EFFECT OF EXOGENOUS GLUTATHIONE ON PHOTOSYSTEM II FUNCTIONING OF RICE (*ORYZA SATIVA* L.) SEEDLINGS UNDER Na_2CO_3 STRESS

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Abstract. Chlorophyll (Chl) a fluorescence as a non-destructive technique was used to assess photosynthetic information when plants were subjected to environmental stress. Glutathione (GSH) is a strong non-enzymatic antioxidant. The purpose of this study was to estimate rice (*Oryza sativa* L.) seedlings' response to Na_2CO_3 stress after spraying exogenous GSH. The results showed that Na_2CO_3 stress had a negative impact on the development of rice seedlings and reduced SPAD values. The changes of specific energy fluxes (ABS/RC, TR_0/RC, DI_0/RC) revealed Na_2CO_3 stress inhibited the activity of photosystem II (PSII) reaction centers (RCs) and chloroplast stability. As shown by the alteration of the polyphasic fluorescence transient (OJIP) parameters, PSII function was less effective due to the reduction of energetic connectivity under Na_2CO_3 stress, which resulted in an imbalance in electron transfer. However, GSH effectively reversed changes in PSII as well as improved the photochemical efficiency. Therefore, the results suggested that exogenous GSH as a regulator of cellular defense against abiotic stress could effectively alleviate the damage to the PSII RC by Na_2CO_3 stress and improve the resistance of rice to salt stress.
Keywords: *salt-alkali stress, Chl a fluorescence, PSII, OJIP test, plant growth regulation*

Introduction

Rice, as a nutrient-rich cereal providing energy for nearly 50% of the population on earth (Hussain et al., 2020) is more sensitive to inter-root salinity than other cereals (Hussain et al., 2017). It is estimated that there are up to 100 million hectares of saline land in China, and around 30% productivity is affected by salinity (Liu et al., 2021). It has been confirmed that alkaline salts ($NaHCO_3$ and Na_2CO_3 are often mentioned) cause a higher damage to plants than neutral salts (Acosta et al., 2018; Zhang et al., 2018). Salt-alkali soils reduce the net photosynthetic rate and increase reactive oxygen species (ROS) in plants (Sheteiwy et al., 2019). Excessive ion accumulation disturbs ion balance (Waqas et al., 2021), which leads to ion poisoning.

GSH (γ-c-glutamyl-cysteinyl-glycine), an essential antioxidant in cellular defense and protection, plays a key role in diverse stress signaling transduction (Dumanović et al., 2021). GSH can scavenge ROS and influence the flow of reduced sulphur to some extent (Ghori et al., 2019). It was demonstrated that GSH/GSSG is an important signal in plant defense, activating or participating in various signaling pathways (Gibson et al., 1985). GSH can accelerate the damage repair rate of PSII protein complex and improve the light energy conversion efficiency of rice seedlings to Na_2CO_3 stress (Hasanuzzaman et al., 2017), and have a positive effect on signaling, epigenetic and maintain cells inefficient inner system against oxidative damages (Boro et al., 2018; Sultana et al., 2020). The previous study has pointed that GSH accelerated AAL toxin-stressed mitigation in

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Arabidopsis (*Arabidopsis thaliana* L.) via salicylic acid mediated inhibition of ethylene (Sultana et al., 2020). Żur et al. (2019) reported GSH seems to protect cells from oxidative stress by enhancing antioxidant activity.

Photosynthesis not only determines the efficiency of light energy acquisition and storage in plants, but is also a probe of stressed plant physiological status (Van et al., 2019). Chl a fluorescence provides sensitively and rapidly reactions in the photosynthetic process (McAusland et al., 2019). The intricate links between fluorescence kinetics and light energy transformation contributes to analysing the physiological mechanisms of photosynthesis (Sayed, 2003). The OJIP test is available to estimate how photosynthetic organs respond to harmful environments.

In this study, we detected Chl a fluorescence after 24 h of Na_2CO_3 treatment, there was no significant difference in plant growth while Chl a fluorescence exhibited an obvious response. This also indicated that Chl a fluorescence could detect plant damage rapidly and sensitively. The objective of this study was to provide a new idea for GSH regulation of salt-alkali tolerance in rice and a theoretical basis for using exogenous substances to mitigate the damage of salt-alkali stress.

Materials and methods

Plant species and culture methods

Rice seeds were sterilized with 1% sodium hypochlorite for 20-25 min, flushed in ultra-clean water 2-3 times. Seeds were soaked and then germinated at 28°C and 30°C for 24 h. After that, the germinating seeds were incubated in Hoagland's solution and grown in growth chamber set at 28°C/26°C light/dark, a 16-h photoperiod, photosynthetic photon flux density (PPFD) of 10000 Lux, and 85% relative humidity (Li et al., 2015).

GSH and Na_2CO_3 treatments

After 7 d of growth, rice seedlings were sprayed with different concentrations of GSH. According to our previous study (Ren et al., 2022), we applied 10 mM Na_2CO_3 concentration in this research. Rice seedlings were subjected to 10 mM Na_2CO_3 stress after 24 h of GSH treatments. Rice seedlings were divided into the following groups: (1) CK, (2)T0: 10 mM Na_2CO_3 stress, (3)T1: 25 μM GSH pretreatment and 10 mM Na_2CO_3 stress, (4)T2: 50 μM GSH pretreatment and 10 mM Na_2CO_3 stress, (5)T3: 75 μM GSH pretreatment and 10 mM Na_2CO_3 stress, (6)T4: 100 μM GSH pretreatment and 10 mM Na_2CO_3 stress. All treatments were repeated thrice.

Growth parameters measurement

After 5 d of Na_2CO_3 treatment, ten seedlings per pot were randomly selected and the shoot and the longest root length as well as fresh weight were recorded. Shoot and root were dried at 80℃ until a steady amount and the dry weight was recorded.

SPAD values measurement

After 24 h of Na_2CO_3 treatment, SPAD values of six seedlings per pot were measured with a portable chlorophyll meter (SPAD-502 Plus, Topp Instrument Co., Ltd, Zhejiang, China).

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Chl a fluorescence measurement

After 24 h of Na_2CO_3 treatment, six seedlings per pot were randomly selected to measure Chl a fluorescence with a portable fluorometer (Pocket PEA, Hansatech, Norfolk, UK). The leaves were dark-acclimated for 25 min, and exposed to the weak modulate measuring beam, and then 3000 μmol m^{-2} s^{-1} saturating light to obtain the initial (F_0) fluorescence yield and the maximum (Fm) fluorescence yield. The transient's graphical illustrations were double-normalized between F_0 and Fm to show the variable fluorescence at time. The following Chl a fluorescence parameters were used: the absorption flux (ABS/RC), the energy trapping (TR_0/RC), and the dissipation energy flux at 0 μs (DI_0/RC) per RC; electron acceptors captured exciton-transferred electrons (ψo), quantum yield (at t = 0) of energy dissipation (φDo), and quantum efficiency (φEo).

Statistical analyses

Each experiment was repeated three replicates, and calculated mean ± standard deviation (SD) using Microsoft Office Excel 2010. Differences among treatments were determined with one-way ANOVA. Means separation was carried out according to LSD's multiple comparison range test at 0.05 probability levels of significance.

Results

Growth parameters

Na_2CO_3 stress significantly limited the plant height of rice seedlings, while GSH pretreatment alleviated the inhibitory effect (*Fig. 1*).

Figure 1. *Effects of exogenous GSH on the growth of rice seedlings under Na_2CO_3 stress*

Under Na_2CO_3 stress, shoot height and root length, shoot fresh weight were decreased remarkably (i.e., 12%, 17.1%, 11.3%, respectively) but root fresh weight had no change (*Fig. 2*).

Under Na_2CO_3 stress, 100 μM GSH treatment significantly improved root length and 25 μM GSH treatment increased root fresh weight of rice seedlings, but GSH treatment did not change shoot height and shoot fresh weight.

SPAD values

Na_2CO_3 stress reduced SPAD values of leaves by 24.7% compared with CK (*Fig. 3*), while spraying different concentrations of GSH significantly improved SPAD values. Of these, 100 μM GSH treatment was the most effective, reaching 32%.

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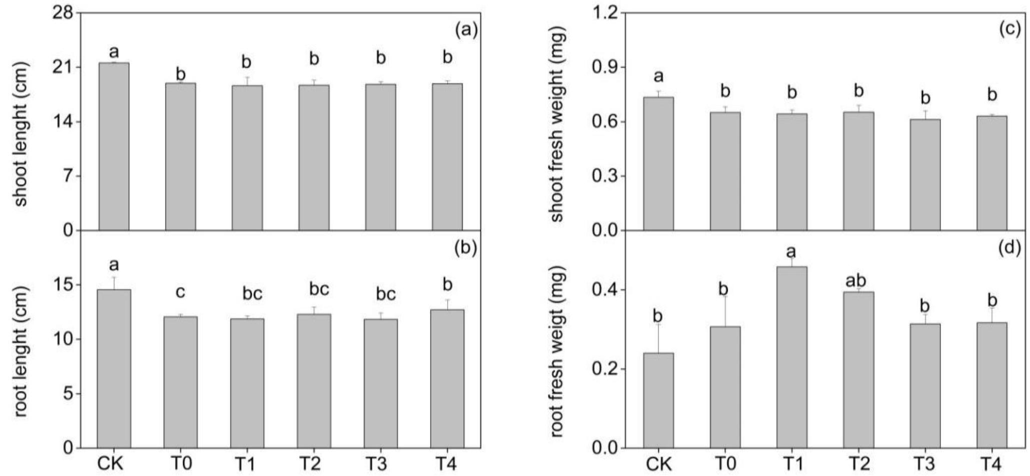

Figure 2. *Effects of exogenous GSH on shoot height (a), root length (b), shoot fresh weight (c) and root fresh weight (d) of rice seedlings under Na_2CO_3 stress. Ten independent set of experiments with three replicates in each value. Different letters on the columns indicate significant difference at $p<0.05$*

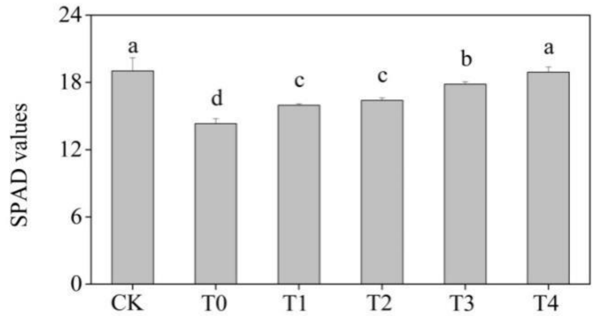

Figure 3. *Effects of exogenous GSH on SPAD values of rice seedlings under Na_2CO_3 stress. Six independent set of experiments with three replicates in each value. Different letters on the columns indicate significant difference at $p<0.05$*

OJIP curve and standardized analysis

Chl a fluorescence rise transients were exhibited the typical OJIP shape, while GSH has a weak effect on the OJIP curve as shown in *Fig. 4a*. OJIP curve (V_t) of rice seedlings with $Fm\text{-}F_0$ normalization and the change in fluorescence difference ΔVt between treatment and control are shown in *Fig. 4b*. There was a sharp decrease between the treatment with Na_2CO_3 + 75 μM GSH and control was the largest in lowering the activity in the oxygen-evolving complex (OEC) (*Fig. 4b*). V_{OK} and V_{OJ} curves normalized between $F_0\text{-}F_k$ and $F_0\text{-}F_J$, respectively, showed the change in fluorescence difference between the different treatments and control (*Fig. 4c,d*). Leaves sprayed with 0 and 25 μM GSH exhibited a positive L-band, whereas leaves sprayed with 50, 75 and 100 μM GSH exhibited a negative L-band, suggesting a higher utilization of this system. Leaves sprayed with 50, 75 and 100 μM GSH exhibited a negative K-band, implying the rice photosynthesized normally. However, leaves sprayed with 0 and 25 μM GSH exhibited a positive K-band, indicating Na_2CO_3 stress inhibited the physiological activity of rice.

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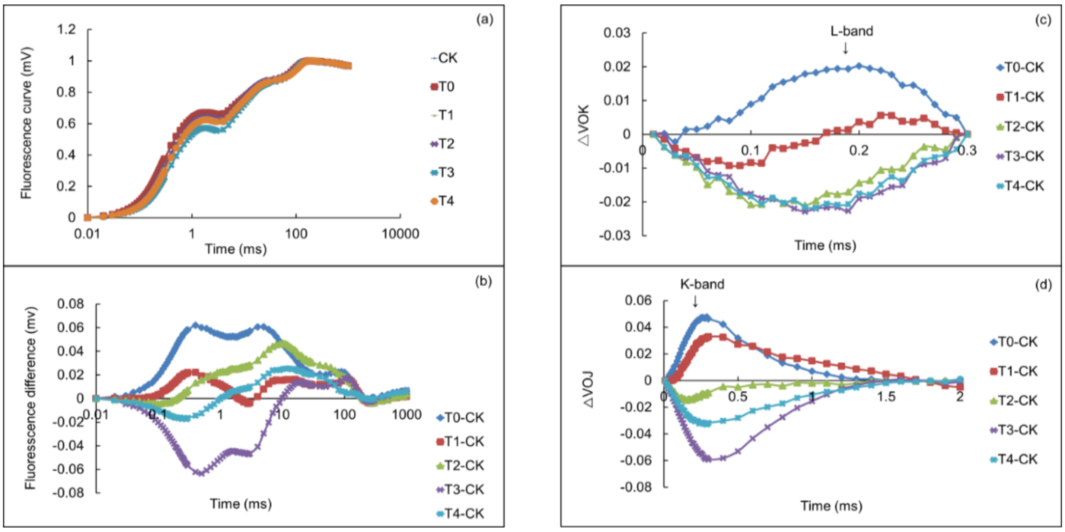

Figure 4. *Effects of exogenous GSH on Chl a fluorescence transient (a), fluorescence difference (b), V_{OK} (c) and V_{OJ} curves (d) of rice seedlings under Na_2CO_3 stress. Six independent set of experiments with three replicates in each value. Curves with rhombus, square, triangles, fork, asterisk, circular logo graphic represents control and 0, 25, 50, 75, and 100 µM GSH, respectively.* $V_{OK} = \frac{Ft-F_0}{F_K-F_0}$ *(Eq.1).* $V_{OJ} = \frac{F_t-F_0}{F_J-F_0}$ *(Eq.2)*

Fluorescence parameters and performance index

Na_2CO_3 stress led to the minimal fluorescence of PSII (F_0) significant increase by 59.7% and the maximal fluorescence of PSII (Fm) significant decrease by 22.4% (*Fig. 5a,b*). Compared to rice subjected to Na_2CO_3, GSH treatment significantly decreased F_0, but significantly increased Fm. Sm was reduced by 17.2% under Na_2CO_3 stress, indicating that the PQ pool was also lowered. GSH effectively mitigated the damage of Na_2CO_3, and 100 µM GSH had the best effect on stress relief with a 13.4% ratio reversion (*Fig. 5c*). A significant increase in W_k under Na_2CO_3 stress indicated that the OEC was damaged, while it reduced after spraying GSH, especially at 75 µM and 100 µM GSH treatment (*Fig. 5d*). ΦPo (Fv/Fm) remarkably declined during Na_2CO_3 stress, while it significantly increased under 25 µM and 75 µM GSH treatment (*Fig. 5e*). The reduction of performance index (PI_{ABS}) is related to overall RC functionality and photonic energy conversion. Na_2CO_3 stress significantly reduced PI_{ABS} by 50.7%, and 75 µM and 100 µM GSH treatment significantly increased PI_{ABS} (*Fig. 5f*).

Specific energy fluxes and quantum efficiency

Na_2CO_3 stress significantly increased the specific energy fluxes ABS/RC, TR_0/RC and DI_0/RC by 20.8%, 14.8%, and 49.2%, respectively. With increasing GSH concentration, ABS/RC, TR_0/RC and DI_0/RC were decreased (i.e., 24.7%, 21.9%, 42.5%, respectively), and the lowest value was at 75 µM GSH treatment. GSH reduced the specific energy fluxes, suggesting that exogenous GSH improves the ability of rice to resist external disturbances and maintain internal homeostasis (*Fig. 6a,b,c*).

Under Na_2CO_3 stress, ψo and φEo were reduced by 28.7% and 31.4%, respectively, while φDo were increased by 19.9%, suggesting that the electron transfers and transitions were inhibited. φEo and ψo were obviously enhanced with 75 µM GSH spraying (*Fig. 6d*), and φDo was decreased with 25-100 µM GSH spraying (*Fig. 6e,f*).

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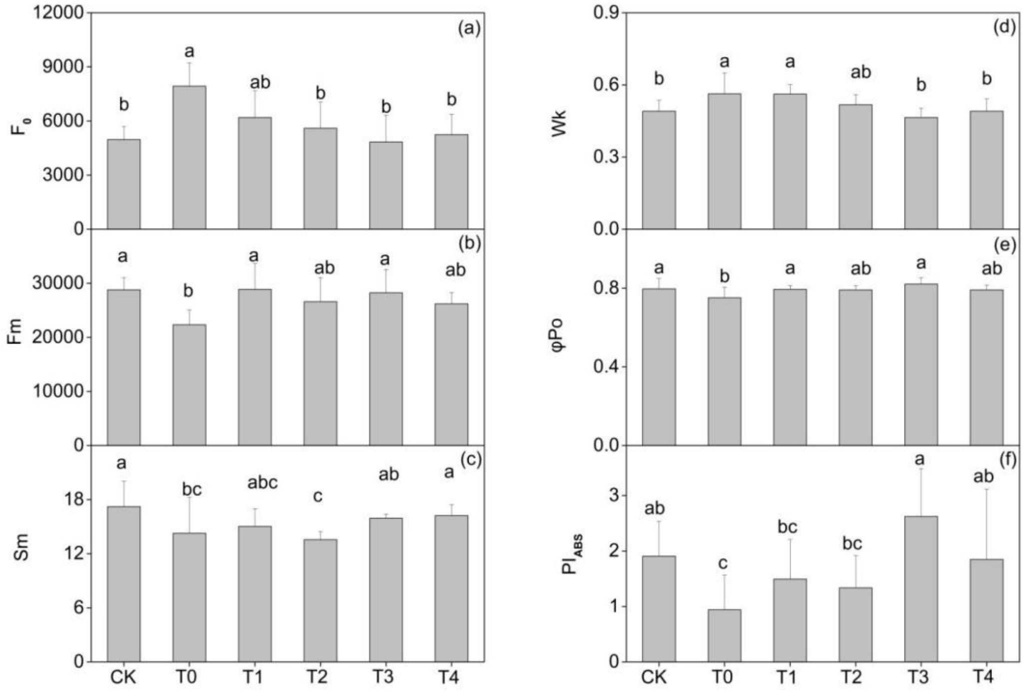

***Figure 5.** Effects of exogenous GSH on F_0 (a), Fm (b), Sm(c), W_k(d), φPo (e) and PI_{ABS}(f) of rice seedlings under Na_2CO_3 stress. Six independent set of experiments with three replicates in each value. Different lowercase letters on the columns indicate significant difference at p<0.05*

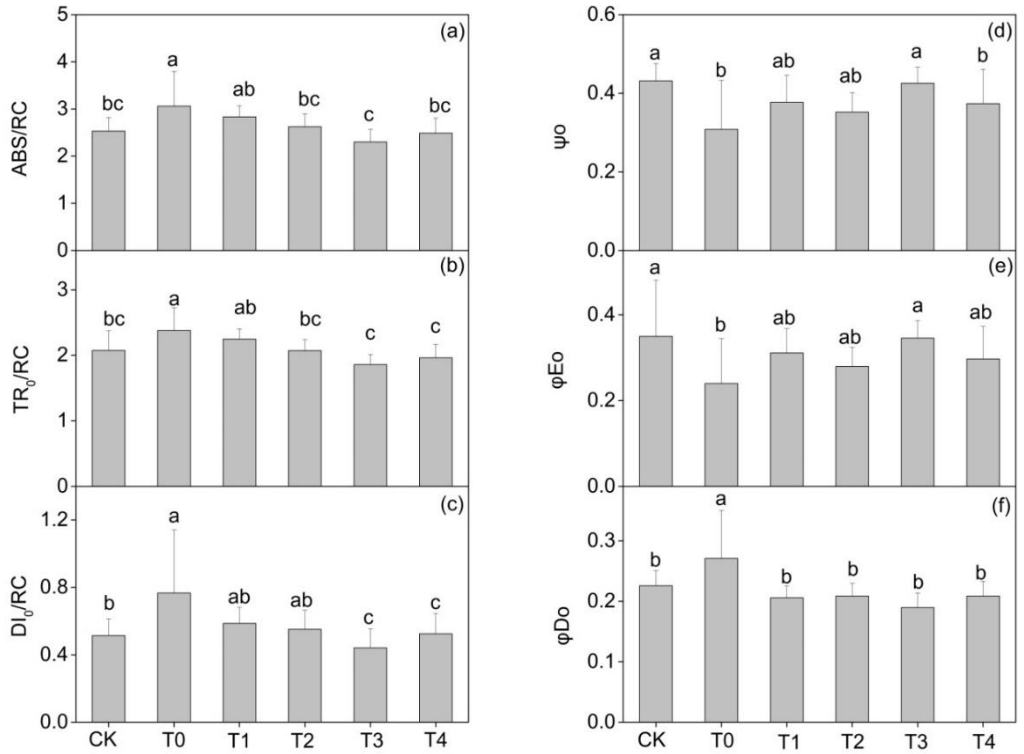

***Figure 6.** Effects of exogenous GSH on ABS/RC (a), TR_0/RC (b), DI_0/RC(c) and quantum efficiency ψo(d), φEo(e), φDo(f) of rice seedlings under Na_2CO_3 stress. Six independent set of experiments with three replicates in each value. Different lowercase letters on the columns indicate significant difference at p<0.05*

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Discussion

Rice is sensitive to salt (Gupta et al., 2020), and salt stress is the second most important factor affecting rice yield after drought. Both increasing Na^+ and CO_3^{2-} affect physiological pathways in plants (Golezani et al., 2017, 2020). Na^+ causes a reduction in leaf water and CO_3^{2-} leads to partial hydrolysis resulting in alkaline medium (Golezani et al., 2020; Evgrashkina et al., 2020). Therefore, studying the physiological changes of rice seedlings under Na_2CO_3 stress contributes to learning the potential mechanism of rice salt-alkali resistance. Salinity has a negative influence on plant growth and productivity (Kumar et al., 2020). In this study, Na_2CO_3 stress inhibited various parameters of rice growth and decreased SPAD value of leaves. Na_2CO_3 stress reduced the expansion pressure of plant cells, thus plants require to ensure cell proliferation by osmoregulation (Fang et al., 2021). Decrease in plants biomass finally led to a reduced intake and a limited area for photosynthesis, resulting in lower productivity (Kapoor et al., 2020). GSH is a simple sulfur-containing tripeptide compound (Shcherbatykh and Chernovyants, 2021), and has redox and nucleophilic properties, which turns on different types of cellular defense mechanisms against xenobiotics, salinity and other toxic effects (Zhou et al., 2017). Exogenous GSH treatment promoted the growth and SPAD values of rice seedlings under Na_2CO_3 stress.

Increased photosynthetic intensity keeps crops growth activity to resist salt-alkali stress (Yin et al., 2022). Effect of stresses on photosynthesis can be evaluated by fast Chl a fluorescence analysis (Ghorbanzadeh et al., 2021), which detectes PSII changes without damaging the plant (He et al., 2018). Goussi et al. (2018) pointed that salinity induced structural disorganization of chloroplast, which caused an inhibition of photosynthetic apparatus. In the present study, each treatment group exhibited a standard OJIP curve shape, indicating that leaves were photosynthetically active. The K-band shows the activity of OEC in stress environment, which is relevant to the size of the PSII antenna (Goussi et al., 2018). It was also reported that the positive K-band was induced by an inhibition of OEC performance and diverse energy distribution (Marečková et al., 2019). Our results exhibited the negative K-band implying that the damage of OEC was alleviated by higher concentrations of GSH. Ayyaz et al. (2020) observed negative K-band in cadmium-stressed canola (*Brassica napus* L.) treated with melatonin. Similarly, the negative K-band was found in *Thellungiella salsuginea* treated with NaCl-$CdCl_2$ interactions (Goussi et al., 2018). We usually use L-band as a specific indicator of how photosynthetic units vary with respect to energy connectivity, which is negative indicating stronger connectivity (Bednaříková et al., 2020). The negative L-band was a sign when plants had a better performance under stress conditions (Zampirollo et al., 2021). The negative L-band in our study suggested that GSH enhanced the energetic connectivity of PSII units and improved photosynthetic efficiency.

Fm decreased while F_0 increased, which revealed that the structure of the RC inactived and partial protein complex of the PSII damaged under Na_2CO_3 stress (Chen et al., 2021). Under Na_2CO_3 stress, exogenous GSH attenuated RC damage of rice seedlings, accelerated the repairing speed of PSII protein complex, improved the efficiency of light energy conversion, which were coincided with earlier reports (Hasanuzzaman et al., 2017). Reduced Sm showed the PQ pool was decreased, and the electronic transmission activities exceeding Q_A in the Na_2CO_3 stressed leaves were suppressed (Chen et al., 2021). In the OJIP curve, the K point refers to the value of Chl fluorescence at 300 μs， and W_k is used to reflect the change of K points: $W_k = (F_k\text{-}F_0) / (F_J\text{-}F_0)$ (Strasser, 1997). When plants are in stress conditions, W_k is an important reference indicator of OEC activity

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reduction and restricted PSII electron transfer (Strasser, 1997). In this study, increased W_k showed Na_2CO_3 stress hurt the OEC, while GSH treatment alleviated the damage of OEC. φPo (Fv/Fm) reflects the maximum photochemical efficiency after dark adaptation (Lu and Zhang, 1998). Under Na_2CO_3 stress, reduced φPo suggested that the electronic transport from Q_A to Q_B was inhibited (Meng et al., 2016). However, exogenous GSH significantly enhanced φPo, indicating that GSH improved photochemical efficiency in stressed rice seedling (Bednaříková et al., 2020). Changes in RC, light energy conversion and electron transfer can be find by the performance index PI_{ABS}, which provides information on internal physiology in plants (Ayyaz et al., 2020). PI_{ABS} increased in GSH-treated leaves under Na_2CO_3 stress confirmed that GSH can improve photochemical efficiency and electron transport (Chen et al., 2021).

Plants develop self-defense mechanisms when subjects to stress, and PSII RCs may degrade, during this process, in addition to ensuring electron transfer, the major assimilated and captured light energy is consumed as heat (Zhang et al., 2020). The rising ABS/RC, TR_0/RC and DI_0/RC under Na_2CO_3 stress reflected electron flow to the final reduced photosystem I (PSI) terminal electron receptors (Snider et al., 2018). In contrast, exogenous GSH reduced specific energy fluxes means that RC is more active (Kumar et al., 2020). The previous studies pointed that the PSII receptor side is a key point of salinity stress (Kan et al., 2017). Under Na_2CO_3 stress, ψo and φEo were declined while φDo was rised, indicating that PSII acceptor side was inhibited (Liu et al., 2021). We found that ψo and φEo were higher, and φDo lower after spraying GSH due to the alleviated inhibitory effect.

Conclusion

This study concluded that Na_2CO_3 stress impair photosynthesis from the PSII donor side to the PSI acceptor side by limiting the terminal acceptor of the electron transport chain. However, exogenous GSH showed a good performance in mitigating the damage of PSII RC, improving the light energy conversion efficiency, thus enhancing the resistance of rice seedlings to Na_2CO_3 stress. It provided a theoretical and applied example for exogenous GSH enhancing rice resistance to salt-alkali stress. Different stress conditions have different stress responses in plants. To further explore the effect of GSH on plants photosynthesis, more studies in the field through undesirable conditions such as drought, high temperature, etc. are needed.

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APPENDIX

ANOVA tables

Table A1. *Effects of exogenous GSH on growth parameters of rice seedlings under Na_2CO_3 stress*

Treatment	shoot length (cm)	root length (cm)	shoot fresh weight (mg)	root fresh weight (mg)
CK	21.54±0.78a	14.55±1.81a	0.73±0.03a	0.24±0.07b
G0	18.95±0.62b	12.06±1.39c	0.65±0.03b	0.31±0.08b
G25	18.62±1.07b	11.88±0.96bc	0.64±0.02b	0.46±0.02a
G50	18.7±0.70b	12.26±1.60bc	0.65±0.03b	0.39±0.01ab
G75	18.82±0.50b	11.83±1.14bc	0.61±0.04b	0.31±0.02b
G100	18.9±0.55b	14.11±2.64b	0.63±0.01b	0.32±0.04b

Different letters between lines indicate significantly different at $p<0.05$

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Table A2. *Effects of exogenous GSH on SPAD values of rice seedlings under Na_2CO_3 stress*

Treatment	SPAD values
CK	19.03±1.60a
G0	14.33±0.87d
G25	15.97±0.53c
G50	16.40±0.46c
G75	17.84±0.51b
G100	18.92±0.94a

Different letters between lines indicate significantly different at p<0.05

Table A3. *Effects of exogenous GSH on fluorescence parameters of rice seedlings under Na_2CO_3 stress*

Treatment	F_0	Fm	Sm	Wk	φPo	PI_{ABS}
CK	4967.60±727.46b	28790.50±2213.74a	17.22±2.82a	0.49±0.05b	0.80±0.05a	1.91±0.63ab
G0	7931.00±1289.72a	22352.33±2741.03b	14.26±3.98bc	0.56±0.08a	0.75±0.05b	0.94±0.62ab
G25	6193.12±1483.95ab	28877.88±4777.70a	15.03±1.95abc	0.56±0.03a	0.79±0.02a	0.94±0.71bc
G50	5602.11±1455.24b	26595.44±4452.10ab	13.56±0.89c	0.52±0.04ab	0.79±0.02ab	0.94±0.58bc
G75	4838.33±1476.03b	28255.25±4305.99a	15.94±0.45ab	0.46±0.04b	0.82±0.03a	0.94±0.86a
G100	5241.11±1121.85b	26205.57±2096.24ab	16.21±1.23a	0.49±0.05b	0.79±0.02ab	0.94±1.27ab

Different letters between lines indicate significantly different at p<0.05

Table A4. *Effects of exogenous GSH on performance index of rice seedlings under Na_2CO_3 stress*

Treatment	ABS/RC	TR_O/RC	DI_O/RC	ψo	φEo	φD
CK	2.53±0.29bc	2.07±0.30bc	0.51±0.10b	0.43±0.04a	0.35±0.13a	0.23±0.02b
G0	3.06±0.74a	2.38±0.34a	0.77±0.38a	0.31±0.12b	0.24±0.10b	0.27±0.08a
G25	2.83±0.24ab	2.38±0.16ab	0.59±0.10ab	0.31±0.07ab	0.31±0.06ab	0.21±0.01b
G50	2.62±0.28bc	2.38±0.17bc	0.55±0.11ab	0.31±0.05ab	0.28±0.05ab	0.21±0.02b
G75	2.30±0.26c	2.38±0.16c	0.44±0.11c	0.31±0.04a	0.35±0.04a	0.19±0.02b
G100	2.49±0.32bc	2.38±0.20c	0.52±0.12c	0.31±0.09b	0.3±0.08ab	0.21±0.02b

Different letters between lines indicate significantly different at p<0.05

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