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Roles of Si and SiNPs in Improving Thermotolerance of Wheat Photosynthetic Machinery via Upregulation of PsbH, PsbB and PsbD Genes Encoding PSII Core Proteins

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Abstract: Photosystem II is extremely susceptible to environmental alterations, particularly high temperatures. The maintenance of an efficient photosynthetic system under stress conditions is one of the main issues for plants to attain their required energy. Nowadays, searching for stress alleviators is the main goal for maintaining photosynthetic system productivity and, thereby, crop yield under global climate change. Potassium silicate (K_2SiO_3, 1.5 mM) and silicon dioxide nanoparticles (SiO_2NPs, 1.66 mM) were used to mitigate the negative impacts of heat stress (45 °C, 5 h) on wheat (*Triticum aestivum* L.) cv. (Shandawelly) seedlings. The results showed that K_2SiO_3 and SiO_2NPs diminished leaf rolling symptoms and electrolyte leakage (EL) of heat-stressed wheat leaves. Furthermore, the maximum quantum yield of photosystem II (Fv/Fm) and the performance index (PI_{abs}), as well as the photosynthetic pigments and organic solutes including soluble sugars, sucrose, and proline accumulation, were increased in K_2SiO_3 and SiO_2NPs stressed leaves. At the molecular level, RT-PCR analysis showed that K_2SiO_3 and SiO_2NPs treatments stimulated the overexpression of *PsbH, PsbB*, and *PsbD* genes. Notably, this investigation indicated that K_2SiO_3 was more effective in improving wheat thermotolerance compared to SiO_2NPs. The application of K_2SiO_3 and SiO_2NPs may be one of the proposed approaches to improve crop growth and productivity to tolerate climatic change.

Keywords: silicon; silicon nanoparticles; photosynthetic performance; *PsbH*; *PsbB* and *PsbD* genes; wheat

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1. Introduction

Photosynthesis is a significant solar energy storage process that sustains life on the Earth. Photosynthesis is significantly sensitive to environmental alterations, particularly temperature [1]. Heat stress influences the functional properties of chloroplasts [2,3], including inhibition of the carbon assimilation system [4], decline in the electron transport chain, which in turn stimulates the production of reactive oxygen species [5,6], as well as the reduction in Rubisco activity [4], consequently reducing the photosynthetic capacity under stress conditions [7]. Both photosystems I and II play vital roles in transformation of light energy to ATP molecules [8]. High temperature directly damages the photosynthetic systems, particularly PSII [9], concomitant with distortion of the membrane integrity [10,11]. Additionally, chlorophyll fluorescence (Fv/Fm; PI_{abs}) information is considered an important parameter for estimating the photosynthetic apparatus damage in heat-stressed leaves [12]. It has been described that heat stress induces reduction in the ratio of Fv/Fm [13,14]. Furthermore, the functions of both PSI and PSII could be described by the performance index (PI_{abs}) [15]. It was stated that PI_{abs} is more susceptible to climatic changes compared to Fv/Fm [16] and could reflect the efficiency of photosynthetic systems

and CO_2 assimilation [17,18]. Notably, PI_{abs} represents quantitative knowledge on the present state of the stressed plant performance [19]. It was observed that PSII is generally more sensitive to temperature stress [20] compared to PSI [21]. Indeed, plants deal with environmental stresses by regulating the expression of some stress-responsive genes [22]. Heat stress induces alterations in protein factors that are involved in de novo assembly and/or degradation and turnover of the of PSII reaction center proteins. It was documented that the assembly of PSII is controlled by several phosphoproteins such as *PsbH*, *PsbB*, and *PsbD* [23]. Meanwhile, *PsbD* encodes the reaction center protein D2 of PSII [24], while the *psbB* gene encodes the photosystem II (PSII) chlorophyll-binding protein of 47 kDa (CP47). It contributes with chlorophyll-binding protein 43 kDa (CP43) in the formation of the inner light-harvesting complex [25]. The PSII subunit H protein (*PsbH*) is essential for PSII activity and is recognized as an 8 kDa phosphoprotein in higher plant chloroplasts [26]. Furthermore, *PsbH* might play a role in regulating PSII assembly/ stability and repair of photodamaged PSII [27,28]. *PsbH* is necessary to protect the PSII core and the thylakoid membrane from oxidative damage [29].

Plants can tolerate heat stress by evolving different defense strategies such as compatible solute accumulation (e.g., soluble sugar, proline), which participate in maintaining cell turgor via the osmotic adjustment [30,31]. Indeed, soluble sugars perform an important role in gene regulation mechanisms, rather than osmotic adjustment [32]. Meanwhile, proline plays highly protective roles in the antioxidant defense system [33].

Generally, majority of the world's population depends upon wheat as a staple food [34]. Wheat is a winter crop, has an optimum growth temperature at 23–25 °C and, consequently, it is sensitive to temperature above 25 °C [35]. Wheat is grown in about 30% of the world cereal area, and about 50% of such area experiences heat stress [36]. It was reported that heat stress above the optimum threshold level (25 °C) over a period is enough to induce irreversible damage to the wheat plant [37]. Hence, it is necessary to search for a potent cheap and ecofriendly alleviator to nullify heat stress hazards. It was reported that silicon (Si) enhances the tolerance of several plants against temperature stress [38,39]. It has been reported that Si deposited in the epidermal and vascular tissue walls of stems and leaves in most plant species, especially monocots, affects the physiological properties of plants [40,41]. In addition, silicon treatment stimulated the overexpression of aquaporin genes associated with improvement in the water status [41], antioxidant activities [42], photosynthetic performance [43], and the expression of antioxidant genes [44]. It was reported that high salinity almost resulted in the disappearance of PSI and PSII complexes; however, Si treatment protected the protein complexes in tomato plants.

Si nanoparticles (SiNPs) are currently considered a novel Si source that can be used to improve plant tolerance against abiotic stresses. A number of studies have explored the effects of seed priming with SiNPs on heat, salinity, and metal-stressed plants [41,45,46]. However, its role in the alleviation of heat stress has not been studied yet. Ultimately, the present investigation explores the effectiveness of Si and SiNPs in the protection of photosynthetic machinery.

2. Materials and Methods

2.1. Materials and Growth Conditions

Wheat (*Triticum aestivum* L.) cv. (Shandawelly) grains were obtained from the Agricultural Research Centre, Giza, Egypt. Potassium silicate (K_2SiO_3) and nanoparticles of silicon dioxide (SiO_2NPs) were obtained from Sigma-Aldrich (Lot 637238, St. Louis, MO, USA). SiO_2NP characteristics were as follows: 99.5% purity and 20–30 nm particle size. The SiO_2NPs were suspended in water by sonicating the silicon particles via an ultrasonicator at 10 MHz for ~40 min resulting in a partially homogeneous solution. This research was conducted at Faculty of Science, Ain Shams University, Egypt, using two controlled growth chambers, modelV3-DM, Vision Scientific Company, Daejeon, Korea. The grains were surface sterilized by immersion in 1% (*w*/*v*) sodium hypochlorite solution for 5 min, then

washed three times with sterile distilled water prior to experimental procedures to prevent fungal contamination.

2.2. Imposition of Treatments

The experiment was conducted in a completely randomized design. There were 4 treatments replicated 10 times. The sterilized wheat grains were divided into three sets; the first set was soaked in water (control), the second and third sets were soaked in SiO_2NPs (1.66 mM) or K_2SiO_3 (1.5 mM), respectively, for 8 h at room temperature (25 ± 2 °C). The soaked grains were then sown in plastic pots (25 × 25 cm) containing 2 kg of mixed soil (clay and sand, 1:1 *w*/*w*). The control growth chamber was maintained at optimum temperature, 24/18 °C day/night temperature, 70% relative humidity, photosynthetic photon flux density (PPFD) of 250 µmol m^{-2} s^{-1}, 16/8 h light/dark photoperiod. The water holding capacity of the soil was maintained at 80% throughout the experiments. Water holding capacity (WHC) of the soil was determined using the modified method adopted by [47]. The proper WHC was kept constant throughout the experiment. All plants were grown in the control chamber for 17 d. The untreated plants were divided into two groups. The first group was kept in the optimal temperature chamber, and the other group as well as the K_2SiO_3 and SiO_2NPs treated plants were moved to the other chamber adjusted at 45 °C for about 5 h. The PPFD and photoperiod settings in the high-temperature treatment were the same as the control chamber. The fully expanded second leaves of all treatments were collected directly after 5h exposure to heat, frozen in liquid nitrogen, and then stored at −80 °C for biochemical analyses.

2.3. Methods

2.3.1. Leaf Rolling Score

Leaf rolling score was determined by the visible recognition of leaf rolling in each pot. All pots were given a mean leaf rolling score, ranging from 1 to 5 scales, with 1 being flat and 5 a tightly rolled leaf [48].

2.3.2. Chlorophyll Fluorescence Measurements

Chlorophyll a fluorescence measurement was assessed in both control and stressed leaves at the morning hours. The intact flag leaves were adapted to darkness for 30 min using light-withholding clips. Leaf chlorophyll fluorescence (Fv/Fm) was measured simultaneously using a pulse amplitude modulation portable fluorometer (Handy PEA, Hansatech, Norfolk, UK). After the adaptation of leaves to darkness, a single strong 1 s light pulse (3500 µmol/m^2/s) was applied. Ten replicates were used for each treatment. The fast fluorescence kinetics (F_0 to Fm) were recorded during 10 µs to 1 s. Plant vitality was characterized by the performance index PI_{abs} parameter [19], which was calculated according to the equations described by [49] as follows:

$$PI_{abs} = \frac{1 - (F_0/F_m)}{M_0/V_J} \times \frac{F_m - F_0}{F_0} \times \frac{1 - V_J}{V_J} \dots$$

where F_0 means fluorescence intensity at 50 µs, Fm represents maximal fluorescence intensity, V_J is relative variable fluorescence at 2 ms (J-step), calculated as follows:

$V_J = (F_2ms - F_0)/(Fm - F_0)$ (4). M_0 represents the initial slope of fluorescence kinetics, which can be derived as $M_0 = 4\ (F300\mu s - F_0)/(Fm - F_0)$. Maximum quantum efficiency of PSII photochemistry (Fv/Fm) was calculated according to the equation $Fv/Fm = (Fm - F_0)/Fm$.

2.3.3. Estimation of Photosynthetic Pigments

The photosynthetic pigments in terms of Chl a, Chl b, and carotenoids were extracted and measured according to the method of [50]. One gram of fresh leaves was homogenized in 85% aqueous acetone for 5 min. The homogenate was centrifuged, and the supernatant was made up to 100 mL with 85% acetone. The extinction was measured against a blank of

pure 85% aqueous acetone at three different wavelengths (452.5, 644, and 663 nm) by using a spectrophotometer (Spectronic 601, Milton Roy Company, Ivyland, PA, USA).

2.3.4. Estimation of Carbohydrates

The soluble carbohydrate contents were determined by reacting 0.1 mL of the ethanolic extract with 3 mL of freshly prepared anthrone reagent (150 mg anthrone + 100 mL 72% H_2SO_4) in a boiling water bath for 10 min. After cooling, the absorbance was measured at 620 nm [51]. Moreover, the method used for the determination of sucrose was described by [52], which is based on extraction of sugars with 80% ethanol followed by destruction of reducing sugars with 30% KOH at 40 °C for 10 min. Further the sucrose content was estimated by the anthrone reagent method.

2.3.5. Determination of Proline

The total free proline was assessed by the method described by [53] using ninhydrin reagent. The leaf tissue (0.5 g) was homogenized with 6 mL of 3% (*w*/*v*) sulfosalicylic acid solution. An aliquot (2 mL) of the filtrate was taken with 2 mL ninhydrin reagent and 2 mL glacial acetic acid, and the mixture was kept in a boiling water bath for one hour. Then, the mixture was cooled in ice and was separated using a separating funnel. The absorbance of the upper phase was read at 520 nm. Proline concentration was determined from a standard curve of proline and calculated as µg/g fresh weight.

2.3.6. Determination of Electrolyte Leakage (EL)

The stress injury was measured by electrolyte leakage as described by [54]. Plant tissue (0.5 g) was incubated with 20 mL of de-ionized water for 24 h at 25 °C. Then, the electrical conductivity of the solution (L_1) was quantified. Samples were then autoclaved at 120 °C for 20 min, and then the final conductivity (L_2) was assessed after equilibration at 25 °C. The EL was determined according to the following equation:

$$EL\ \% = (L_1/L_2) \times 100$$

2.3.7. Estimation of Lipid Peroxidation Products (Conjugated Dienes, CD)

The lipid peroxidation conjugated dienes were extracted by homogenization of 100 mg of plant tissue in 5 mL of 96% (*v*/*v*) ethanol. The absorbance of the supernatant was assayed by estimating the increase in absorption at 234 nm [55]. The CD was calculated from the extinction coefficient of 2.74 × 104 M^{-1} cm^{-1} [56].

2.3.8. Quantitative Real-Time PCR (qRT-PCR) Analysis

The total RNA was separated from plant tissue (100 mg) with 30% PEG6000 using the RNeasy Plant Mini Kit (Qiagen, Amsterdam, The Netherlands). The total RNA (1 µg) was transformed into cDNA by reverse transcription using the c.DNA Kit (TaKaRa) following the instructions of the manufacturer. qRT-PCR was conducted on an ABI 7500 system (Applied Biosystems, New York, NY, USA) using TransStart™ Green qRT-PCR Super Mix Kit (TransGen, Beijing, China). TaActin rRNA (GenBank Accession: AB181991.1) was used as a reference gene to standardize the relative transcriptional abundance and to minimize different copy numbers of cDNA templates [57]. All data were investigated from three replicates based on the $2^{-\Delta\Delta Ct}$ method [58]. The primers of the *PsbH*, *PsbB*, and *PsbD* genes (Table 1) used in the qRT-PCR excluded the highly conserved protein domain and had high efficiency and specificity.

2.4. Statistical Analysis

The experimental data presented in this work were statistically analyzed by analysis of variance (ANOVA) using SPSS v20.0 (SPSS Inc., Chicago, IL, USA) software. Statistical significances of the means were compared with the Duncan test at $p \leq 0.05$ levels, and the

standard error (SE) of the means are shown in tables and figures as mean ± SE, the number of degrees of freedom (n) = 3.

Table 1. The primers used for real-time PCR analysis.

Primer Name	Primer Sequence 5′-3′	Accession Number	Gene Name
PsbHF	TGGCTACACAAACCGTTGAA	NC_002762.1 (70762..70983) NC_002762.1 (68672..70198) NC_002762.1 (8995..10056)	Photosystem II Reaction center protein H
PsbHR	CCGTCCAGTAAAACGGAAGA		
PsbBF	GGTTTGCCTTGGTATCGTGT		
PsbBR	TCCACATTGGATCCAGAACA		
PsbDF	CGCTTTAGGGGGTGTGTTTA		
PsbDR	GCCCCCATAGTAGCAACAAA		
TaActinF	TGCTATCCTTCGTTTGGACCTT	AB181991.1	
TaActin R	AGCGGTTGTTGTGAGGGAGT		

3. Results

The unstressed wheat leaves remained unrolled during the day, with score = 0. Meanwhile, imposition of heat stress induced a significant full leaf rolling (about score = 5 about 100%) as compared with those exposed to normal growth temperature (Figures 1 and 2a). Leaf rolling symptoms were detected firstly in heat-stressed wheat leaves after about 5 h from imposition of the temperature (45 °C) regime. On the other hand, K_2SiO_3 and SiO_2NPs treated leaves were almost unrolled, measuring about ≥1 and 2 (5 and 20%), respectively, Figures 1 and 2a) as compared with that of heat-stressed leaves.

Moreover, results showed that the electrolyte leakage level increased in wheat leaves exposed to 45 °C. The greatest increase in electrolyte leakage was measured in stressed leaves. Pretreatment with K_2SiO_3 or SiO_2NPs significantly reduced the electrolyte leakage of wheat leaves (Figure 2b). Heat stress increased the EL concomitant with increments in the lipid peroxidation product CD (Figure 2b,c) compared with those of control unstressed plants.

Likewise, heat-stressed leaves exhibited reductions in Chl a, Chl b, total chlorophyll content, as well as the carotenoid content (Table 2). However, the ratios of Chl a/b of heat-stressed leaves were markedly increased. Interestingly, results showed that K_2SiO_3 and SiO_2NPs treatment enhanced Chl a, Chl b, Chl a + b, total chlorophyll content, and carotenoids concomitant with decline in the Chl a/b ratio as compared with those of stressed, untreated leaves (Table 2).

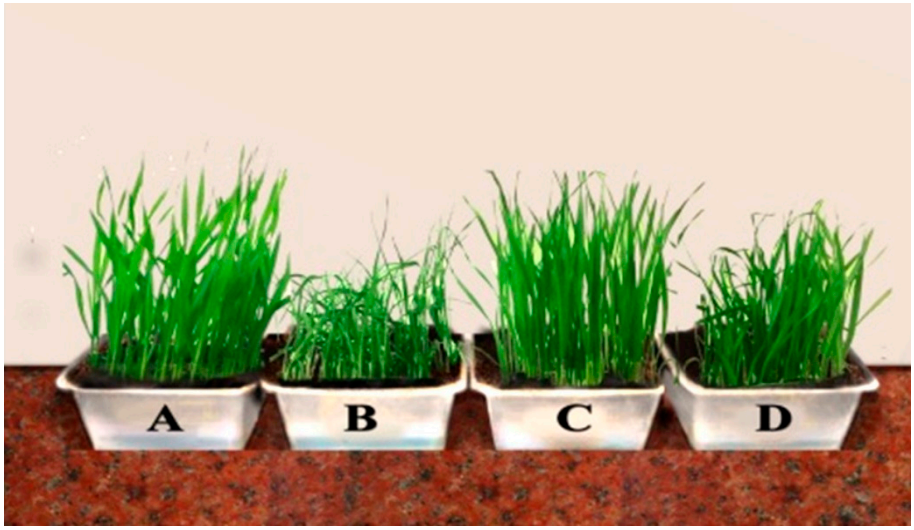

Figure 1. Wheat seedlings exposed to room temperature (**A**) and 45 °C for 5 h in absence (**B**) and presence of K_2SiO_3 (**C**) or SiO_2NPs (**D**).

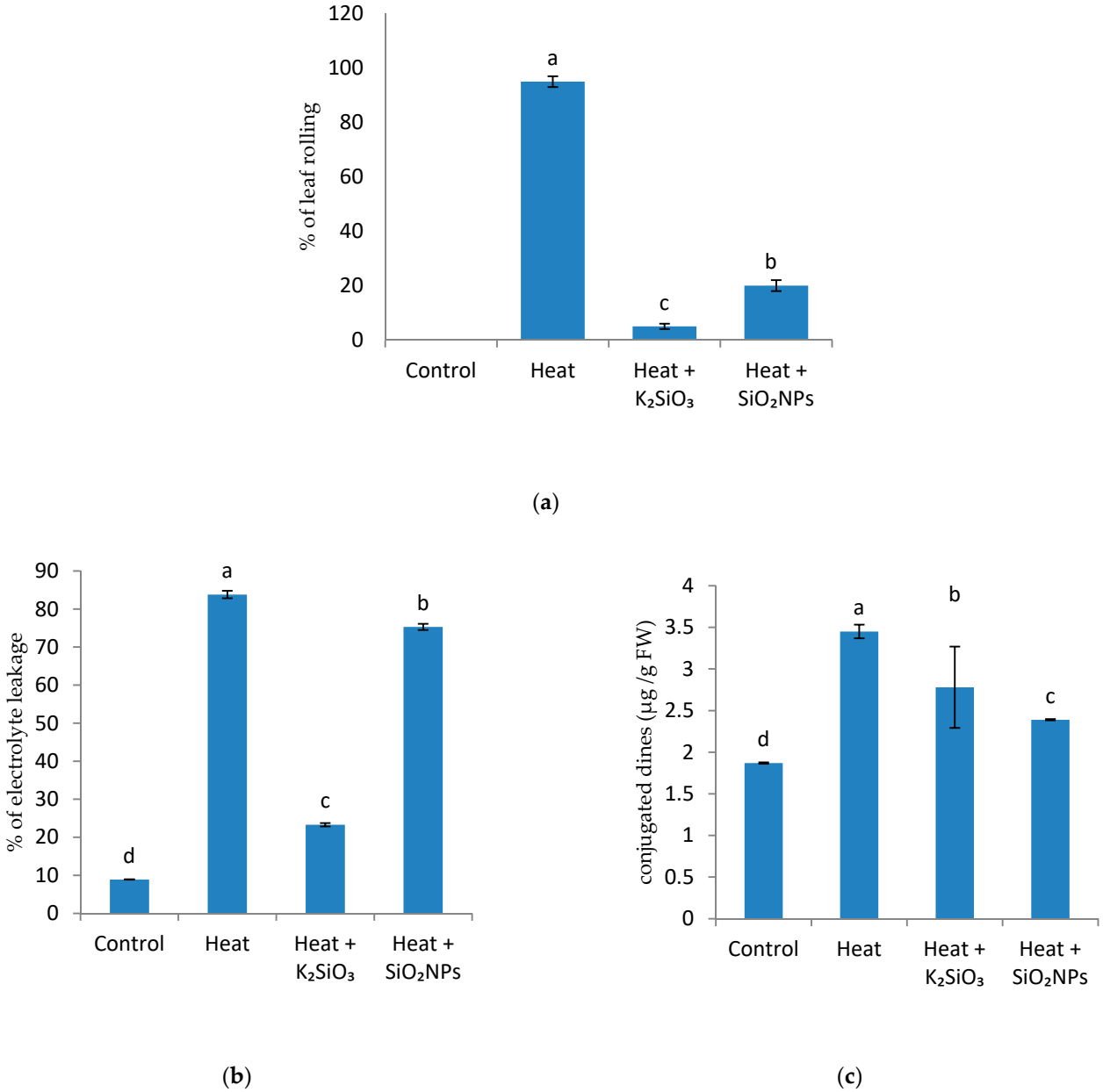

Figure 2. Effect of K_2SiO_3 and SiO_2NPs treatments on (**a**) leaf rolling, (**b**) the percentage of electrolytes leakage (EL), and (**c**) conjugated dines (CD) (µg/g FW) of heat-stressed wheat leaves. Each value is the mean of three replicates ± SE. Columns with different letters are significantly different at $p < 0.05$.

Table 2. Effect of K_2SiO_3 or SiO_2NPs on photosynthetic pigments (µg/g FW) of wheat leaves exposed to heat stress. Data are means of three replications ± SE.

Treatments	Chl (a)	Chl (b)	Chl (a + b)	Carotenoids	Chlorophyll a/b Ratio
Control	11.3 ± 0.17 a	5.5 ± 0.17 a	16.8 ± 0.12 a	2.5 ± 0.06 a	2.05 ± 0.08 b
Heat	6.5 ± 0.12 c	2.1 ± 0.06 c	8.6 ± 0.12 d	1.6 ± 0.05 c	3.0 ± 0.15 a
Heat+ K_2SiO_3	10.8 ± 0.17 a	5.3 ± 0.13 a	16.1 ± 0.12 b	2.2 ± 0.52 b	2.3 ± 0.17 b
Heat+ SiO_2NPs	9.8 ± 0.23 b	4.3 ± 0.17 b	14.1 ± 0.04 c	2 ± 0.11 b	2.03 ± 0.09 b

Columns with different letters are significantly different at $p < 0.05$.

Furthermore, thermal stress induced significant reduction in Fv/Fm values of stressed wheat leaves (Figure 3a) concomitant with a significant decrease in PI_{abs} (Figure 3b). Notably, K_2SiO_3 and SiO_2NPs pretreatment enhanced Fv/Fm values and the PI_{abs} values

of heat-stressed leaves compared with that of the untreated stressed ones. The greatest increments in Fv/Fm and PI_{abs} were attained in K_2SiO_3-stressed leaves compared with SiO_2NPs ones.

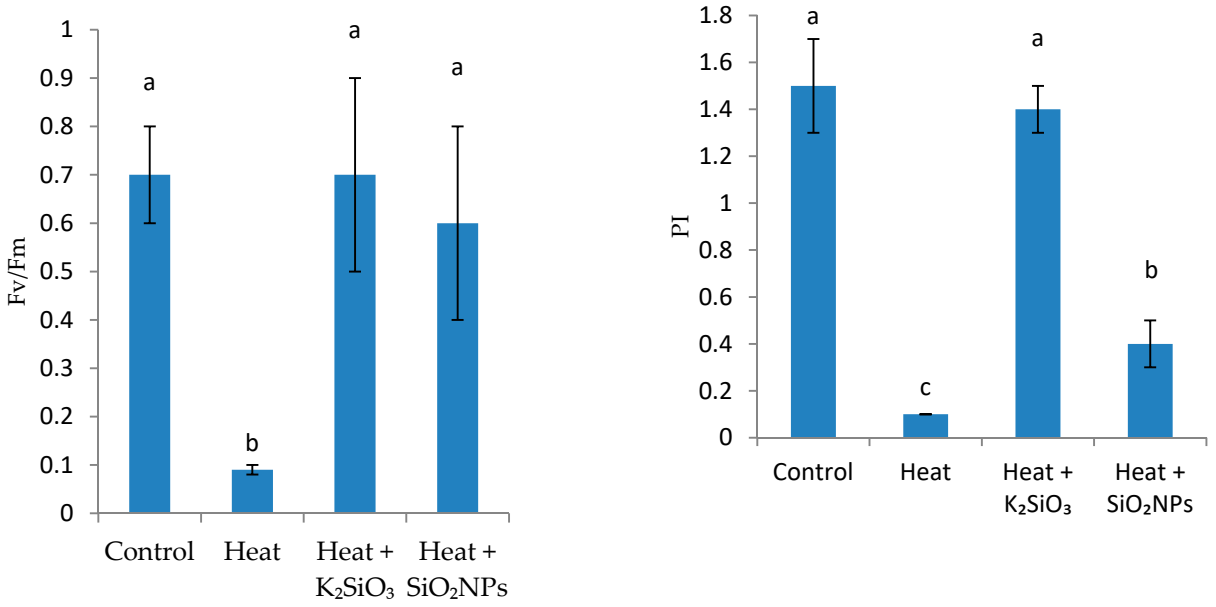

Figure 3. Effect of K_2SiO_3 and SiO_2NPs treatments on (**a**) the maximal photochemical efficiency of primary photochemistry (Fv/Fm), (**b**) on the performance index of heat-stressed wheat leaves. Each value is the mean of three replicates ± SE. Columns with different letters are significantly different at $p < 0.05$.

The current data also revealed that imposition of heat stress significantly increased the accumulation of total soluble sugar and sucrose content (Table 3). The accumulation of total soluble sugars as well as sucrose content in heat-treated wheat seedlings increased significantly by 96.7% and 33.6%, respectively, as compared with their respective controls. Meanwhile, K_2SiO_3 and SiO_2NPs treated seedlings exhibited further increases in the total soluble sugars and sucrose accumulation.

Table 3. Effect of K_2SiO_3 or SiO_2NPs on total soluble sugars (µg glucose equivalent $g^{-1\ F.wt}$), sucrose (µg glucose equivalent $g^{-1\ F.wt}$), and proline (µg proline $g^{-100\ F.wt}$) of wheat leaves exposed to heat stress. Data are means of three replications ± SE.

Treatments	Total Soluble Sugars	Sucrose	Proline
Control	113.6 ± 0.036 d	2.29 ± 0.030 d	26.8 ± 0.69 d
Heat	223.5 ± 0.034 c	3.06 ± 0.040 c	46 ± 0.28 c
Heat+ K_2SiO_3	330.0 ± 0.06 a	5.54 ± 0.037 a	86 ± 0.90 a
Heat+ SiO_2NPs	328.6 ±0.04 b	5.28 ± 0.028 b	57 ± 0.40 b

Columns with different letters are significantly different at $p < 0.05$.

Moreover, in this study, heat stress obviously induced a marked increase in proline accumulation compared to the control (Table 3). It was clearly shown that the accumulation of proline level increased significantly in wheat seedlings exposed to heat stress (Table 3). The pretreatment of wheat with K_2SiO_3 and SiO_2NPs enhanced the accumulation of proline in stressed wheat seedlings as compared to those of the controls.

In addition, RT-PCR analysis showed that heat stress downregulated *PsbH*, *PsbB*, and *PsbD* expressions (Figure 4a–c). Meanwhile, K_2SiO_3 and SiO_2NPs treatments stimulated the overexpression of *PsbH*, *PsbB*, and *PsbD* of heat-stressed wheat leaves compared to

those of unstressed leaves (Figure 4a–c). Notably, K_2SiO_3 treatment attained the greatest *PsbH*, *PsbB*, and *PsbD* gene overexpression in wheat leaves exposed to heat stress.

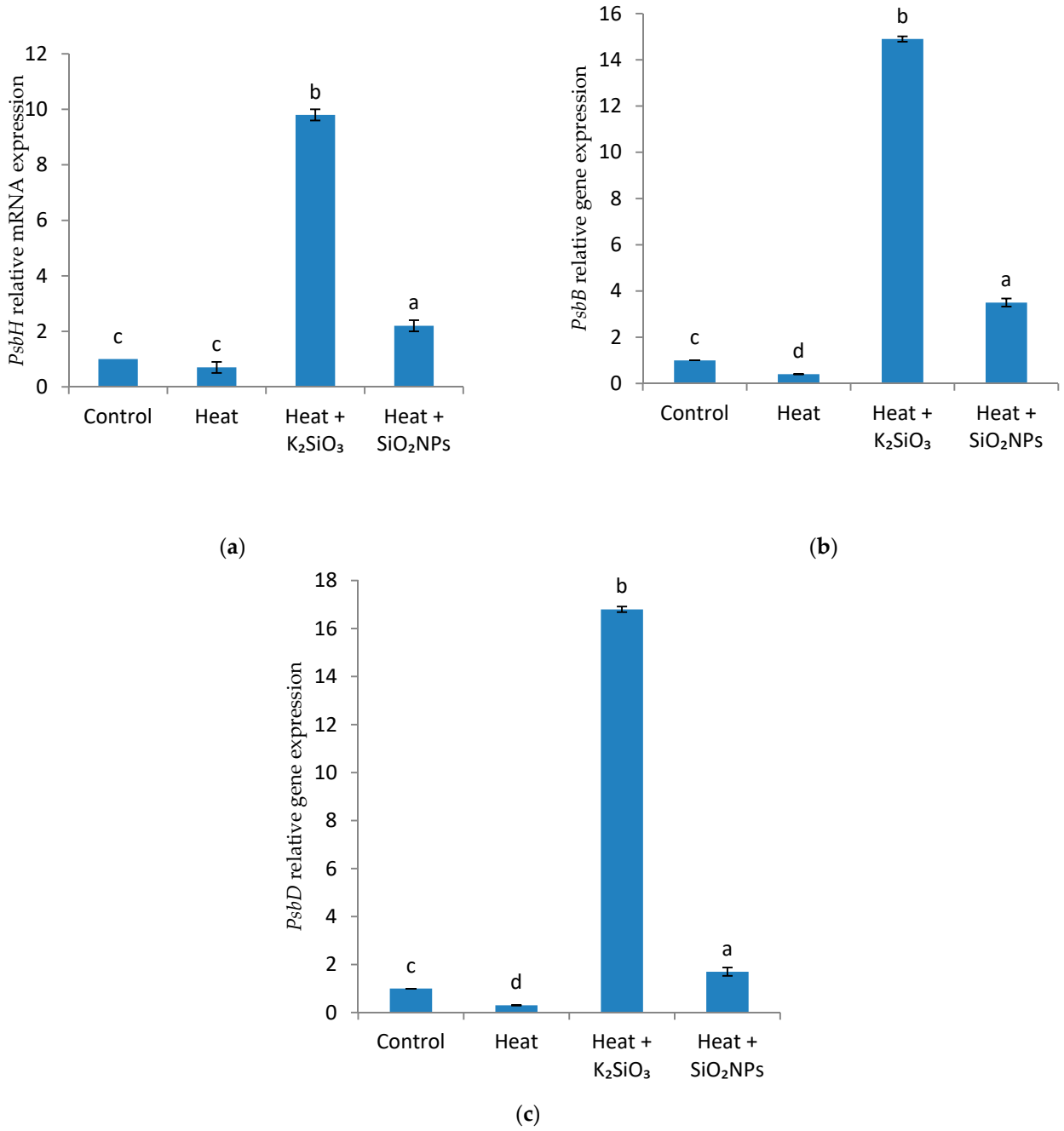

Figure 4. Effect of K_2SiO_3 and SiO_2NPs treatments on mRNA expression of photosystem II reaction center proteins (**a**) H (*PsbH*), (**b**) (*PsbB*), and (**c**) (*PsbD*) of heat-stressed wheat leaves. Each value is the mean of three replicates ± SE. Columns with different letters are significantly different at $p < 0.05$.

4. Discussion

Heat is an important environmental determinant that could affect the morphological and physiological parameters of plants. Thus, imposition of heat stress induced a significant full leaf rolling of wheat seedlings as compared with those exposed to normal growth temperature (Figure 2a). Leaf rolling symptoms were detected firstly in heat-stressed wheat leaves after about 5 h from imposition of the temperature (45 °C) regime (Figure 1). Leaf rolling is a heat avoidance strategy that reduces the interception of solar radiation

and thereby decreases leaf temperature and water loss via transpiration [59]. However, K_2SiO_3 and SiO_2NPs treated leaves displayed lower rolling symptoms compared with heat-stressed leaves (Figure 2a). The impedance of leaf rolling by Si pretreatments may be attributed to the development of a double layer of cuticle silica, which consequently reduces the transpiration rate, decreasing the opening of stoma and limiting the loss of water [60,61]. Moreover, deposition of Si in the plant cell wall enhanced its rigidity and consequently increased leaf erectness [62,63]. In addition, the ameliorative effect of SiO_2NPs might be due to the increments in the irregular deposition of SiO_2NPs in the epicuticular layer of stressed leaves [64] as well as in the cell walls, the intercellular space, cytoplasm, and in cell organelles. Thus, deposition of SiNPs reduced the cuticular transpiration and maintained plant water use efficiency [65].

Likewise, cell membrane distortion is one of the major damages caused by heat stress, thereby stimulating cell permeability and electrolyte leakage [66]. Heat stress increased the EL concomitant with increments in the lipid peroxidation product CD (Figure 2b,c) as compared with those of control unstressed leaves. Meanwhile, pretreatment with K_2SiO_3 or SiO_2NPs significantly reduced the EL and CD (Figure 2b,c) as compared with stressed wheat seedlings. This finding matches those investigated by [67] on stressed rice. Si treatment enhanced the thermal stability of cell membranes via preventing the distortion of cell membranes and ion leakage and, therefore, increased plant resistant against stresses [61,68,69]. The alterations in the membrane systems induced by environmental stresses were concomitant with leaf rolling, inadequate light interception, and consequent reduction in photosynthetic activities [70]. Indeed, estimation of chlorophyll content is usually taken as an indicator that reflects a plant's resistance to stress. Estimation of the photosynthetic pigment contents showed that heat stress significantly reduced Chl a, Chl b, and the total chlorophyll content (Table 2). Such effect might be attributed to the injury of thylakoid membranes, which may lead to chlorophyll loss [71,72]. Likewise, the impact of heat stress on the pigments and other photosynthetic performances has been reported by [73] on sorghum, [74] on maize, and [75] on wheat plants. Meanwhile, the decrease in Chl a+b content by heat may be attributed to the reduction in biosynthesis and/or to the degradation of the pigments [76]. The reduction in carotenoids in wheat leaves could have serious consequences for the effect of heat on chlorophyll pigments [77]. The observed increments in Chl a/b in heat-stressed seedlings may be related to the degradation of chlorophyll b by high temperature, which in turn may reduce the light-harvesting chlorophyll a/b-binding proteins (LHC) [78]. Interestingly, the present study showed that K_2SiO_3 and SiO2NPs treatment could notably enhance Chl a, Chl b, total chlorophyll content, and carotenoids concomitant with decline in the Chl a/b ratio, thereby resulting in higher survival rates in stressed wheat seedlings (Figure 1). Si and SiNP treatments can delay chlorophyll degradation and improve photosynthetic capacity in plants under abiotic stresses [42]. The increases in both chl. a+b and carotenoid content were concomitant with reductions in Chl a/b ratios in both K_2SiO_3 and SiO_2NPs stressed leaves as compared with those of stressed untreated leaves (Table 2). Such effect may be attributed to the role of Si in protecting chlorophyll from oxidation by carotenoids [79]. Carotenoids act as an important antioxidant, protecting pigments from the oxidation induced by stressful conditions [80]. Similarly, it was stated that Si-mediated improvement of the chloroplast ultrastructure and chlorophyll contents enhanced the photosynthetic efficiency in stressed plants [42,81,82]. Likewise, SiNPs increased the photosynthetic parameters of stressed cherry tomatoes and strawberry plants [62,83]. SiNPs stimulated stabilization of the epicuticular wax structure, chlorophyll content, as well as carotenoid content [64]. Moreover, SiNPs are characterized by their high reactivity and can directly bind with PSII and stabilize the photosynthetic activity [84] under stress conditions.

Moreover, quantitative information on the current state of plant performance under stress conditions could be explored via PI_{abs} and Fv/Fm measurements [14,19]. The observed reduction in the values of Fv/Fm in stressed wheat leaves (Figure 3a) may result from the conformational changes of the reaction center of PSII [85], which causes imbalance

between the generation and utilization of electrons [11,12]. Meanwhile, it was observed that PI_{abs} decreased in heat-stressed wheat leaves (Figure 3b). High temperatures might induce cleavage and aggregation of reaction center proteins [86,87] and, consequently, the conversion of excitation energy to electron transport [88]. Interestingly, K_2SiO_3 and SiO_2NPs pretreatment enhanced both Fv/Fm and the PI_{abs} values of heat-stressed leaves compared with that of the untreated stressed ones. The greatest increases in the Fv/Fv and the PI_{abs} values were achieved in K_2SiO_3 treated leaves (Figure 3a,b). Such increases in Fv/Fm and PI_{abs} values in K_2SiO_3 treated leaves may be related with the enhanced density of the PSII active reaction centers [89], which indicates that Si was capable to nullify the harmful effects of heat stress on the photochemical reactions [90,91].

Meanwhile, the tolerant mechanism was associated with osmotic adjustment. This process involves accumulation of some osmolytes such as total soluble sugars, sucrose, and proline, which reduced the cell osmotic potential, reduced water losses, and resulted in the increase in leaf turgor [92]. The current data revealed that imposition of heat stress significantly increased the total soluble sugar and sucrose contents (Table 3), which may be ascribed to the impairing of sucrose-metabolizing enzymes [93]. Soluble sugars and sucrose serve as osmo-protectants, carbon sources, and free radical scavengers [94], and so it may be an Si strategy in improving wheat tolerance against heat stress. Furthermore, proline accumulation is another strategy that has been implicated in the tolerance mechanism of several stressed plant species. In the present investigation, heat stress obviously induced a marked accumulation of proline compared to the control (Table 3). Such obtained results are concomitant with those reported by [95] on rice and [96] on sugar cane. The accumulation of proline under heat stress could also serve as chaperones, stabilizing and protecting the structure of enzymes and proteins, maintaining membrane integrity and scavenging ROS, and as nitrogen and carbon pools [96,97]. Pretreatment of wheat with K_2SiO_3 and SiO_2NPs alleviated the adverse effects of heat stress via increasing photosynthetic pigments and organic solutes, including soluble sugars, sucrose, and proline content (Table 3). Similarly, the application of silicon significantly increased sucrose and fructose levels in salt-stressed sorghum [98]. The improving effect of Si treatment on carbohydrates may be attributed to enhancement of the plant performance via adjusting the leaf position and as well its light interception [99]. The greatest increase in proline accumulation in Si-treated plants may be evidence for the alleviation of stress damage. Similarly, proline was accumulated in stressed maize plants [100]. Such an increment in proline level may be due to increased proline synthesis or reduction in proline degradation [101]. It was reported that the improvement in rice tolerance to salt stress by Si and SiNPs treatments was concomitant with increases in photosynthetic rate, antioxidants, proline, and water use efficiency traits [102].

In addition, photosystem II is extremely sensitive to high-temperature stress. It has been reported that high temperatures disrupt the water-oxidizing complex (WOC), light-harvesting complex, and PSII reaction center [103]. It has been suggested that there is a link between chloroplast gene expression and environmental stress responses [22]. In the current study, RT-PCR analysis showed that heat stress repressed the expressions of *PsbH*, *PsbB*, and *PsbD* genes, which were associated with inactivation of PSII as revealed by the reduction in Fv/Fm and the photosynthetic performance index (PI_{abs}). Such negative effects of heat stress were concomitant with reductions in the photosynthetic pigment levels and organic solutes, including soluble sugars, sucrose, and proline (Tables 2 and 3). The chloroplast gene *PsbD* encodes the reaction center protein D2 of PSII [104]. Meanwhile, the *psbB* gene encodes the photosystem II (PSII) chlorophyll-binding protein (CP47) [25]. The PSII subunit H protein (*PsbH*) is also an important phosphoprotein for PSII activity in the chloroplasts. The phosphorylation of PSII proteins regulated the stability, degradation, and turnover of the reaction center proteins [105]. However, dephosphorylation of these proteins was stimulated under stressful conditions [106,107]. It was reported that salt stress caused the destruction of D1 protein (encoded by the *PsbA* gene) in *Avena sativa* plants concomitant with downregulation of *PsbA*, *PsbB*, *PsbC*, and *PsbD* [108]. Phosphorylation and dephosphorylation of PSII are the main regulatory aspects, and they play a major role

in PSII repair [109]. However, no attention has been paid to explore the roles of Si and SiNPs on chloroplast phosphorylation and dephosphorylation of PSII at the molecular level, particularly in response to high-temperature stress. The effects of K_2SiO_3 and SiO_2NPs on the core PSII proteins were estimated via RT-PCR analysis of *PsbB* (CP47 subunit of PSII), *PsbD* (D2 subunit of PSII), and *PsbH* (8 kDa phosphoprotein) expressions. RT-PCR results indicated that *PsbB*, *PsbD*, and *PsbH* genes encoding extrinsic and intrinsic PSII proteins were upregulated in K_2SiO_3 and SiO_2NPs treated stressed leaves (Figure 4a–c). Notably, the expression of the investigated genes was highly remarkable in K_2SiO_3 treated leaves compared with SiO_2NPs leaves. Si greatly ameliorated the hazards induced by heat stress. Similarly, various stresses are known to cause damage to the D2 protein (encoded by the *PsbD* gene) [110,111], which is associated with the substantial lower abundance of *PsbD* transcripts in heat-treated plants. *PsbH* plays a vital role in activating the heat stress transcription network [112]. In addition, the upregulation of *PsbH* expression in the Si or SiNPs treated leaves could participate in the stability and repair of PSII after exposure to heat stress [113]. It has been observed that *PsbH* is involved in photoprotection against the damaging effects of reactive oxygen species [112,114]. K_2SiO_3 regulates *PsbH*, *PsbB*, and *PsbD* expressions under high-temperature stress conditions (Figure 4a–c) and may accelerate the repair of PSII, thus decreasing the damage of the photosynthetic apparatus induced by heat stress (reviewed by [109]) and improving the repair of photosystem II (PSII) by maintaining overexpression of *PsbH*, *PsbB*, and *PsbD*, establishing a consistent flow of electrons. Consequently, the overexpression of *PsbH*, *PsbB*, and *PsbD* by K_2SiO_3 treatment might protect the photosynthetic machinery under heat stress conditions, thereby improving the photosynthetic system efficiency and alleviating the harmful effects of heat. Further studies are required to elucidate the roles of K_2SiO_3 and SiO_2NPs in regulation of *PsbH*, *PsbB*, and *PsbD* expressions at the transcriptional level.

5. Conclusions

The results of the current investigation provide molecular and physiological evidence supporting the vital roles of K_2SiO_3 and SiO_2 NPs in sustaining photosynthetic activity in heat-stressed wheat seedlings, which may have a beneficial impact in horticultural crop management to tolerate climatic fluctuations. The obtained results revealed that K_2SiO_3 and SiO_2 NPs alleviated the injuries caused by thermal stress through physical protection and biochemical strategies, which contribute to the protection of the photosystems as well as photosynthetic activity. Such a protective effect was evident by the obtained reductions in photosynthetic parameters of chlorophyll fluorescence (PI_{abc}, Fv/Fm), EL, and CD accumulation. Notably, K_2SiO_3 was more effective than SiO_2 NPs in improving wheat thermotolerance. Meanwhile, K_2SiO_3 highly induced the expression of PSII-related genes (*PsbH, PsbB, PsbD*), which might be involved in the regulation of a complex network that confers protection of photosystem II from heat-induced oxidative damages and thereby enhances the photosynthetic capacity of wheat leaves. The greater enhancement effects of K_2SiO_3 compared to SiO_2NPs are perhaps due to the synergistic effects of Si and K forming K_2SiO_3. Further work will be conducted to study the synergistic effects of some minerals with biogenic SiNPs, which can be used as a nanofertilizer. There are no investigations exploring the synergetic effects of SiNPs with K. Therefore, further in-depth biochemical and molecular studies are necessary to understand the key interactions of signaling pathways concerning the role of either Si or SiNPs in alleviation of stress hazards.

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Abbreviations

Chl a	chlorophyll a
Chl b	chlorophyll b
EL	electrolyte leakage
Fv/Fm	maximum quantum yield of photosystem II
PI_{abs}	performance index
PsbH, *PsbB*, and *PsbD*	photosystem II reaction center protein H, B, and D
PSII	photosystem II
Si	silicon
SiNPs	silicon nanoparticles
WHC	water holding capacity

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