments [22, 23]. Furthermore, carbohydrates can help to remove reactive oxygen species (ROS) created during stress and oxidized sugar synthesis [24]. During freezing stress, several enzymes regulate soluble carbohydrate metabolism, through which sucrose phosphate synthase (SPS) converts glucose and fructose into sucrose. The breakdown of sucrose into glucose and fructose is catalyzed by acid invertase (AI) and neutral invertase (NI), which play a critical role in plant growth [25, 26]. Sucrose synthase (SS) mainly functions in the direction of cleavage despite playing a dual role in both synthesis and hydrolysis [27]. There is a positive association between SPS and SS activities and sucrose content [28].

Silicon (Si) is the second most common element on Earth and has several beneficial effects on plant growth and metabolism, particularly under alleviating stress conditions [29]. The exogenous application of Si mitigates the negative impact of freezing stress by regulating macronutrient homeostasis and hormone balancing [30, 31]. Several studies have reported that the exogenous application of Si under low-temperature stress regulates the antioxidant defense system and alleviates freeze damage [32]. Si improves leaf and stem strength by maintaining cell wall polysaccharides and lignin polymers. Si affects a variety of plant physiological activities, including photosynthetic rate, stomatal conductance, water use efficiency, stomatal size and number, transpiration rate, and the hydraulic conductance of roots in many crops [33, 34]. The ameliorative effect of Si against freezing has been reported in numerous crops, including grapes [35], mangoes [36], cucumber [37], tomato [38], barley [39], wheat [40], alfalfa [41] and turfgrass [42]. Potassium (K) is another major element in plants that plays an important role in mitigating different abiotic stresses such as drought, heat, and cold [43, 44]. It is also revealed that the adequate supply of K effectively protects the plants against the adverse effects of freezing temperatures [43]. It also stimulates the translocation of sugars and regulates carbohydrate and sucrose metabolism [45]. Several physiological and metabolic processes are transformed due to the lower availability of K, which affects plant photosynthetic activity, carbon fixation, electron transport, and stomatal conductance, and are the primary targets of freezing stress in plants [46, 47] grapevines [48], olives [49], and pistachio [50]. Therefore, K application in plants helps maintain physiological functions at their optimum level to survive under adverse environmental conditions.

Under stress conditions, carbohydrate accumulation can play an important role in osmotic adjustments, osmoprotectants, carbon storage, and radical scavenging [51]. So, due to the beneficial role of Si and K in regulating plant growth under freezing stress, we hypothesized that they may have a regulatory effect on plant carbohydrate metabolism. This study aimed to identify the individual

and combined effect of Si and K on the cold hardiness of citrus and to investigate their effects on the plant photosynthetic activity and carbohydrate metabolism in leaves under freezing stress.

2 Material and Methods

2.1 Plant Material and Treatment Plan

Two-year-old Satsuma mandarin (cv. Owari) plants (*Citrus unshiu* Marc.) grafted onto Rich 16–6 (*Poncirus trifoliata* L.) rootstock were used for this study and grown in the greenhouse facility of Horticultural Science Department at North Florida Research and Education Center, University of Florida, Quincy, FL (27° 25' 32.6604" N; 80° 24' 18.7092" W). The plants were grown in Citri pots (10.16 × 10.16 × 34.29 cm) filled with coconut fiber growing media. The plants were grown in a controlled greenhouse, maintaining an average day and night temperature of 25 °C ± 2 °C, with 80% relative humidity and a photoperiod of 12 h. The experiment was performed in a randomized complete block design (RCBD) with six treatments, and each treatment consisted of four replicates with ten plants per replicate. The six treatments were as follows: (T1) control; plants treated with distilled water, (T2) Si 50 ppm, (T3) Si 100 ppm, (T4) K 50 ppm, (T5) K 100 ppm, (T6) combination of Si and K with 50 ppm of both.

2.2 Silicon and Potassium Application Strategy

Silicon dioxide (SiO₂) provided by Dyna-Gro (Pro-TeKt) Richmond, California, United States, was used for the Si treatments, and potassium sulphate 99% (K₂SO₄) by Thermo Scientific Waltham, Massachusetts, United States, was used for the K treatment. Since the silicon product used in this study also contains 3% potassium, both silicon treatments Si 50 ppm and Si 100 ppm, include some additional K by 16 and 32 ppm, respectively, and K treatments were adjusted accordingly. The foliar application of planned treatments was applied to plants daily for twenty-one days. After the treatments, the plants were moved to a programmed walk-in freezing chamber with a photoperiod of 14 h of daylight and 10 h of dark with 96% relative humidity. Initially, plants were maintained at 25 °C for 24 h, then the temperature gradually decreased from 25 °C to -6 °C for 24 h; then, the plants were subject to continuous exposure at -6 °C for 24 h. Leaf samples were collected before and after the freezing treatment.

2.3 Determination of Photosynthesis Activity

The net photosynthetic rate of fully expended leaves was measured using a portable photosynthesis system (Li-6800 Licor Inc, Lincoln, NE, United States). All the observations were taken under both temperature conditions. Three fully matured leaves from each plant of individual replicate were selected, and photosynthesis was measured from the leaf's different leaf positions, i.e., leaf center, side of the leaf [52]. In addition, the photosynthesis instrument conditions were as follows: flow (400 µmol s⁻¹), relative humidity (55%), CO₂ (420 µmol mol⁻¹), temperature (25 °C), and light intensity (500 µmol m⁻² s⁻¹).

2.4 Measurement of Sugars and Starch Contents

Soluble sugars were determined by using the High-performance liquid chromatography (HPLC) system (HPLC, Shimadzu, Kyoto, Japan) following the method described by Chow and Dong [53, 54] with some modifications. Briefly, the collected leaf samples were taken, and soluble sugars were extracted with 80% ethanol (v/v) with four replicates at 75 °C for 10 min. After evaporating the supernatant, the residues were redissolved in distilled water, filtered through a 0.45 µm membrane HPLC filter (Whatman, Maidstone, UK), and used for soluble sugar analysis. After soluble sugars extraction, the leftover residues were used for starch content determination according to the method described by Orak and Yang [55, 56]. Briefly, the leaf samples were grounded, mixed with distilled water, and shaken well using a vortex mixer. The sample was homogenized by adding 50 ml of distilled water and put in the hot bath for 10 min. After that, 100 ml water was added and mixed with ultrasonic water bath through shaking and later cooled at room temperature. Samples were filtered through 0.45 µm membranes (Sartorius, minisat RC25), and 20 µL of the extract was injected into HPLC. The starch concentration was determined by external standard calibration using extra pure soluble starch. The chromatographic conditions used for this analysis were as follows: detector (Hewlett-Packard HP 1047 A refractive index detector), column: (BIO-RAD, aminex HPX-87, 300 mm × 7.8 mm), mobile phase (100% ultra distilled water obtained from Millipore Synergy-185), water quality (18.2 MΩ cm), flow rate (0.6 ml/min), column compartment temperature (85 °C), and injection volume (20 µL).

2.5 Enzyme Extraction and Activity Assay

Enzymatic activity was quantified by using the method described by Iqbal [52]. 0.5 g of frozen leaf tissues were taken to determine the activities of enzymes involved in carbohydrate metabolism. Leaves were homogenized with 50 mM Tris-hydrochloric acid added with magnesium

chloride (5 mM), mercaptoethanol (5 mM), ethylenediaminetetraacetic acid (1 mM), ethylene glycol-bis- β -aminoethyl ether (1 mM), 2-hydroxyl-1-ethanethiol (0.1 mM), and glycerol (15%). The homogeneous mixture was centrifuged at 17,000 g for 15 min at 4 °C. The supernatant was used to determine the different enzymatic activities. For total acid invertase (AI), the reaction mixture (1 mL) contained 0.2 mL extract, sodium acetate (0.1 M, pH 4.8), and sucrose (0.1 M). For neutral invertase (NI), the reaction mixture was comprised of dipotassium phosphate (0.1 M), sodium citrate (0.1 M), and sucrose (0.1 M). The reaction mixture was added with 0.2 mL of enzyme extract for AI and NI and incubated at 37 °C for 40 min. The reaction mixture was then stopped by adding dinitro salicylic acid (1 mL). The activity of AI and NI was determined by a spectrophotometer at the wavelength of 540 nm.

Sucrose phosphate synthase (SPS) and sucrose synthase (SS) enzymes were obtained using the methods used by Rufty and Huber [57]. The SPS activity for SS and SS activity for sucrose cleavage were evaluated by Bird's method [58]. Fructokinase was examined by the method of [59]. The reaction mixture contained magnesium chloride (5 mM), Tris-hydrochloric acid (0.1 M, pH 8.5), nicotinamide adenine dinucleotide (0.5 M), fructose (10 mM), glucose-6-phosphate dehydrogenase (2 U) and adenosine triphosphate (2 mM). Hexokinase (HK), phosphofructokinase (PFK), and pyruvate kinase (PK) activities were determined using enzyme kits (Sigma-Aldrich, USA) by following the manufacturer's protocols and using a spectrophotometer at the wavelength of 340 nm.

2.6 Statistical Analysis

The data were analyzed through Statistix 10.0 (Analytical Software, USA) using ANOVA and LSD test for significant differences. The graphs were generated using Sigmaplot (v 15.0) software. Each treatment consisted of four replicates with 10 plants per replicate in RCBD. The principal component analysis (PCA) and correlation analysis were performed using R (v 4.3.3).

3 Results

3.1 Photosynthetic Activity

Freezing stress significantly reduced photosynthetic activity, while the exogenous application of Si and K improved the photosynthetic activity. Plants under the normal temperature showed a photosynthesis rate between 20.74 to 23.78 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ with no significant difference among treatments. When the plants were exposed to freezing stress (-6 °C), all the treatments showed a decrease in photosynthesis

rate compared to the control of normal temperature. Plants treated individually with Si (100 ppm) and K (100 ppm) under freezing stress showed a maximum photosynthesis rate (17.92 and 14.36 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), respectively, followed by the plants treated with the combination of Si and K (Si-50 ppm + K-50 ppm) which is 11.98 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. However, the plants treated with K (50 ppm) showed the lowest photosynthesis rate among all treatments during freezing stress (Fig. 1).

3.2 Soluble Sugars and Starch Contents

In this study, a noticeable increase was observed in the concentration of glucose and fructose under freezing stress (-6 °C) compared to the control plants. The application of Si and K significantly affects these concentrations. A maximum concentration of 21.29 and 5.88 mg g^{-1} DW (Dry weight) glucose and fructose, respectively, was observed when the plants were treated individually with Si (100 ppm). The combined application (Si-50 ppm + K-50 ppm) also increased the glucose and fructose content (8.38 and 6.16 mg g^{-1} DW), followed by K (100 ppm) i.e., 7.31 and 3.67 mg g^{-1} DW, respectively. Meanwhile, the K (50 ppm) treatment exhibited the lowest concentration (7.01 and 3.17 mg g^{-1} DW), respectively, among all treatments (Fig. 2A and B).

Similarly, total soluble sugars (TSS), sucrose, and starch concentration revealed a higher concentration under freezing stress than control plants. The higher concentrations of TSS, sucrose, and starch were recorded when the plants were treated with Si (100 ppm) individually, i.e., 125.89, 19.83, and 82.37 mg g^{-1} DW, respectively. The combined effect of Si and K (50 ppm of both) also showed an increase in these concentrations (103.28, 19.65, and 77.03 mg g^{-1} DW), followed by

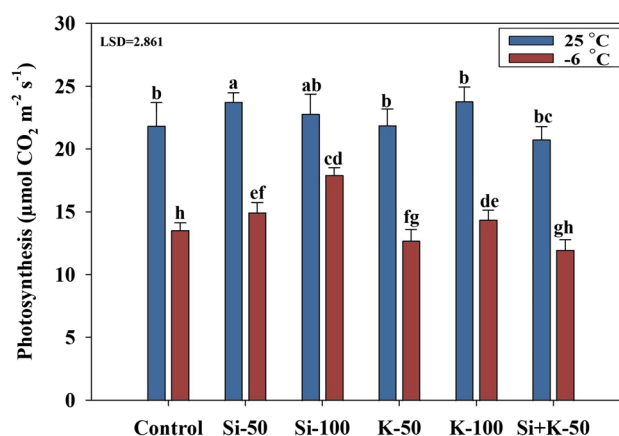


Fig. 1 Effect of Si and K on the photosynthesis rate of satsuma mandarin under normal and freezing temperatures. The lower-case letters on graphs represent significant differences using the least significant difference (LSD) at $p < 0.05$, while vertical bars represent the mean standard error ($n = 4$)

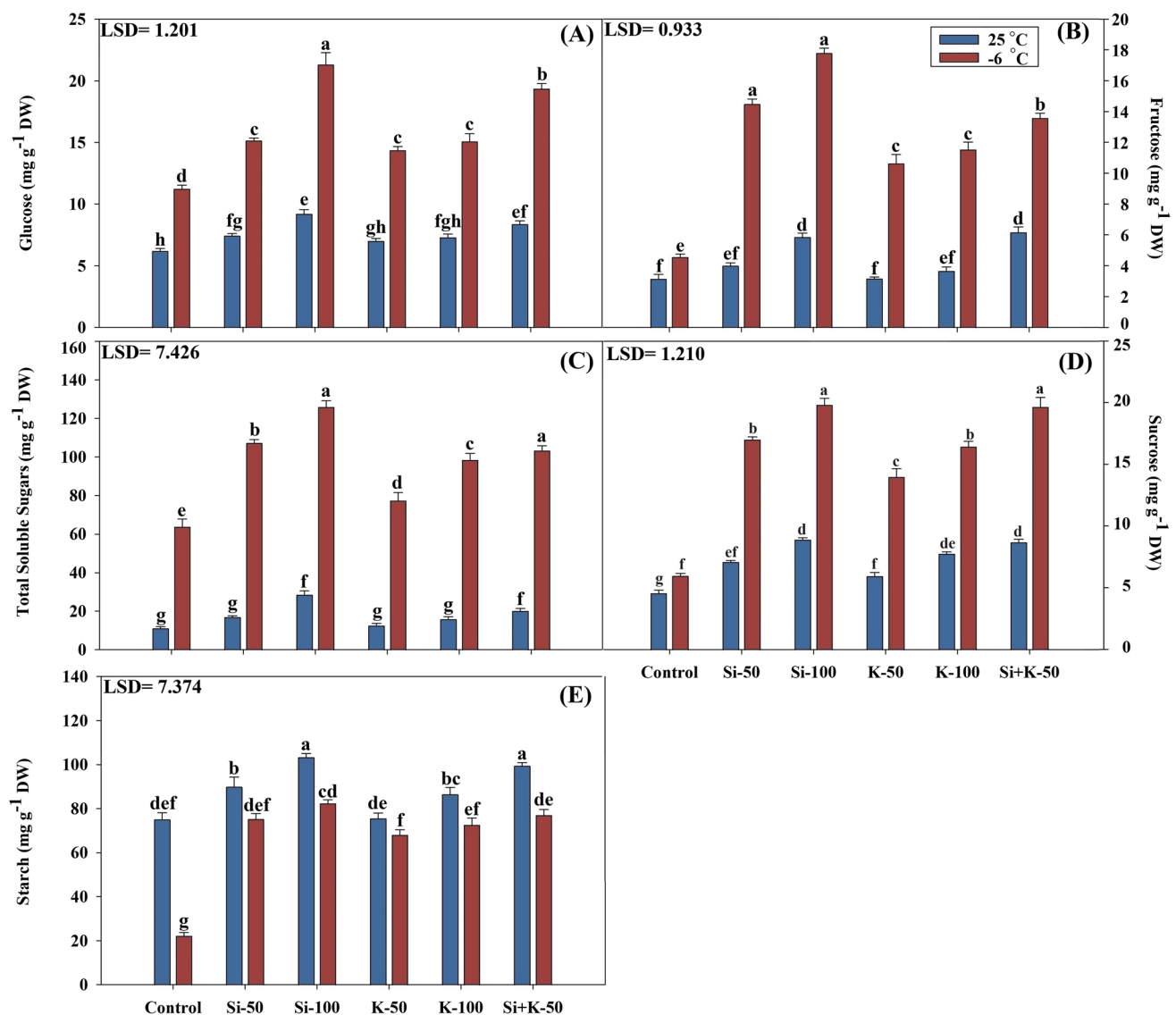


Fig. 2 Effect of Si and K on glucose (A), fructose (B), total soluble sugars (C), sucrose (D), and starch (E) concentration of satsuma mandarin under normal and freezing temperatures. The lower-case letters

on graphs represent significant differences using the least significant difference (LSD) at $p < 0.05$, while vertical bars represent the mean standard error ($n=4$)

K (100 ppm) and K (50 ppm) i.e. 98.42, 16.44, 72.66 mg g^{-1} DW, and 77.31, 13.99, 68.11 mg g^{-1} DW, respectively (Fig. 2C, D and E). Notably, the concentration of soluble sugars and starch contents significantly increased during freezing stress in response to Si and K application compared to those plants grown under normal temperatures.

3.3 Enzymes of Carbohydrate Metabolism

3.3.1 Fructokinase and Hexokinase

Freezing stress significantly increases the activities of fructokinase (FK) and hexokinase enzymes (HK). The enzymatic

activity of FK significantly increased in both Si- and K-treated plants in freezing stress compared to the normal temperature treatment plants. At the same time, HK showed significant differences in some treatments compared to the control. Under freezing stress, the maximum activity of FK ($21.01 \mu\text{mol min}^{-1} \text{mg}^{-1}$) was recorded in the plants applied with the Si (100 ppm), followed by plants treated with the blend of Si with K ($20.21 \mu\text{mol min}^{-1} \text{mg}^{-1}$) and an individual K (100 ppm) application ($12.14 \mu\text{mol min}^{-1} \text{mg}^{-1}$) (Fig. 3A). The maximum activity of HK (1.56 U mg^{-1}) was recorded in control plants, followed by plants treated with Si (100 ppm) and the blend of Si with K i.e. 1.50 and 1.42 U mg^{-1} , respectively (Fig. 3B).

Fig. 3 Effect of Si and K on the enzymatic activity of fructokinase (A) and hexokinase (B) in carbohydrate metabolism of satsuma mandarin under normal and freezing temperatures. The lower-case letters on graphs represent significant differences using the least significant difference (LSD) at $p < 0.05$, while vertical bars represent the mean standard error ($n = 4$)

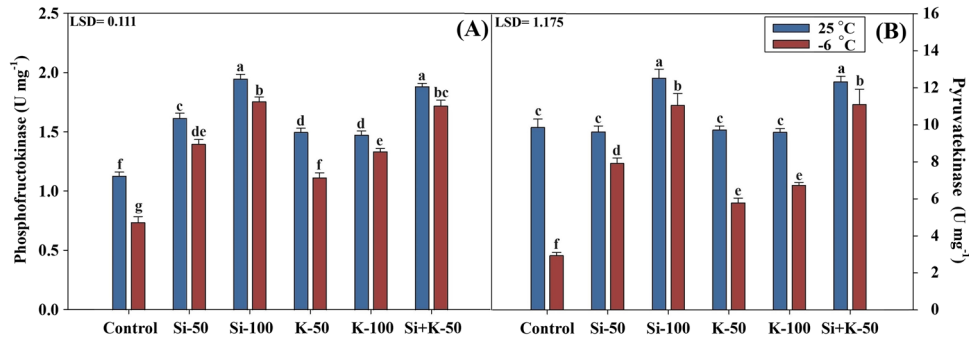
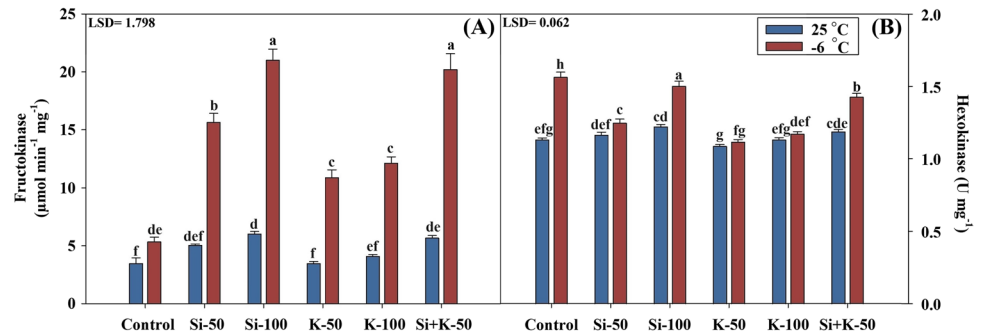


Fig. 4 Effect of Si and K on the enzymatic activity of phosphofructokinase (A) and pyruvatekinase (B) in carbohydrate metabolism of satsuma mandarin under normal and freezing temperatures. The

lower-case letters on graphs represent significant differences using the least significant difference (LSD) at $p < 0.05$, while vertical bars represent the mean standard error ($n = 4$)

3.3.2 Phosphofructokinase and Pyruvatekinase

Freezing stress caused a significant reduction in the activities of phosphofructokinase (PFK) and pyruvatekinase (PK). The activities of PFK and PK were increased in response to the foliar application of Si and K under freezing stress. While comparing the control of freeze-stressed and normal plants, the plants under freeze-stress gradually decreased the PK concentration from 9.86 to 2.95 $\mu\text{mol min}^{-1} \text{mg}^{-1}$. The activity of PFK under freezing stress (-6°C) and normal conditions (25°C), didn't show a significant difference, while the activity of PK showed a significant difference under the control condition of both normal and freeze-stressed plants. The maximum activities of PFK and PK were recorded in the plants treated with Si (100 ppm) application (1.75 U mg^{-1} and 11.05 $\mu\text{mol min}^{-1} \text{mg}^{-1}$, respectively), followed by the combined (Si and K) and individual (K) applications. The lowest activities (1.11 U mg^{-1} and 5.79 $\mu\text{mol min}^{-1} \text{mg}^{-1}$) of PFK and PK were observed in plants treated with K (50 ppm) among Si and K treatments (Fig. 4A and B).

3.3.3 Sucrose Synthase and Sucrose Phosphatesynthase

Plants grown under freezing stress showed higher sucrose synthase (SS) and sucrose phosphatesynthase (SPS)

activities than those grown under normal temperature i.e., 25°C . However, the enzymatic activities of SS and SPS were further increased by the exogenous application Si and K application. The highest activity of SS and SPS was recorded in the plants treated with Si (100 ppm) (0.94 and 0.29 $\mu\text{mol min}^{-1} \text{mg}^{-1}$), followed by those plants treated with the combined application of Si and K (0.90 and 0.30 $\mu\text{mol min}^{-1} \text{mg}^{-1}$) and individual K (100 ppm) applications (0.74 and 0.23 $\mu\text{mol min}^{-1} \text{mg}^{-1}$), respectively. The plants applied with a lower concentration of K (50 ppm) had the minimum SS and SPS activities but were higher in those activities compared to the control plants (Fig. 5A and B).

3.3.4 Acid Invertase and Neutral Invertase

Plants under freezing stress exhibited higher acid invertase (AI) and neutral invertase (NI) activities than those grown under normal temperatures. The exogenous application of Si and K significantly enhanced the AI and NI activities under freezing stress. The activity of AI and NI (0.83 and 0.18 $\mu\text{mol min}^{-1} \text{mg}^{-1}$, respectively) were measured in plants treated with higher concentrations of Si (100 ppm) under freezing conditions. The plants applied with a blend of Si and K (50 ppm of each) also showed an increase in AI and NI activities (0.86 and 0.18 $\mu\text{mol min}^{-1} \text{mg}^{-1}$) under freezing stress but comparatively less than plants treated

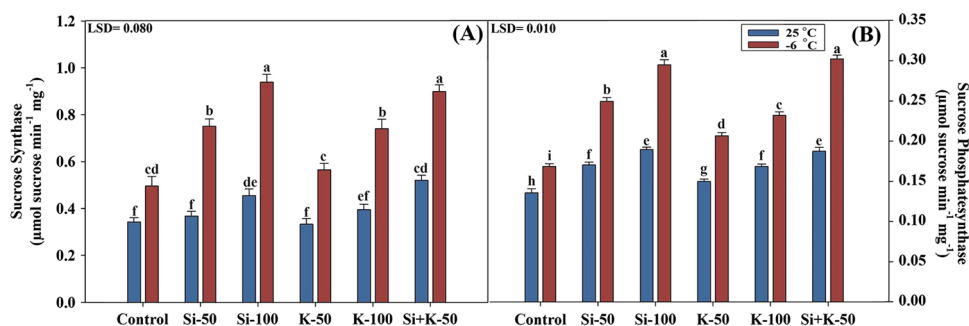


Fig. 5 Effect of Si and K on the enzymatic activity of sucrose synthase (A) and sucrose phosphatesynthase (B) in carbohydrate metabolism of satsuma mandarin under normal and freezing temperatures.

The lower-case letters on graphs represent significant differences using the least significant difference (LSD) at $p < 0.05$, while vertical bars represent the mean standard error ($n = 4$)

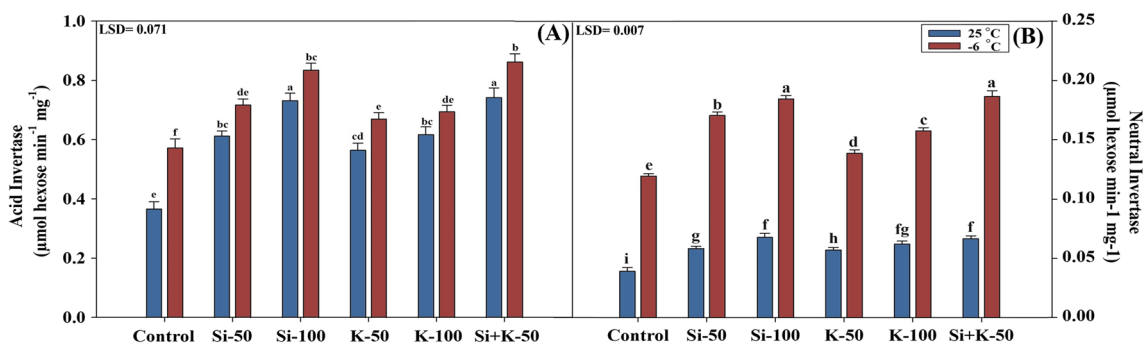


Fig. 6 Effect of Si and K on the enzymatic activity of acid invertase (A) and neutral invertase (B) in carbohydrate metabolism of satsuma mandarin under normal and freezing temperatures. The lower-case

letters on graphs represent significant differences using the least significant difference (LSD) at $p < 0.05$, while vertical bars represent the mean standard error ($n = 4$)

with a higher concentration of Si i.e., 100 ppm. Overall, the application of a higher level of Si (100 ppm) followed by the combined application of Si and K (50 ppm of each) was found to be more effective in improving the activities of AI and NI (Fig. 6A and B).

3.4 Principal Component Analysis and Correlation

Principal component analysis (PCA) was performed to recognize the uncorrelated variables and identify patterns of factors in high-dimensional datasets. The PCA of this study represents the PC1 and PC2 components of the explained variables i.e., 21.4 and 38.3%, respectively. The variables close to each other are correlated, while those far from them are dissimilar (Fig. 7A). The variables starch, PK, FPK, and AI are closer to each other, so they lie in one group, while other variables, such as SP, TSS, HK, glucose, etc. lie together and make one group. So, the variables presented in the PC plot are similar. On the other hand, the correlation plot showed the relationship and their association in the data (Fig. 7B). The correlation analysis showed the degree of correlation among variables and differentiated based on

Pearson's value. The correlation among variables is characterized by color and significance level as $p < 0.05$ (* significant), $p < 0.01$ (** more significant), and $p < 0.001$ (***) highly significant).

4 Discussion

In North Florida and the Gulf Coast states, i.e., Alabama, Georgia, Louisiana, and Mississippi, unexpected severe freezing events in the winter and spring are the most restrictive factors for cold hardy citrus production [60, 61]. The cold hardy citrus industry is emerging in the southeastern United States; however, early and late freeze events threaten successful citrus production. Growers of cold hardy citrus regions continually seek cost-effective methods to enhance the resilience of their groves and minimize damage from freezes. Phytohormones and plant-beneficial nutrients are tools used to improve the cold hardiness in agricultural crops. Silicon (Si) and potassium (K) are reported to be effective in improving plant resilience to different abiotic

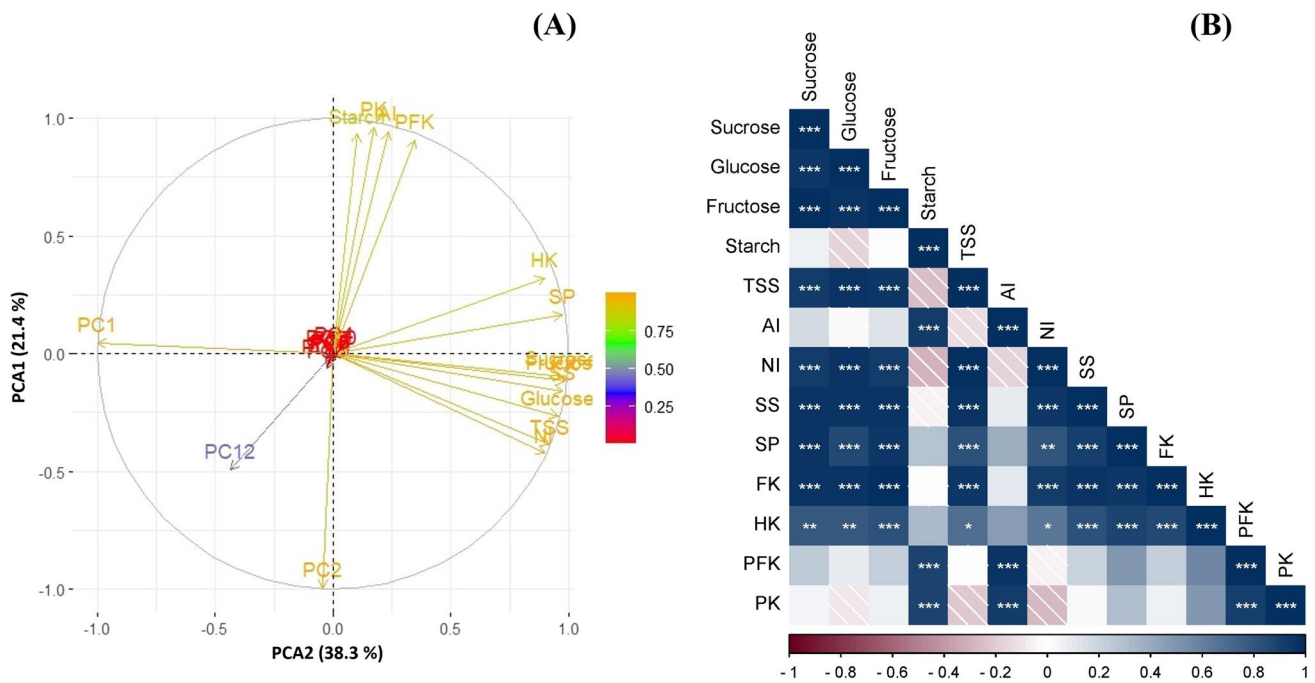


Fig. 7 Principal component analysis (PCA) (A) and correlation analysis (B) of all parameters used in this study of satsuma mandarin under normal and freezing temperatures. The X-axis and Y-axis show the PC1 and PC2 values, respectively. Parameters in cluster showing more closely to each other In the correlation graph, the positive and

negative correlation is shown by the color strip given on the right side of the graph. The asterisk (*) showed the significance level as $p < 0.05$ (* significant), $p < 0.01$ (** more significant), and $p < 0.001$ (***) highly significant)

stresses, including freezing stress [62, 63]. In our study, the exogenous application of Si and K significantly improved plant growth and carbohydrate metabolism, resulting in enhanced photosynthetic activities under freezing stress, which is consistent with the earlier findings in barley [39], maize [64], tomato [65], and beans [66].

Photosynthesis is one of the main processes that significantly contribute to plant growth by providing energy in the form of carbohydrates, which helps the plant regulate several metabolic processes for optimum vegetative and reproductive development [67]. Freezing stress exhibited an adverse effect on photosynthesis in plants [68], as it affects various photosynthetic components like electron transport, carbon and nitrogen metabolism, and translocation [69, 70]. Si has shown a protective role in plant growth, specifically photosynthetic preservation under different abiotic and biotic stress conditions [52, 71, 72] that allow the plants to grow better under adverse conditions. Silicon has been reported to be very effective in alleviating the destructive effect caused by various stress factors on the photosynthetic apparatus in multiple species, including strawberries [73], tomatoes [74], rice [75], and maize [76]. Similarly, potassium (K) also plays a significant role in photosynthesis and carbohydrate metabolism by regulating chlorophyll inflorescence and stomatal conductance [77]. The present study also showed decreased photosynthesis when exposed to freezing stress. In contrast,

applying Si and K helps the plants maintain optimal photosynthetic activity under freezing stress. Our findings align with the previous studies reported in plantain and sugarcane, which described that Si and K improve photosynthetic activity under different biotic and abiotic stress conditions [45, 78]. The findings of this study also provided evidence that the application of Si and K improved the photosynthetic performance of citrus trees under freezing temperatures, which might allow the plants to have a constant supply of assimilates between source and sink, resulting in improved tolerance to freezing temperatures.

Soluble carbohydrates can be easily identified in plants under freezing stress and play an important role in osmoregulation, cryoprotection, and signaling [79]. Sugars function as protective, signaling, and regulatory molecules in plants under various biotic and abiotic stresses. Sucrose is one of the main components in carbohydrate metabolism [80]; maintaining balance among sucrose production, translocation, and usage is important for sustaining normal plant growth. However, the accumulation of soluble sugars such as glucose, fructose, and sucrose can be observed under various environmental stresses [81–83]. Soluble sugars such as sucrose, glucose, and fructose serve as an immediate source of energy for plant metabolism; their concentration in low amounts can lead to decreased growth, reduced stress tolerance, and imbalanced nutritional status [84]. Si showed

beneficial effects under various conditions on plant growth has been extensively reported [85, 86]. The present study indicated that plants under normal conditions had lower levels of soluble sugars while applying Si and/or K improved concentrations of soluble sugars under freezing stress. In the present study, Si and K perform best under freezing stress, helping plants accumulate soluble sugars at optimum levels, resulting in a balanced energy level within plant tissues. These results suggested that Si and K positively affect various enzymatic activities in plants involved in carbohydrate biosynthesis. In addition, a higher concentration of sucrose can promote starch biosynthesis, serve as a main carbohydrate store in higher plants, and can be speedily degraded to release soluble sugars for plant utilization [87, 88]. In this study, under freezing stress, silicon and potassium reduced the starch biosynthesis, thereby decreasing the starch accumulation, suggesting that Si and K improved sugar synthesis from the source.

The activity of acid invertase (AI) and neutral invertase (NI) are important for plant metabolism as they help the plant catalyze the hydrolysis of sucrose into glucose and fructose [89]. Few studies have reported the Si supplementation effect on NI, but it has been reported to alter plant carbohydrate metabolism, potentially impacting invertase activity [90, 91]. In this study, Si and K-mediated plants showed an increase in invertase activity during freezing stress, which helped the plants improve their carbohydrate metabolism and translocate to different plant parts. Similarly, the level of sucrose synthase (SS) and sucrose phosphatesynthase (SPS) also improved with Si and K-mediated plants under freezing stress, indicating that Si has a significant role in mediating carbohydrate metabolism under freezing stress. So, the baseline level of sucrose accumulation is directly associated with higher SPS activity.

Our present results support earlier studies that suggest increased metabolic activities that could enhance the resilience of plants to tolerate adverse stress conditions [9, 92]. In plant metabolic processes, glycolysis is a critical enzymatic reaction that provides energy to various plant parts and is regulated by enzymes like FK, HK, PFK, and PK [93]. In this study, we observed an increase in the enzymes of carbohydrate metabolism in response to Si and K application under freezing stress. Our findings align with other studies confirming the significant role of enzymatic enzymes in improving plant growth and development [94]. The PCA result of this study also showed a similar group between the enzymatic activities and solubles sugars showing their interaction with each other, improving plant internal metabolism and overall growth.

5 Conclusion

Our findings demonstrate the beneficial effect of Si in mitigating the freezing stress on photosynthetic activity and carbohydrate metabolism in citrus plants. Applying both Si and/or K improved the photosynthetic activity, subsequently helping the plants produce soluble sugars to provide energy to plants for optimum growth and development. The exogenous application of Si and K might activate the plant's internal metabolism and effectively trigger its biosynthesis process, thereby improving its ability to tolerate freezing stress. The findings of this study suggest that foliar application of Si (100 ppm) could be an effective and eco-friendly strategy to improve cold hardiness in citrus plants.

Acknowledgements The author acknowledges all participants of this study for their outstanding contributions. Researchers supporting project (RSPD2024R751), King Saud University, Saudi Arabia are also acknowledged.

Author Contributions S.I wrote the manuscript and did data analysis; R.M.B assisted in statistical analysis, M.F.S assisted the graphical work, M.M provided resources, editing, and review, J.M.C. did the statistical analysis, review, and editing, M.A.S. conceptualized the study, data curation, project administration, supervision and had the main responsibility for the final content. All authors have proofread and approved the final draft of the manuscript.

Funding We are grateful to Hatch project FLA-NFC-006212 for supporting this study. The study was funded by researchers supporting the project (RSPD2024R751), King Saud University, Riyadh, Saudia Arabia.

Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing Interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

1. Thomashow MF (1998) Role of cold-responsive genes in plant freezing tolerance. *Plant Physiol* 118(1):1–8
2. Attaway JA (1997) History of Florida citrus freezes

3. Hasanuzzaman M, Gill SS, Fujita M (2012) Physiological role of nitric oxide in plants grown under adverse environmental conditions, Plant acclimation to environmental stress. Springer, pp. 269–322
4. Solanke AU, Sharma AK (2008) Signal transduction during cold stress in plants. *Physiol Mol Biol Plants* 14:69–79
5. Yadav SK (2010) Cold stress tolerance mechanisms in plants. A review. *Agron Sustain Dev* 30(3):515–527
6. Adhikari L, Baral R, Paudel D, Min D, Makaju SO, Poudel HP, Acharya JP, Missaoui AM (2022) Cold stress in plants: Strategies to improve cold tolerance in forage species. *Plant Stress* 4:100081
7. Herrmann HA, Schwartz J-M, Johnson GN (2019) Metabolic acclimation—a key to enhancing photosynthesis in changing environments? *J Exp Bot* 70(12):3043–3056
8. Adam S, Murthy S (2014) Effect of cold stress on photosynthesis of plants and possible protection mechanisms. *Approaches to plant stress and their management* 219–226
9. Chen TH, Murata N (2008) Glycinebetaine: an effective protectant against abiotic stress in plants. *Trends Plant Sci* 13(9):499–505
10. Liu Y, Dang P, Liu L, He C (2019) Cold acclimation by the CBF–COR pathway in a changing climate: Lessons from *Arabidopsis thaliana*. *Plant Cell Rep* 38:511–519
11. Verslues PE, Agarwal M, Katiyar-Agarwal S, Zhu J, Zhu JK (2006) Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *Plant J* 45(4):523–539
12. Wang H, Gong M, Xin H, Tang L, Dai D, Gao Y, Liu C (2018) Effects of chilling stress on the accumulation of soluble sugars and their key enzymes in *Jatropha curcas* seedlings. *Physiol Mol Biol Plants* 24:857–865
13. Olien C, Smith MN (1977) Ice adhesions in relation to freeze stress. *Plant Physiol* 60(4):499–503
14. Demidchik V, Straltsova D, Medvedev SS, Pozhvanov GA, Sokolik A, Yurin V (2014) Stress-induced electrolyte leakage: the role of K⁺-permeable channels and involvement in programmed cell death and metabolic adjustment. *J Exp Bot* 65(5):1259–1270
15. Cao S, Yang Z, Zheng Y (2013) Sugar metabolism in relation to chilling tolerance of loquat fruit. *Food Chem* 136(1):139–143
16. Ruelland E, Vaultier M-N, Zachowski A, Hurry V (2009) Cold signalling and cold acclimation in plants. *Adv Bot Res* 49:35–150
17. Van den Ende W, Valluru R (2009) Sucrose, sucrosyl oligosaccharides, and oxidative stress: scavenging and salvaging? *J Exp Bot* 60(1):9–18
18. Slugina MA, Meleshin AA, Kochieva EZ, Shchennikova AV (2020) The opposite effect of low temperature on the Pho1a starch phosphorylase gene expression in *Solanum tuberosum* L. tubers and *Petota* species leaves. *Am J Potato Res* 97:78–87
19. Sicher R (2011) Carbon partitioning and the impact of starch deficiency on the initial response of *Arabidopsis* to chilling temperatures. *Plant Sci* 181(2):167–176
20. Rosa M, Prado C, Podazza G, Interdonato R, González JA, Hilal M, Prado FE (2009) Soluble sugars: Metabolism, sensing and abiotic stress: A complex network in the life of plants. *Plant Signal Behav* 4(5):388–393
21. Knaupp M, Mishra KB, Nedbal L, Heyer AG (2011) Evidence for a role of raffinose in stabilizing photosystem II during freeze–thaw cycles. *Planta* 234:477–486
22. Miura K, Furumoto T (2013) Cold signaling and cold response in plants. *Int J Mol Sci* 14(3):5312–5337
23. Smeekens S, Ma J, Hanson J, Rolland F (2010) Sugar signals and molecular networks controlling plant growth. *Curr Opin Plant Biol* 13(3):273–278
24. Couée I, Sulmon C, Gouesbet G, El Amrani A (2006) Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. *J Exp Bot* 57(3):449–459
25. Borsani J, Budde CO, Porrini L, Lauxmann MA, Lombardo VA, Murray R, Andreo CS, Drincovich MF, Lara MV (2009) Carbon metabolism of peach fruit after harvest: changes in enzymes involved in organic acid and sugar level modifications. *J Exp Bot* 60(6):1823–1837
26. Lombardo VA, Osorio S, Borsani J, Lauxmann MA, Bustamante CA, Budde CO, Andreo CS, Lara MV, Fernie AR, Drincovich MF (2011) Metabolic profiling during peach fruit development and ripening reveals the metabolic networks that underpin each developmental stage. *Plant Physiol* 157(4):1696–1710
27. Fallahi H, Scofield GN, Badger MR, Chow WS, Furbank RT, Ruan Y-L (2008) Localization of sucrose synthase in developing seed and siliques of *Arabidopsis thaliana* reveals diverse roles for SUS during development. *J Exp Bot* 59(12):3283–3295
28. Ni Z, Zhang Z, Gao Z, Gu L, Huang L (2011) Effects of bagging on sugar metabolism and the activity of sugar metabolism related enzymes during fruit development of Qingzhong loquat. *Afr J Biotech* 10(20):4212–4216
29. Etesami H, Jeong BR (2018) Silicon (Si): Review and future prospects on the action mechanisms in alleviating biotic and abiotic stresses in plants. *Ecotoxicol Environ Saf* 147:881–896
30. Hajiboland R (2022) Silicon-mediated cold stress tolerance in plants, Silicon and Nano-Silicon in Environmental Stress Management and Crop Quality Improvement. Elsevier, pp. 161–180
31. Moradtalab N, Weinmann M, Walker F, Höglinger B, Ludewig U, Neumann G (2018) Silicon improves chilling tolerance during early growth of maize by effects on micronutrient homeostasis and hormonal balances. *Front Plant Sci* 9:342862
32. Ma JF, Miyake Y, Takahashi E (2001) Silicon as a beneficial element for crop plants. *Stud Plant Sci* 8:17–39
33. Chen D, Yin L, Deng X, Wang S (2014) Silicon increases salt tolerance by influencing the two-phase growth response to salinity in wheat (*Triticum aestivum* L.). *Acta Physiol Plant* 36:2531–2535
34. Xie Z, Song R, Shao H, Song F, Xu H, Lu Y (2015) Silicon improves maize photosynthesis in saline-alkaline soils. *Sci World J* 2015
35. Habibi G (2015) Effects of soil-and foliar-applied silicon on the resistance of grapevine plants to freezing stress. *Acta Biologica Szegediensis* 59(2):109–117
36. Gooda A, El-Hamid A, Abo-Hadid A, El-Behari O (2018) Impact of irrigation water management and silicon spraying on avoiding cold injury and improvement yield of kitt mangos. *Arab Univ J Agric Sci* 26(2):501–517
37. Luan H, Niu C, Nie X, Li Y, Wei M (2022) Transcriptome and physiological analysis of rootstock types and silicon affecting cold tolerance of cucumber seedlings. *Plants* 11(3):445
38. Bashir S, John R (2023) Alleviation of chilling stress by supplementation of brassinosteroid and silicon in *Solanum lycopersicum* L. *Plant Soil* 486(1):165–181
39. Joudmand A, Hajiboland R (2019) Silicon mitigates cold stress in barley plants via modifying the activity of apoplasmic enzymes and concentration of metabolites. *Acta Physiol Plant* 41(2):29
40. Fan Q-H, Sun W-C, Li Z-J, Liang Y-C (2009) Effects of silicon on photosynthesis and its major relevant enzyme activities in wheat leaves under short-term cold stress. *J Plant Nutr Fertilizers* 15(3):544–550
41. Rahman MA, Song Y, Hasan MM, Jahan MS, Siddiqui MH, Park HS, Lee S-H, Singh D, Corpas FJ, Kabir A.H (2023) Mechanistic basis of silicon mediated cold stress tolerance in alfalfa (*medicago sativa* L.). *Silicon* 1–13
42. He Y, Xiao H, Wang H, Chen Y, Yu M (2010) Effect of silicon on chilling-induced changes of solutes, antioxidants, and membrane stability in seashore *paspalum* turfgrass. *Acta Physiol Plant* 32:487–494

43. Römheld V, Kirkby EA (2010) Research on potassium in agriculture: needs and prospects. *Plant Soil* 335:155–180
44. Wang M, Zheng Q, Shen Q, Guo S (2013) The critical role of potassium in plant stress response. *Int J Mol Sci* 14(4):7370–7390
45. Ho L-H, Rode R, Siegel M, Reinhardt F, Neuhaus HE, Yvin J-C, Pluchon S, Hosseini SA, Pommerrenig B (2020) Potassium application boosts photosynthesis and sorbitol biosynthesis and accelerates cold acclimation of common plantain (*Plantago major* L.). *Plants* 9(10):1259
46. Cakmak I (2005) The role of potassium in alleviating detrimental effects of abiotic stresses in plants. *J Plant Nutr Soil Sci* 168(4):521–530
47. Waraich EA, Rashid F, Ahmad Z, Ahmad R, Ahmad M (2020) Foliar applied potassium stimulate drought tolerance in canola under water deficit conditions. *J Plant Nutr* 43(13):1923–1934
48. Karimi R (2017) Potassium-induced freezing tolerance is associated with endogenous abscisic acid, polyamines and soluble sugars changes in grapevine. *Sci Hortic* 215:184–194
49. Saadati S, Baninasab B, Mobli M, Gholami M (2021) Foliar application of potassium to improve the freezing tolerance of olive leaves by increasing some osmolyte compounds and antioxidant activity. *Sci Hortic* 276:109765
50. Norozi M, ValizadehKaji B, Karimi R, Solgi M (2020) Potassium and zinc-induced frost tolerance in pistachio flowers is associated with physiological and biochemical changes. *Trees* 34:1021–1032
51. Krasavina MS, Burmistrova NA, Raldugina GN (2014) The role of carbohydrates in plant resistance to abiotic stresses, Emerging technologies and management of crop stress tolerance. Elsevier, pp. 229–270
52. Iqbal S, Hussain M, Sadiq S, Seleiman MF, Sarkhosh A, Chater JM, Shahid MA (2024) Silicon nanoparticles confer hypoxia tolerance in citrus rootstocks by modulating antioxidant activities and carbohydrate metabolism. *Heliyon* 10(1)
53. Chow PS, Landhäusser SM (2004) A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiol* 24(10):1129–1136
54. Dong C-J, Wang X-L, Shang Q-M (2011) Salicylic acid regulates sugar metabolism that confers tolerance to salinity stress in cucumber seedlings. *Sci Hortic* 129(4):629–636
55. Orak HH (2006) Determination of starch and maltose content in some hybrid maize varieties by using HPLC. *Cereal Res Commun* 34:1261–1266
56. Yang J, Zhang J, Wang Z, Zhu Q (2001) Activities of starch hydrolytic enzymes and sucrose-phosphate synthase in the stems of rice subjected to water stress during grain filling. *J Exp Bot* 52(364):2169–2179
57. Ruffy TW Jr, Huber SC (1983) Changes in starch formation and activities of sucrose phosphate synthase and cytoplasmic fructose-1, 6-bisphosphatase in response to source-sink alterations. *Plant Physiol* 72(2):474–480
58. Bird IF, Cornelius MJ, Keys AJ, Whittingham CP (1974) Intracellular site of sucrose synthesis in leaves. *Phytochemistry* 13(1):59–64
59. Zrenner R, Salanoubat M, Willmitzer L, Sonnewald U (1995) Evidence of the crucial role of sucrose synthase for sink strength using transgenic potato plants (*Solanum tuberosum* L.). *Plant Journal* 7(1):97–107
60. Spiers JD, Jenda CA, Farrell BS (2017) History of gulf coast citrus. *HortScience* 52(6):806–813
61. Yelenosky G (1996) An overview of Florida citrus freeze survival, Proceedings-Florida State Horticultural Society. Florida State Horticultural Society, pp. 118–123
62. Mir RA, Bhat BA, Yousuf H, Islam ST, Raza A, Rizvi MA, Charagh S, Albaqami M, Sofi PA, Zargar SM (2022) Multidimensional role of silicon to activate resilient plant growth and to mitigate abiotic stress. *Front Plant Sci* 13:819658
63. Swain R, Sahoo S, Behera M, Rout GR (2023) Instigating prevalent abiotic stress resilience in crop by exogenous application of phytohormones and nutrient. *Front Plant Sci* 14:1104874
64. Xu J, Guo L, Liu L (2022) Exogenous silicon alleviates drought stress in maize by improving growth, photosynthetic and antioxidant metabolism. *Environ Exp Bot* 201:104974
65. Zhang Y, Liang Y, Zhao X, Jin X, Hou L, Shi Y, Ahammed GJ (2019) Silicon compensates phosphorus deficit-induced growth inhibition by improving photosynthetic capacity, antioxidant potential, and nutrient homeostasis in tomato. *Agronomy* 9(11):733
66. Hafez EM, Osman HS, El-Razek UAA, Elbagory M, Omara AE-D, Eid MA, Gawayed SM (2021) Foliar-applied potassium silicate coupled with plant growth-promoting rhizobacteria improves growth, physiology, nutrient uptake and productivity of faba bean (*Vicia faba* L.) irrigated with saline water in salt-affected soil. *Plants* 10(5):894
67. Yamori W, Shikanai T (2016) Physiological functions of cyclic electron transport around photosystem I in sustaining photosynthesis and plant growth. *Annu Rev Plant Biol* 67:81–106
68. Rastogi A, Yadav S, Hussain S, Kataria S, Hajihashemi S, Kumari P, Yang X, Brestic M (2021) Does silicon really matter for the photosynthetic machinery in plants...? *Plant Physiol Biochem* 169:40–48
69. Huner N, Dahal K, Hollis L, Bode R, Krol M, Ivanov AG (2012) Chloroplast redox imbalance governs phenotypic plasticity: the “grand design of photosynthesis” revisited. *Front Plant Sci* 3:34453
70. Kalaji H, Rastogi A, Živčák M, Brestic M, Daszkowska-Golec A, Sitko K, Alsharafa K, Lotfi R, Stypiński P, Samborska I (2018) Prompt chlorophyll fluorescence as a tool for crop phenotyping: an example of barley landraces exposed to various abiotic stress factors. *Photosynthetica* 56(3):953–961
71. Hussain M, Iqbal S, Shafiq M, Balal RM, Chater J, Kadyampakeni D, Alferez F, Sarkhosh A, Shahid MA (2023) Silicon-induced hypoxia tolerance in citrus rootstocks associated with modulation in polyamine metabolism. *Sci Hortic* 318:112118
72. Mostofa MG, Rahman MM, Ansary MMU, Keya SS, Abdelrahman M, Miah MG, Phan Tran L-S (2021) Silicon in mitigation of abiotic stress-induced oxidative damage in plants. *Crit Rev Biotechnol* 41(6):918–934
73. Avestan S, Ghasemnezhad M, Esfahani M, Byrt CS (2019) Application of nano-silicon dioxide improves salt stress tolerance in strawberry plants. *Agronomy* 9(5):246
74. Al-aghabary K, Zhu Z, Shi Q (2005) Influence of silicon supply on chlorophyll content, chlorophyll fluorescence, and antioxidative enzyme activities in tomato plants under salt stress. *J Plant Nutr* 27(12):2101–2115
75. Lal B, Gautam P, Mohanty S, Raja R, Tripathi R, Shahid M, Panda B, Baig M, Rath L, Bhattacharyya P (2015) Combined application of silica and nitrogen alleviates the damage of flooding stress in rice. *Crop Pasture Sci* 66(7):679–688
76. Sayed S, Gadallah M (2014) Effects of silicon on Zea mays plants exposed to water and oxygen deficiency. *Russ J Plant Physiol* 61:460–466
77. Sardans J, Peñuelas J (2021) Potassium control of plant functions: Ecological and agricultural implications. *Plants* 10(2):419
78. Verma KK, Song X-P, Verma CL, Malviya MK, Guo D-J, Rajput VD, Sharma A, Wei K-J, Chen G-L, Solomon S (2021) Predication of photosynthetic leaf gas exchange of sugarcane (*Saccharum* spp) leaves in response to leaf positions to foliar spray of potassium salt of active phosphorus under limited water irrigation. *ACS Omega* 6(3):2396–2409

79. Welling A, Palva ET (2006) Molecular control of cold acclimation in trees. *Physiol Plant* 127(2):167–181
80. Choudhury B, Mitra S, Biswas AK (2010) Regulation of sugar metabolism in rice (*Oryza sativa* L.) seedlings under arsenate toxicity and its improvement by phosphate. *Physiol Mol Biol Plants* 16:59–68
81. Gil R, Boscaiu M, Lull C, Bautista I, Lidón A, Vicente O (2013) Are soluble carbohydrates ecologically relevant for salt tolerance in halophytes? *Funct Plant Biol* 40(9):805–818
82. Richter JA, Erban A, Kopka J, Zörb C (2015) Metabolic contribution to salt stress in two maize hybrids with contrasting resistance. *Plant Sci* 233:107–115
83. Wei W, Li Q-T, Chu Y-N, Reiter RJ, Yu X-M, Zhu D-H, Zhang W-K, Ma B, Lin Q, Zhang J-S (2015) Melatonin enhances plant growth and abiotic stress tolerance in soybean plants. *J Exp Bot* 66(3):695–707
84. Hellmann HA, Smeekens S (2014) Sugar sensing and signaling in plants. *Front Plant Sci* 5:86866
85. Liang Y, Sun W, Zhu Y-G, Christie P (2007) Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. *Environ Pollut* 147(2):422–428
86. Rizwan M, Ali S, Ibrahim M, Farid M, Adrees M, Bhawana SA, Zia-ur-Rehman M, Qayyum MF, Abbas F (2015) Mechanisms of silicon-mediated alleviation of drought and salt stress in plants: a review. *Environ Sci Pollut Res* 22:15416–15431
87. Tetlow IJ, Morell MK, Emes MJ (2004) Recent developments in understanding the regulation of starch metabolism in higher plants. *J Exp Bot* 55(406):2131–2145
88. Yuan Y, Zhong M, Shu S, Du N, He L, Yuan L, Sun J, Guo S (2015) Effects of exogenous putrescine on leaf anatomy and carbohydrate metabolism in cucumber (*Cucumis sativus* L.) under salt stress. *J Plant Growth Regul* 34:451–464
89. Sturm A (1999) Invertases. Primary structures, functions, and roles in plant development and sucrose partitioning. *Plant physiology* 121(1):1–8
90. Liang Y, Nikolic M, Bélanger R, Gong H, Song A (2015) Silicon in agriculture. LIANG, Y. et al. Silicon-mediated tolerance to salt stress. Springer Science 123–142
91. Ma JF, Yamaji N (2006) Silicon uptake and accumulation in higher plants. *Trends Plant Sci* 11(8):392–397
92. Gupta AK, Kaur N (2005) Sugar signalling and gene expression in relation to carbohydrate metabolism under abiotic stresses in plants. *J Biosci* 30(5):761–776
93. Dhal S, Pal H (2022) Regulation of Glycolysis and Krebs Cycle during Biotic and Abiotic Stresses, Photosynthesis and Respiratory Cycles during Environmental Stress Response in Plants. Apple Academic Press, pp. 263–308
94. Vega M, Riera A, Fernández-Cid A, Herrero P, Moreno F (2016) Hexokinase 2 is an intracellular glucose sensor of yeast cells that maintains the structure and activity of Mig1 protein repressor complex. *J Biol Chem* 291(14):7267–7285

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.