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Impacts of silicon and silicon nanoparticles on leaf ultrastructure and *TaPIP1* and *TaNIP2* gene expressions in heat stressed wheat seedlings

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Abstract

Heat stress is one of the most crucial factors affecting crop growth and productivity worldwide. So, searching for a potent eco-friendly heat stress alleviator is the main issue nowadays. The current study was conducted to assess the ameliorative effects of 1.5 mM potassium silicate (K_2SiO_3 , further only Si) or 1.66 mM silicon dioxide nanoparticles (SiNPs) on wheat (*Triticum aestivum* L.) seedlings exposed to heat stress (45 °C, 4 h). The observations show that Si or SiNPs treatments significantly restored the heat stress-provoked ultrastructural distortions of cellular organelles, particularly chloroplasts and the nucleus. Further, both Si and SiNPs enhanced the photosynthetic capacity as revealed by increments in the photochemical efficiency of photosystem II and the performance index as well as the content of photosynthetic pigments. A reduction in malondialdehyde accumulation in Si and SiNPs treated plants was positively related to their membrane stability index. The reverse transcription PCR analysis showed that Si treatment but not SiNP treatment stimulated the overexpressions of both *Triticum aestivum* plasma membrane intrinsic protein (*TaPIP1*) and *Triticum aestivum* nodulin 26-like intrinsic protein (*TaNIP2*) aquaporin genes parallelly with an improvement in the relative water content. This investigation reveals that Si was more effective than SiNPs in restoring the heat stress injuries. To the best of our knowledge, this is the first investigation exploring the effects of Si and SiNPs in improving thermotolerance of wheat seedlings.

Additional key words: aquaporins, chlorophyll content, chlorophyll fluorescence, membrane stability, photosystem II, *Triticum aestivum*.

Introduction

Heat stress is one of the main challenges induced abiotic stresses. Plants exposed to high temperatures experience metabolic changes which may lead to cell death (Ihsan *et al.* 2019). Heat stress provokes ultrastructural deformations of cellular organelles, particularly chloroplasts and mitochondria (Liu *et al.* 2013). It has been reported that heat stress induces damage to the chloroplasts including, excessive swelling of the outer chloroplast membrane, distortion of the stroma and intergranal lamellar system, disorganization of thylakoids concomitant with reduction in grana stacking thickness, decline in starch granules size, and increments in the numbers of plastoglobuli (Liu *et al.* 2013, Wang *et al.* 2018). The disorganization of thylakoid membranes concomitant with the loss of the thylakoid membrane integrity was reported as an important marker in many heat-sensitive plants (Dankov *et al.* 2015, Li *et al.* 2016). Similarly, the photosynthetic performance

index (PI_{abs}), photochemical efficiency of photosystem II (F_v/F_m), chlorophyll biosynthesis, and the net photosynthetic rate (P_N) are thermally labile parameters that reflect the efficiency of the photosynthetic system (Djanaguiraman *et al.* 2018, Wang *et al.* 2019b). It was reported that, the photochemical efficiency was reduced by heat stress resulting in the production of reactive oxygen species (ROS) which in turn trigger lipid peroxidation in wheat plants (Wang *et al.* 2014).

Water status is the main physiological parameter of plants influenced by heat stress (Christou *et al.* 2014). Generally, heat stimulates water loss via enhancement of transpiration rate and thereby impairing plant water balance (Fahad *et al.* 2017). Plants tolerate heat stress by evolving different biochemical and molecular strategies, including regulation of water/osmotic homeostasis, ion balance, as well as controlling water transport via aquaporins (AQPs; Kapilan *et al.* 2018, Khan *et al.* 2019). The AQPs are the main membrane transporters of water (Yanef *et al.* 2016).

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Abbreviations: AQP - aquaporin; Car - carotenoids; Chl - chlorophyll; DPPH - 2,2-diphenyl-1-picrylhydrazyl; F_v/F_m - variable to maximum chlorophyll fluorescence ratio (photochemical efficiency of photosystem II); MDA - malondialdehyde; MSI - membrane stability index; NIP - nodulin 26-like intrinsic protein; PI_{abs} - performance index; PIP - plasma membrane intrinsic protein; PS II - photosystem II; RWC - relative water content; SiNP - silicon dioxide nanoparticle; TEM - transmission electron microscope/microscopy. **Acknowledgments:** Authors acknowledge the partial support of the community service and environmental development affairs sector of Ain Shams University.

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It was reported earlier that, the transfer of water *via* AQPs is sensitive to temperature (Ionenko *et al.* 2010). Plant AQPs are also involved in the transport of small molecules such as Si, B, O₂, CO₂, NH₄, and H₂O₂ (Maurel *et al.* 2015, Zwiazek *et al.* 2017). The plant AQPs family is divided into five subfamilies according to amino acid sequence homology and protein subcellular localization (Hussain *et al.* 2011): plasma membrane intrinsic proteins (PIPs), tonoplast membrane intrinsic proteins (TIPs), nodulin 26-like intrinsic proteins (NIPs), small basic intrinsic proteins, and uncharacterized-intrinsic proteins (XIPs) (Gupta and Sankararamakrishnan 2009, Park *et al.* 2010). Changes in aquaporin genes expression in response to heat stress have been reported in various plant species including strawberry and spinach (Chen and Arora 2014, Christou *et al.* 2014).

Wheat (*Triticum aestivum*) is one of the most important food crops worldwide (Curtis and Halford 2014). However, the growth and productivity of wheat is affected by environmental stresses such as drought, salinity, and extreme temperature. Wheat as a temperate crop has an optimum growth temperature at 23 - 25 °C and is generally injured at temperatures above 25 °C (Blum 1986). Wheat is grown in about 30 % of the world cereal area and about 50 % of such area experiences high temperature stress (Cossani and Reynolds 2012). Nowadays, it is crucial to enhance wheat thermotolerance to meet the global food demands. So, searching for potent and ecofriendly heat alleviators play an important role in enhancing wheat productivity under stressful condition.

Different forms of silicon can be used as environmental stress alleviators (Asgari *et al.* 2018). Silicon has numerous beneficial effects in enhancing plant growth and development, particularly under stressful conditions (Alzahrani *et al.* 2018, Asgari *et al.* 2018). It has been reported that silicon can be deposited in the walls of the epidermal and vascular tissues of stems and leaves in most plant species, especially monocots, thereby affecting the physiological properties of plants (Gong and Chen 2012, Rios *et al.* 2017). In addition, application of silicon was capable to enhance the plant water uptake and transport (Yavaş and Aydın 2017), the antioxidant activities (Ma *et al.* 2016), the photosynthetic performance (Wang *et al.* 2019b), and the expression of antioxidant genes (Abdel Latef and Tran 2016). Silicon dioxide nanoparticles (SiNPs) are currently considered a novel silicon source that can be used to increase plant tolerance to stress. Several studies have explored the effects of SiNPs on salt-stressed and metal-stressed plants (Alsaeed *et al.* 2018, Hussain *et al.* 2019). However, the impact of SiNPs on mitigation of heat stress has not been investigated yet. Nanoparticles influence plant metabolism *via* utilization as micronutrients (Liu and Lal 2015), regulation of gene expressions (Nair and Chung 2014), or affection of different antioxidants (Hossain *et al.* 2015). However, no clear comparative studies concerning the mechanisms of K₂SiO₃ and SiNPs alleviation of heat stress were done. Thus, the present study was conducted to compare their protective roles against heat stress by improving water status and the photosynthesis capacity, and by maintaining ultrastructure of wheat seedling leaves.

Materials and methods

Plants and growth conditions: Wheat (*Triticum aestivum* L.) cv. Gemeza11 grains were obtained from the Agricultural Research Centre, Giza, Egypt, and kept in the dark at 4 °C before use. Potassium silicate and SiNPs were obtained from *Sigma-Aldrich* (St. Louis, USA). The SiNPs characteristics were: 99.5 % purity and 20 - 30 nm particle size. The SiNPs was suspended in water by sonicating the silicon particles at 10 MHz for ~40 min resulting in a partially homogeneous solution. The grains were surface sterilized by immersion in 1 % (m/v) sodium hypochlorite solution for 5 min. The grains were then rinsed thoroughly with running distilled water. 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 SiNPs (1.66 mM) or K₂SiO₃ (1.5 mM), respectively, for 8 h at room temperature (25 ± 2 °C). The soaked grains were immediately sown in plastic pots (20 × 20 cm) containing 2 kg of soil composed of sieved air-dried clay and washed sand (1:1, m/m). The experiment was conducted using a completely randomized design at Faculty of Science, Ain Shams University, Egypt using two controlled growth chambers (model *V3-DM*, *Vision Scientific Company*, Daejeon-Si, Korea) which were maintained at 24/18 °C day/night temperatures, a 70 % relative humidity, a photosynthetic photon flux density (PPFD) of 250 µmol m⁻² s⁻¹, and a 16-h photoperiod; a water holding capacity of the soil was maintained at 70 %. Water holding capacity (WHC) of the soil was determined using a modified method of Bajwa *et al.* (2017). To restore the appropriate WHC, each pot was weighed using an electronic digital balance every day and appropriate quantity of water was added to the soil. After 14 d, the untreated seedlings (control) were then split into two further subsets. The first subset was kept in the optimal temperature chamber to serve as a control. The second subset and the SiNPs- or K₂SiO₃ (further only Si)-treated seedlings were kept in the second chamber and exposed to high temperature of 45 °C for 4 h. Then, the fully expanded leaves from the second internodes of two-weeks-old were collected, frozen in liquid nitrogen and then stored at -80 °C for biochemical analyses.

Leaf ultrastructure: Samples (1 cm²) from the middle of the second flag leaves were collected, then sliced and fixed immediately in 4 % (m/v) glutaraldehyde in 100 mM sodium cacodylate buffer, at pH 7.2 overnight. Following glutaraldehyde fixation, samples were post-fixed in 2 % (m/v) osmium tetroxide in the same cacodylate buffer, stained in 1 % (m/v) aqueous uranyl acetate for 8 h, dehydrated in acetone, and embedded in Spurr's resin (Spurr 1969). The samples (ultrathin sections, 300 nm) were then examined under transmission electron microscope (TEM; *JEM 1011*, *JEOL*, Tokyo, Japan) at 80 kV, and photographed for image analysis with an *AMT* digital image capture system.

Pigment content and chlorophyll fluorescence: The photosynthetic pigments chlorophyll (Chl) *a*, Chl *b*, and

carotenoids (Car) were extracted in 80 % (v/v) acetone and measured spectrophotometrically (*Spectronic 601*, Milton Roy Company, Rochester, NY, USA) according to Metzner *et al.* (1965).

Leaf Chl *a* fluorescence was measured using a pulse amplitude modulation portable fluorometer (*Handy PEA*, Hansatech, Norfolk, UK). The leaves were dark adapted in a leaf clip for 30 min. For all treatments, 30 measurements (three replicates of 10 leaves from different plants) were recorded. The data obtained were used to calculate the maximum efficiency of PS II (F_v/F_m) and performance index on absorption basis (PI_{abs}) (Strasser *et al.* 2004) according to the equations reviewed by (Stirbet and Govindjee 2011)

Lipid peroxidation was evaluated by measuring the production of malondialdehyde (MDA) by the thiobarbituric acid (TBA)-based colorimetric method as described by Heath and Packer (1968).

Membrane stability index (MSI) was determined according to Sairam *et al.* (2002). Leaf samples (200 mg) were immersed in 10 cm³ of deionized water and divided into 2 sets. One set was kept at 40 °C for 30 min and its conductivity recorded (C1) using a conductivity meter (*HI 8733*, Hanna Instruments, Woonsocket, RI, USA). The second set was kept in a boiling water bath (100 °C) for 15 min and its conductivity was also recorded (C2). The MSI was calculated as: $MSI = [1 - (C1/C2)] \times 100$.

Relative water content (RWC) was measured according to the method described by Pieczynski *et al.* (2013). Leaf discs were taken from the fully expanded and uniform leaves. The fresh mass (FM) was recorded, and then samples were placed in a Petri dish with distilled water for 4 h. The water saturated mass (WSM) was then recorded, and the leaf samples were placed in an oven at 80 °C for 48 h, to determine the dry mass (DM). Leaf RWC was calculated as: $RWC [\%] = [(FM - DM)/(WSM - DM)] \times 100$.

Activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging was determined using the method described by Hatano *et al.* (1988). A volume of 4 cm³ of each sample was added to 1 cm³ of methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.2 mM). The mixture was kept at room temperature for 30 min before measuring the changes in absorbance at 517 nm. The DPPH radical scavenging activity was calculated as $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the DPPH solution and A_1 is the absorbance of the sample.

Gene expression analysis: Total RNA was extracted from wheat leaves of all treatments using the *RNeasy Plant Mini* kit (*Qiagen*, Amsterdam, The Netherlands). Total RNA (1 µg) from each sample was converted into cDNA by reverse transcription using the RNA PCR kit (*TaKaRa*, Tokyo, Japan) according to the manufacturer's instructions. The RT-qPCR was conducted on an *ABI 7500* system (*Applied Biosystems*, New York, NY, USA) using

a *TransStart™ Green qRT-PCR Super Mix* kit (*TransGen*, Beijing, China). A *TaActin* rRNA (GenBank accession No. AB181991) was used as a reference gene to normalize the relative transcription and to minimize different copy numbers of cDNA templates. The control sample was conferred a value of 1. All data were calculated and analyzed from three independent samples based on the 2^{-ΔΔC_t} method (Livak and Schmittgen 2001). The primers of the *Triticum aestivum* plasma membrane intrinsic protein *TaPIP1* and *Triticum aestivum* nodulin 26-like intrinsic protein *TaNIP2* genes (Table 1 Suppl.) used in quantitative reverse transcription PCR excluded the highly conserved protein domain and had a high efficiency and specificity.

Statistical analysis: Analysis of variance (ANOVA) for the current data were performed using *SPSS v. 20.0* (*SPSS*, Chicago, USA) software. Statistical significances of the means were compared by the Duncan's test at $P \leq 0.05$.

Results

The leaf ultrastructure of normal wheat cells was found to have a densely packed nucleus with an intact envelope (Fig. 1A). The exposure of wheat seedlings to heat stress induced ultrastructural distortion of the nucleus (Fig. 1B). Chromatin was unequally dispersed and agglutinated with heterochromatin. In addition, there was no distinct boundary between the nucleolus and chromatin (Fig. 1B). Meanwhile, the treatment with either Si or SiNPs maintained to some extent the integrity of the nuclear envelope and markedly dispersed chromatin appearance (Fig. 1C,D).

The results also showed that the chloroplasts have an ellipsoid shape with an intact membrane, concomitant with clearly arranged thylakoid segments in control unstressed leaves (Fig. 2A). However, heat stress imposed several changes in chloroplast ultrastructure including their swelling, expanding of matrix zone, and loosening of lamella structure (Fig. 2B). In addition, the number and size of plastoglobuli markedly increased in heat-stressed leaves compared with unstressed ones (Fig. 2B). As shown in Fig. 2C,D, Si or SiNPs treatments alleviated the heat induced deleterious effects as reflected by less occurrence of irregular grana lamellae and less damaged chloroplast envelopes. Additionally, the heat-stressed leaves exhibited reductions in Chl *a*, Chl *b*, and total Chl content as well as in Chl/Car ratio. However, the Car content markedly increased in the stressed wheat leaves (Table 1). Furthermore, heat stress induced apparent reduction in PI_{abs} and F_v/F_m values (Fig. 3A,B). Notably, Si or SiNPs treatments improved these parameters. The greater alleviation of negative effects of heat stress on Chl content, PI_{abs} , and F_v/F_m was in Si-treated leaves compared with SiNPs ones (Table 1, Fig. 3A,B).

Membrane ultrastructure of control wheat cells appeared normal in shape with large intercellular junctions (Fig. 4A). On the other hand, observations revealed damaged in plasma membranes and cell walls of heat-stressed wheat leaves; the plasma membranes seemed to

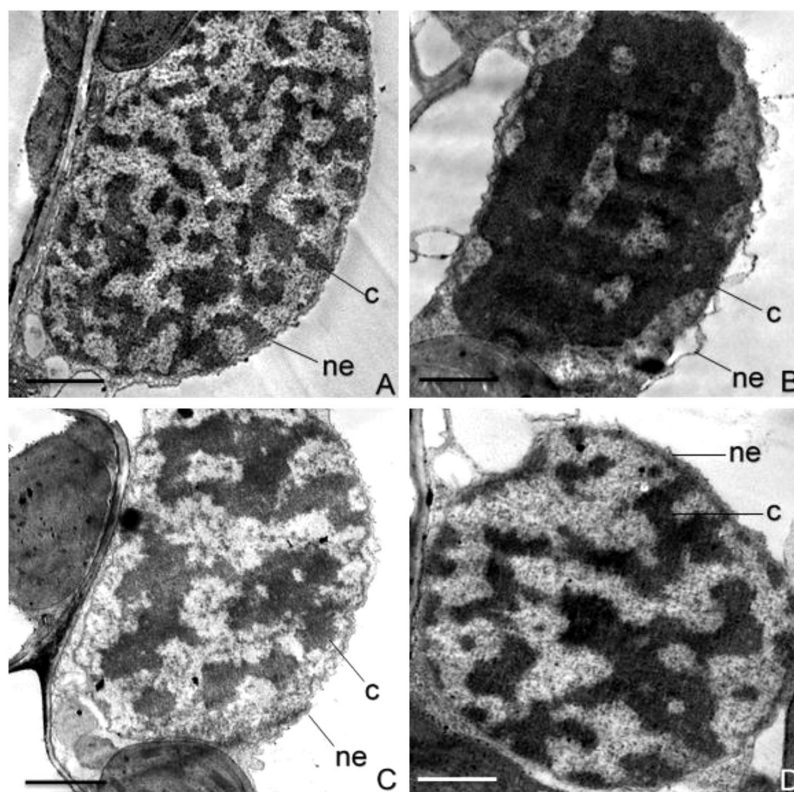


Fig. 1. Effect of potassium silicate (Si) or silicon dioxide nanoparticles (SiNPs) on nucleus ultrastructure of mesophyll cells from wheat leaves. *A* - control, normal temperature, *B* - heat stress (45 °C for 4 h), *C* - Si pretreatment + heat stress, *D* - SiNP pretreatment + heat stress; ne - nuclear envelope, c - chromatin; bars are 500 nm.

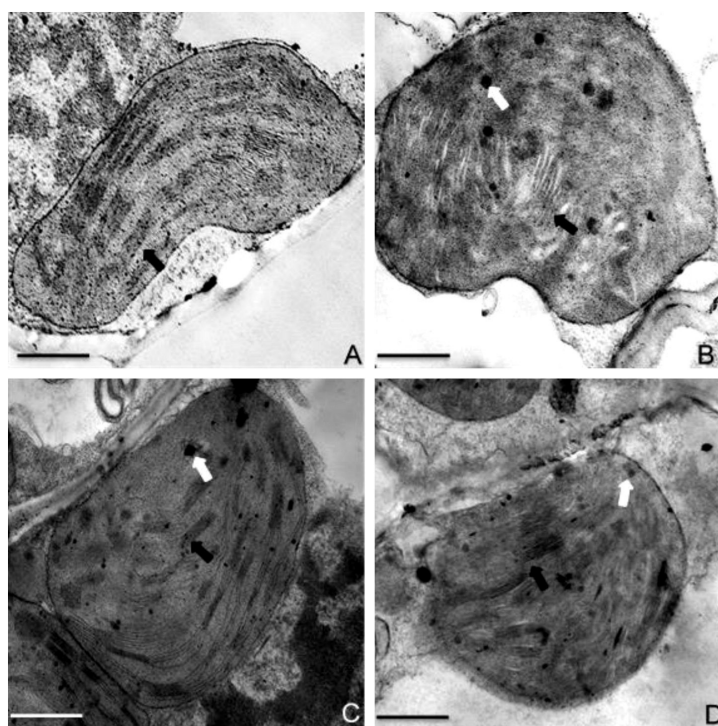


Fig. 2. Effect of potassium silicate (Si) or silicon dioxide nanoparticles (SiNPs) on chloroplast ultrastructure of mesophyll cells from wheat leaves. *A* - control, normal temperature, *B* - heat stress (45 °C for 4 h), *C* - Si pretreatment + heat stress, *D* - SiNP pretreatment + heat stress; black arrows point to grana lamellae, white arrows point to plastoglobuli; bars are 500 nm.

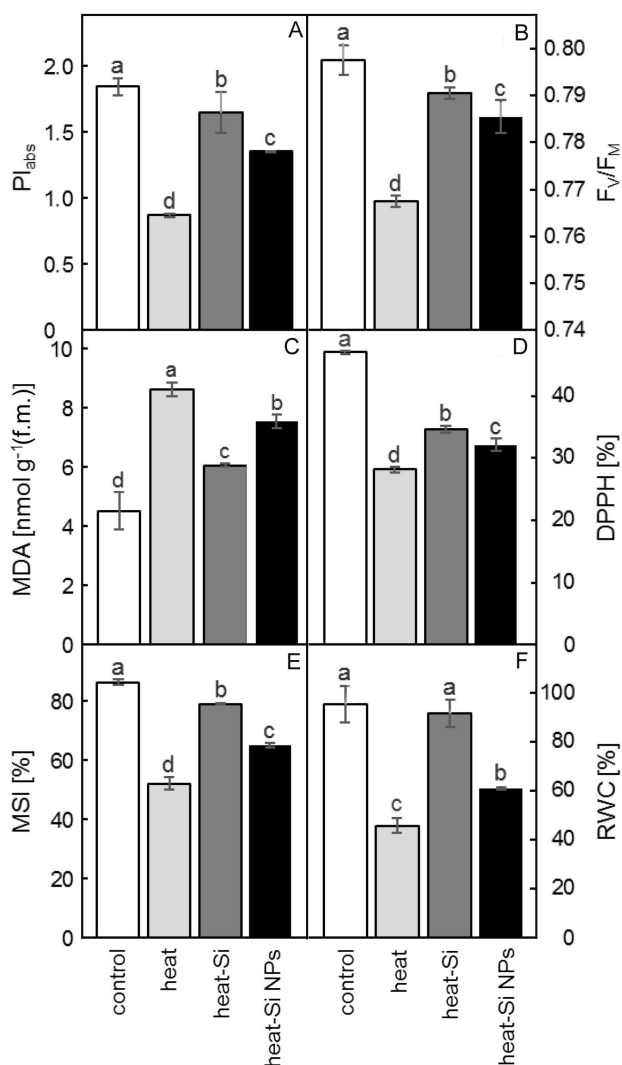


Fig. 3. Effect of heat stress and potassium silicate (Si) or silicon dioxide nanoparticle (SiNP) pretreatment on: *A* - performance index (PI_{abs}), *B* - maximum efficiency of photosystem II (F_v/F_m), *C* - malondialdehyde (MDA) content, *D* - 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, *E* - membrane stability index (MSI), and *F* - relative water content (RWC) of wheat seedlings. Means \pm SEs, $n = 3$, different letters indicate significant differences at $P \leq 0.05$.

be detached from the cell wall, and the intercellular spaces of cells appeared more compacted (Fig. 4B). The plasma

membrane distortion was associated with a significant increment in the MDA content (about 2-fold) of heat stressed leaves (Fig. 3C). However, the mesophyll cells of Si- or SiNPs-treated leaves maintained an integrated structure and relatively thick cell wall (Fig. 4C,D). In addition, both Si and SiNPs treatments alleviated extensive membrane damage, as shown by increasing MSI and nullifying membrane lipid peroxidation as observed by the reduction of MDA content (Fig. 3C,E). The DPPH scavenging activity was significantly increased in Si and SiNPs treated leaves as compared to that in non-treated heat-stressed leaves (Fig. 3D). Such effects were more pronounced in Si-treated leaves. The present results also showed that the RWC significantly decreased in heat stressed leaves. The Si treatment significantly increased RWC by about two folds compared to the untreated stressed leaves (Fig. 3F). In addition, the RT-PCR analysis showed that the *TaPIP1* and *TaNIP2* expressions were downregulated in heat-stressed leaves compared to the unstressed control ones (Fig. 5). However, both genes were significantly upregulated in Si-stressed leaves (Fig. 5). On the other hand, the expressions of the both genes were relatively unchanged in SiNPs-treated leaves as compared with heat-stressed ones (Fig. 5).

Discussion

The environmental stresses, particularly heat stress, induced metabolic disruptions which are recognized by the ultrastructure distortions of cell organelles. The electron microscopy analysis showed that imposition of heat stress caused the damages of chloroplasts (swollen thylakoids, increased abundance of plastoglobuli) and nucleus (condensation of chromatin) in wheat mesophyll cells (Figs. 1, 2, and 4). The observed changes of nuclear chromatin organization might be attributed to the modification in the chromatin configurations (Lang-Mladek *et al.* 2010). Similar disruptions in chromatin under heat stress have been reported by Santos *et al.* (2011) in rice and by Pecinka *et al.* (2010) in *Arabidopsis*. However, the pretreatment of wheat with Si or SiNPs minimized the heat stress-induced adverse ultrastructural alterations as revealed by maintaining the integrity of nuclear envelope and the normal dispersion of chromatin (Fig. 1C,D). In fact, it is evident that silicon possesses multifaceted role in the regulation of genes involved in stress resistance

Table 1. Effect of silicon or silicon dioxide nanoparticles (SiNPs) on content of chlorophylls (Chl) and carotenoids (Car) [$\mu\text{g g}^{-1}(\text{d.m.})$] in wheat leaves exposed to heat stress alone or after pretreatment with 1.5 mM K_2SiO_3 ; (Si) or 1.66 mM SiNPs. Means \pm SEs, $n = 3$, different letters indicate significant differences at $P \leq 0.05$.

Treatment	Chl <i>a</i>	Chl <i>b</i>	Chl <i>a+b</i>	Car	Chl <i>a+b</i> /Car
Control	546.672 \pm 6.7 ^a	338.573 \pm 5.9 ^a	885.248 \pm 0.8 ^a	69.461 \pm 2.0 ^d	4.44 \pm 6.7 ^a
Heat	376.964 \pm 0.4 ^d	218.078 \pm 3.2 ^d	595.042 \pm 3.6 ^d	106.817 \pm 2.6 ^a	1.96 \pm 0.5 ^d
Heat + Si	497.924 \pm 0.9 ^b	305.407 \pm 5.2 ^b	803.331 \pm 6.2 ^b	65.975 \pm 1.2 ^c	4.27 \pm 1.4 ^b
Heat + SiNPs	413.084 \pm 0.6 ^c	241.087 \pm 1.4 ^c	654.171 \pm 0.7 ^c	89.397 \pm 0.4 ^b	2.55 \pm 0.6 ^c

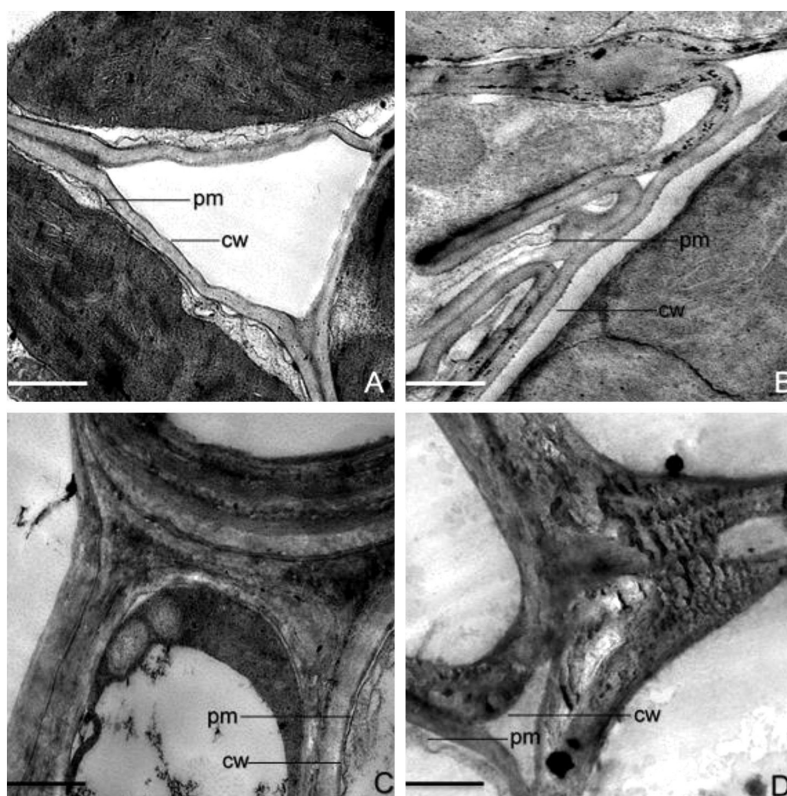


Fig. 4. Effect of heat stress and pretreatment with potassium silicate (Si) or silicon dioxide nanoparticle (SiNPs) on walls and membrane ultrastructure of mesophyll cells from wheat leaves. *A* - control, normal temperature, *B* - heat stress (45 °C for 4 h), *C* - Si pretreatment + heat stress, *D* - SiNP pretreatment + heat stress; cw - cell wall, pm - plasma membrane, bars are 500 nm.

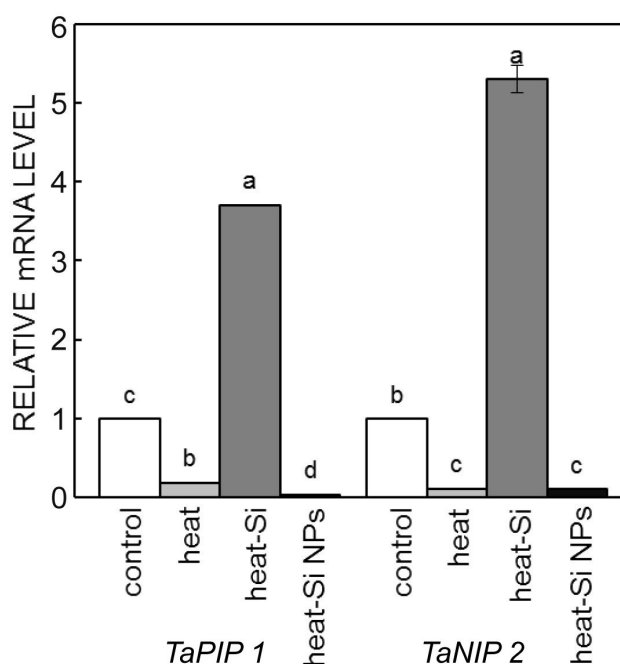


Fig. 5. Effect of heat stress and potassium silicate (Si) or silicon dioxide nanoparticle (SiNP) pretreatment on *Triticum aestivum* plasma membrane intrinsic protein *TaPIP1* and *Triticum aestivum* nodulin 26-like intrinsic protein *TaNIP2* gene expressions in wheat seedling leaf tissue. Means \pm SEs, $n = 3$, different letters indicate significant differences at $P \leq 0.05$.

(Manivannan *et al.* 2016).

The TEM results showed that heat stress induced changes in the shape and ultrastructure of chloroplast (Fig. 2*B*). The chloroplast grana were more sensitive than stroma and their disintegration might be attributed to the photooxidative damages resulted from ROS accumulation (Djanaguiraman *et al.* 2018). Meanwhile, grana decay was concomitant with an increase in the number of plastoglobuli occurred in chloroplast stroma suggesting the decomposition of thylakoid membrane lipids (Lutz *et al.* 2015). The distortion of the thylakoid membranes under heat stress leads to a significant inhibition of the photochemical reactions (Djanaguiraman *et al.* 2014). Assessment of the fluorescence parameters in terms of F_v/F_m and PI_{abs} reflects the photochemical efficiency of PS II and offers evidence about the functional and structural integrity of thylakoids (Kalaji *et al.* 2016). It was reported that the values of F_v/F_m and PI_{abs} altered under stress condition before the detection of the visible stress signs on the leaves (Christen *et al.* 2007). The current data showed that, heat stress induced a marked reduction in PI_{abs} and F_v/F_m values (Fig. 3*A,B*) concomitant with significant decreases in the Chl content in wheat leaves (Table 1). Indeed, the distortion of thylakoid membranes may also lead to chlorophyll loss as reported by Chuartzman *et al.* (2008). The pronounced reduction in Chl *a*, Chl *b*, total Chl content, as well as Chl/Car ratio may be due to either decreased biosynthesis of the chlorophyll and/or

increased its degradation (Dutta *et al.* 2009). Moreover, the decrease in Chl *a*, Chl *b*, and total Chl content in response to heat stress had earlier been ascribed to photo-oxidation of chlorophyll (Guo *et al.* 2007). Likewise, the reduction in the F_v/F_m values in stressed wheat leaves may resulted from the conformational changes and damages of the reaction center of photosystem (PS) II (Colom and Vazzana 2003) which causes imbalance between the generation and utilization of electrons, leading to photoinhibition (Li *et al.* 2016). Similar impacts of heat stress on the pigments associated with other photosynthetic performance has been reported by Yüzbaşıoğlu *et al.* (2017) on maize and Djanaguiraman *et al.* (2018) on wheat. However, PI_{abs} and F_v/F_m were improved in the Si- or SiNPs treatment concomitant with the preservation of chloroplast ultrastructure (Fig. 2C,D) as well as the content of photosynthetic pigments (Table 1). Notably, Si was more effective than SiNPs in this regard. The effect of Si on the photosynthetic apparatus can be attributed to its positive impact on plant water status (Fig. 3F), as well as on the possible uptake and accumulation of both macro and micronutrients (Savić and Marjanović-Jeromela 2013). Also, Si can induce the synthesis of new pigments and help in the maintenance of previously existing Chl *a* (Silva *et al.* 2012). Tuna *et al.* (2008) and Ma *et al.* (2016) obtained similar results on stressed wheat. Meanwhile the increasing PI_{abs} value by Si treatment may be related to the increase in the density of the active reaction centers of PS II (Ghassemi-Golezani and Lotfi 2015). Also SiNPs are characterized by their high reactivity and thereby they can bound with PS II and so stabilize the photosynthetic activity under stress conditions (Noji *et al.* 2010).

The distortions of the plasma membrane and cell walls were detected by TEM in heat-stressed wheat leaves (Fig. 4B). Such effect is attributed to osmotic stress concomitant with thermal stress (Lima *et al.* 2013), as well as the alterations of membrane proteins and lipids induced by high temperature (Somerville and Browse 1991, Agarie *et al.* 1998). Meanwhile, Si or SiNPs treatments maintained the integrity of both cell walls and cell membranes (Fig. 4C,D). Such impact on the cell walls was resulted from the deposition of either Si or SiNPs at the interface of the plasma membrane with the cell wall and/or in the intercellular spaces (Bauer *et al.* 2011, Sun *et al.* 2014, Asgari *et al.* 2018). In addition, SiNPs deposited in cytoplasm and in cell organelles such as the vacuoles (Sun *et al.* 2014). Both Si and SiNPs mitigated the adverse effects of heat stress, but with different intensities.

Interestingly, the observed increase in MDA content (Fig. 3C) was positively related to the abundance of plastoglobuli in the thylakoids of stressed leaves (Fig. 2B). Similar results have been obtained by Shah *et al.* (2013). The present investigation also showed that Si or SiNPs treatment reduced MDA content concomitant with increments in membrane stability index (Fig. 3C,E). Such result might resulted from the enhanced DPPH scavenging capacity (Fig. 3D). Similarly, the enhancement of the antioxidant defense systems in plants was reported by Kim *et al.* (2017), and Merwad *et al.* (2018) in different Si- treated plants. Furthermore, SiNPs stimulated the

antioxidant system in stressed tomato and squash plants (Haghighi *et al.* 2012, Siddiqui *et al.* 2014).

Notably, maintenance of greater leaf water content under heat stress is one of the remarkable effects of Si and SiNPs (Fig. 3F) and maximum increase in RWC was displayed in Si-treated leaves. Such effect may be due to the greater deposition of Si compared to SiNPs in the leaf tissues (Fig. 4C,D), decreasing water loss and thus protecting plants from water desiccation induced by heat stress (Ma 2004, Coskun *et al.* 2019). Despite the role of Si or SiNPs in maintenance of water content, it is interesting to explore the role of Si or SiNPs in improving the water status in heat stressed plants *via* AQPs performance. The regulation of AQP coding genes expressions contributes in the stress adaptation mechanisms. However, their role in the improvement of plant tolerance against heat stress remains to be a challenge (Kapilan *et al.* 2018). The results of the current work showed a reduction in *TaPIP1* and *TaNIP2* gene expressions in heat stressed wheat seedlings (Fig. 5). Meanwhile, Si treatment induced the overexpression of *TaPIP1* and *TaNIP2* (Fig. 5). Such increase in gene expression may be at the transcriptional level (Hu *et al.* 2012). Our finding is supported by those of Zhu *et al.* (2015) who reported that silicon increased the expression of members of the *PIP2* subfamily resulting in an increase in water uptake. In fact, AQPs act as multi-functional channels that transport water and many small molecules such as silicon (Fox *et al.* 2017). Silicon compounds can be translocated by *TaPIPs* and *TaNIPs* transporters, however, the SiNPs, due to their small size, can diffuse through the cell walls (Asgari *et al.* 2018). The current data proved that unlike Si treatment, *TaPIP1* and *TaNIP2* genes expression in the SiNPs-treated stressed leaves was relatively unaffected (Fig. 5). The present observations assumed that, the flow of SiNPs is independent on the *TaPIP1* and *TaNIP2* transporters. Meanwhile, SiNPs improved thermotolerance *via* maintaining water status which achieved by the mechano-physical strategy only. Additional experimental work is needed to explore the molecular mechanism of Si and SiNPs in improving thermotolerance.

In conclusion, the current observations inferred that imposition of high temperature caused reduction in RWC, distortion in the cellular structures, decrease in photosynthetic system performance, and membrane damages. The results also illustrated that soaking of wheat grains in either Si or SiNPs solutions could ameliorate some heat stress induced injuries including decrease in RWC, ultrastructural changes of mesophyll cells, MSI index, parameters of chlorophyll fluorescence (PI_{abs} , F_v/F_m), and MDA accumulation as well as they enhanced the DPPH scavenging activity. Notably, Si was more effective than SiNPs in alleviation of adverse effects of heat. Meanwhile, maintaining greater RWC in heat stressed wheat leaves is one of the remarkable effects of Si which was associated with the significant overexpression of *TaPIP1* and *TaNIP2* compared with the relatively unaffected ones in SiNPs-treated wheat leaves. Therefore, the data of current investigation added innovative knowledge for the improvement of thermotolerance and gave additional

support to the proposed roles of Si or SiNPs in plant responses to heat stress.

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