biologia plantarum
an international journal for experimental botany

BIOLOGIA PLANTARUM (2021) 65: 323-332 DOI: 10.32615/bp.2021.044

Potassium silicate combined with glycine betaine improved salt tolerance in *Dalbergia odorifera*

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Abstract

Salinity has a huge negative impact on plant growth and development by increasing sodium ions accumulation and potassium ions loss that deeply disturbs the plant cell homeostasis and can lead to plant cell death. The imbalance between Na^+ and K^+ could be solved by applying potassium silicate (K_2SiO_3). The glycine betaine (GB) is well-known to play a crucial role against oxidative stress in plants by improving the antioxidant machinery. Thus, this research aimed to apply K_2SiO_3 (1 mM) and GB (10 mM) alone or in combination against 200 mM NaCl-induced damages in *Dalbergia odorifera*. The results showed a significant amelioration of negative effects of salt stress on the phenotypic traits, chlorophyll content, net photosynthetic rate, stomatal conductance, transpiration rate, and water use efficiency by applied substances. The contents of saccharides and proline were down-regulated by K_2SiO_3, GB, and K_2SiO_3-GB, whereas the proteins content was increased by these treatments. The contents of lipid peroxidation, superoxide anion, hydrogen peroxide were reduced by exogenous substances under stress. The activities of antioxidant enzymes (superoxide dismutase, peroxidase, and catalase) and the accumulation of antioxidants (glutathione and ascorbate) were enhanced by exogenous substances. The K_2SiO_3-GB combination mostly showed better effects on antioxidant machinery compared to a single treatment.

Keywords: antioxidants, chlorophyll, *Dalbergia odorifera*, glycine betaine, oxidative stress, photosynthesis, potassium silicate, redox homeostasis, salt tolerance.

Introduction

Salt stress restrains plant growth and diminishes water and nutrient uptake, which causes water-deficit and osmotic stress. Salinity induces ion toxicity in a plant cell, which is due to the accumulation of Na^+ in plant cells and tissues, and has negative influences on plant growth and development (Assaha *et al*. 2017, Temme *et al*. 2020). The accumulation of Na^+ in plants under salinity disturbs the balance of K^+/Na^+ in the cell membranes, and Na^+ over-accumulation can be highly deleterious to plants homeostasis (Shabala and Cuin 2008). Indeed, the augmentation of Na^+ content in a plant cell is one of the major components of salt stress, and supplying a plant with a plethora of potassium can be a shield for the plant cells to face the deleterious effects of Na^+ during salinity (Qi and Spalding 2004). Potassium is a prominent element that plays a major role in many biochemical and physiological processes in plant growth and metabolism under both non-stressed and stress conditions (Wang *et al*. 2013). The application of K^+ to

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Received 29 January 2021, *last revision* 5 July 2021, *accepted* 30 July 2021.

Abbreviations: APX - ascorbate peroxidase; AsA - ascorbic acid; Car - carotenoid; CAT - catalase; Chl - chlorophyll; c_i - intercellular CO_2 concentration; E - transpiration rate; GB - glycine betaine; GPX - glutathione peroxidase; g_S - stomatal conductance; GSH - reduced glutathione; MDA - malondialdehyde; O_2^- - superoxide anion radical; OH - hydroxyl radical; P_N - net photosynthetic rate; POD - peroxidase; RC - relative conductivity; ROS - reactive oxygen species; RWC - relative water content; SOD - superoxide dismutase; WP - water potential; WUE - water use efficiency.

Acknowledgements: The present work was supported by the Hainan Provincial Natural Science Foundation of China (320RC507, 421QN192, and 317052), the National Natural Science Foundation of China (No. 32060240 and 31660165), and the Scientific Research Starting Foundation of Hainan University to Fan Yang (kyqd1573).

+These authors contributed equally.

Conflict of interest: The authors declare that they have no conflict of interest.

peanut cultivars promoted salt tolerance by improving Na^+-exclusion and K^+-accumulation resulting in an osmotic adjustment and ionic balance (Chakraborty *et al.* 2016). Beyond doubt, a crucial character of salt tolerance is the regulation of Na^+ uptake and transport and high cytosolic K^+/Na^+ ratios (Shabala and Pottosin 2014, Assaha *et al.* 2017).

Some other elements such as silicon (Si) can play a key role to mitigate salt stress in diverse plant species by up-regulating the expression of genes encoding maize ZmSOS$_1$ Na^+ exporter in root tissues, or *via* significant sequestration of Na^+ into the vacuoles of leaf mesophyll cells in maize (Bosnic *et al.* 2018). Si is the second richest element found in soil and is present in plants in amounts similar to those of such nutrients as phosphorus, calcium, or magnesium (Epstein 1999). Furthermore, severe salinity generated critical accumulation of reactive oxygen species (ROS) such as H_2O_2, $O_2^{\bullet-}$, and $^{\bullet}OH$, which can disrupt the cell homeostasis and functioning (Demidchik 2015). The use of exogenous Si in soybean plants can significantly down-regulate genes related to ROS production and increase the antioxidant enzyme activities [catalase (CAT) and ascorbate peroxidase (APX)] and contents of non-enzymatic antioxidants (reduced glutathione (GSH) and ascorbate acid (AsA)] to confer salt tolerance (Chung *et al.* 2020). But an earlier study reported that exogenous Si promoted salt tolerance in okra plants by improving the antioxidant enzyme activities [APX and peroxidase (POD)] (Abbas *et al.* 2017). Indeed, the effect of exogenous Si application in plants on antioxidant machinery is reliant on time, the concentration of Si, the severity of salinity, and plant species (Khan *et al.* 2019). Moreover, previous studies reported the role of exogenous glycine betaine (GB) and Si in the up-regulation of AsA and GSH content in plants under other environmental stresses (Ali *et al.* 2020, Hasanuzzaman *et al.* 2014). Indeed exogenous Si enhanced AsA and GSH accumulation under drought stress in *Brassica napus* seedlings which massively inhibited lipid peroxidation (Hasanuzzaman *et al.* 2018b).

The oxidative stress caused by salinity has negative impacts on photosynthesis and cellular respiration. To alleviate salt-induced damages, diverse plants can accumulate an osmoprotectant called glycine betaine (GB), which is a quaternary ammonium compound (McNeil *et al.* 1999). It is well-known to enhance antioxidant activities and stabilize photosynthetic machinery under stress (Annunziata *et al.* 2019). Further, GB is a non-toxic molecule that can raise osmotic pressure and protect some macromolecular structures against denaturation in plants under stress (Timasheff 1992). Naturally, GB accumulation occurs in barley plants under severe salinity, drought, or cold stress to provide stress tolerance (Jagendorf and Takabe 2001). The GB application is widely known to enhance stress tolerance in various plant species. Under salinity, exogenous GB can decrease the lipid peroxidation and ROS accumulation in tobacco plants (Banu *et al.* 2010), and it can also increase and protect the photosynthetic performance in barley and tomato plants (Park *et al.* 2006, Oukarroum *et al.* 2012).

In view of the key roles of GB, Si, and potassium in salt stress tolerance in various plant species, it is interesting to elucidate their effects on a plant when they are combined. Thus in the present study, a woody tree endemic to China *Dalbergia odorifera* T. Chen was chosen and exposed to NaCl-induced salinity and GB, K_2SiO_3, and a combination of K_2SiO_3 and GB were used to ameliorate salt stress. *D. odorifera* is an endangered semi-deciduous tree belonging to the *Leguminosae* family (Liu *et al.* 2019) and there is a huge lack of study of its response to abiotic stresses particularly to salinity. The phenotypic traits, water status, redox homeostasis, antioxidant activities, and photosynthetic performance in *D. odorifera* were determined.

Materials and methods

Plants: *Dalbergia odorifera* T.C. Chen belongs to protected plant species; the trade of its commercialized seedlings is permitted and legal in China. Thus, we settled that no specific permissions for the seedlings collection in this location were required by the Forestry Bureau of Hainan Province, China. The three-month-old *D. odorifera* seedlings were collected from nursery garden in Ledong county (18° 44′ 52″ 93 N, 109° 17′ 31″ E), Hainan province of China in March. The seedlings were transplanted into pots with 10 cm in height and 12 cm in diameter containing an equal volume of mixed substratum (2/3 red soil and 1/3 sand). The seedlings were grown and well-watered daily for 5 weeks with clean water in the greenhouse in natural conditions from March to April.

Experimental design and growth conditions: Healthy seedlings with almost the same size were chosen to perform the experiment. This study used a completely randomized design using two factors (salinity and exogenous substances) and 4 replicates for each treatment. Each treatment was composed of at least 12 seedlings. The treatments were: *1*) Ck: control, well-watered conditions; *2*) K_2SiO_3: well-watered and 1 mM K_2SiO_3; *3*) GB: well-watered and 10 mM GB; *4*) K_2SiO_3-GB: well-watered and 10 mM GB + 1 mM K_2SiO_3; *5*) S: 200 mM NaCl solution; *6*) S+K_2SiO_3; *7*) S+GB; *8*) S+K_2SiO_3-GB. The salt-treated seedlings were irrigated twice (in two days) with 200 mM of NaCl solution. A week before the salt application, the GB and K_2SiO_3 were supplied in the soil to the selected seedlings. After 22 d of treatment, plants were harvested for physiological and biochemical analyses.

Growth and water status: At the end of the treatments, leaf number, leaf area, and plant height increments were determined. The leaf area was measured *via* a portable area meter *LI-3000C* (*Li-COR*, Lincoln, USA). A fully expanded non-senescent leaf was used to determine the relative water content (RWC), relative conductivity (RC), and water potential (WP). The RWC was measured based on the method described by Yang *et al.* (2010) and calculated *via* the formula: RWC [%] = [(fresh mass - dry mass)/fresh mass] × 100. A dew-point measurement by *Potential Meter WP4C* (*Gene Company*, Pullman, USA) was used to estimate the WP. About 10 fresh leaf discs and 20 cm^3 of deionized water were put into test tubes. After that, the conductivity (C1) was measured, and then the test tubes were boiled (90 °C) for 30 min to measure the conductivity again (C2). To determine the RC, the following formula was used RC [%] = C1/C2 × 100.

Photosynthetic pigments and gas exchange: The chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids (Car) were quantified on fully expanded leaf tissue after extraction in 80 % (v/v) acetone. The content of Chl *a*, Chl *b*, and Car was determined at absorbance (A) of 663, 646, and 470 nm and calculated according to the formulas described by Lichtenthaler and Wellburn (1983).

Gas exchange was determined from 9:00 to 12:00 in May with *LI-COR 6400* portable photosynthesis system (*LI-COR*). Measurements were carried out with terminal lateral leaves according to Yang *et al.* (2010). The measured characteristics were: net photosynthetic rate (P_N), transpiration rate (E), water use efficiency (WUE), stomatal conductance (g_S), and intercellular carbon dioxide concentration (c_i).

Analysis of lipid peroxidation and ROS accumulation: The lipid peroxidation (MDA) was estimated in fully expanded leaf tissue that was finely powdered in liquid nitrogen containing 0.1 % (m/v) trichloroacetic acid. The mixture was centrifuged at 10 000 *g* for 10 min, and then 1 cm^3 of the aliquot was mixed with 4 cm^3 of 20 % trichloroacetic acid and 0.5 % (m/v) thiobarbituric acid, and incubated at 100 °C for 30 min. The reaction was stopped by incubating on an ice bath, and the reaction mixture was centrifuged at 10 000 *g* and 4 °C for 10 min. The absorbance of the supernatant was read at 530 nm and corrected at 600 nm. The content of MDA was calculated according to Yang *et al.* (2010).

The hydrogen peroxide (H_2O_2) content in *D. odorifera* leaves was determined by the colorimetric method according to Yang *et al.* (2010) with some modifications. Approximately 200 mg of leaf material was grounded and homogenized in 0.1 % trichloroacetic acid. The extract has been centrifuged at 10 000 *g* and 4 °C for 10 min. The reaction mixture was composed of 1 cm^3 of 10 mM potassium buffer, 1 cm^3 of potassium iodide, and 1 cm^3 of the supernatant. The absorbance was determined at 390 nm and the content of H_2O_2 was calculated *via* a standard curve.

The production rate of the superoxide anion ($O_2^{\bullet -}$) was measured spectrophotometrically using an assay kit (*Nanjing Jiancheng Bioengineering Institute*, Nanjing, China) and absorbance was measured at 550 nm. The phosphate buffer solution was employed to extract the $O_2^{\bullet -}$.

Sugars, proteins, and proline content: The content of soluble sugars was determined by the anthrone method as described by Yemm and Willis (1954). Leaf samples were extracted in 3 % (m/v) sulfosalicylic acid and approximately 1 cm^3 of the extract reacted with 2 cm^3 of 0.2 % (m/v) anthrone. The absorbance was determined at 630 nm and a standard graph was used to quantify the content of soluble sugar in each sample.

The Bradford (1976) method using bovine serum albumin as a standard was used to determine the soluble protein content in fresh leaves of *D. odorifera*. About 100 mg of leaf samples were ground and homogenized in 2 cm^3 of potassium phosphate buffer (pH 7.8). The reaction mixture was composed of 1 cm^3 of Coomassie Brilliant Blue G-250 reagent and 100 mm^3 of supernatant from the extract. The absorbance was read at 595 nm and the content of soluble protein was calculated *via* a standard curve.

A modified method of Bates *et al.* (1973) was used to determine the content of proline in *D. odorifera* leaves. Frozen leaf material (250 mg) was ground to powder in liquid nitrogen and then homogenized with 5 cm^3 of 3 % (m/v) aqueous sulfosalicylic acid. The reaction mixture was constituted by 2 cm^3 of the supernatant, 2 cm^3 of ninhydrin reagent, and 2 cm^3 of glacial acetic acid. The mixture was incubated at 100 °C for approximately 1 h, and then ice water was used to stop the reaction. About 4 cm^3 of toluene was added to the mixture to obtain the chromophore-containing phase and the absorbance was read at 520 nm.

Antioxidants: Frozen leaf material (100 mg) was ground to a fine powder in liquid nitrogen and then homogenized with 1 cm^3 of phosphate buffer solution. The antioxidant accumulations and activities were determined by using antioxidant assay kits from *Nanjing Jiancheng Bioengineering Institute*.

The peroxidase (POD) activity was determined with the peroxidase assay kit by measuring the change in absorbance at 420 nm. The superoxide dismutase (SOD) activity was quantified with a total SOD assay kit according to the manufacturer's instructions using the hydroxylamine method. The SOD activity was measured at 550 nm. The catalase (CAT) activity was determined with a CAT assay kit and the absorbance was read at 405 nm. The AsA and GSH accumulations were measured at 536 nm and 405 nm, respectively, *via* the GSH assay kit and AsA assay kit.

Subordinate function and principal component analysis: To assess and compare the influence of K_2SiO_3, GB, and K_2SiO_3-GB on *D. odorifera* seedlings under drought stress we used the subordinate function analysis as described by Cao *et al.* (2015). The calculation for the subordinate function analysis was made by the following formula U (X*i*) = (X*i* - X*min*) / X*max* -X*min*. The U (X*i*) corresponds to the value of subordinate function analysis, X*i* is the parameter's value, X*min* is the minimum value of this parameter, and X*max* is the maximum value. Furthermore, a principal component analysis (PCA) was used to draw a heat map and biplot to display the correlation between different measured parameters *via GraphPad prism 9.0.0* and the loading data.

Statistical analyses: The data presented in this work are mean of four replicates at least and the level of significance was determined at $P \leq 0.05$ using two-way *ANOVA* and

Tukey's HSD (honestly significant difference) test. The data were expressed as means ± standard errors and *Graph Pad prism 8.0.2* software was used to draw the graphs and analyze the data.

Results

Under both non-stressed and salt-stress conditions, the growth traits were significantly ($P \leq 0.05$) promoted by an application of GB, K_2SiO_3 or K_2SiO_3-GB (Fig. 1). A single application of K_2SiO_3 did not show a better increase in phenotypic indexes compared to GB or K_2SiO_3-GB treatments under salinity. Under salt stress, the maximum values were found in seedlings treated with K_2SiO_3-GB concerning the plant height increment; whereas the GB-treated seedlings showed a greater leaf area increment. Under normal conditions, the plant height was higher in K_2SiO_3-treated seedlings, meanwhile, GB-treated plants showed a greater leaf area increment. The number of leaves was not statistically different in seedlings treated with GB, K_2SiO_3 and K_2SiO_3-GB.

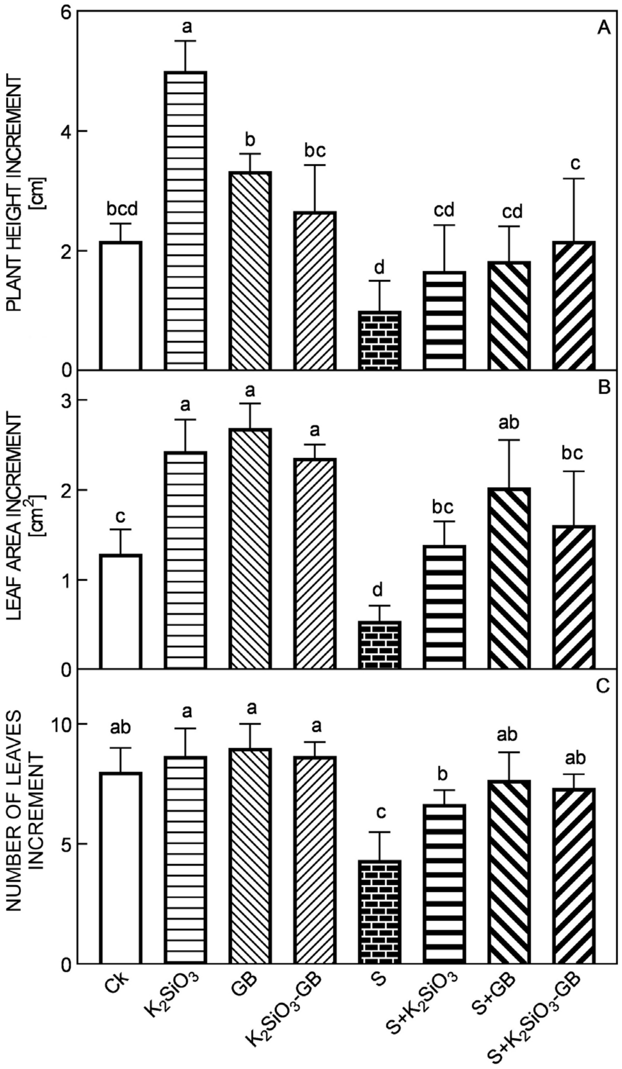

Fig. 1. Increments of plant height (*A*), leaf area (*B*) and leaf number (*C*) in *Dalbergia odorifera* under salt stress and exposed to GB, K_2SiO_3 and K_2SiO_3-GB. Means ± SEs, $n = 4$, different letters indicate significant differences according to Tukey's multiple comparison test ($P < 0.05$), Ck - control, GB - glycine betaine, S - salt.

Salinity had huge impacts on RWC, WP, and RC in *D. odorifera* seedlings (Fig. 2). Under non-stressed conditions, exogenous substances did not affect significantly the RWC and WP, whereas the RC was significantly increased by their application. The RWC and WP were similar in seedlings treated with GB, K_2SiO_3 and K_2SiO_3-GB under salt stress. The RC was significantly

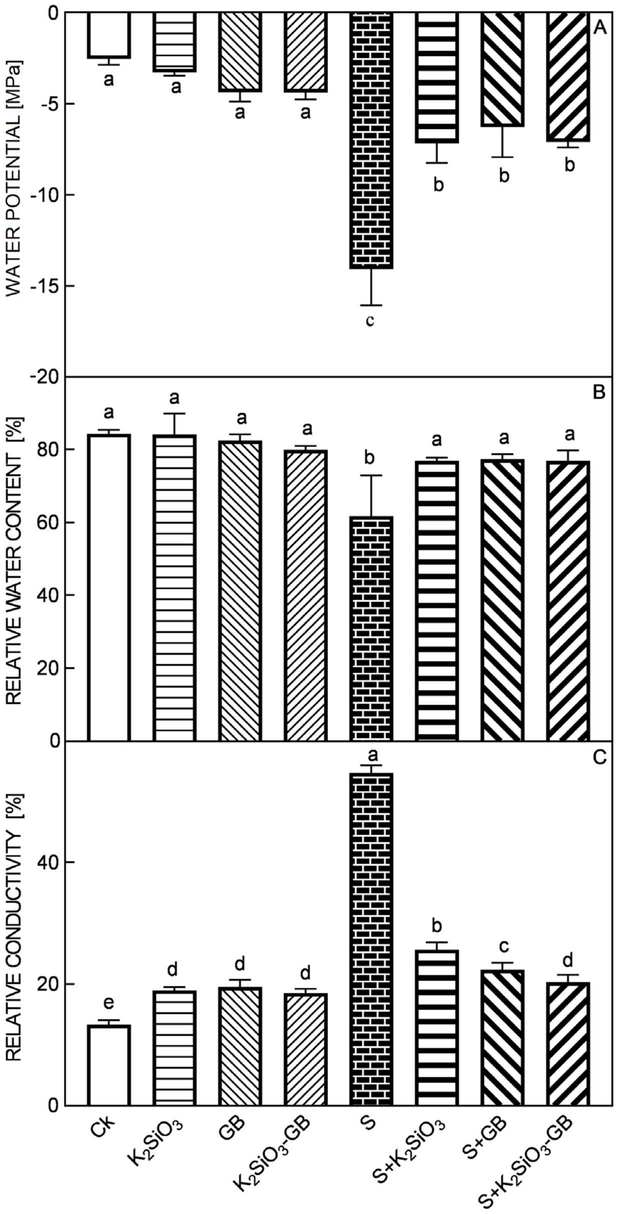

Fig. 2. Leaf water potential (*A*), relative water content (*B*), and relative conductivity (*C*) in *D. odorifera* under salinity with GB, K_2SiO_3 and K_2SiO_3-GB. Means ± SEs, $n = 4$, different letters indicate significant differences according to Tukey's multiple comparison test ($P < 0.05$), Ck - control, GB - glycine betaine, S - salt.

Table 1. Changes of the photosynthetic pigments Chl *a*, Chl *b* and Car [µg g^{-1}(f.m.)] and gas exchange parameters P_N [µmol m^{-2} s^{-1}], WUE [µmol $mmol^{-1}$], g_S [mol m^{-2} s^{-1}], c_i [µmol mol^{-1}], and E [mmol m^{-2} s^{-1}] in *D. odorifera* leaves under salt stress and exposed to GB, K_2SiO_3 and GB-K_2SiO_3. Means ± SEs, *n* = 4. The different letters indicate significant differences between treatments ($P < 0.05$) based on Tukey's multiple comparison test.

Parameters/ Treatments	Chl *a*	Chl *b*	Car	P_N	WUE	g_S	c_i	E
Ck	453±25.56c	162.5 ±8.43b	109.07±6.51c	2.762±0.012d	2.29±0.007d	0.130±0.0005c	246.5±0.23c	1.23±0.007d
K_2SiO_3	948±27.6a	293.0±7.92a	239.0±4.72a	3.388±0.023c	2.44±0.019c	0.190±0.0001b	248.5±0.45c	1.38±0.008c
GB	950±2.09a	278.0±18.72a	231.0±15.37a	5.170±0.012b	2.26±0.003d	0.220±0.0004a	260.8±0.13b	2.28±0.004a
K_2SiO_3+GB	580± 17.03b	168.5±13.55b	149.0±17.77b	5.630±0.027a	2.55±0.006b	0.223±0.0005a	250.3±0.14c	2.20±0.005b
Salt	215±16.34d	89.0±8.09d	55.0±4.58d	0.021±0.001h	0.16±0.007g	0.007±0.0004g	381.4±1.20a	0.12±0.006h
Salt+K_2SiO_3	428±14.02c	143.0±4.45bc	113.0±6.33c	0.390±0.005g	1.66±0.025e	0.014±0.0003f	264.3±0.48b	0.23±0.005g
Salt+GB	389±16.30c	141.0±15.16bc	105.0±8.83c	0.440±0.004f	1.57±0.020f	0.016±0.0001e	263.6±0.93b	0.28±0.001f
Salt+K_2SiO_3+GB	380±7.74c	117.0±18.68cd	89.0±9.29c	1.623±0.008e	3.37±0.052a	0.035±0.0001d	231.7±1.20d	0.48±0.005e

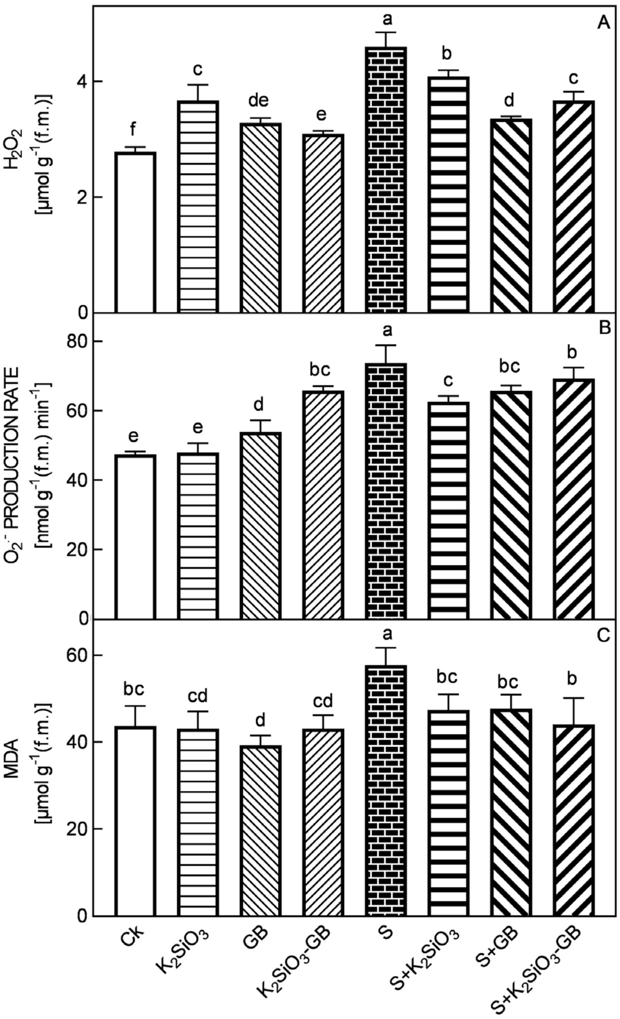

Fig. 3. Content of H_2O_2 (*A*), $O_2^{\bullet-}$ production rate (*B*), and content of MDA (*C*) in *D. odorifera* seedlings under salinity and treated with GB, K_2SiO_3 and K_2SiO_3-GB. Means ± SEs, *n* = 4, different letters indicate significant differences according to Tukey's multiple comparison test ($P < 0.05$), Ck - control, GB - glycine betaine, S - salt.

decreased by the combination of GB and K_2SiO_3 regardless of the single application of GB or K_2SiO_3 under stress.

The content of photosynthetic pigments varied markedly with exogenous substances application under normal conditions (Table 1). The content of Chl *a*, Chl *b*, and Car was significantly higher in seedlings treated with a single K_2SiO_3 or GB compared to K_2SiO_3-GB treatment under non-stressed-conditions. The salt stress decreased massively the photosynthetic pigments in *D. odorifera* seedlings. However, the addition of GB, K_2SiO_3 or K_2SiO_3-GB improved significantly the pigment contents in seedlings under salt stress.

Negative effects of salt stress on P_N, E, g_S, and WUE were significantly less apparent in seedlings treated with GB, K_2SiO_3 and GB-K_2SiO_3 compared to the seedlings exposed only to NaCl solution (Table 1). Generally, based on the P_N, E, g_S, and WUE, the seedlings treated with GB, K_2SiO_3 and GB-K_2SiO_3 showed a better photosynthesis performance compared to the control group. Moreover, the GB-K_2SiO_3 presented higher values of P_N, E, and g_S compared to the other treatments under non-stressed conditions. Under salt stress, the combination between K_2SiO_3 and GB showed a better ability to increase the P_N, E, g_S, and WUE than a single treatment.

A further consequence of salt stress on *D. odorifera* seedlings was a massive increase of MDA, H_2O_2, and $O_2^{\bullet-}$ (Fig. 3). Application of GB, K_2SiO_3 and GB-K_2SiO_3 reduced significantly the contents of MDA, H_2O_2 and $O_2^{\bullet-}$ under salinity. Under non-stressed conditions, the content of H_2O_2, and production rate of $O_2^{\bullet-}$ were increased by K_2SiO_3, GB and K_2SiO_3-GB treatments compared to the control, and the greater values were found in K_2SiO_3-treated seedlings for H_2O_2, and in GB-treated plants for $O_2^{\bullet-}$. Under salt stress, the content of H_2O_2 was significantly lower in seedlings treated with a single GB application than under other treatments. Meanwhile, K_2SiO_3-treated seedlings showed a better $O_2^{\bullet-}$ scavenge compared to GB or K_2SiO_3-GB treatments. The MDA content was not statistically different in seedlings treated with K_2SiO_3 and GB, while it was significantly lower in K_2SiO_3-GB-treated seedlings under salt stress. Further, under normal

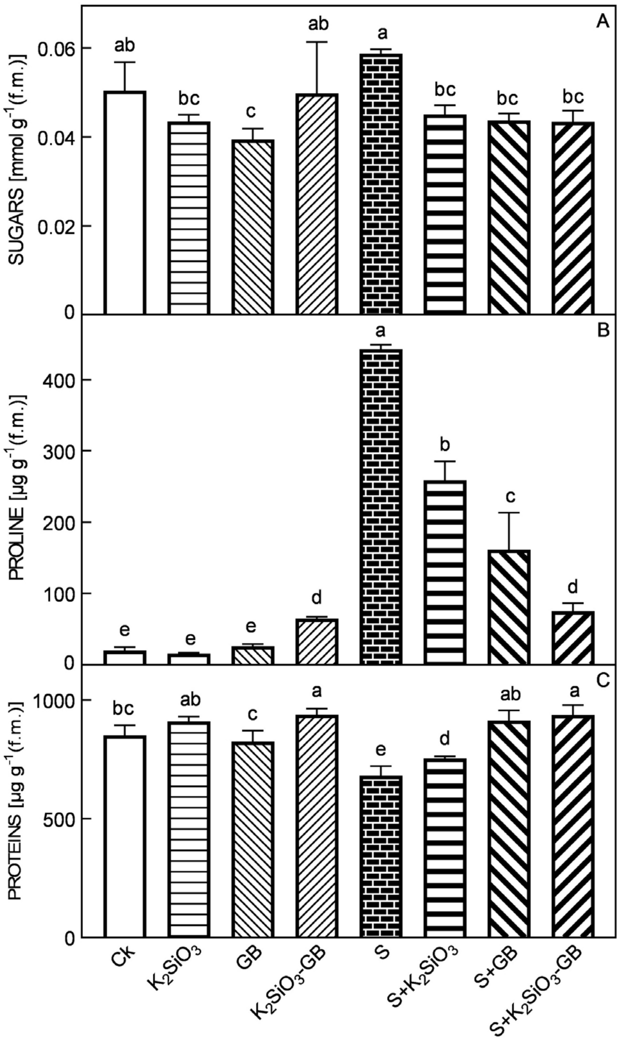

Fig. 4. Content of sugars (*A*), proline (*B*), and proteins (*C*) in *D. odorifera* seedlings under salinity and treated with GB, K_2SiO_3 and K_2SiO_3-GB. Means ± SEs, *n* = 4, different letters indicate significant differences according to Tukey's multiple comparison test ($P < 0.05$), Ck - control, GB - glycine betaine, S - salt.

conditions, the lipid peroxidation was decreased by exogenous substances compared to the control group, and the GB-treated seedlings showed the lowest values.

Although the osmoprotectants in *D. odorifera* seedlings treated with a single salt solution increased strongly, proline and sugar content was markedly reduced by GB, K_2SiO_3, and GB-K_2SiO_3 under salt stress (Fig. 4*A*,*B*). The sugar content was similar in seedlings treated with GB, K_2SiO_3, and K_2SiO_3-GB under salt stress; however, the K_2SiO_3 treatment caused a greater proline content than GB and K_2SiO_3-GB treatments.

Salinity also had a marked effect on the content of proteins in *D. odorifera* seedlings; indeed the proteins were significantly degraded by severe salt stress (Fig. 4*C*). The application of GB, K_2SiO_3, and K_2SiO_3-GB ameliorated strongly the effect of NaCl on protein content and the higher values were found in seedlings treated with K_2SiO_3-GB. Under normal conditions, K_2SiO_3 and K_2SiO_3-GB treatments increased significantly the content of protein compared to the control and the GB treatment.

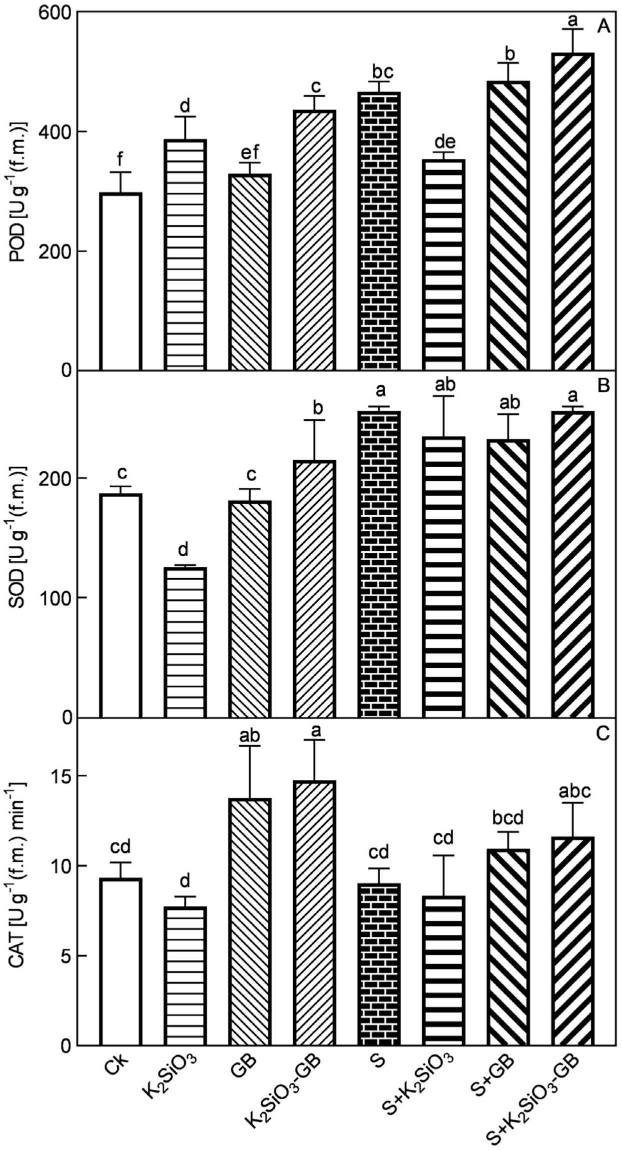

Fig. 5. Peroxidase, POD (*A*), superoxide dismutase, SOD (*B*), and catalase, CAT (*C*) activities in *D. odorifera* under salt stress and subjected to GB, K_2SiO_3 and K_2SiO_3-GB. Means ± SEs, *n* = 4, different letters indicate significant differences according to Tukey's multiple comparison test ($P < 0.05$), Ck - control, GB - glycine betaine, S - salt.

In the absence of NaCl, the activities of POD and CAT were strongly enhanced by exogenous substances (GB and K_2SiO_3-GB) compared to the control, excepted for the CAT activity in K_2SiO_3-treated seedlings. Meanwhile, the SOD activity was increased only by K_2SiO_3-GB treatment (Fig. 5). Moreover, the GSH content was significantly increased by K_2SiO_3-GB, K_2SiO_3, and GB treatments (Fig. 6*A*), meanwhile the AsA content was significantly decreased by these substances compared to the control (Fig. 6*B*).

The GSH content was higher in K_2SiO_3-treated in *D. odorifera* seedlings under both non-stressed and salt-

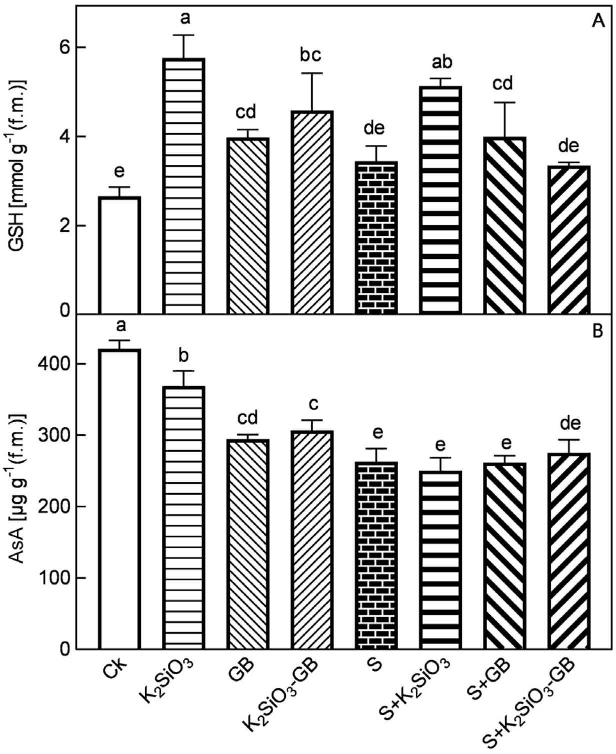

Fig. 6. Reduced glutathione, GSH (*A*) and ascorbic acid, AsA (*B*) accumulation in *D. odorifera* under salinity and exposed to GB, K_2SiO_3 and K_2SiO_3-GB. Means ± SEs, $n = 4$, different letters indicate significant differences according to Tukey's multiple comparison test ($P < 0.05$), Ck - control, GB - glycine betaine, S - salt.

stressed conditions. Under salt stress, the seedlings treated with K_2SiO_3 and GB showed a greater GSH accumulation compared to the control and K_2SiO_3-GB treatment. The GSH content in seedlings treated only with salinity or NaCl combined with K_2SiO_3-GB were statistically similar. The AsA accumulation was slightly enhanced by K_2SiO_3-GB treatment under stress in comparison to control, K_2SiO_3 or GB treatments. Under stress conditions, the activities of CAT and POD were increased by GB and K_2SiO_3-GB treatments. The SOD activities were similar in seedlings treated with a single salinity and those exposed to NaCl combined with K_2SiO_3-GB. But the seedlings submitted to K_2SiO_3 and GB showed a decrease in SOD activities under stress conditions. Under salinity, the antioxidant enzyme activities and AsA accumulation were higher in the seedling treated with K_2SiO_3-GB than under other treatments.

The score from the subordinate function analysis showed a greater value in seedlings treated with a single substance (K_2SiO_3 and GB) compared to K_2SiO_3-GB under normal conditions. The highest score was found in seedlings treated with K_2SiO_3 under non-stressed conditions. Under salt stress, a greater score was found in seedlings exposed to K_2SiO_3-GB treatment. The results suggested that under natural conditions K_2SiO_3 had the best impact on *D. odorifera*. Whereas, under stress conditions, the combination between K_2SiO_3 and GB provided a better stress tolerance in *D. odorifera* seedlings (Table 2).

The two first PCAs (PC1 and PC2) from loading data were set to generate a biplot that exhibited the associations

Table 2. Subordinate function analysis of exogenous GB, K_2SiO_3 and K_2SiO_3-GB treatments under normal conditions and salt stress in *D. odorifera* seedlings.

Indexes	Well-watered conditions				Stressed conditions			
	Ck	K_2SiO_3	GB	K_2SiO_3-GB	Salt	Salt+K_2SiO_3	Salt+GB	Salt+K_2SiO_3-GB
Plant height	0.24	1.00	0.51	0.19	0.00	0.08	0.16	0.16
Leaf area	0.29	1.00	0.71	0.58	0.00	0.34	0.58	0.42
Leaf number	0.79	0.95	1.00	0.53	0.00	0.58	0.68	0.63
RWC	0.87	1.00	0.74	0.71	0.00	0.60	0.60	0.59
WP	1.00	0.95	0.85	0.85	0.00	0.58	0.77	0.61
Chl *a*	0.29	1.00	1.00	0.50	0.00	0.32	0.24	0.22
Chl *b*	0.25	1.00	0.93	0.39	0.00	0.36	0.25	0.14
Car	0.26	1.00	0.96	0.51	0.00	0.31	0.27	0.18
Total Chl	0.28	1.00	0.99	0.47	0.00	0.33	0.24	0.21
P_N	0.49	0.60	0.92	1.00	0.00	0.07	0.07	0.29
g_s	0.40	0.42	1.00	1.00	0.00	0.03	0.04	0.13
E	0.51	0.58	1.00	0.96	0.00	0.05	0.07	0.16
WUE	0.86	0.95	0.88	1.00	0.00	0.63	0.59	1.34
SOD	0.36	0.22	0.24	0.07	0.00	0.56	0.61	1.00
GSH	0.22	1.00	0.38	0.56	0.00	0.70	0.29	0.19
RC	1.00	0.87	0.86	0.88	0.00	0.72	0.78	0.84
Proline	1.00	1.00	0.99	0.95	0.00	0.74	0.86	0.94
Sugars	0.54	0.79	1.00	0.79	0.00	0.76	0.74	0.74
Proteins	0.70	1.00	0.58	0.95	0.00	0.38	0.96	1.00
Mean	0.55	0.86	0.82	0.68	0.00	0.43	0.46	0.52

among the antioxidants, osmoprotectants, lipid peroxidation, and reactive oxygen species (Fig. 1 Suppl.). On the other hand, to draw a correlation heat map (Fig. 2 Suppl.), the three first PCAs were used (PC1, PC2, and PC3). Under natural conditions, the total cumulative proportion of variance of PC1, PC2, and PC3 was equal to 89.78 % (PC1 = 45.05; PC2 = 27.89; PC3 = 16.84), while it was 80.59 % under salt stress (PC1 = 40.81; PC2 = 29.06; PC3 = 10.72).

In both conditions, their eigenvalues were higher than 1. Under non-stressed conditions, it was found a strong correlation between proline, CAT, POD, $O_2^{\bullet -}$, GSH, H_2O_2, AsA and MDA (Figs. 1 and 2 Suppl.). The profile of correlation between these indexes was different under salt stress; indeed the Figs. 1 and 2 Suppl. exhibited a great association between proline and H_2O_2; between AsA and CAT, and among MDA, SOD, and $O_2^{\bullet -}$.

Discussion

The stress resistance of many plants increased by the addition of different substances particularly Si, potassium, calcium, melatonin, spermidine, or GB. The effects of a single application of these substances on the plants under abiotic stresses were mentioned in numerous reports. The Si, potassium and GB protect plant photosynthesis and growth by counteracting in various plant species with oxidative stress induced by environmental stresses (Hasanuzzaman *et al*. 2018a, Annunziata *et al*. 2019, Chung *et al*. 2020). It is well-established that salt stress influences negatively the physiological, molecular, and morphological processes in plants (Pailles *et al*. 2020). The present study demonstrated the positive effects of the combination between K_2SiO_3 and GB on plant growth and development of *D. odorifera* seedlings under salinity. Moreover, despite the fact that the subordinate function analysis suggested that K_2SiO_3-GB showed a mostly better ability to increase salt tolerance in *D. odorifera* seedlings, the single treatment of seedlings with K_2SiO_3 or GB showed better salt tolerance at certain conditions compared to K_2SiO_3-GB. Indeed, the GB, one of the most well-known osmoprotectants that can play a major role in plant stress tolerance, can counterattack abiotic stress *via* maintaining the oxygen-evolving activity of the PS II protein complexes (Papageorgiou and Murata 1995). Additionally, GB can relieve the damages induced by oxidative stress *via* its capability to scavenge directly the ROS (Banu *et al*. 2010). The major sites in which the salt can provoke over-accumulation of ROS in plant cells are the chloroplast and mitochondria (Demidchik 2015). These two organelles are the headquarters where take place two of the most crucial processes in the plant (photosynthesis and cellular respiration). Exogenous GB application in various plants ameliorates negative effects of stress on photosynthesis by scavenging the ROS and *via* activation of antioxidant activities. It was reported an increase of antioxidant activities in *Oryza sativa* under drought (Farooq *et al*. 2008) or in *Glycine max* under salt stress (Malekzadeh 2015). The present study confirmed the ability of GB to enhance the activities and accumulation of various antioxidants in *D. odorifera*. Furthermore, the combination between GB and K_2SiO_3 induced a better antioxidant activities compared to a single application of GB in plants under salinity.

The disturbance of plant cell homeostasis by salinity is directly related to the imbalance between K^+ and Na^+ (Zhu 2003, Farooq *et al*. 2018). The combination between GB and K_2SiO_3 could maintain the water status and plant cell redox-homeostasis *via* the accumulation of K^+ caused by a supply of exogenous K_2SiO_3. Moreover, various studies demonstrated that Si and K^+ can indirectly increase environmental stress tolerance *via* modulation of the antioxidant system. Indeed Si confers salt tolerance in soybean and cowpea plants by enhancing photosynthesis performance and regulating antioxidant activities which affect positively the phenotypic traits (Ahmad *et al*. 2019, Chung *et al*. 2020). There is fewer amount of studies about the effects of exogenous K^+ on antioxidant and photosynthesis under stress, while more often was reported an increase of salt tolerance *via* osmotic adjustment and ionic balance which affect positively the essential processes, *e.g*., in peanut cultivars under salinity (Chakraborty *et al*. 2016).

The Si applied in maize under salinity increases the expression of *ZmSOS1* and *ZmSOS2* genes in the roots which promotes the regulation of Na^+ exclusion and decreases the negative effects of Na^+ over-accumulation on photosynthetic processes (Bosnic *et al*. 2018). The role of Si and K in the regulation of Na^+ accumulation under salinity could be a key point of the ability of K_2SiO_3-GB treatment to provide salt tolerance in plants. Indeed, it was not reported yet a direct implication of GB on Na^+ sequestration in plants under salinity, thus exogenous GB can be used to counteract salt stress using its combination with K_2SiO_3. In this present work, it was important to notice that a single application of K_2SiO_3 under salinity in *D. odorifera* decreased the activities of CAT, POD, and SOD. However, it is well-established the enhancement of antioxidant activities by a single Si application in various species under stress. In *Brassica napus*, exogenous Si increases significantly activities of monodehydroascorbate reductase, CAT, or glutathione peroxidase under salinity (Hasanuzzaman *et al*. 2018b). Furthermore, Si application enhances the activities of SOD, POD, and CAT in *Abelmoschus esculentus* under salt stress (Abbas *et al*. 2017). Therefore, the results of the present work are different to the above-mentioned reports. However, in a recent work, it was reported that Si decreases the enzymatic and non-enzymatic antioxidants to provide salt tolerance in *Glycine max* (Chung *et al*. 2020). Indeed the expressions of antioxidant-related genes *GmCAT1*, *GmCAT2*, and *GmAPX1* drop several hours after a supply of Si to *Glycine max* plants under salinity. The role of Si and K_2SiO_3 in the antioxidant machinery appears to be more complex and inquisitive; meanwhile, the ability of GB to increase the antioxidant activities is almost unanimous.

In conclusion, this present work exhibited a clear improvement of salt stress tolerance in *D. odorifera* seedlings *via* an application of K_2SiO_3, GB, or GB-K_2SiO_3.

The combination between GB and K_2SiO_3 is a very promising method to mitigate salt stress in plants. The key role of this combination was associated with the greater ability of GB-K_2SiO_3 treatment to increase the antioxidant activities and accumulation in *D. odorifera* under salinity in comparison to a single GB or K_2SiO_3 treatment.

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