

Article

Exogenous Silicon Application Improves Chilling Injury Tolerance and Photosynthetic Performance of Citrus

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Abstract: Low-temperature stress is an important limiting factor affecting citrus growth and fruit yields. Therefore, increasing citrus cold stress tolerance may enhance the growth, yield, and quality of citrus production in marginal areas. The objective of this study was to determine the efficacy of silicon (Si) fertilizer application on cold-tolerance enhancement in citrus. Two citrus cultivars (Delta and Nules) were subjected to Si fertilization (control, 1000 mg L⁻¹) and cold-stress temperature treatments (control and 0 °C for 72 h) using a 2 × 2 factorial treatment structure with six replications. Leaf gas exchange and chlorophyll fluorescence parameters, such as net photosynthetic rate (A), stomatal conductance (g_s), transpiration rate (Tr), internal CO₂ concentration (C_i), intrinsic water-use efficiency (iWUE), minimal fluorescence (F_o), maximum fluorescence (F_m), maximum quantum efficiency of PSII primary photochemistry of dark-adapted leaves (F_v/F_m), maximum quantum efficiency of PSII primary photochemistry of dark-/light-adapted leaves (F'_v/F'_m), electron transport rate (ETR), non-photochemical quenching (NPQ), and the relative measure of electron transport to oxygen molecules (ETR/A), were measured. The application of Si drenching to trees that were subsequently exposed to cold stress reduced g_s, Tr, and C_i but improved iWUE and F_o in both cultivars compared to the Si-untreated trees. In addition, specific adaptation mechanisms were found in the two citrus species; NPQ and ETR were improved in Si-treated Valencia trees, while A, F_m, and ETR/A were improved in Clementine trees under chilling stress conditions. The current research findings indicate the potential of Si application to enhance cold stress tolerance in citrus, which can provide a strategy for growing citrus in arid and semi-arid regions that may experience cold stress. Overall, after the application of silicon drenching, the cold-sensitive citrus Valencia cultivar became as cold-tolerant as the cold-tolerant Clementine cultivar.

Keywords: abiotic stress; citrus; chlorophyll fluorescence; cold tolerance; physiological performance



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1. Introduction

Silicon (Si) is a non-essential element in higher plants, but it is known to be involved in the growth and developmental processes of plants subjected to biotic (e.g., diseases) and abiotic (e.g., heat, drought, and low temperature) stresses [1–3]. The mechanisms for Si-mediated increases in tolerance to drought and low-temperature stresses include physiological, biochemical, and physical aspects. These include promoting photosynthetic enzymatic activities, photochemical efficiency, and the photosynthetic rate, maintaining

nutrient balance, and improving water retention by decreasing water loss from leaves and increasing water uptake by roots [4].

Numerous studies have demonstrated that fertilizing plants with Si mitigates chilling stress and reduces frost damage in both Si accumulator and non-accumulator crops [5]. For example, in wheat (*Triticum aestivum* L.), the photosynthetic rate and water-use efficiency were significantly inhibited under cold stress but improved after Si application [6]. Similarly, Si application improved the stomatal conductance and photosynthetic rate of bean plants (*Phaseolus vulgaris* L.) under saline stress conditions [7]. In a study conducted on cucumber (*Cucumis sativum* L.) seedlings under osmotic stress, Si application significantly improved the photosynthetic rate [8]. Ma et al. [9] also demonstrated that the application of Si to cucumber plants under drought stress enhanced their photosynthetic rate and significantly reduced their transpiration rate and stomatal conductance.

Other studies have reported that Si application significantly increased the water content and dry mass of tomato (*Solanum lycopersicum* L.) plants grown under saline conditions [10]. Similarly, exogenous Si application improved the leaf water potential in rice (*Oryza sativa* L.) and wheat under drought-stressed conditions [11,12]. Si improves leaf water potential by reducing water loss due to transpiration because most of the silicon is deposited in the outer walls of the epidermal cells on both sides of the plant leaf. This effectively forms a silicon-enriched cuticle in a double layer, which reduces the transpiration rate [12].

Citrus is an important fruit crop that is grown commercially in more than 100 countries [13]. The crop thrives in varied climatic zones, including tropical, subtropical, arid, and semi-arid zones. The optimal temperature for efficient leaf photosynthesis in citrus is between 25 and 30 °C [14]. Subtropical and tropical crops, including citrus, are prone to low-temperature stress [15]. Chilling stress is one of the most important limiting factors for citrus fruit production [16,17]. Low temperatures also affect flower induction, resulting in reduced fruit yield and quality [18,19]. This affects the revenue of citrus producers because the market price of fruit is dependent on its quantity and quality [20]. Si fertilization of citrus plants has been documented to cause a significant increase in the root and shoot masses of germinating grapefruit seedlings (*Citrus paradisi*) subjected to cold stress because of the impact of Si fertilization on cold stress tolerance [21]. Other studies have shown that Si fertilization applied to young citrus trees improved plant height, branching, and fruit yield [22,23]. The effects of Si application on physiological processes linked to low-temperature stress tolerance are not well understood and have been sparsely investigated in citrus. However, the application of Si may be a viable and cost-effective approach for boosting citrus production in frost-prone regions [24].

Major determining factors for the location of citrus production are low temperatures and frost due to the sensitivity of citrus to cold stress. Semi-arid and arid production areas, such as the Nile Delta region in Egypt, Florida and California in the USA, Valencia in Spain, Sicily in Italy, the Cukurova region in Turkey, and the Hartvaals area of the Northern Cape Province of South Africa, represent the majority of citrus producers. These regions are ideal for citrus production due to the cool night temperatures and high relative humidity [15–17]. However, these areas are also prone to low-temperature stress events. Therefore, the ability to mitigate low-temperature stress would enhance citrus production and reduce the risk of frost damage associated with citrus in these regions.

Chlorophyll fluorescence is a widely used tool for determining the activity of the photosynthetic apparatus of plants, and it allows for the assessment of photo-protection mechanisms involved in plant responses under abiotic and biotic stresses [25–29]. Parameters such as the maximum quantum efficiency of PSII primary photochemistry (F_v/F_m), qP (photochemical quenching), non-photochemical quenching (NPQ), stomatal conductance (g_s), transpiration rate (T), photosynthetic rate, and intercellular CO₂ concentration (C_i) are important indicators of photosynthetic performance in crops subjected to stress factors [25,30–32]. Analysis of leaf gas exchange and chlorophyll fluorescence can aid in the identification and selection of cultivars with tolerance to biotic and abiotic

stresses [26,27,30,33,34]. The objective of this study was to determine the efficacy of the application of silicon (Si) fertilizer on cold-tolerance enhancement in citrus.

2. Materials and Methods

2.1. Plant Material

Two citrus cultivars, Valencia 'Delta' (*Citrus sinensis* L.) and Clementine 'Nules' (*Citrus reticulata* L.), were used for the study. These citrus cultivars are widely cultivated on a commercial scale in South Africa. These two cultivars were selected for the study to represent two genetically distinct citrus species, namely, orange and soft citrus (mandarins) [35].

2.2. Determination of Root Si Application Rates

A preliminary experiment was conducted under glasshouse conditions at the Experimental Farm of the University of Pretoria (25°44' S, 28°15' E). The aim of this experiment was to determine the optimal Si application rates for citrus trees for subsequent use in chilling stress studies. The experiment was performed on two-year-old 'Delta' Valencia oranges and 'Nules' Clementine trees. The two cultivars were grafted onto Carrizo citrange rootstock by DuRoi Nursery, Limpopo, South Africa (23.8625° S, 30.3837° E). The trees were grown in 10 L polyethylene pots. Potassium silicate solution was applied as a drench fortnightly over a three-month period at 0, 100, 500, 1000, and 2000 mg L⁻¹ of Si, with four replications and 3 trees per replicate. The optimal Si application rate was determined to be 1000 mg L⁻¹ [35,36] and was selected for the subsequent experiment (i.e., the field trial).

2.3. Chilling Injury Experiment

An artificial chilling injury treatment was applied using a cold chamber situated at the Rosaly Commercial Farm, near Krugersdorp. After 3 months of Si application through drenching (i.e., 0 and 1000 mg L⁻¹), the young citrus trees were subjected to chilling stress at 0 °C for 72 h. Non-stressed trees were kept in the glasshouse conditions at ±25 °C for the whole year and served as the control. Following the chilling treatment, the trees were kept in the open for three weeks before being measured for a range of leaf gas exchange and chlorophyll fluorescence parameters.

The experiment was conducted with a 2 × 2 × 2 factorial treatment structure, with two-year-old citrus cultivars that were approximately 2 m tall (Nules and Delta) and Si concentrations represented by the control and 1000 mg L⁻¹. The spacing for citrus orchards in South Africa for orange and clementine is typically 3 m × 6 m, resulting in 555 plants per ha. One liter of potassium silicate was applied per plant at a Si concentration of 0.55 kg ha⁻¹. The chilling stress (control, cold stress) was used as a treatment factor. The 8 treatment combinations were organized in a completely randomized design (CRD) with 6 replications and three trees per replicate, resulting in a total of 144 experimental units.

3. Data Collection

3.1. Leaf Gas Exchange Measurements

Leaf gas exchange measurements were performed on three fully expanded leaves per tree (6 trees/treatments) using a field-portable LICOR 6400-40 leaf chamber pulse-amplitude-modulated fluorometer attached to the gas analyzer sensor head of the infrared LICOR-6400 XT (LI-COR Inc., Lincoln, NE, USA). The portable photosynthesis system was fitted with an LED (light-emitting diode) light source that used mixed LEDs to deliver both red and blue light to leaves in the chamber. The CO₂ concentration of the reference air entering the leaf chamber was adjusted with a CO₂ mixer control unit such that the "sample" air entering the chamber contained 400 μmol CO₂ mol⁻¹. This resulted in a CO₂ concentration in the reference air of approximately 400 μmol CO₂ mol⁻¹. The flow rate of H₂O was set to 500 μmol m⁻² s⁻¹. The quantum flux density was adjusted according to daily ambient conditions and was fixed at 1000 μmol m⁻² s⁻¹. The leaf temperature and relative humidity in the chamber were set to 25 °C and 50%, respectively. The following leaf gas exchange parameters were measured: stomatal conductance (g_s), intercellular CO₂

concentration (C_i), transpiration (E), and net photosynthesis assimilation rate (A) [31,32]. Intrinsic water-use efficiency (iWUE) was measured as the ratio of A and g_s [37].

3.2. Chlorophyll Fluorescence Measurements

Chlorophyll fluorescence parameters were also determined using a LICOR-6400 XT Portable Photosynthesis System fitted with a chamber pulse-amplitude-modulated fluorometer (6400-40). Four leaves from each tree were dark-adapted for 30 min by covering the leaves with aluminum foil. The aluminum foil was then removed, and the leaves were exposed to a 0.8 s light flash. Following this, the dark-adapted leaves were light-adapted for one hour, and the leaves were exposed to actinic illumination for 6 s to excite PSI and force the electron to drain from PSII. Photosystem II activity was measured with the following parameters: the minimal level of fluorescence F_o was obtained with low-intensity-modulated light. The value of F_m was obtained with 6 s pulses of saturating light, the maximum quantum efficiency of the primary photochemistry of dark-adapted (F_v/F_m) and light adapted (F'_v/F'_m) leaves in photosystem II, the electron transport rate (ETR), and non-photochemical quenching (NPQ) [31,38]. The relative measure of electron transport to oxygen molecules was determined by ETR/A [39].

4. Statistical Analysis

Analysis of variance was performed using R Version 4.2.1 (R Core Team, 2018). Differences between treatments were determined using Fisher's least significant difference (LSD) at a 5% level of significance.

5. Results

5.1. Summary of the Analysis of Variance

The ANOVA for evaluated gas exchange and chlorophyll fluorescence parameters indicated that the effect of chilling stress was significant in most parameters: A, Cond, Tr, C_i , F'_v/F'_m , F_v/F_m , and F_o (Table 1). All leaf gas exchange parameters indicated a significant chilling stress and Si interaction except for C_i . Meanwhile, only F_o indicated a significant interaction with respect to chilling stress and Si in chlorophyll fluorescence parameters (Table 1). Variety type significantly influenced the following leaf gas exchange parameters A, Cond and Tr. In chlorophyll fluorescence parameters F_o and F'_m were significantly influenced by the variety.

The interaction between chilling stress, variety and Si was significant in the following gaseous exchange parameters: A, Cond and iWUE. Meanwhile, in chlorophyll fluorescence parameters only F'_m demonstrated a significant interaction between chilling stress, variety and Si.

5.2. Effect of Si Drenching on the Photosynthetic Performance of Citrus Trees after a Chilling Temperature Stress of 0 °C

5.2.1. Changes in Chlorophyll Fluorescence Parameters in Response to Chilling Stress and Si Drenching

The effective quantum yield of light-adapted Valencia leaves (F'_v/F'_m) did not improve in Si-treated trees compared to Si-untreated trees, regardless of the growing conditions. Overall, the interaction between chilling stress and high-light conditions resulted in a considerable reduction in F'_v/F'_m . With the chilling stress at 0 °C, the F'_v/F'_m values of Si-treated trees showed a negligible difference from Si-untreated trees (Figure 1A).

The maximum quantum yield of dark-adapted Valencia leaves (F_v/F_m) was significantly reduced in Si-treated trees (0.719) compared to Si-untreated trees (0.862) after the chilling stress at 0 °C (Figure 1B). A non-significant difference was observed in the Clementine F_v/F_m value for Si-treated trees compared to Si-untreated trees after the chilling stress at 0 °C (Figure 1B).

With respect to the chlorophyll fluorescence parameters after the chilling stress at 0 °C, the level of F_o in Si-treated Valencia trees was 27% higher than that in Si-untreated

trees (Figure 1C). After the chilling stress at 0 °C, the F_o values of Clementine trees were significantly larger than those after Si drenching (1175.86) compared to those of the Si-untreated trees (409) (Figure 1C).

The minimal fluorescence (F_m) of Valencia leaves was reduced by 44% in Si-treated trees compared to Si-untreated trees after the chilling stress at 0 °C (Figure 1D), whereas in Clementine, the F_m value of Si-untreated trees was reduced by 30% under stressed conditions compared to non-stressed conditions (Figure 1D). The value of F_m for Clementine was significantly higher in Si-treated trees (4291) than in Si-untreated (1875) trees after the chilling stress at 0 °C (Figure 1D & Table 1).

The NPQ of Valencia was reduced by 24% in Si-drenched trees compared to Si-untreated trees after the chilling stress at 0 °C (Figure 2A). The non-photochemical quenching (NPQ) of Clementine leaves under non-stressed conditions was reduced by 99% in Si-treated trees compared to that in Si-untreated trees. However, the NPQ values of Si-treated Clementine trees did not provide evidence of a positive effect of the Si treatment after chilling stress conditions.

Table 1. Summary of the analysis of variance showing mean squares and significant tests for leaf gas exchange and chlorophyll fluorescence parameters in two citrus varieties in the Si treatment subjected to chilling stress.

Leaf Gas Exchange Measurements						
Source of Variation	df	A	Cond	Tr	C_i	iWUE
Time	1	3.9411 ***	0.0586 ***	0.4995 NS	1437886 ***	0.1806 NS
Variety	1	0.0307 *	0.0153 ***	1.1836 **	5094 NS	0.0948 NS
Si	1	0.0253 NS	0.0281 ***	1.6567 ***	116591 NS	0.4679 *
Time × Variety	1	0.0284 *	0.0305	3.2384 ***	20014 NS	0.7024 **
Time × Si	1	0.032 *	0.0305 ***	2.2549 ***	185324 NS	0.3782 *
Variety × Si	1	0.0073 NS	0.0183 ***	0.1378 NS	65471 *	0.2821 *
Time × Variety × Si	1	0.0044 *	0.0198 ***	0.1171 NS	17197 NS	0.4193 **
Residual	35	0.228	0.001	0.1234	33019	0.0555
Lsd Value	-	0.0473	0.0189	0.2058	106.49	0.138
CV Value	-	7.3	46	34	63	17
p Value	-	0.01322	0.000106	0.00014	0.02348	0.009
Chlorophyll Fluorescence Measurements						
Source of Variation	F'_v/F'_m	F_v/F_m	F_o	F'_m	NPQ	ETR
Time	0.0485 *	0.195 ***	440740 **	89612 NS	0.005 NS	2719 NS
Variety	0.0091 NS	0.0007 NS	577593 **	3101270 *	0.0668 NS	1762 NS
Si	0.0060 NS	0.00068 NS	290549 *	1662320 NS	0.032 NS	
Time × Variety	0.0017 NS	0.0009 NS	198419 NS	2535637 *	0.0019 NS	2079 NS
Time × Si	0.019 NS	0.0026 NS	499401 **	1334069 NS	0.0204 NS	6861 NS
Variety × Si	0.0096 NS	0.0028 NS	204565 NS	4275174 **	0.0023 NS	714 NS
Time × Variety × Si	0.0082 NS	0.0005 NS	220400 NS	5391827 **	0.0207 NS	72197 NS
Residual	0.0085	0.0051 NS	53834	468052	0.0262	7080 **
Lsd Value	0.135	0.053	170.594	503.018	0.168	61.64
CV Value	11.27	11.075	50	28	51	34.33
p Value	0.0883	0.0094	0.788	0.0052	0.2967	0.8822

*, ** and *** denote significance at the 5, 1% probability levels and highly significant at 1%, respectively. NS, non-significant.

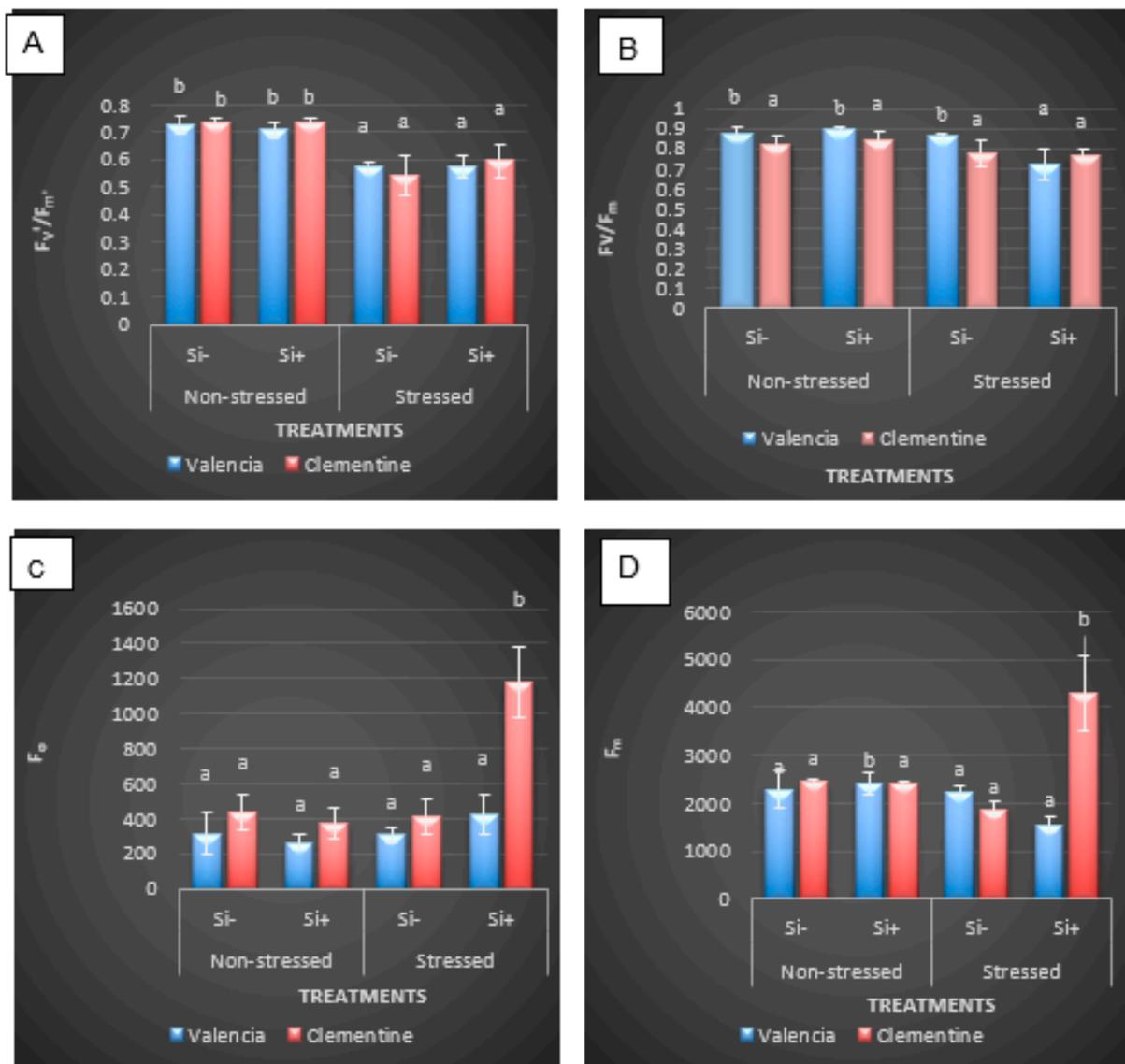


Figure 1. The effect of Si treatments (Si+ (1000 mg L⁻¹) and Si- (0 mg L⁻¹)) on (A) the effective quantum yield of photosystem II, (B) the maximum quantum yield of the efficiency of photosystem II, (C) the minimum fluorescence of dark-adapted leaves, and (D) the maximum fluorescence of dark-adapted leaves of ‘Valencia’ and ‘Clementine’ after chilling injury stress at 0 °C. Data are means ± standard errors. Error bars sharing a letter are not significantly different.

The electron transport rate (ETR) of Valencia leaves in Si-untreated trees was reduced by 23.4% under stressed conditions compared to that under non-stressed conditions (Figure 2B). The ETR was increased by 21% in Si-treated Valencia trees compared to the control trees after the chilling stress at 0 °C (Figure 2B). This could be linked to the reduction in heat dissipation (NPQ) of 24% in Si-treated Valencia trees subjected to chilling stress at 0 °C (Figure 2A). The ETR of Si-treated Valencia trees was increased by 26% after chilling stress compared to that in the non-stressed conditions. The electron transport rate (ETR) of Clementine leaves subjected to Si treatment was reduced by 35% after the chilling stress at 0 °C compared to that in non-stressed conditions.

In trees of both cultivars exposed to chilling stress at 0 °C, the increase in photorespiration (ETR/A) could be linked to a reduction in the carbon assimilation rate (A) (Figure 2C). The ETR/A of Si-treated Valencia trees was significantly reduced compared to that of Si-untreated trees after a chilling stress at 0 °C (Figure 2C). With Clementine trees, the ETR/A

was also significantly reduced in Si-treated trees (21.61) compared to that in Si-untreated trees (56.84) after the chilling stress at 0 °C (Figure 2C). This reduction could be linked to the increase in A of 51% in Si-treated Clementine trees subjected to chilling stress at 0 °C (Figure 3).

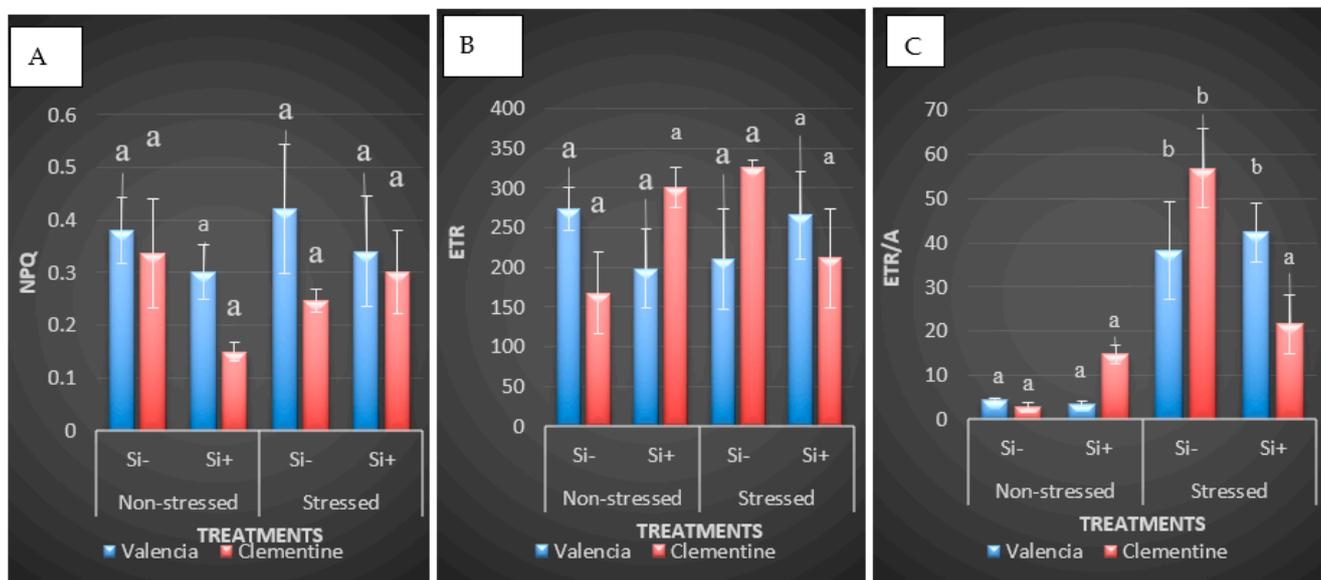


Figure 2. The effect of Si treatments (Si+ (1000 mg L⁻¹) and Si- (0 mg L⁻¹)) on (A) non-photochemical quenching, (B) electron transport rate, and (C) photorespiration (ETR/A) of ‘Valencia’ and ‘Clementine’ leaves during chilling injury stress at 0 °C. Data are means ± standard errors. Error bars sharing a letter are not significantly different.

5.2.2. Changes in Leaf Gas Exchange in Response to Chilling Stress and Si Drenching

The leaf gas exchange parameters of citrus cultivars treated with Si drenching after chilling stress of 0 °C are shown in Figure 3. The photosynthetic rate (A) was significantly higher in non-stressed trees than in chilling-stressed trees in both citrus cultivars (Figure 3A). In Clementine trees, a significant increase in A was observed in Si-treated trees (9.6 μmol CO₂ m⁻²s⁻¹) compared to Si-untreated trees (7.7 μmol CO₂ m⁻²s⁻¹) under chilling stress conditions. Clementine trees had a significantly higher photosynthetic rate than that of Valencia trees under chilling stress. Under chilling stress, the photosynthetic rate of Si-treated Clementine trees was significantly higher than that of Si-untreated trees (Figure 3A).

The stomatal conductance was significantly reduced in Si-treated Valencia trees (0.0574 mol H₂O m⁻²s⁻¹) compared to that in non-Si-treated trees (0.236 mol H₂O m⁻²s⁻¹) under optimal conditions (Figure 3B). Similarly, after chilling stress, the stomatal conductance was reduced in Valencia trees after Si treatment (0.0281 mol H₂O m⁻²s⁻¹) compared to that in non-Si-treated trees (0.0246 mol H₂O m⁻²s⁻¹). A reduction in stomatal conductance was observed in Clementine trees after Si treatment compared to that in non-Si-treated trees under optimal conditions and chilling stress.

The transpiration rate (Tr) was reduced in Valencia trees for both Si-treated and Si-untreated trees subjected to chilling stress compared to those in non-stressed conditions (Figure 3C). In addition, there was a slight increase in the transpiration rate in Si-treated trees compared to that in non-Si-treated trees under chilling stress. In Clementine trees, Tr was significantly reduced in the Si-drenched trees relative to that in the Si-untreated trees under optimal growth conditions (Figure 3C). Meanwhile, a considerable increase in Tr was observed in Si-drenched trees compared to that in Si-untreated trees after chilling stress.

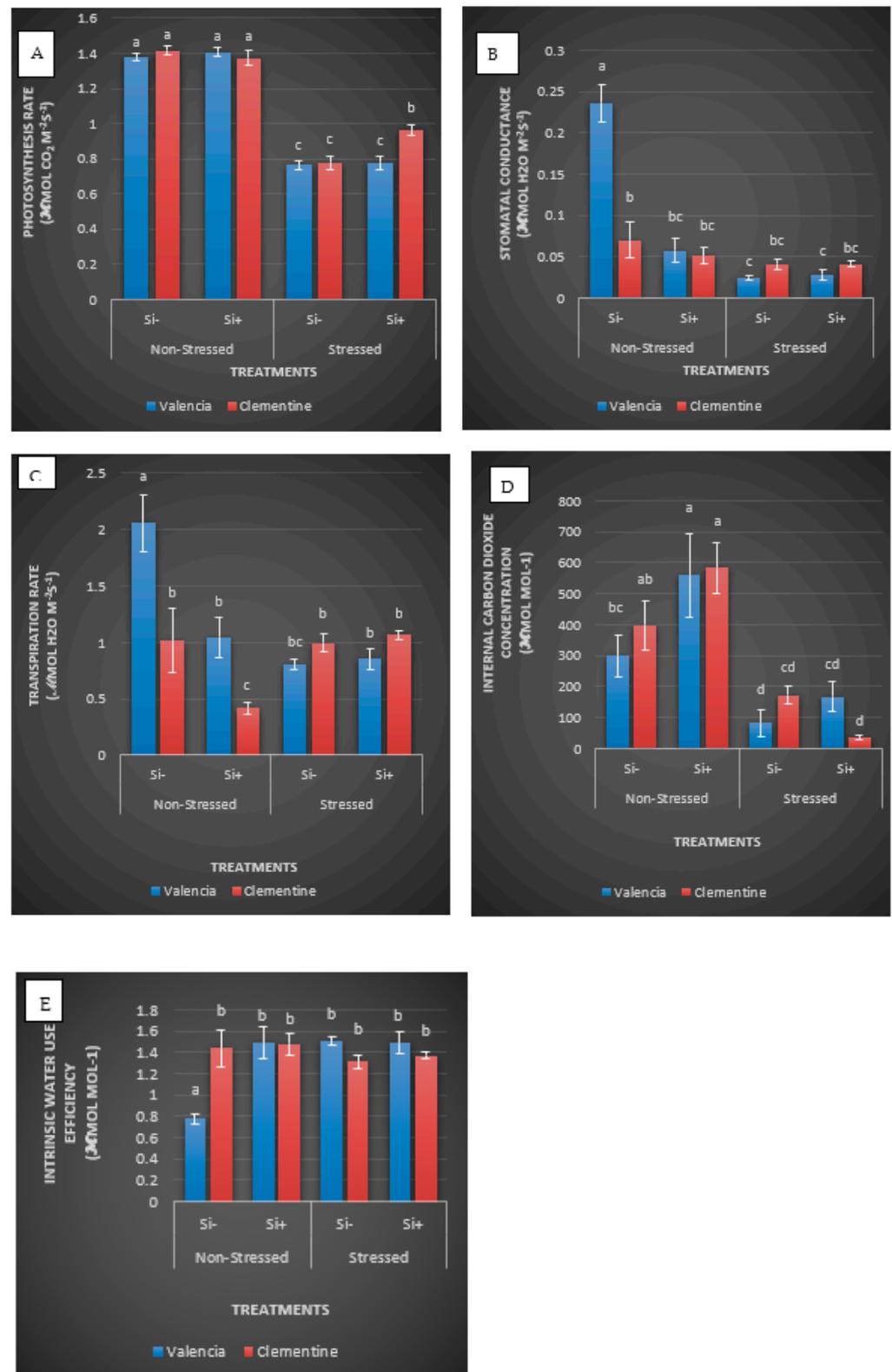


Figure 3. The effect of Si treatments (Si+ (1000 mg L^{-1}) and Si- (0 mg L^{-1})) on (A) the photosynthetic rate, (B) the stomatal conductance, (C) the transpiration rate, (D) the internal carbon dioxide concentration, and (E) the intrinsic water-use efficiency of ‘Valencia’ and ‘Clementine’ leaves after chilling injury stress at 0°C . Data are means \pm standard errors. Error bars sharing a letter are not significantly different.

The intercellular CO₂ concentration (C_i) of Valencia trees was significantly higher ($559 \mu\text{mol mol}^{-1}$) after Si application when subjected to non-stressed conditions as opposed to chilling stress conditions ($167 \mu\text{mol mol}^{-1}$) (Figure 3D). The intercellular CO₂ concentration was increased by 105% after exposure to chilling stress in Si-treated trees compared to that in Si-untreated Valencia trees. In contrast, a considerable reduction of 80% in C_i was observed in Clementine trees subjected to Si drenching compared to the control under chilling stress (Figure 3D). In both cultivars, the C_i responses followed a similar trend to that of g_s , whereby reductions compared to the response in non-stressed conditions were observed in Si-drenched and Si-untreated trees subjected to chilling stress. Overall, a similar trend of reduction in A was observed in g_s and Tr regarding chilling-stressed trees compared to the non-stressed trees in both cultivars. Another trend observed was an overall reduction in g_s , Tr, and A in Si-drenched trees compared to those in Si-untreated trees, regardless of their chilling stress status.

In Valencia trees, a significant increase in intrinsic water-use efficiency (iWUE) was observed in trees exposed to chilling stress compared to that in non-stressed trees, regardless of Si status (Figure 3E). In contrast, in Clementine trees, a reduction in iWUE was observed in trees exposed to chilling stress compared to that in non-stressed trees, regardless of Si status (Figure 3E). Additionally, there was an increase in iWUE in both cultivars after Si drenching treatment compared to the control, irrespective of the chilling conditions (Figure 3E). The improvement in iWUE in Si-treated trees subjected to chilling stressed could be linked to the increase in A (photosynthetic rate) in Si-treated trees subjected to chilling stress.

6. Discussion

Citrus is an important horticultural commodity requiring optimum temperatures between 25 and 30 °C for maximum fruit set and yield potential. However, in recent years, climate change has caused extended durations of winter months characterized by cold temperatures. For example, in major citrus-growing areas of South Africa, the months of August and September have recently been characterized by low temperatures, which have impacted flowering, growth, fruit set, and yield [21]. Therefore, the development of cold stress management strategies for enhancing the fruit yield of citrus is essential. The present study determined the efficacy of silicon application for enhancing cold-stress tolerance in citrus through leaf gas exchange and chlorophyll fluorescence measurements (Table 1). Si application is known to improve photosynthesis and gas exchange in plants subjected to abiotic stresses, such as cold, drought, and heat [25,26,40,41]. The maximum quantum yield of photosystem II (F'_v/F'_m and F_v/F_m) is indicative of the improved photosynthetic capacity of a plant [28], suggesting that Si improved the photosynthetic capacity of Valencia trees, as observed in the present study (Figure 1A). The maximum quantum yield of photosystem II (F_v/F_m) under chilling stress conditions was in the normal range, albeit with a reduction (Figure 1B), suggesting that Si application promoted a more robust photoprotective mechanism and a healthy photosynthetic apparatus [42].

The considerable reduction in photosystem II efficiency (F'_v/F'_m) in both cultivars under low-temperature stress alone or after Si application was due to the reduction in electron transport efficiency, which led to photoinhibition, and this was more pronounced in the presence of high light intensity [43]. Si application failed to improve the efficiency of photosystem II under low-temperature stress in both cultivars (Figure 1 A,B). The possibility exists that the expected reduction in reactive oxygen species in Si-treated trees was masked due to the plants' inherent ability to increase their antioxidant activities under low-temperature stress conditions [44,45].

Photoinhibition has been linked to an increase in F_0 in stressed plants [25,46]. The increase in F_0 in Si-treated trees was observed in both cultivars under chilling stress (Figure 1C), which implied that photoinhibition was employed as a protective mechanism during chilling stress [41,47].

The maximal fluorescence (F_m) level is defined as the level of fluorescence reached after the application of a high-intensity flash, and its increase is linked to reduced heat dissipation [25,31]. The reduction in F_m by 30% observed in Si-untreated Clementine trees subjected to chilling stress at 0 °C could have been due to the protein deactivation in the chloroplast structure [25]. However, Si application triggered an increase in F_m due to the reduction in heat dissipation, which led to an improvement in the efficiency of photosystem II in chilling-stressed plants [28,30].

Plants have developed several protective mechanisms, including non-photochemical quenching (NPQ), which quench the excitation of chlorophyll within the light-harvesting structure of PSII by converting excitation energy into thermal energy, which is dissipated as heat and protects the system from photodamage [48]. The current study demonstrated that the moderate increase in NPQ in Si-treated Valencia trees was associated with improved photoprotection after exposure to cold stress (Figure 2A). This corroborates findings in wheat plants that demonstrated Si-supplied plants experienced reduced energy dissipation [49]. Contrastingly, in Clementine trees, Si application failed to induce photoprotection under chilling stress conditions.

The electron transport rate (ETR) is an indication of the capacity of a plant to protect the PSII reaction centers from oxidative damage [50]. In the present study, Si application reduced free radical development, which typically occurs under stress conditions [51]. On the other hand, the reduced ETR observed under chilling stress in Valencia trees could be linked to stomatal closure, which triggered an increase in NPQ as a photoprotective mechanism for the avoidance of the over-excitation of PSII against photoinhibition [28,52]. On the contrary, Si application failed to improve the ETR efficiency in Clementine trees subjected to chilling stress. The differences in the ETR and NPQ responses between the two cultivars can be explained by the fact that plant tolerance to chilling injuries varies greatly between species [53]. Another explanation is the existence of specific physiological and biochemical responses to photoinhibition under stress conditions in each citrus species [54].

Chilling injury/cold stress result in a reduction in CO₂ fixation [55]. This was also observed in another study conducted on bottle gourd (*Lagenaria siceraria* (Molina) Standl.), which demonstrated a reduction in the CO₂ assimilation rate under water stress conditions [55]. In the present study, the observed improved photosynthetic activity in Si-treated Clementine trees after chilling stress was linked to an improvement in iWUE [9,56].

The reduction in stomatal conductance due to chilling injury in the present study concurred with the findings of Ribeiro et al. [28], who reported a significant reduction in stomatal conductance under low-temperature stress due to stomatal closure. This can be explained by the reduction in CO₂ availability for Rubisco synthesis because of reduced stomatal conductance, which subsequently leads to a reduction in the photosynthetic capacity of citrus trees under chilling stress [57,58]. The reduction in stomatal conductance observed in the current study after exposure to chilling stress and Si treatment agreed with the findings of Lobato et al. [59], who reported a reduction in stomatal conductance in sweet pepper (*Capsicum annum* L.) under water stress conditions despite the application of Si as an adaptation mechanism. Similarly, a reduction in stomatal conductance attributed to Si application was reportedly due to the thickening of the cuticle layer in maize (*Zea mays* L.) [42,60]. Moreover, Hussain et al. [61] substantiated that chilling stress results in a reduction in the CO₂ assimilation rate and a reduction in stomatal conductance, which would lead to a disruption in photosynthesis and electron transport through the thylakoid membrane.

The reduction in the transpiration rate of trees under chilling stress that occurred in both cultivars suggested that there is a mechanism for protecting citrus leaves from photo-damage [58]. The reduction in the transpiration rate in Valencia trees after Si application could be attributed to the deposition of Si around the cell walls, the formation of silica bodies, and the thickening of the cuticle layer [60,62,63]. Transpiration from the leaves of some plants can be considerably reduced through Si application [64–67]. This effect has

been explained by the development of a layer of silica gel associated with the cellulose in epidermal cell walls [68].

On the other hand, the increase in transpiration observed in Si-treated Clementine trees after chilling stress could be attributable to the improvement in leaf water status via increased water uptake [61]. This could be linked to the improvement in photosynthetic activities in chilling-stressed Clementine trees subjected to Si application. Furthermore, the decrease in stomatal conductance in Si-treated trees could be linked to a reduced transpiration rate as a photoprotective response to chilling stress [62].

The sensible increase in C_i observed in Valencia trees after exposure to chilling stress could be explained by acclimation. The reduction in C_i during chilling stress was because Valencia trees are cold-sensitive and unable to maintain an optimal internal carbon dioxide concentration. This agrees with the findings of a previous study that reported that the effects of Si were detectable only in plants grown under severe abiotic and biotic stress conditions [69]. The significant decrease in C_i chilling stress in Clementine trees despite the application of Si may have been attributable to the reduction in stomatal conductance [70].

In both cultivars, a decrease in water-use efficiency suggested susceptibility to chilling stress. Similar studies have reported similar effects in Kentucky bluegrass (*Poa pratensis* L.) [43] and bottle gourd (*Lagenaria siceraria* L.) [30] under drought stress conditions. This reduction in iWUE caused by chilling stress was caused by reductions in stomatal conductance and the transpiration rate, and these were attributed to the decrease in leaf water potential [37]. In Valencia trees, Si treatment alone or the combination of Si treatment with chilling stress triggered an increase in iWUE. This highlights evidence of adaptation processes taking place within this species. The increase in iWUE observed in Si-treated Clementine trees under chilling stress can be explained by the improvement in the CO_2 assimilation rate. Improvements in iWUE in relation to Si treatment have also been observed in strawberry (*Fragaria* sp. L.) [64], Kentucky bluegrass [43], maize [42], and tomato [10].

Photorespiration is defined as the process through which Rubisco binds to oxygen molecules, and the reaction deviates from the regular metabolic pathway; therefore, no sugar and ATP molecules are synthesized [70]. It is quantified by using the ratio of the electron transport rate to photosynthetic assimilation (ETR/A) [40]. In both cultivars, there was a significant increase in ETR/A under stressed conditions, implying that there was an increase in the photorespiration rate (Figure 2C). The increase in photorespiration rate was triggered by the decreased stomatal conductance as the stomatal CO_2 concentration declined, hence increasing O_2 in stressed plants [64]. Photorespiration is one of the dissipating mechanisms adopted by plants as a form of photoprotection against oxidative damage due to inefficiencies in electron transport under chilling stress conditions [39]. The efficiency of the photosynthetic activity in Si-treated Clementine trees subjected to chilling stress was improved to the point that photorespiration was significantly reduced, given that chlorophyll fluorescence is closely correlated to photosynthesis [38,39].

7. Conclusions

The present study evaluated the efficacy of silicon application for enhancing cold stress tolerance in citrus. The application of silicon enhanced photosynthetic function in both Valencia and Clementine trees despite their genotypic differences. In addition, silicon application enhanced photoprotection in the two citrus cultivars subjected to prolonged cold stress. The present findings are useful to citrus growers by providing an efficient strategy for citrus cultivation in cold-prone areas. Further trials with mature trees are needed to show the financial links between yield reductions due to cold stress and the potential for Si fertilization to enhance the income of citrus farmers in frost-prone regions of the sub-tropics around the world.

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