Effects of γ-aminobutyric acid (GABA) seed priming on salt stress alleviation in pepper and associated physiological and biochemical changes: Postprint

Authors: Lin Xinqi, Wei Qianya, Liang Lamei, Zhongwei Qin, Li Yingzhi

Date: 2023-06-05T00:00:00+00:00

Abstract

Seed priming is an effective method for improving salt tolerance during crop growth; however, the effect and mechanism of γ-aminobutyric acid (GABA) seed priming on salt tolerance in pepper (Capsicum annuum) remain unclear. This study used 'Maoshu 360' upward-pointing pepper as material to analyze the effects of seed priming with different concentrations of γ-aminobutyric acid (0, 1.0, 2.0, 4.0, 6.0, 8.0 mol•L-1) on plant biomass, osmotic adjustment substances, antioxidant capacity, photosynthetic system, and potassium and sodium ion absorption under 100 mmol•L-1 NaCl stress at the 4–6 leaf stage. The results showed that: (1) Seed priming significantly increased the biomass of pepper plants under salt stress, with the 6.0 mol•L-1 GABA priming treatment showing the optimal effect. (2) Seed priming treatment increased the contents of soluble sugars, soluble proteins, and proline in plants under salt stress, while the contents of O2•- and MDA decreased; the activities of antioxidant enzymes (SOD, POD, CAT, and APX) were enhanced, leaf chlorophyll content increased, leaf chlorophyll fluorescence parameters were less affected by stress, and chlorophyll fluorescence indices including Fv /Fm , qP_{Lss}, QY_{Lss}, NPQ_{Lss}, and Rfd all increased. The K+ content and K+/Na+ ratio in roots and stems decreased. (3) Grey relational analysis indicated that GABA seed priming alleviated salt stress damage to pepper plants primarily by increasing POD and CAT activities and osmotic adjustment substance contents. In summary, 6.0 mol•L-1 GABA seed priming can effectively improve salt tolerance in pepper seedlings, and its mechanism of action may involve enhancing the antioxidant capacity and osmotic adjustment ability of pepper plants under salt stress.

Full Text

Effects of γ-Aminobutyric Acid (GABA) Seed Priming on Alleviating Salt Stress and Associated Physiological-Biochemical Changes in Pepper (Capsicum annuum)

LIN Xinqi, WEI Qianya, LIANG Lamei, QIN Zhongwei, LI Yingzhi*

College of Coastal Agricultural Sciences, Guangdong Ocean University, Zhanjiang 524088, Guangdong, China

Abstract

Seed priming is an effective method for enhancing crop salt tolerance during the growth period, yet the effects and mechanisms of γ-aminobutyric acid (GABA) seed priming on pepper (Capsicum annuum) salt tolerance remain unclear. This study used "Maoshu 360" Chaotian pepper as experimental material to investigate the effects of seed priming with different GABA concentrations (0, 1.0, 2.0, 4.0, 6.0, and 8.0 µmol $\cdot$ L^{-1}) on plant biomass, osmotic adjustment substances, antioxidant capacity, photosynthetic system, and potassium/sodium ion uptake in plants under 100 mmol $\cdot$ L^{-1} NaCl stress at the 4–6 leaf stage. The results demonstrated: (1) Seed priming significantly increased pepper biomass under salt stress, with 6.0 µmol $\cdot$ L^{-1} GABA treatment showing the optimal effect. (2) Seed priming enhanced the contents of soluble sugars, soluble proteins, and proline while decreasing superoxide anion ($\cdot \mathrm{O_2}^-$) and malondialdehyde (MDA) contents. Antioxidant enzyme activities (SOD, POD, CAT, and APX) were strengthened, leaf chlorophyll content increased, and chlorophyll fluorescence parameters showed reduced stress impact, with improvements in Fv /Fm , qP_{Lss}, QY_{Lss}, NPQ_{Lss}, and Rfd. K$^+$ content and K$^+$/Na$^+$ ratio in roots and stems decreased. (3) Grey correlation analysis revealed that GABA seed priming primarily alleviated salt stress damage in pepper by enhancing POD and CAT activities and increasing osmotic adjustment substance content. In conclusion, 6.0 µmol $\cdot$ L^{-1} GABA seed priming effectively improves salt tolerance in pepper seedlings, likely by enhancing antioxidant capacity and osmotic adjustment ability under salt stress.

Keywords: γ-aminobutyric acid (GABA), pepper, seed priming, salt tolerance, grey correlation analysis

---

Introduction

Approximately 1.1×10^9 hectares of global land surface have been affected by soil salinization, with China's total saline-alkali soil area reaching 3.69×10^7 hectares. Salinization has become a major obstacle to sustainable agricultural production (Yang et al., 2022). As an abiotic stress factor, soil salinity adversely affects crop yields by reducing seed germination rates, impairing plant vigor, disrupting cellular ion homeostasis, and altering metabolic pathways (Shabala et al., 2016; Elbadri et al., 2021). Pepper (Capsicum annuum) is an important solanaceous vegetable crop with cultivation area exceeding 2.1 million hectares in China (Zou et al., 2020). Pepper exhibits sensitivity or moderate sensitivity to salinity, with salt stress during growth and development leading to reduced yield and fruit quality (Hu et al., 2022).

Extensive research has investigated salt stress mechanisms, which primarily involve direct or indirect overaccumulation of reactive oxygen species (ROS) and oxidative stress (Abdel & He, 2014), resulting in ion toxicity, nutritional imbalance, restricted water absorption, decreased photosynthetic efficiency, and ultimately poor growth or plant death (Cuartero et al., 2006; Afzal et al., 2008; Nouman et al., 2014). Plants employ multiple mechanisms to cope with salt stress, including stomatal regulation, maintenance of cell membrane integrity, hormonal balance modification, activation of antioxidant systems, osmotic potential adjustment, and toxic ion exclusion (Neto et al., 2005; Abdel et al., 2019).

Seed priming is an emerging seed treatment technology that enhances stress resistance during plant growth and development. Although its mechanism remains incompletely understood, seed priming likely works by controlling limited seed activation or stress acclimation, altering gene expression patterns to prepare plants for subsequent stress exposure (Li et al., 2016). Seed priming can activate stress response systems, conferring “cross-tolerance” when seeds encounter future stress conditions (Bhanuprakash & Yogeesha, 2016). As a practical, economical, low-risk cultivation measure without biosafety concerns, seed priming not only improves germination and seedling emergence under stress (Migahid et al., 2019) but also enhances stress resistance during growth through memory effects that may even be heritable (Margarete et al., 2019).

γ-Aminobutyric acid (GABA) is a non-protein, four-carbon amino acid produced from glutamate decarboxylation or polyamine degradation by diamine oxidase, representing a novel plant growth regulator (Wang et al., 2014). Under stress conditions, GABA rapidly accumulates in plant cells and enhances stress adaptation by improving antioxidant responses, regulating carbon-nitrogen metabolism and cytoplasmic pH, and participating in osmotic adjustment and signal transduction (Jia et al., 2014; Zhang et al., 2020). In salt stress contexts, GABA functions as a signaling molecule or temporary nitrogen reservoir, significantly influencing plant salt tolerance by modulating antioxidant capacity and improving photosynthetic characteristics (Li et al., 2016; Ramesh et al., 2017; Kaspal et al., 2021).

This study focused on seed priming and pepper salt tolerance, employing biomass and physiological-biochemical analyses to investigate: (1) the effects of GABA seed priming on pepper salt tolerance; (2) the optimal GABA concentration for seed priming; and (3) the underlying mechanisms through which GABA seed priming enhances pepper salt tolerance.

Materials and Methods

1.1 Experimental Materials and Treatments The experimental material was "Maoshu 360" Chaotian pepper, and γ-aminobutyric acid (GABA) was purchased from Sangon Biotech.

1.1.1 Seed Priming Treatment Six GABA priming concentrations were established: 0 (distilled water, T0), 1.0 (T1), 2.0 (T2), 4.0 (T4), 6.0 (T6), and 8.0 (T8) $\mu mol \cdot L^{-1}$. Uniform, plump pepper seeds (1.8 g, approximately 100 seeds) were placed in different GABA solutions at a seed mass (g) to solution volume (mL) ratio of 1:5 (Wu et al., 2017) and primed at 20 °C in darkness for 24 h. After priming, seeds were rinsed with distilled water to remove residual GABA, surface moisture was blotted dry, and seeds were returned to their original weight in a forced-air drying oven at 28 °C. The experiment was repeated three times.

1.1.2 Salt Stress Treatment Primed and unprimed seeds were germinated in normal substrate and sown in 15-cell trays under normal management, with unprimed plants serving as controls (CK). When plants reached the 4–6 leaf stage, 50 mL of 100 $mmol \cdot L^{-1}$ NaCl solution was applied to each plant every two days. Samples were collected for analysis after 14 days. Each treatment included 20 plants, with three replications.

1.2 Measurement Indicators

1.2.1 Plant Growth Assessment Five pepper plants were randomly selected from each treatment to measure plant height and fresh and dry weights of roots, stems, and leaves.

1.2.2 Enzyme Activity and Metabolite Content Determination Five plants per treatment were randomly selected, and fully expanded upper leaves were harvested for analysis. Soluble protein content was determined using the Bradford (1976) method. Soluble sugar content, malondialdehyde (MDA) content, and ascorbate peroxidase (APX, $U \cdot (g \cdot min)^{-1}$ FW) activity were measured using the anthrone colorimetric method, thiobarbituric acid method, and the method of Nakano and Asada (1981), respectively, as described by Li (2012). Reduced ascorbic acid (ASA) and dehydroascorbate (DHA) contents were determined according to Yang et al. (2018). Proline (Pro), hydrogen peroxide (H_2O_2), superoxide anion ($\cdot O_2^-$) contents, and activities of superoxide dismutase (SOD, $U \cdot g^{-1}$ FW), peroxidase (POD, $U \cdot g^{-1}$ FW), catalase (CAT, $U \cdot g^{-1}$ FW), and glutathione reductase (GR, $U \cdot g^{-1}$ FW) were measured using commercial kits (Solarbio Science & Technology Co., Ltd.), where one unit (U) represents the amount of enzyme catalyzing the conversion of 1 μmol substrate per minute under optimal conditions.

1.2.3 Chlorophyll Content Measurement Five plants per treatment were randomly selected, and the second to fifth fully expanded leaves from the growing point were used to determine chlorophyll content with a SPAD-502 meter (Konica Minolta) (Wakiyama, 2016).

1.2.4 Chlorophyll Fluorescence Parameter Determination Chlorophyll fluorescence parameters were measured using a FluorPen chlorophyll fluorescence analyzer (Photon Systems Instruments, Czech Republic). Six plants per treatment were randomly selected, and fully expanded leaves at the plant apex were used to measure Fv/Fm values. After dark adaptation for 20 min, other chlorophyll fluorescence parameters were determined: potential maximum quantum yield (Fv /Fm), steady-state photochemical quenching (qP_{Lss}), steady-state non-photochemical quenching (NPQ_{Lss}), steady-state quantum yield (QY_{Lss}), and fluorescence decline ratio (Rfd).

1.2.5 Na^+ and K^+ Content Determination Five plants per treatment were randomly selected, washed with distilled water, and separated into roots, stems, and leaves for drying. Dried samples were digested using the concentrated H_2SO_4-H_2O_2 method (Bao, 2000), and potassium and sodium contents in the filtrate were determined by flame atomic absorption spectrometry (Bao, 2000).

1.3 Data Analysis Data were processed routinely in Excel 2019. One-way ANOVA was performed using SPSS 25.0, with Duncan's multiple comparison test for post-hoc analysis. Grey correlation analysis was conducted using SPSSAU.

Results

2.1 Effects of Different GABA Seed Priming Concentrations on Pepper Growth Under Salt Stress The effects of different GABA seed priming concentrations on pepper plant growth under salt stress are shown in [Figure 1: see original paper]. GABA seed priming increased fresh and dry weights of roots, stems, and leaves, as well as plant height. Different GABA concentrations produced varying effects, with the T6 treatment (6.0 $\mu mol \cdot L^{-1}$ GABA) showing the best performance, followed by T4 and T2 treatments. These three treatments showed no significant differences except in stem dry weight and plant height, and all were significantly higher than the unprimed control (CK). Compared with CK, T6 treatment increased root, stem, and leaf fresh weights by 72.4%, 163.9%, and 94.3%, respectively; root, stem, and leaf dry weights by 1.20-fold, 2.22-fold, and 1.56-fold, respectively; and plant height by 0.92-fold. Compared with the distilled water treatment, T6 increased root, stem, and leaf fresh weights by 62.2%, 138.5%, and 88.3%, respectively; dry weights by 1.03-fold, 2.14-fold, and 1.28-fold, respectively; and plant height by 0.86-fold. These results indicate that GABA seed priming promoted better growth and greater biomass accumulation in pepper plants under salt stress.

2.2.1 Effects on Soluble Sugar, Soluble Protein, and Proline Contents in Pepper Leaves The effects of different GABA seed priming concentrations on soluble sugar, soluble protein, and proline contents in pepper leaves under salt stress are presented in [Figure 2: see original paper]. GABA seed priming increased the contents of all three substances to varying degrees. Maximum values were observed at 6.0 $\mu mol \cdot L^{-1}$ GABA, which were significantly higher than in unprimed plants: soluble sugar content increased by 1.28-fold, soluble protein by 1.72-fold, and proline by 1.04-fold. Compared with the distilled water treatment, soluble sugar, soluble protein, and proline contents increased by 0.94-fold, 0.97-fold, and 0.79-fold, respectively. The T4 treatment showed no significant difference from T6 in soluble sugar and protein contents (decreasing by only 0.83% and 11.8%, respectively), while T6 proline content was 1.27-fold higher than T4 and significantly different from other treatments. These findings demonstrate that GABA seed priming enhanced physiological activity and accumulation of osmotic adjustment substances in pepper plants under salt stress.

2.2.2 Effects on H_2O_2, Superoxide Anion ($\cdot O_2^-$), and MDA Contents in Pepper Leaves As shown in [Figure 3: see original paper], compared with unprimed plants, GABA seed priming increased H_2O_2 content while decreasing superoxide anion ($\cdot O_2^-$) and MDA contents in pepper leaves. Under salt stress, H_2O_2 content was highest in the T6 treatment, showing significant differences from other treatments. Compared with unprimed plants, T6 increased H_2O_2 content by 2.1-fold and by 1.04-fold compared with the distilled water treatment. Superoxide anion ($\cdot O_2^-$) and MDA contents were lowest in the T6 treatment, decreasing by 63.6% and 73.6% compared with unprimed plants, and by 73.0% and 68.8% compared with the distilled water treatment, respectively, with no significant differences among GABA priming treatments (T1, T2, T4, T8). These results indicate that GABA seed priming effectively alleviated reactive oxygen species accumulation and cell membrane oxidative damage in pepper leaves under salt stress.

2.2.3 Effects on SOD, POD, CAT, APX, and GR Enzyme Activities in Pepper Leaves [Figure 4: see original paper] shows that under salt stress, GABA seed priming significantly increased the activities of SOD, POD, CAT, and APX in pepper leaves, with peak activities observed at 6.0 $\mu mol \cdot L^{-1}$ GABA. However, GR activity was not significantly affected [Figure 4E: see original paper]. Except for POD activity in the T4 treatment, significant differences existed between T6 and other GABA priming treatments in SOD, POD, CAT, and APX activities. Compared with unprimed plants, T6 increased

SOD, POD, CAT, and APX activities by 0.44-fold, 4.09-fold, 7.22-fold, and 1.35-fold, respectively. Compared with the distilled water treatment, these activities increased by 0.32-fold, 3.30-fold, 1.13-fold, and 1.04-fold, respectively. No consistent differences in GR activity were observed among unprimed, water-primed, and GABA-primed treatments. These findings demonstrate that GABA seed priming enhanced the antioxidant capacity mediated by SOD, POD, CAT, and APX enzymes in pepper leaves under stress.

2.2.4 Effects on ASA, DHA Contents and ASA/DHA Ratio in Pepper Leaves [Figure 5: see original paper] shows that GABA seed priming increased ASA content and the ASA/DHA ratio while decreasing DHA content in pepper leaves. ASA content was highest in the T8 treatment, which was significantly different from T4 and 1.40-fold higher than T4. Compared with unprimed leaves, ASA content increased by 62.3% and by 2.02-fold compared with the distilled water treatment. The higher ASA content and lower oxidized product (DHA) content in GABA-primed leaves indicate enhanced antioxidant capacity or reduced free radical stress.

2.3 Effects of Different GABA Seed Priming Concentrations on Chlorophyll Content in Pepper Leaves The effects of different GABA seed priming concentrations on chlorophyll content in pepper leaves under salt stress are shown in [Figure 6: see original paper]. Only the 6.0 µmol$\cdot$L^{-1} GABA priming treatment showed significantly higher chlorophyll content compared with unprimed and water-primed treatments, with increases of 8.78% and 8.41%, respectively. At the highest GABA concentration (T8), chlorophyll content was significantly lower than in the T6 treatment, decreasing by 16.0%. These results indicate that appropriate GABA priming concentrations can alleviate chlorophyll degradation in pepper leaves under salt stress.

2.4 Effects of Different GABA Seed Priming Concentrations on Chlorophyll Fluorescence Characteristics The effects of different GABA priming concentrations on chlorophyll fluorescence characteristics in pepper leaves under salt stress are presented in [Figure 7: see original paper]. Compared with unprimed and water-primed treatments, GABA seed priming decreased Fo values while showing no significant differences in Fv/Fm values. However, Fv /Fm , qP_{Lss}, NPQ_{Lss}, QY_{Lss}, and Rfd values all increased significantly. Compared with unprimed plants, the T6 treatment showed the lowest Fo value (5.04% reduction) and the highest Fv /Fm , qP_{Lss}, NPQ_{Lss}, QY_{Lss}, and Rfd values, increasing by 10.5%, 22.3%, 40.0%, 14.5%, and 36.1%, respectively. Compared with the water-primed treatment, Fo decreased by 14.6%, while Fv /Fm , qP_{Lss}, NPQ_{Lss}, QY_{Lss}, and Rfd increased by 5.47%, 29.5%, 10.4%, 26.0%, and 27.0%, respectively. These results demonstrate that GABA seed priming maintained a relatively stable photosynthetic system and promoted normal photosynthesis under salt stress.

2.5 Effects of Different GABA Seed Priming Concentrations on Na^+, K^+ Contents and K^+/Na^+ Ratio The effects of different GABA seed priming concentrations on potassium and sodium ion contents in pepper roots, stems, and leaves under salt stress are shown in [Figure 8: see original paper]. Potassium content increased sequentially from roots to stems to leaves in pepper plants, while Na^+ content showed no significant differences among plant parts. GABA seed priming decreased K^+ content in roots and stems but had no significant effect on leaf K^+ content. Priming did not significantly affect Na^+ content in roots, though some treatments increased Na^+ content in stems (T0) and leaves (T8). The T1 treatment decreased K^+/Na^+ ratios in roots and stems but did not significantly affect the leaf K^+/Na^+ ratio.

2.6 Grey Correlation Analysis of Different GABA Seed Priming Treatments on Pepper Physiological Indices Under Salt Stress Thirty-eight physiological indices were treated as a grey system, with different treatments as reference sequences and physiological indices as comparative sequences. Initial value standardization was applied to comprehensively analyze their correlations. As shown in , correlation values ranged from 0.556 to 0.755, with higher values indicating stronger correlation with the reference value. POD activity showed the highest comprehensive evaluation (correlation coefficient of 0.755), followed by CAT activity (0.692) and soluble protein content (0.688). These results suggest that GABA seed priming primarily alleviated salt stress damage in pepper plants by increasing POD and CAT activities and enhancing osmotic adjustment substance content.

Discussion and Conclusion

Numerous studies have demonstrated that seed priming enhances crop tolerance to salt stress during growth and development (Chen et al., 2021; Zafar et al., 2022; Adhikari et al., 2022). In pepper, KCl, hydrogen peroxide, and 5-aminolevulinic acid have been used as priming agents to improve tolerance to drought, salinity, or low temperature (Korkmaz & Korkmaz, 2009; Rinez et al., 2018; Gammoudi et al., 2020; Solichatun et al., 2022). GABA is recognized as a novel plant growth regulator associated with plant stress adaptation (Jia et al., 2014; Zhang et al., 2020). GABA priming of white clover (Trifolium repens) and black pepper (Piper nigrum) seeds increased biomass under water or osmotic stress (Vijayakumari & Puthur, 2016; Zhou et al., 2021). Adding GABA to hydroponic nutrient solution promoted growth of salt-stressed maize seedlings, increasing plant height and fresh/dry weights of roots, stems, and leaves (Wang, 2016), and enhanced growth of tomato (Lycopersicon esculentum) roots, stems, and leaves under salt stress (Luo et al., 2011). However, GABA seed priming in pepper has not been previously reported. This study demonstrates that different GABA seed priming concentrations effectively improved growth of salt-stressed pepper plants, increasing plant height and fresh/dry weights of roots, stems, and leaves, with 6.0 µmol $\cdot$ L^{-1} GABA (T6) showing the best results.

Osmotic stress is the most direct response of crops to salt stress, manifested as reduced water uptake capacity and leaf wilting. Soluble sugars and proteins reflect plant physiological activity and function as osmotic adjustment substances along with proline (Xue et al., 2018; Li et al., 2021). In this study, all GABA seed priming treatments significantly increased leaf soluble sugar and protein contents compared with unprimed or water-primed treatments, indicating enhanced physiological activity in salt-stressed pepper plants. The T4 and T6 priming treatments increased leaf proline content above that of unprimed, water-primed, and other concentration treatments, but the high concentration (T8) treatment significantly decreased proline content compared with unprimed or water-primed treatments. This aligns with the optimal priming concentration determined by biomass measurements and suggests that higher GABA concentrations may adversely affect pepper physiological metabolism. Previous studies have shown that GABA can directly act as an osmoprotectant or maintain stable osmotic potential by increasing osmotic adjustment substance content (Bai et al., 2022). Adding 0.5 g $\cdot$ L^{-1} GABA to culture medium increased soluble sugar, soluble protein, and free proline contents in blueberry (Vaccinium corymbosum) plantlets, alleviating vitrification (Zhang et al., 2021). GABA seed priming increased soluble sugar, protein, and proline contents in water-stressed white clover (Zhou et al., 2021). Foliar GABA application promoted proline accumulation in salt-stressed Siberian Nitraria (Nitraria sibirica), enhancing salt tolerance (Wang, 2021). GABA-induced salt tolerance in creeping bentgrass (Agrostis stolonifera) was associated with increased soluble sugar and polyamine accumulation (Li et al., 2020). However, some studies reported that GABA seed priming decreased proline content in stressed rice (Sheteiwy et al., 2019). Therefore, GABA seed priming can increase osmotic adjustment substance content in pepper plants, maintaining cellular osmotic balance and reducing salt stress impact.

Reactive oxygen species accumulation is a plant response to salt stress, and scavenging capacity reflects crop salt tolerance (Abdel et al., 2014). In this study, GABA seed priming decreased superoxide anion ($\cdot O_2^-$) and MDA contents in salt-stressed pepper leaves, indicating reduced oxidative stress and intact cell membranes. Although leaf H_2O_2 content increased, activities of antioxidant enzymes (SOD, POD, CAT, and APX) were enhanced, enabling timely removal of peroxides and oxygen radicals, alleviating oxidative stress and improving pepper salt tolerance. Research has shown that H_2O_2 can function as a signaling molecule in plant cells, participating in systemic acquired resistance (SAR) and hypersensitive response (HR) processes (Li et al., 2007). Therefore, GABA seed priming may activate the plant antioxidant system through H_2O_2 accumulation, thereby enhancing pepper salt resistance. Additionally, GABA itself possesses ROS scavenging capacity (Deng et al., 2010; Liu et al., 2011). Exogenous GABA application can promote transcription of antioxidant enzyme-related genes (Li et al., 2017; Zhang et al., 2022) and increase SOD, POD, CAT, and APX activities in rice, black pepper, and perennial ryegrass (Krishnan et al., 2013;

Nayyar et al., 2014; Vijayakumari & Puthur, 2016). GABA rice seed priming controlled oxygen radical levels by inducing antioxidant enzyme activities and gene transcription under stress (Sheteiwy et al., 2019).

As a terminal oxidase, ascorbate peroxidase scavenges reactive oxygen species by catalyzing ascorbic acid oxidation to dehydroascorbate (Li et al., 2013), while ASA also functions as a non-enzymatic antioxidant to scavenge oxygen radicals (Akashi et al., 2004). In this study, GABA seed priming increased APX activity and ASA content without increasing the oxidized product (DHA), suggesting that enhanced salt tolerance may be independent of this pathway or that downstream DHA pathways participate in ROS scavenging. Li et al. (2016) also reported that foliar GABA application significantly increased ASA content and ASA/DHA ratio in heat-stressed creeping bentgrass leaves. Therefore, GABA seed priming likely enhanced oxygen radical scavenging capacity in salt-stressed pepper plants through similar mechanisms.

Changes in photosynthetic capacity under stress reflect plant stress tolerance. Abiotic stress can affect photosynthetic performance, chlorophyll fluorescence parameters, and chlorophyll content (Brugnoli & Lauteri, 1991; Garg et al., 2002). This study showed that GABA seed priming affected chlorophyll content in salt-stressed pepper leaves, with only the T6 concentration significantly higher than unprimed and water-primed treatments, while the highest concentration (T8) was significantly lower than other treatments. Similar results have been reported, where foliar GABA application increased chlorophyll content in salt-stressed Siberian Nitraria (Wang et al., 2019). Regarding chlorophyll fluorescence parameters, GABA-primed plants were not inferior or were superior to unprimed plants under salt stress, indicating that GABA seed priming mitigated damage to the pepper photosynthetic system. Few studies have reported GABA seed priming effects on plant photosynthetic systems, but research has shown that GABA addition to nutrient solution increased Fv/Fm, ETR, ΦPS , and qP in salt-stressed tomato seedlings (Luo, 2011), and GABA priming of broad bean (Vicia faba) seeds increased Fv/Fm and decreased NPQ in salt-stressed leaves, alleviating adverse effects on the photosynthetic system (Shomali et al., 2021).

Salt stress causes Na^+ accumulation and Na^+/K^+ imbalance in plants (Flowers & Colmer, 2015), and plants can enhance salt tolerance through selective Na^+ uptake or efflux (Niu et al., 2018). This study showed that under salt stress, potassium accumulated most in pepper leaves, followed by stems, with less accumulation in roots, similar to normal growth conditions (Wu et al., 2019). GABA seed priming decreased K^+ content in roots and stems but did not significantly affect leaf K^+ content. Priming did not significantly affect Na^+ content in roots and stems, though the high concentration treatment (T8) significantly increased leaf Na^+ content compared with unprimed treatment. Although some studies reported that nutrient solution GABA addition decreased Na^+ flux and content in tomato (Wu et al., 2020) and that GABA seed priming promoted Na^+/K^+ transport and accumulation in white clover seedlings (Cheng et al., 2018), this study suggests that enhanced pepper salt tolerance by GABA seed priming may not involve selective Na^+/K^+ uptake or transport.

Grey correlation analysis is widely used as a comprehensive evaluation method in crop resistance research (Gao et al., 2021). Given the complex effects of GABA on plant physiological activities, this study employed grey correlation analysis to investigate mechanisms of GABA seed priming effects on pepper salt tolerance. The results indicate that GABA priming primarily alleviated salt stress damage by increasing antioxidant enzyme activities (POD and CAT) and enhancing osmotic adjustment substance content.

In summary, 6.0 $\mu mol \cdot L^{-1}$ GABA seed priming effectively promoted pepper plant growth under salt stress and can be recommended as a practical seed treatment method. The mechanisms underlying enhanced salt tolerance likely involve promoted physiological metabolism, increased contents of soluble sugars, soluble proteins, and the osmotic adjustment substance proline, enhanced antioxidant capacity, reduced reactive oxygen species levels and membrane lipid peroxidation damage, and maintained normal photosynthetic system operation.

References

ABDEL LATEF AAH, HE CX, 2014. Does inoculation with Glomus mosseae improve salt tolerance in pepper plants? [J]. J Plant Growth Regul, 33(3): 644-653.

ABDEL LATEF AAH, MOSTOFA MG, RAHMAN M, et al., 2019. Extracts from yeast and carrot roots enhance maize performance under seawater-induced salt stress by altering physio-biochemical characteristics of stressed plants[J]. J Plant Growth Regul, 38(3): 966-979.

ADHIKARI B, OLORUNWA OJ, BARICKMAN TC, 2022. Seed priming enhances seed germination and morphological traits of Lactuca sativa L. under salt stress[J]. Seeds, 1(2): 74-86.

AFZAL I, RAUF S, BASRA S, et al., 2008. Halopriming improves vigor, metabolism of reserves and ionic contents in wheat seedlings under salt stress[J]. Plant Soil Environ, 54(9): 382-388.

AKASHI K, NISHIMURA N, ISHIDA Y, et al., 2004. Potent hydroxyl radical-scavenging activity of drought-induced type-2 metallothionein in wild watermelon[J]. Biochem Biophys Res Commun, 323(1): 72-78.

BAI MY, LU SS, XIA XY, 2022. Effects of γ-aminobutyric acid on the growth and related physiological characteristics of Vaccinium plantlets in vitro[J]. Plant Physiol J, 58(3): 577-586.

BAO SD, 2000. Soil Agrochemical Analysis. 3rd ed. [M]. Beijing: China Agricultural Press.

BHANUPRAKASH K, YOGEESHA HS, 2016. Seed priming for abiotic stress tolerance: an overview[J]. Abiotic Stress Physiology of Horticultural Crops, 103-117.

BRADFORD MM, 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding[J]. Anal Biochem, 72(1/2): 248-254.

BRUGNOLI E, LAUTERI M, 1991. Effects of Salinity on Stomatal Conductance, Photosynthetic Capacity, and Carbon Isotope Discrimination of Salt-Tolerant (Gossypium hirsutum L.) and Salt-Sensitive (Phaseolus vulgaris L.) C3 Non-Halophytes. [J]. Plant Physiol, 95(2): 628-635.

CHEN XF, ZHANG RD, XING YF, et al., 2021. The efficacy of different seed priming agents for promoting sorghum germination under salt stress[J]. PLoS ONE, 16(1): e0245505.

CHENG BZ, LI Z, LIANG LL, et al., 2018. The γ-aminobutyric acid (GABA) alleviates salt stress damage during seeds germination of white clover associated with Na+/K+ transportation, dehydrins accumulation, and stress-related genes expression in white clover[J]. Int J Mol Sci, 19(9): 2520.

CUARTERO J, BOLARIN MC, ASINS MJ, et al., 2006. Increasing salt tolerance in the tomato[J]. J Exp Bot, 57(5): 1045-1058.

DENG Y, XU LJ, ZENG X, et al., 2010. New perspective of GABA as an inhibitor of formation of advanced lipoxidation end-products: it's interaction with malondiadehyde[J]. J Biome Nano, 6(4): 318-324.

ELBADRI AM, BATOOL M, WANG CY, et al., 2021. Selenium and zinc oxide nanoparticles modulate the molecular and morpho-physiological processes during seed germination of Brassica napus under salt stress[J]. Ecotoxicol Environ Safety, 225: 112695.

FLOWERS TJ, COLMER TD, 2015. Plant salt tolerance: adaptations in halophytes [J]. Ann Bot, 115(3):327-331.

GAMMOUDI N, KARMOUS I, ZERRIA K, et al., 2020. Efficiency of pepper seed invigoration through hydrogen peroxide priming to improve in vitro salt and drought stress tolerance[J]. Hortic Environ Biotechnol, 61(4): 703-714.

GAO AJ, LIU TT, ZHOU ML, et al., 2021. Effects of exogenous melatonin on growth and physiological characteristics of tartary buckwheat seedlings under drought stress[J]. J S Agric, 52 (11): 3003-3012.

GARG AK, KIM JK, OWENS TG, et al., 2002. Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses[J]. Proc Natl Acad Sci USA, 99(25): 15898–15903.

HU HR, DU L, ZHANG RH, et al., 2022. Research progress in the adaptation of hot pepper (Capsicum annuum L.) to abiotic stress[J]. Biotechnology Bulletin, 38(12):1-15.

JIA Y, ZHAO HW, WANG JG, et al., 2014. Research Progress on γ-aminobulyric acid metabolism and function of crops under stress[J]. Crops, (5): 9-15.

KASPAL M, KANAPADDALAGAMAGE MH, RAMESH SA, 2021. Emerging Roles of γ-Aminobutyric Acid (GABA) Gated Channels in Plant Stress Tolerance[J]. Plants, 10(10): 2178.

KORKMAZ A, KORKMAZ Y, 2009. Promotion by 5-aminolevulenic acid of pepper seed germination and seedling emergence under low-temperature stress[J]. Sci Hort-Amsterdam, 119(2): 98-102.

KRISHNAN S, LASKOWSKI K, SHUKLA V, et al., 2013. Mitigation of drought stress damage by exogenous application of a non-protein amino acid γ–aminobutyric acid on perennial ryegrass[J]. J Amer Soc Hortic Sci, 138(5): 358-366.

LI H, TANG X, YANG X, et al., 2021. Comprehensive transcriptome and metabolome profiling reveal metabolic mechanisms of Nitraria sibirica Pall. to salt stress[J]. Sci Reports, 11(1): 1-19.

LI HS, 2012. Modern Plant Physiology. 3rd ed. [M]. Beijing: Higher Education Press.

LI J, XU JG, LIN C, et al., 2016. Effect of priming on germination and physiological characteristics of different types of corn seeds under low-temperature stress[J]. Plant Physiol J, 52(2): 157-166.

LI MF, GUO SJ, YANG XH, et al., 2016. Exogenous gamma-aminobutyric acid increases salt tolerance of wheat by improving photosynthesis and enhancing activities of antioxidant enzymes[J]. Biol Plant, 60(1): 123-131.

LI SW, XUE LG, FENG HY, et al., 2007. Hydrogen Peroxide Signaling and Its Biological Importance in Plants[J]. Chin J Biochem Mol Biol, 23:(10): 804-810.

LI Y, FAN Y, MA Y, et al., 2017. Effects of exogenous γ-aminobutyric acid (GABA) on photosynthesis and antioxidant system in pepper (Capsicum annuum L.) seedlings under low light stress[J]. Plant Growth Regul, 36: 1–14.

LI Z, YU JJ, PENG Y, et al., 2016. Metabolic pathways regulated by γ-aminobutyric acid (GABA) contributing to heat tolerance in creeping bentgrass (Agrostis stolonifera)[J]. Scientific Reports, 6(1): 1-16.

LI Z, CHENG B, PENG Y, et al., 2020. Adaptability to abiotic stress regulated by γ-aminobutyric acid in relation to alterations of endogenous polyamines and organic metabolites in creeping bentgrass[J]. Plant Physiol Biochem, 157: 185-194.

LI ZQ, LI JX, ZHANG GF, 2013. Expression regulation of plant ascorbate peroxidase and its tolerance to abiotic stresses[J]. HEREDITAS, 35(1): 45-54.

LIU CL, ZHAO L, YU GH, 2011. The dominant glutamic acid metabolic flux to produce γ-amino butyric acid over proline in Nicotiana tabacum leaves under water stress relates to its significant role in antioxidant activity[J]. J Integr Plant Biol, 53(8): 608-618.

LUO HY, 2011. Effects of three kinds of exogenous substances on reactive oxygen species metabolism and chlorophyll fluorescence in tomato seedlings under NaCl stress[D]. Baoding: Agricultural University of Hebei.

LUO HY, GAO HB, XIA QP, et al., 2011. Effects of exogenous GABA on reactive oxygen species metabolism and chlorophyll fluorescence parameters in tomato under nacl stress[J]. Sci Agric Sin, 44(4):753-761.

MARGARETE B, ANDRAS B, ANDREAS P, et al., 2019. Preparing plants for improved cold tolerance by priming[J]. Plant Cell Environ, 42(3): 782-800.

MIGAHID MM, ELGHOBASHY RM, BIDAK LM, et al., 2019. Priming of Silybum marianum (L.) Gaertn seeds with H2O2 and magnetic field ameliorates seawater stress[J]. Heliyon, 5(6): e01886.

NAKANO Y, ASADA K, 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts[J]. Plant Cell Physiol, 22(5): 867-880.

NAYYAR H, KAUR R, KAUR S, et al., 2014. γ-Aminobutyric acid (GABA) imparts partial protection from heat stress injury to rice seedlings by improving leaf turgor and upregulating osmoprotectants and antioxidants[J]. J Plant Growth Regul, 33(2): 408-419.

NETO ADDA, PRISCO JT, ENÉAS-FILHO J, et al., 2005. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes[J]. Environ Exp Bot, 56(1): 87-94.

NIU ML, HUANG Y, SUN ST, et al., 2018. Root respiratory burst oxidase homologue-dependent H2O2 production confers salt tolerance on a grafted cucumber by controlling Na+ exclusion and stomatal closure[J]. J Exp Bot, 69(14): 3465-3476.

NOUMAN W, BASRA SMA, YASMEEN A, et al., 2014. Seed priming improves the emergence potential, growth and antioxidant system of Moringa oleifera under saline conditions[J]. Plant Growth Regul, 73(3): 279-287.

RAMESH SA, TYERMAN SD, GILLIHAM M, et al., 2017. γ-Aminobutyric acid (GABA) signalling in plants[J]. Cell Mol Life Sci, 74(9): 1577-1603.

RINEZ I, GHEZAL N, RINEZ A, et al., 2018. Improving salt tolerance in pepper by bio-priming with Padina pavonica and Jania rubens aqueous extracts[J]. Int J Agric Biol, 20(3): 513-523.

SHABALA L, ZHANG JY, POTTOSIN I, et al., 2016. Cell-type-specific H+-ATPase activity in root tissues enables K+ retention and mediates acclimation of barley (Hordeum vulgare) to salinity stress[J]. Plant Physiol, 172(4): 2445-2458.

SHETEIWY MS, SHAO H, QI W, et al., 2019. GABA-alleviated oxidative injury induced by salinity, osmotic stress and their combination by regulating cellular and molecular signals in rice[J]. Int J Mol Sci, 20(22): 5580.

SHOMALI A, ALINIAEIFARD S, DIDARAN F, et al., 2021. Synergistic effects of melatonin and gamma-aminobutyric acid on protection of photosynthesis system in response to multiple abiotic stressors[J]. Cells, 10(7): 1631.

SOLICHATUN S, PUTRI TA, MUDYANTINI W, et al., 2022. Effect of seed priming using KCL on the growth and proline accumulation of paprika (Capsicum annuum) growing at different water availability[J]. Asian J Trop Biotechnol, 19(1): 1-6.

VIJAYAKUMARI K, PUTHUR JT, 2016. γ-Aminobutyric acid (GABA) priming enhances the osmotic stress tolerance in Piper nigrum Linn. plants subjected to PEG-induced stress[J]. Plant Growth Regul, 78(1): 57-67.

WAKIYAMA Y, 2016. The Relationship between SPAD Values and Leaf Blade Chlorophyll Content throughout the Rice Development Cycle[J]. Jpn Agric Res Q JARQ, 50, 329–334.

WANG CY, FAN LQ, GAO HB, et al., 2014. Polyamine biosynthesis and degradation are modulated by exogenous gamma-aminobutyric acid in root-zone hypoxia-stressed melon roots[J]. Plant Physiol Biochem, 82: 17-26.

WANG H, 2021. Regulatory effects of exogenous GABA on polyamines and proline metabolisms, and GABA shunt in Nitraria sibirica Pall. under salt stress[D]. Harbin: Northeast Agricultural University.

WANG X, YAN YQ, YIN Y, et al., 2019. Effect of exogenous γ-aminobutyric acid (GABA) on photosynthetic characteristics of Nitraria sibirica pall under salt stress[J]. Jiangsu J Agric Sci, 35(5): 1032-1039.

WANG YC, 2016. Mechanism of aminobutyric acid (GABA) regulating maize seed germination and seeding growth under salt stress[D]. Harbin: Northeast Agricultural University.

WU GQ, LI H, LEI CR, et al., 2019. Effects of additional KCl on growth and physiological characteristics of sainfoin (Onobrychis viciaefoia) under high salt stress[J]. Acta Pratacult Sin, 28(6):45-55.

WU LY, Li M, YAO DW, 2017. Effects of seed priming on seed germination and emergence of pepper and eggplant at different temperature[J]. Acta Agric Shanghai, 33(3): 37–40.

WU XL, JIA QY, JI SX, et al., 2020. Gamma-aminobutyric acid (GABA) alleviates salt damage in tomato by modulating Na+ uptake, the GAD gene, amino acid synthesis and reactive oxygen species metabolism[J]. BMC Plant Biol, 20(1): 1-21.

XUE TX, REN ZB, REN SF, 2018. Impacts of NaCl stress on physiological characteristics of Forsythia intermedia[J]. Jiangsu Agric Sci, 46(11): 104-108.

YANG JS, YAO RJ, WANG XP, et al., 2022. Research on salt-affected soils in China: history, status Quo and prospect[J]. Acta Pedol Sin, 59(1):10–27.

YANG YL, LU LR, Li J, et al., 2018. Comparison of ascorbic acid-glutathione cycle in two wheat seedlings under salt stress[J]. Journal of Northwest Normal University (Nat Sci Ed), 54 (3): 65-70.

ZAFAR S, PERVEEN S, KAMRAN K M, et al., 2022. Effect of zinc nanoparticles seed priming and foliar application on the growth and physio-biochemical indices of spinach (Spinacia oleracea L.) under salt stress[J]. PLoS ONE, 17(2): e0263194.

ZHANG HH, BAI MY, XIA XY, 2021. Effect of γ-aminobutyric acid on growth and physiological metabolism of Vaccinium corymbosum plantlets in vitro under hyperhydricity stress[J]. Plant Physiol J, 57 (3): 623–631.

ZHANG HL, CHEN YY, YANG LX, et al., 2020. Regulation of γ-aminobutyric acid on plant growth and development and stress resistance[J]. Plant Physiol J, 56(4): 600-612.

ZHANG MR, LIU ZY, FAN YT, et al., 2022. Characterization of GABA-transaminase gene from mulberry (Morus multicaulis) and its role in salt stress tolerance[J]. Genes, 13(3): 501.

ZHOU M, HASSAN MJ, PENG Y, et al., 2021. γ-Aminobutyric Acid (GABA) Priming Improves Seed Germination and Seedling Stress Tolerance Associated With Enhanced Antioxidant Metabolism, DREB Expression, and Dehydrin Accumulation in White Clover Under Water Stress [J]. Front Plant Sci, 12: 641397.

ZOU XX, MA YQ, DAI XZ, et al., 2020. Spread and industry development of pepper in China [J]. Acta Hortic Sin, 47(9): 1715-1726.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv — Machine translation. Verify with original.