Mechanisms of Salt Tolerance in *Brassica napus*, Soybean and *Chenopodium quinoa*: Implications for Sustainable Agriculture in Saline-Alkali Soils

Hu Liu [1, 2, 3, a], Jian Wang [1, 2, b], Qi Wang [1, 2, c], Hongfang Li [1, 2, d], Hui Zhou [1, 2, e], Weidong Xi [1, 2, f], and Lei Gao [1, 2, g]

[1]Yinshanbeilu Grassland Eco-Hydrology National Field Observation and Research Station, China Institute of Water Resources and Hydropower Research, Beijing, China;

[2] Institute of Water Resources for Pastoral Area Ministry of Water Resources&China Institute of Water Resources and Hydropower Research, Hohhot, China;

[3] State Key Laboratory of Eco-hydraulics in Northwest Arid Region. Xian University of Technology, Xi'an, China.

[a] liuh@iwhr.com, [b] wangjian@iwhr.com, [c] qwnmg2014@163.com, [d] hongfangli2021@126.com,

[e] 792606382@qq.com, [f] 20201100432@imut.edu.cn, [g] 1215164583@qq.com

Abstract. This study investigated the physiological responses of three salt-tolerant crops (*Brassica napus*, soybean, and *Chenopodium quinoa*) to varying salt stress levels (control, mild, moderate, severe) under controlled conditions. Experimental data revealed a pronounced suppression of both germination and early seedling development in all tested species when exposed to saline conditions, with *Chenopodium quinoa* exhibiting superior tolerance, maintaining higher germination rates and biomass under severe stress. Physiological analyses revealed elevated malondialdehyde (MDA) content, indicating oxidative damage, alongside increased proline and soluble protein levels for osmotic adjustment. *Chenopodium quinoa* uniquely upregulated catalase (CAT) and ascorbate peroxidase (APX) activities under high stress, suggesting enhanced antioxidant capacity. These findings highlight *Chenopodium quinoa*'s potential for saline-alkali land restoration and sustainable crop production under climate change. The study provides critical insights for breeding salt-tolerant varieties and optimizing agricultural practices in marginal ecosystems.

Keywords: salt stress; seed germination; physiological traits; antioxidant enzymes; osmotic regulation.

1. Introduction

With the intensification of global climate change, soil salinization has become an increasingly severe issue, posing significant challenges to agricultural production and ecosystem stability [1]. Salt stress inhibits seed germination, disrupts plant physiological metabolism, and reduces biomass, thereby significantly affecting crop growth and productivity [2]. Such challenges have made varietal screening and the development of tolerant genotypes essential components in strategies addressing both land restoration and food security in saline environments [3].

This study focuses on three salt-tolerant crops (*Brassica napus*, soybean, and *Chenopodium quinoa*) and analyzes the effects of salt stress on their seed germination, physiological indicators, and antioxidant enzyme systems. The aim is to reveal the salt tolerance mechanisms of different crops and provide a theoretical basis for agricultural practices in saline-alkali soils. *Chenopodium quinoa*, as an emerging salt-tolerant crop, has attracted particular attention for its performance under high-salt conditions [4]. These results provide valuable implications for both rehabilitating degraded saline ecosystems and developing climate-resilient crop varieties.

2. Materials and Methods

2.1 Materials and Treatments

2.1.1 Experimental Materials

The tested varieties included *Brassica napus*, soybean, and *Chenopodium quinoa*.

2.1.2 Simulation of Salt Sterss Conditions

Severe saline surface water from Wuyuan County, Inner Mongolia, was diluted to create four salinity gradients. The salinity, electrical conductivity (EC), and pH of each treatment are listed in Table 1.

Table 1. Salinity, EC, and pH of Solutions for Salt Stress Treatment

Treatment	Salinity (%)	EC (us/cm)	pH
Severe salt	0.6	11200	7.57
Moderate salt	0.4	7500	7.55
Mild salt	0.2	3800	7.54
Control	0.01	260	7.26

2.1.3 Seed Germination Experiment

(1) Seed sterilization (5% NaClO, 15 min; 3 × distilled water rinse)

(2) Placement of 50 seeds on dual-layer filter paper in 9 cm Petri dishes

(3) Application of 5 mL treatment solutions (four salinity gradients in triplicate)

(4) Incubation under controlled environment (4000 lux, 25±1°C, 16/8h photoperiod) for one week.

Treatment solutions were replaced daily at 10:00 to maintain consistent concentrations. Germination counts began on the second day, and germination was considered complete if no additional seeds germinated for three consecutive days. On day 7, seedlings were collected to measure root length, biomass, MDA content, soluble protein, proline levels, and antioxidant enzyme activities.

2.2 Measurement Indicators and Methods

2.2.1 Seed Germination Rate

Germination was defined as the emergence of a radicle at least twice the seed length. The germination rate (GP) was calculated as:

$$\text{GP} = (\text{Number of germinated seeds on day 5} / \text{Total seeds}) \times 100\%$$ [5]

2.2.2 Root Length and Biomass of Seedlings

Root length was measured using ImageJ software, with 10 seedlings per treatment averaged. Fresh weight was determined by weighing 10 seedlings per treatment.

2.3 Measurement Indicators and Methods

All experimental data were processed using Microsoft Office Excel (v2019) for visualization and preliminary analysis. Statistical significance was determined through one-way ANOVA followed by Duncan's multiple range test ($P < 0.05$) in SPSS Statistics (Version 19.0, IBM Corp.).

3. Results and Analysis

3.1 Effects of Salt Stress on Crop Seed Germination and Growth

As shown in Table 2, salt stress inhibited crop growth, with the degree of inhibition increasing significantly as salinity levels rose. The germination rate of *Brassica napus* decreased significantly under salt stress ($P < 0.05$), with no significant difference between moderate and severe stress. For soybean and *Chenopodium quinoa*, no significant differences in germination rates were observed among the control, mild, and moderate salt stress treatments, but germination rates decreased significantly under severe salt stress ($P < 0.05$). *Chenopodium quinoa* exhibited higher germination rates across all salt stress treatments compared to *Brassica napus* and soybean, indicating stronger salt tolerance.

Table 2. Effects of Salt Stress on Crop Seed Germination

Crop	Salt treatment			
	Control	Mild salt	Moderate salt	Severe salt
Brassica napus	51.34±4.73a	30.00±1.00b	19.34±2.08c	9.34±1.16c
soybean	64.66±1.53a	58.66±1.53a	54.00±1.00a	26.66±4.16b
Chenopodium quinoa	97.34±2.31a	81.34±1.16a	81.34±1.16a	42.00±1.00b

The biomass of *Brassica napus* seedlings was significantly higher under moderate salt stress than under other treatments. Soybean seedlings showed the highest biomass under mild salt stress and the lowest under severe salt stress. *Chenopodium quinoa* seedlings had significantly lower biomass under severe salt stress, with no significant differences among other treatments. These results demonstrate that salt stress significantly inhibits the growth of crops.

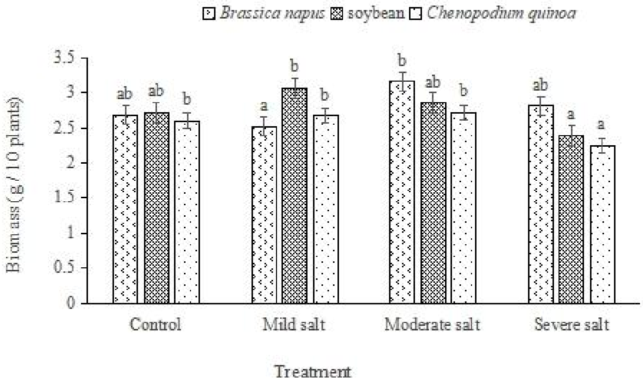

Fig. 1 Impact of Salinity Gradients on Seedling Biomass Accumulation in Tested Crops

Mean separation was performed using Duncan's multiple range test ($\alpha = 0.05$). Within each measurement, treatment means followed by different lowercase letters differ significantly ($P < 0.05$). This notation convention applies to all subsequent figures and tables.

3.2 Effects of Salt Stress on MDA Content During Germination

As shown in Figure 2, the MDA content of *Brassica napus* seedlings increased with rising salinity levels. Under mild salt stress, MDA content was lower than the control but not significantly different. Under moderate and severe salt stress, MDA content was significantly higher than in the mild salt stress and control groups ($P < 0.05$). For soybean, MDA content under mild and moderate salt stress was significantly higher than under severe stress and the control ($P < 0.05$). MDA levels in *Chenopodium quinoa* remained stable across mild and moderate salinity treatments ($P > 0.05$), yet showed significant elevation under both control and severe stress conditions ($P < 0.05$).

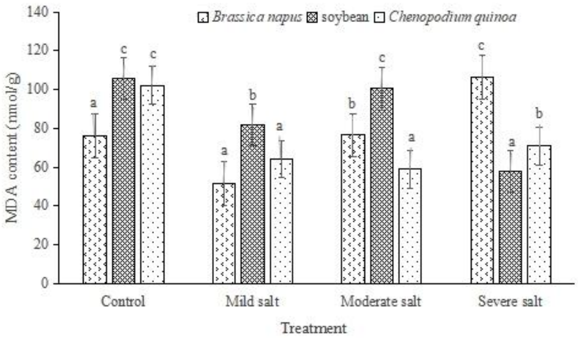

Fig. 2 Comparative MDA Content in Crops Under Varying Salinity Levels

3.3 Effects of Salt Stress on Osmotic Regulators

As shown in Figure 3A, compared to the control, the free proline content of crop seedlings increased under salt stress. For *Brassica napus*, the order of free proline content was severe > moderate > mild > control, with significant differences between moderate/severe and mild/control treatments ($P < 0.05$). Soybean seedlings showed significantly higher proline content under severe salt stress than under mild, moderate, and control treatments ($P < 0.05$), with no significant differences among the latter. *Chenopodium quinoa* had significantly higher proline content under mild salt stress than under other treatments, with the order being mild > severe > moderate > control.

Species-specific patterns emerged in soluble protein accumulation: *Brassica napus* displayed peak levels under moderate stress ($P < 0.05$ vs other treatments); soybean maintained elevated concentrations across all stress levels ($P < 0.05$ vs control); both species showed minimal protein content under severe stress and control conditions, respectively (as shown in Figure 3B).

the soluble protein content of *Brassica napus* seedlings was significantly higher under moderate salt stress ($P < 0.05$) and lowest under severe stress. Soybean seedlings showed no significant differences in soluble protein content among mild, moderate, and severe salt stress treatments, but all were significantly higher than the control ($P < 0.05$). For *Chenopodium quinoa*, soluble protein content increased with salt stress intensity under control, mild, and moderate treatments, but no significant difference was observed under severe stress compared to the control.

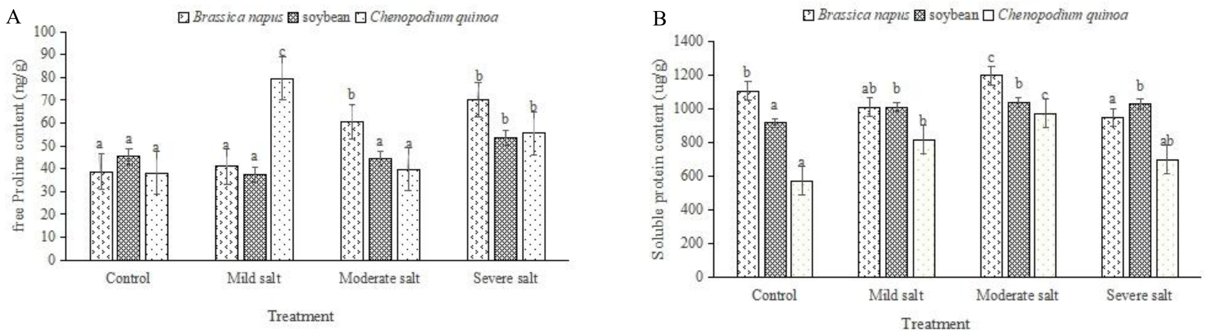

Fig. 3 Free Proline and Soluble Protein Responses to Salinity Gradients

3.4 Effects of Salt Stress on Antioxidant Enzyme Systems

Superoxide dismutase (SOD) activity revealed contrasting stress-response patterns (Fig. 4A): *Brassica napus* exhibited a U-shaped response with peaks at both stress extremes (0.2% and 0.6% salinity) Soybean displayed unimodal activation, maximized at 0.2% before progressive decline Statistical groupings: *Brassica napus* (mild = severe > control = moderate); Soybean (mild > control > severe > moderate) at $P < 0.05$. *Chenopodium quinoa* showed significantly higher SOD activity under moderate stress and control than under mild and severe stress ($P < 0.05$).

As shown in Figure 4B, POD activity in *Brassica napus* seedlings was significantly higher under mild salt stress than under other treatments ($P < 0.05$), with no significant differences among control, moderate, and severe stress. Peroxidase (POD) activity displayed distinct species-specific patterns: Soybean: severe (0.6%) = control > mild (0.2%) = moderate (0.4%) ($P < 0.05$) , *Chenopodium quinoa*: control = mild = severe > moderate ($P < 0.05$), demonstrating fundamentally different oxidative stress management strategies.

As shown in Fig. 4C, CAT activity in *Brassica napus* seedlings first decreased and then increased, being significantly higher under moderate stress than under other treatments ($P < 0.05$), with the order being moderate > severe > control > mild. For soybean, CAT activity was significantly higher under control than under other treatments, increasing with salt stress intensity ($P < 0.05$). *Chenopodium quinoa* showed increasing CAT activity with stress intensity, with the order being severe > moderate > mild ($P < 0.05$), and no significant difference between moderate and control.

As shown in Fig. 4D, APX activity in *Brassica napus* seedlings decreased with salt stress, being significantly higher in the control than under other treatments ($P < 0.05$), with no differences among mild, moderate, and severe stress. APX activity showed contrasting patterns: Soybean: progressive increase with salinity (mild = moderate = severe > control; $P < 0.05$); C. quinoa: U-shaped response (control = severe > mild = moderate; $P < 0.05$), highlighting species-specific antioxidant strategies.

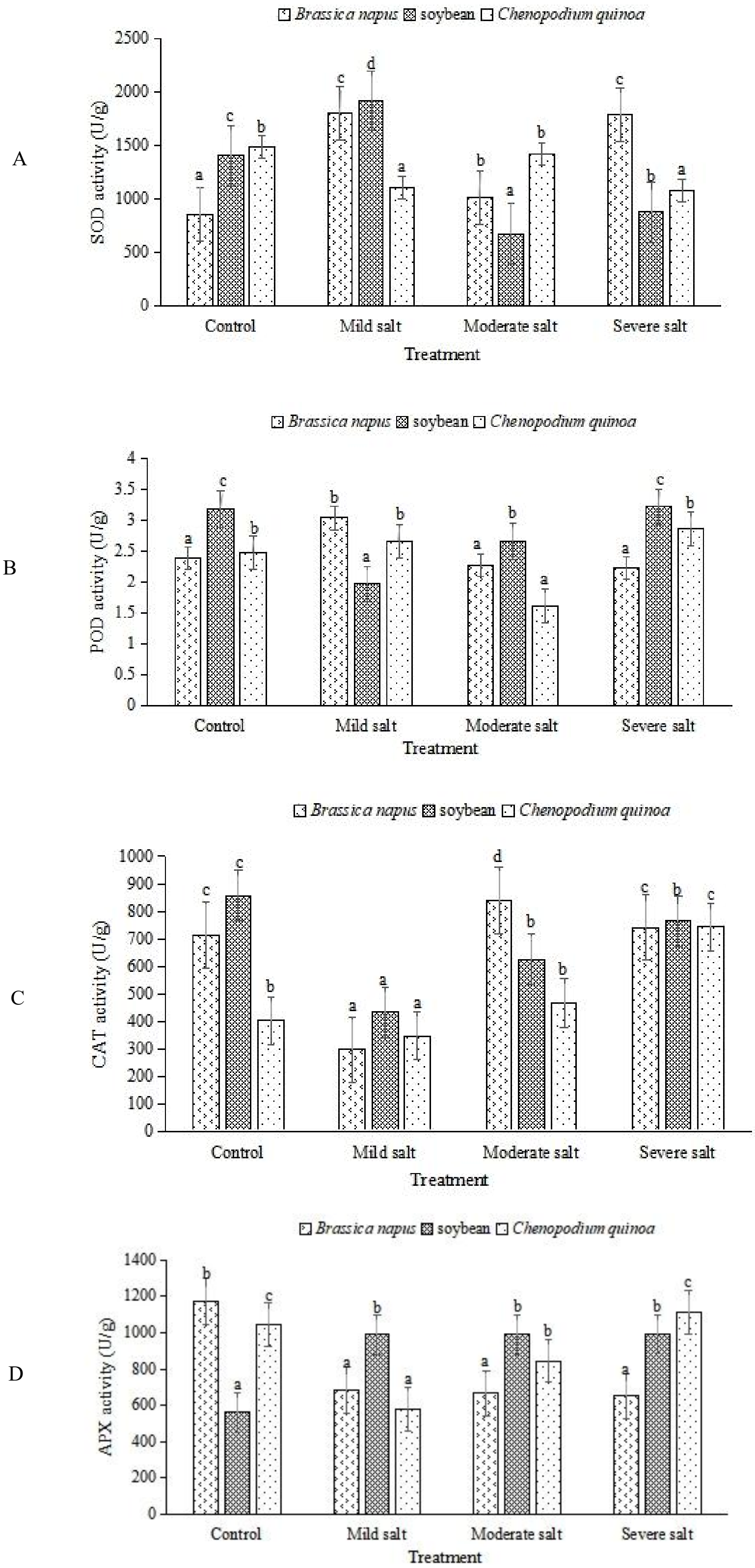

Fig. 4 Effects of Salt Stress on the Antioxidant Enzyme System of Crops

4. Discussion

All three species exhibited dose-dependent suppression of germination and early growth under salinity stress, though with varying tolerance thresholds, with quinoa exhibiting stronger salt tolerance, as evidenced by higher germination rates and biomass under high-salt conditions compared to *Brassica napus* and soybean (Table 2; Figure 1). This finding aligns with the research of Adolf et al. (2013)[4], suggesting that quinoa possesses unique adaptive mechanisms to salt stress. Additionally, salt stress led to increased malondialdehyde (MDA) content in plants (Figure 2), indicating aggravated membrane lipid peroxidation, which is closely associated with oxidative damage induced by salt stress [5].

Regarding osmotic adjustment substances, the free proline and soluble protein content in all three crops increased significantly under salt stress (Fig. 3). This suggests that plants accumulate these substances to maintain cellular water balance [6]. Notably, *Chenopodium quinoa* showed the highest proline content under mild-salt stress, implying that it may rapidly activate osmotic adjustment mechanisms in response to salt stress.

The antioxidant enzyme profiles revealed distinct defense strategies of the different crops (Fig. 4). The enzyme activities of *Brassica napus* and soybean fluctuated under salt stress, while *Chenopodium quinoa* exhibited a significant increase in CAT and APX activities with rising salt stress intensity, indicating a more efficient antioxidant defense system [7]. These results support the potential of *Chenopodium quinoa* as an ideal crop for saline-alkali soils.

5. Summary

Salt stress significantly inhibited crop growth. As salt stress intensity increased, seedling germination rates and biomass generally decreased. Compared to the control, soluble protein and free proline contents increased under salt stress. The antioxidant enzyme system showed an overall increasing trend with rising salt stress, indicating that crops enhance enzyme activity to counteract stress. These analyses demonstrate that *Chenopodium quinoa* exhibits stronger salt tolerance than *Brassica napus* and soybean.

Acknowledgments.

This work was supported by Open Research Fund Program of State Key Laboratory of Eco-hydraulics in Northwest Arid Region, Xi’an University of Technology,(Grant No.2023KFKT-7).

References

[1] Li, R., Shi, F., & Fukuda, K. Effects of salt stress on plant growth, antioxidant capacity, and photosynthetic characteristics. Journal of Arid Land, 2020, 12(1): 1-12.

[2] Munns, R., & Tester, M. Mechanisms of salinity tolerance. Annual Review of Plant Biology, 2008, 59: 651-681.

[3] Zhu, J. K. Abiotic stress signaling and responses in plants. Cell, 2016, 167(2): 313-324.

[4] Adolf, V. I., Jacobsen, S. E., & Shabala, S. Salt tolerance mechanisms in quinoa (Chenopodium quinoa Willd.). Environmental and Experimental Botany, 2013, 92, 43-54.

[5] Hasegawa, P. M., Bressan, R. A., Zhu, J. K., & Bohnert, H. J. (2000). Plant cellular and molecular responses to high salinity. Annual Review of Plant Biology, 2000, 51(1): 463-499..

[6] Ashraf, M., & Foolad, M. R. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany, 2007, 59(2): 206-216.

[7] Bose, J., Rodrigo-Moreno, A., & Shabala, S. ROS homeostasis in halophytes in the context of salinity stress tolerance. Journal of Experimental Botany, 2014, 65(5): 1241-1257.