



Effect of silica extracted from rice straw on wheat plants under salinity stress

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Abstract

In the present study, we aimed to assess the effects of exogenous silica (extracted from rice straw) on the biochemical responses in wheat under salt stress (0 and 100 mM NaCl). Results showed that silica significantly alleviated the harmful effects of salinity on plants biochemical activities. Silica application under saline conditions increased JA production which induces signal molecules. These signals molecules initiate signal transduction to activate the defense system. This defense system is represented in the activation of the enzymatic and the non-enzymatic antioxidants. These antioxidants protect silica treated plants from the toxic effect of the reactive oxygen species. This protection appeared in the reduction of the lipid peroxidation and maintained the plasma membrane stability. This active metabolic status was ensured with the sharp decrease in shikimic acid in silica treated plants. Therefore treated plants can withstand the salinity, while non-treated plants could not withstand against salt stress.

Keywords: Antioxidant enzymes; phenols; phytohormones; shikimic acid

1. Introduction

Wheat (*Triticum aestivum* L. em Thell.) is considered as the first important cereal crop worldwide. Wheat is considered as one of the healthiest and the richest nutritive foods. Its production is the highest in other grain crop such as rice, maize, etc. It is cultivated over a wide range of climatic conditions. Hence, the understanding of metabolic status under stress conditions is very important improving the wheat withstanding against the various environmental conditions^[1].

Salinity is the most common abiotic stress all over the world^[2]. Worldwide about 20% (62 million hectares) of the total cultivated land is under salt stress and this percent increased annually with 1-2%^[3]. Various researches worked on the effect of salt stress on plants growth showed that salinity obviously reduces plants growth, interacts with metabolic status affecting the essential nutrients uptake and transportation and initiates oxidative stress in plant cells. Different researches were developed as solutions to overcome salinity and improve the tolerance of plants against it. The past few decades using Si as a tool for improvement of plants tolerance against salinity has got a great attention^[4].

Silicon is defined as “non-essential element”, which raised a great attention in scientific researches. However, silicon is still not imported in most plant growth-media formulations; it is now widely used as plants fertilizer particularly under stresses such as drought and salinity^[5]. Ibrahim et al.^[1] study the effect of the silicon on wheat plants under salinity stress and results indicated that Si application improved wheat growth and yield under salt treatment, through improving the photosynthetic pigments, N, P, and K contents under salinity stress. Also Sapre and Vakharia^[6] ensured the critical role of silicon as a mechanical and a physiological barrier. Beside this role, silicon increases osmolytes and antioxidant enzymes contents which are the main defense mechanism under salt stress. Xu et al.^[7] indicated that silicon-nutrition significantly induce aloe

plants to get rid of the toxic effects induced by NaCl. This induction achieved through maintenance the homeostasis of ions in aloe leaves under salt stress.

Si positive effect on plant responses to water stress is accompanied with enhancement the hormonal and molecular signals. Abscisic acid (ABA) mainly initiates the changes in physiological processes under stress because of its role in the regulation of stomata closing, and this is the most efficient way to reduce water lose under water shortage stress^[8]. However, highly specialized network of water stress signaling has been identified, such as jasmonic acid (JA), participating in control ABA biosynthesis which controlling salt stress signaling and physiological and biochemical plant responses^[8,9].

The rapid population increase nowadays creates a dangerous problem in food availability for all these people especially in developing countries. Especially with the shortage in water supply available for agriculture, this giving the investigation of crop growth improvement under water stress is urgent need. The challenge is to improve new safe and cheap ways for crop growth improvement under stress. The present study aimed to assess the biochemical effects of exogenous Silica (extracted from rice straw) application on wheat plants growth under saline conditions as cheap way for silicon fertilization.

2. Materials and methods

2.1. Silica Extraction from rice straw:

Silica was extracting according to Adam and Fua^[10]. Thirty grams of dried rice straw were put into a plastic container then added with 600 mL of 1 M HNO₃ and incubated on shaker for 24 h in order to get rid of undesired metals. After incubation the straw was filtered and cleaned with distilled water till pH (4.5–5.0), then dried in oven at 60°C till constant weight, after that the cleaned dried rice straw was incubated with 500 mL of 1 M NaOH on skaking for 24 h. The mixture was filtered to obtain sodium silicate which was kept in a covered plastic container for use and the

silicon concentration in the prepared solution was determined by atomic absorption spectrophotometer.

2.2 Green House experiment

Wheat (*Triticum aestivum* L.; cultivar Skha 96) grains were got from Agricultural Research Center, Giza, Egypt.

Peat moss soil Plastic (2 kg) were filled in plastic pots (20 cm diameter) and divided into two groups. The first one (W), the soil kept as it is without any treatment (the control). The second one (WS), 50 mL of the prepared sodium silicate solution (150mg/mL) was introduced to the peat moss soil. Seeds were washed with 30% sodium hypochlorite solution, and then washed with sterile distilled water. The seeds were planted in the plastic pots. The pots were arranged on greenhouse benches in two groups (W: Non treated group, WS: Silica treated groups), under natural photoperiod (12 to 13 h) and temperature (28±4°C). After 20 days from planting, the groups were subdivided into two groups according to salt application as following; 0, 100 mM NaCl solution. The samples were collected for biochemical analysis 9 days of salt treatment.

2.3 Biochemical studies

2.3.1 Determination of photosynthetic pigment content

Pigment content was evaluated on the basis of Metzner et al. [11].

2.3.2 Determination of elements

Elements were extracted from plant leaves on the basis of Chen et al. [12]. The concentrations of sodium and silicon were obtained with atomic absorption spectrometer (SpectrAA220FS). A control (no leaf sample) was used to eliminate errors.

2.3.3 Oxidative stress markers

The determination of malondialdehyde (MDA) content was carried out following the method described by Li [13].

The plasma membrane permeability was expressed as the change in electrolytes leakage from cells following the method of Pooviah and Leopold [14].

H₂O₂ content was evaluated on the basis of Alexievia et al. [15]. Fresh leaves (1 g) were ground with 0.1% trichloroacetic acid, then it was centrifuged at 3800 g for 10 min. The assay mixture was 0.5mL 100mM K-phosphate buffer (pH 6.8) and 2mL 1M KI were added to 0.5mL of the prepared leaves extract supernatant. Samples were kept at room temperature in dark for 1h, after incubation the optical density was read at 390 nm. Hydrogen peroxide content expressed as µg. g⁻¹ fresh weight (FW).

2.3.4 Enzymatic antioxidants

Enzyme extraction: Five grams of fresh leaves was ground with 0.05 M cold phosphate buffer (pH 6.5) containing 1 mM EDTA, Na₂ and centrifuged at 3800 g for 10 min. The supernatant was completed to a known total volume and used as enzyme source (Superoxide dismutase (SOD), Catalase (CAT) CAT, Polyphenol oxidase (PPO), and peroxidases (POX)) [16]. The residue was well cleaned through washing with dist. water and centrifuged several times, then incubated with 10mL of 1M NaCl for 1h to get

cell wall bound POX. After centrifugation at 3800g for 10min, the supernatant was used as the wall bound POX source [17].

Enzyme assays: SOD activity was performed by the nitro blue tetrazolium reduction method [18]. CAT activity was determined following the method of Kar and Mishra [19]. PPO activity was performed according to Beyer and Fridovich [18]. Soluble and bound POX activity was determined according to Upadhyaya et al. [20].

2.3.5. Non-enzymatic antioxidants

Free phenols were obtained from fresh samples on the basis of Campbell and Ellis [21]. Samples were macerated with liquid nitrogen, 50% methanol were added to the homogenate with ratio 2:1 (v:v), and then kept in water bath at 80°C for 1.5 h. after incubation the samples were subjected to centrifugation for 5 min at 3800 g and the supernatant was completed to a known volume and used for the Folin-Ciocalteu assay for soluble phenol determination. The remaining pellets was used for cell wall bound phenols determination according to Funk and Brodelius [22] method as following; with 4mL of 0.5M NaOH for each 1g of original sample for 24h at room temperature. The mixtures were neutralized with 2 M HCl, centrifuged for 5 min at 3800g, and the supernatants also used for Folin-Ciocalteu assay for cell wall bound phenol determination.

2.3.6. Metabolic status marker:

Shikimic acid content was evaluated on the basis of Zelya et al. [23].

2.3.6 Hormones

Hormone extraction was performed according to Shindy and Smith [24] and reported by Hashem [25]. Abscisic acid content was determined using gas chromatography analysis, the samples and standards were methylated before GC according to Vogel [26]. Jasmonic acid was evaluated on the basis of Kramell [27] with NUCLEODEX beta-PM, 200 mm and 4 mm ID column, flow rate was kept at 1mL. min⁻¹ and read at UV 210 nm.

3. Results

3.1 Plant photosynthetic pigment contents

Pigment analysis showed a decrease in pigments contents under salinity conditions (Table 1). Interestingly, the application of silica alleviated the detrimental effects of salinity on plant morphology; no toxicity symptoms were observed. Indeed, all pigments were maintained at the same content of the control. Moreover, the application of silica to plants non-treated with NaCl significantly enhanced their pigment contents.

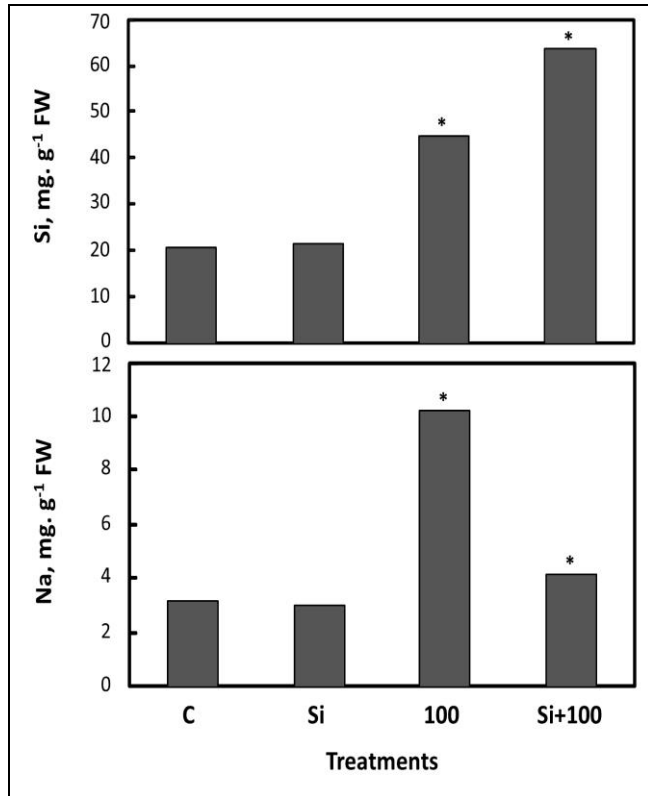
3.3 Elements content

Si application reduced Na uptake, its contents minimized by 58% compared to salt-treated plants without silicon application. In addition, silica was highly absorbed, as shown in Figure (1) their content was 63mg g⁻¹ FW under saline conditions, while under non-saline conditions it was only 21 mg. g⁻¹(Figure 1).

Table 1: Effect of silica application on Chlorophyll *a* (Chla), chlorophyll *b* (Chlb) and carotenoid (Car) contents in wheat plants under two different salinity levels

NaCl, mM	0		100	
	W	WS	W	WS
Chla	1.415	1.69*	0.58*	1.592
Chlb	0.713	0.96*	0.29*	0.940
Car	1.230	1.379	0.51*	1.280

Data are means of 3 replicates. Means with asterisks significantly differ from the control based on paired-samples *t* test.

**Fig 1:** Effect of silica application (C without silica application, Si with silica application) on sodium and silicon contents in wheat plants under two different salinity levels: 0, 100 mM NaCl. Data are means of 3 replicates. Columns with asterisks significantly differ from the control based on paired-samples *t* test.

3.4 Oxidative stress indicators

Malonyl di aldehyde (MDA) content increased by 23 times in the non-treated plants under salt application (Table 2). While, silica application significantly reduced MDA content under no salt application, therefore its content was the same as in the control under salt application.

Plasma membrane permeability (PMP) was significantly disturbed by salinity in the non-treated plants, as the electrolyte leakage significantly increased in these plants under salinity. While, the electrolyte leakage in Si treated plants under salinity was the same in the control, thus Si decreased the negative salt effect on PMP (Table 2).

Salinity conditions significantly increased H₂O₂ content, but silica treatment can reduced this significant increase in H₂O₂ content induced under salinity treatment (Table 2)

Table 2: Effect of silica application on Malonyl dialdehyde (MDA), plasma membrane permeability (PMP) and H₂O₂ content in wheat plants under two different salinity levels

NaCl, mM	0		100	
	W	WS	W	WS
MDA (μmol. g ⁻¹ FW)	0.328	0.26*	7.32*	0.263
PMP (%)	13.94	11.99	70.0*	12.23
H ₂ O ₂ (μmol. g ⁻¹ FW)	0.230	0.210	2.80*	0.304

Data are means of 3 replicates. Means with asterisks significantly differ from the control based on paired-samples *t* test.

3.5 Antioxidant enzyme activities

Under salinity, all studied antioxidant enzyme showed highly significant elevated activities in silica treated plants. Low elevation in SOD, CAT, and soluble POX activities was also recorded in the non-treated plants under salt application. Interestingly the cell wall bound POX activity was significantly decreased in non-treated plants under salinity, while in silica treated plants its activity significantly increased (Table 3).

Table 3: Effect of silica application on SOD, CAT, soluble POX, cell wall bound POX and PPO activities in wheat plants under two different salinity levels

NaCl, mM	0		100	
	W	WS	W	WS
SOD (%)	10.99	11.13	16.22*	50.32*
CAT (μmol H ₂ O ₂ . g ⁻¹ FW. min ⁻¹)	0.22	0.228	0.698*	5.002*
Soluble POX (change in O.d. g ⁻¹ FW. min ⁻¹)	0.005	0.005	0.007*	0.06*
Cell wall bound POX (change in O.d. g ⁻¹ FW. min ⁻¹)	0.0021	0.002	0.001*	0.010*
PPO (change in O.d. g ⁻¹ FW. min ⁻¹)	0.0092	0.009	0.024*	0.191*

Data are means of 3 replicates. Means with asterisks significantly differ from the control based on paired-samples *t* test.

3.6 Non-enzymatic antioxidant system

Free soluble phenol content was doubled under NaCl and quadrated under NaCl/Si conditions (Table 4). The content of cell wall bound phenol compounds elevated under the effect of salinity and silica application (Table 4). The cell wall bound phenol in plants under silica application showed slight increase under normal conditions and the content was increased in high ratio about 275% under salinity conditions and Si application.

3.7. Metabolic activity marker

Under normal conditions silica application decreased shikimic acid content by 35% in comparison with the control. Salt treatment also decreased shikimic acid either in the non-treated plants or in the silica treated one, but in the silica treated plant the decrease ratio was the highest about 59% in comparison with the control (Table 4).

Table 4: Effect of silica application on Shikimic acid, free soluble phenols, cell wall bound phenols in wheat plants under two different salinity levels

NaCl, mM	0		100	
	W	WS	W	WS
Shikimic acid mg. g ⁻¹ FW)	49.0	32.0*	45.96*	13.04*
Free soluble phenols (mg pyrogallol. g ⁻¹ FW)	12.63	12.93	20.3*	42.4*
Cell wall bound phenols (mg pyrogallol. g ⁻¹ FW)	3.00	4.22*	5.96*	15.2*

Data are means of 3 replicates. Means with asterisks significantly differ from the control based on paired-samples *t* test.

3.8 Hormone content

Salinity significantly increased the jasmonic acid (JA) content. This increase was the highest under Si application. Abscisic acid content increased under salinity. In contrast to JA, the increase in ABA was the highest in the non-treated plants (Figure 2).

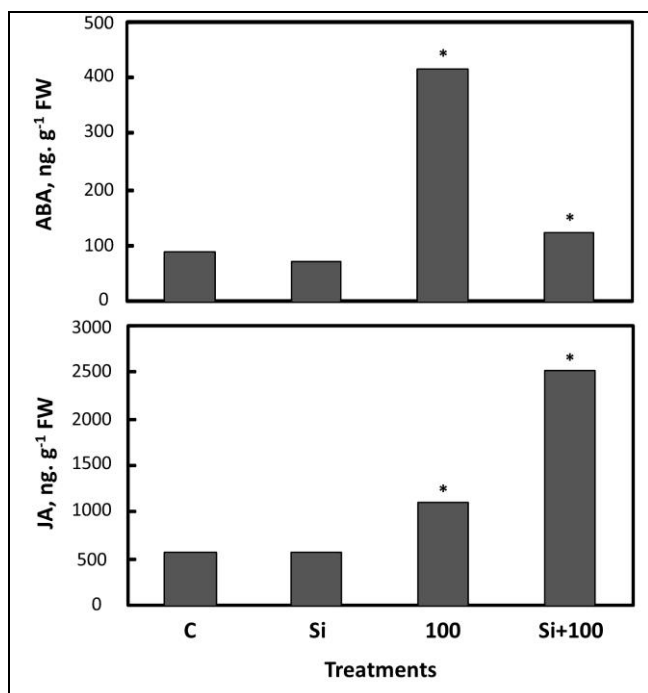


Fig 2: Effect of silica application (C without silica application, Si with silica application) on Abscisic (ABA) and jasmonic acid (JA) contents in wheat plants under two different salinity levels: 0, 100 mM NaCl. Data are means of 3 replicates. Columns with asterisks significantly differ from the control based on paired-samples *t* test.

4. Discussion

Silicon has variable roles in plants especially under stress conditions, however it is considered as a non-essential nutrient for most plants [28]. In the present study, silica application protected wheat plants under saline conditions. This can be attributed to many biochemical changes caused during silica application under salinity as reported in the present study. Silica treatment protected the photosynthetic

pigments under salinity stress. The protective role of Si for pigments under stress conditions was well reported [29-31], as found in the present study.

The protective role of silicon also appeared in improvement of the mineral ions uptake, as shown in the present results silica application decreased the sodium concentration. This decreased is due to the decreasing in the transpirational bypass flow. Beside this silica application enhances of the affinity of transporters for K⁺ which decreases Na⁺ absorption. This reduction in the Na⁺ absorption enable the silica treated plants to overcome the negative effect of the salt on the minerals uptake. This negative effect is that Na⁺ reduces the uptake of the cations and Cl⁻ reduces the uptake of the anions by transporters in plant roots [32]. Similarly, several studies showed that plant supply with nutrients under salt stress improves its growth under salt stress [33].

Silicon application improves the antioxidant machinery and protected the plants from the toxic effect of the elevated concentrations of the reactive oxygen species (ROS) generated under the saline conditions. One of the main toxic effects caused due to the high generation of ROS is the lipid peroxidation. It decreases membrane fluidity which increases electrolyte leakage [34]. Our results showed that Si application protects the plants the oxidative damage caused under saline conditions as lipid peroxidation indicators (MDA and PMP) showed no difference from control in silica treated plants under saline conditions, and H₂O₂ content showed lower increase. This is explained according Liu et al. [35] who showed that silica application has a role in protein structure stabilization and strengthen of the cell wall through enhancement the lignin deposition on the cell wall. Silicon deposition in cell membrane makes it harder and reduces electrolytic leakage. Similarly Kim et al. [32] ensured that silica enhances the antioxidant machinery, which enable the cells to keep the ROS in the safe limit, which protect cell membranes from lipid peroxidation. Our results confirmed the positive role of silica on the antioxidant systems either enzymatic or non-enzymatic one (Tables 3 and 4).

H₂O₂ content significantly increased under salinity without silicon application, while under silica application the H₂O₂ content was near to that in the plants under normal conditions. This is due to the elevated activities of the antioxidant system. This clarifies the absence of lipid peroxidation and electrolyte leakage symptoms in silicon treated plants [5]. This highly metabolic active status is ensured with the sharp decrease in the shikimic acid in the silicon treated plants. Shikimic acid is considered as the precursors of many secondary metabolites used in stress tolerance such as phenols [36, 37].

Phytohormones play an important role in protection of plants against different stresses through initiation various adaptive responses [38]. Abscisic acid (ABA) is a well-known hormone which accumulates in plants under water stress due to its role in controlling the stomata opening and closure [8]. ABA has a dual role, as its serves as signal transduction which activate many defense response, while its very high levels results in the cell death and leaves abscission [9]. This fact is well clarified in the present investigation as ABA content increased under saline conditions in both groups, but its increase in the non-treated plants was highly significant, while its content in silica treated plants was slightly increase (Figure 2). Other hormones such as jasmonic acid (JA) participate in controlling ABA content and so protect plants under water

(8). In present study, the significant increase in jasmonic acid (JA) in salt-treated plants supplied with Si, ensured the important role of JA in abiotic stress response, which is mainly attributed to the induction of the antioxidant pathway^[39].

Conclusion

Silica protected wheat plants from the deteriorious effect of salinity. This protection role is explained as silica application under saline conditions increased JA production. JA induces the cell juvenility, bioactivity and signal molecules (ABA and H₂O₂). These signals molecules initiate signal transduction to activate the defense system. This defense system represented in the activation of the antioxidant machinery, these antioxidants protect silicon treated plants from the toxic effect of the reactive oxygen species generated by the stress. This protection appeared in the reduction of the lipid peroxidation and maintained the plasma membrane stability. This active metabolic status was ensured with the sharp decrease in shikimic acid in Si-treated plants.

Disclosure of interest

I declare that I have no conflict of interests.

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