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EXOGENOUS MELATONIN ENHANCES RADISH SEED GERMINATION UNDER SALT STRESS

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Abstract

This study investigated the effects of different NaCl concentrations (0–200 mmol L^{-1}) and 250 μmol L^{-1} melatonin treatments on radish seed germination under salt stress. Results demonstrated that salt stress adversely affected germination-related parameters in a concentration-dependent manner. Germination potential, rate, index, and vigor index decreased progressively with increasing NaCl, with severe reductions observed at 200 mmol L^{-1}. Fresh weight, dry weight, hypocotyl length, root length, and shoot length also declined significantly under high salt stress. In contrast, melatonin application mitigated salt-induced inhibition, particularly at moderate to high salt concentrations (100–200 mmol L^{-1}), though mild inhibition occurred at 50 mmol L^{-1}. Melatonin increased germination potential by 47%, germination rate by up to 117%, and vigor index by as much as 817% under 200 mmol L^{-1} salt stress, demonstrating a concentration-dependent promotion effect. Seedling biomass parameters such as fresh weight, dry weight, and organ lengths (hypocotyl, root, shoot) were significantly enhanced by melatonin under moderate to high salt conditions, while showing mild inhibition only at 50 mmol L^{-1}. These findings reveal the protective function of melatonin in preserving seed germination under salt stress.

Key words: Radish; Melatonin; Salt stress; Seed germination.

Introduction

Radish (*Raphanus sativus* L.) is an important root vegetable cultivated worldwide for its edible fleshy taproot, which is a biennial or annual herbaceous plant belonging to the Brassicaceae family (Xu *et al*., 2023). With a long cultivation history, radish holds significant value in both agricultural production and dietary culture (Kim *et al*., 2016). Nutritionally, radish is rich in minerals, dietary fiber, and vitamins (Knez *et al*., 2022). Given its high nutritional value and diverse functionalities, developing radish cultivars with enhanced stress tolerance, superior quality, and high yield is a key agricultural objective.

Soil salinization has emerged as a critical global environmental issue, severely impacting agricultural production and ecological systems (Chele *et al*., 2021). This phenomenon leads to progressive soil degradation and significantly inhibits plant growth, potentially causing root death and complete plant mortality under severe conditions (Zhao *et al*., 2021). Numerous studies have shown that salt stress suppresses seed germination in various plant species. Studies on *Sarcozygium xanthoxylon* showed that salt stress delays germination and reduces germination rate, germination potential, and germination index, with complete inhibition occurring at salt concentrations exceeding 20 g L^{-1} (Jia *et al*., 2023). For pumpkin seeds, both germination rate and germination potential decreased significantly under NaCl treatments compared to controls (Liu *et al*., 2024b). These findings collectively demonstrate the systemic nature of salt stress impacts on plants.

Melatonin, an indole heterocyclic compound secreted by the pineal gland, plays crucial regulatory roles in various plant physiological processes including seed germination, root development, flowering, and responses to biotic or abiotic stresses (Cheng *et al*., 2021; Mannino *et al*., 2021; Sun *et al*., 2021). Extensive research has demonstrated that melatonin-treated seeds exhibit significant advantages during germination by enhancing stress resistance, such as in *Cucumis sativus*, *Brassica oleracea*, *Oryza sativa*, and *Medicago sativa* (Huang 2021; Li 2022; Li *et al*., 2022; Jiang 2023; Liu *et al*., 2024a). Collectively, these findings confirm that appropriate melatonin application can effectively promote seed germination, with particularly pronounced effects under stress conditions.

However, most of these studies have focused on staple crops or leafy vegetables, while root vegetables such as radish remain largely underexplored, particularly regarding the interactive effects of melatonin and salt stress on seed germination. This study investigated the potential of melatonin to alleviate salt stress during radish seed germination, aiming to enhance germination performance and improve economic viability of radish cultivation under salt stress. The findings provide a scientific foundation for increasing yield and quality of radish grown in salt-affected soils, while offering practical solutions to mitigate the negative impacts of salinity stress on radish production.

Materials and Methods

Plant materials and reagents: The experiment utilized ‘Qingpicui’ radish seeds obtained from Jilin Hongyu Seed Co., Ltd. as plant materials. Melatonin (CAS No. 73-31-4)

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was purchased from Macklin Biochemical Co., Ltd. (Product No. M813985-500g), while other chemicals used were of analytical grade from domestic suppliers.

Experimental design and methodology: The experimental setup consisted of five treatment groups, each containing 30 seeds of 'Qingpicui' radish with three biological replicates. The experimental procedure comprised two sequential phases: initial screening to determine the effects of different NaCl concentrations on seed germination, subsequent evaluation of combined salt stress and 250 μmol L^{-1} melatonin treatments. Germination parameters were assessed following previously published methodologies (Huang 2021; Li 2022). All incubations were conducted under a 16/8 h light/dark photoperiod with 65 ± 5% relative humidity.

Experimental procedures: Prior to the experiment, uniform and healthy 'Qingpicui' radish seeds were selected and surface-sterilized with 5% sodium hypochlorite for 10 min, followed by thorough rinsing with sterile distilled water and air-drying under aseptic conditions. Sterilized seeds were sown on filter paper in petri dishes and subjected to NaCl solutions at concentrations of 0, 50, 100, 150, and 200 mmol L^{-1}. All dishes were labeled and incubated at 25°C for germination observation. Subsequently, seeds were treated with a combination of 250 μmol L^{-1} melatonin used in previous studies (Cao *et al*., 2025; Lang *et al*., 2025) and the same NaCl concentrations following identical procedures to evaluate the potential mitigating effects of melatonin on salt stress during germination. All experiments were conducted three biological replicates.

Statistical analysis: Statistical analysis was performed with SPSS software (v19.0). One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was employed to determine significant differences among treatments (p<0.05). Data visualization and organization were performed using Microsoft Office 2021. Data are presented as mean ± SE (n = 3).

Results

Effects of different salt stress concentrations on radish seed germination: The germination potential of radish seeds displayed a significant downward trend as salt concentration increased, with the 50 mmol L^{-1} treatment being the exception (Fig. 1A). At 50 mmol L^{-1}, germination potential decreased by 5% relative to the control; at 100 and 150 mmol L^{-1}, it dropped by 30% and 54%, respectively, with the 150 mmol L^{-1} group showing a germination potential just one-quarter of the control value. Under varying salt stress conditions, the germination rate exhibited a progressive decline (Fig. 1B). Mild reductions of 6% and 9% occurred at 50 and 100 mmol L^{-1}, respectively, while a more pronounced 25% decrease was observed at 150 mmol L^{-1}. Exposure to the highest salt concentration (200 mmol L^{-1}) resulted in a germination rate that was 40% lower than the control, representing a 57% reduction.

Salt stress also induced a gradual decline in the germination index (Fig. 1C), with a 7% reduction recorded at 50 mmol L^{-1}, a significant 30% decrease at 100 mmol L^{-1}, and sharp declines of 57% and 82% at 150 and 200 mmol L^{-1}, respectively; the lowest value occurred at 200 mmol L^{-1}. The vigor index of radish seeds decreased progressively with increasing NaCl concentration (Fig. 1D), showing a minor 13% decrease at 50 mmol L^{-1}, substantial declines of 36% and 67% at 100–150 mmol L^{-1}, and a drastic reduction to 77.43 at 200 mmol L^{-1}. This latter value represents a 92% decrease and only one-tenth of the control group's vigor index. Collectively, these results highlight the escalating inhibitory effects of salt stress on multiple germination-related parameters, with the most severe reductions observed at higher NaCl concentrations.

Fresh weight exhibited a continuous downward trend as NaCl concentration increased (Fig. 1E), with an 8% reduction observed at 50 mmol L^{-1}; this was followed by progressively greater decreases of 39%, 59%, and 79% at 100–200 mmol L^{-1}, respectively. The 200 mmol L^{-1} treatment group recorded a fresh weight just one-fifth of the control value. Dry weight decreased steadily with rising salt concentration (Fig. 1F), demonstrating mild reductions of 12% and 17% at 50–100 mmol L^{-1} and more substantial decreases of 31% and 53% at 150–200 mmol L^{-1}.

Hypocotyl length shortened significantly with increasing salt levels (Fig. 1G), showing a 12% decrease at 50 mmol L^{-1}, drastic reductions of 62% and 88% at 100 and 150 mmol L^{-1}, and complete inhibition at 200 mmol L^{-1}. Root length experienced a moderate 25% decrease at 50 mmol L^{-1}, followed by sharp declines of 63% and 80% at 100–150 mmol L^{-1} (Fig. 1H). Shoot length reached its maximum in the control group, decreased by a non-significant 22% at 50 mmol L^{-1}, and exhibited significant declines of 63%, 82%, and 94% at 100–200 mmol L^{-1} (Fig. 1I). These results collectively indicate that elevated NaCl concentrations progressively impair seed generation, with high-salt conditions causing severe morphological deficits.

Effects of melatonin on radish seed germination under salt stress: Based on previous studies showing that 250 μmol L^{-1} melatonin significantly promotes seed germination in horticultural crops (Cao *et al*., 2025; Lang *et al*., 2025), this concentration was used in the experiment. Melatonin significantly improved the germination potential of radish seeds under salt stress conditions compared to the single salt stress group, with the magnitude of increase escalating with higher salt concentrations (Fig. 2A). Specifically, a moderate 14% increase was observed under 50 mmol L^{-1} salt stress, while notable increases of 83%, 238%, and 47% were recorded under 100–200 mmol L^{-1} salt stress, demonstrating a concentration-dependent promotion effect. The promotive effect of melatonin on germination increased in a salt-concentration-dependent manner, with moderate improvements (2-19%) at 50-150 mmol L^{-1} NaCl and a peak enhancement of 117% at 200 mmol L^{-1} NaCl compared to the untreated control, indicating that melatonin effectively mitigates the inhibitory impact of high-concentration salt stress on germination rate (Fig. 2B).

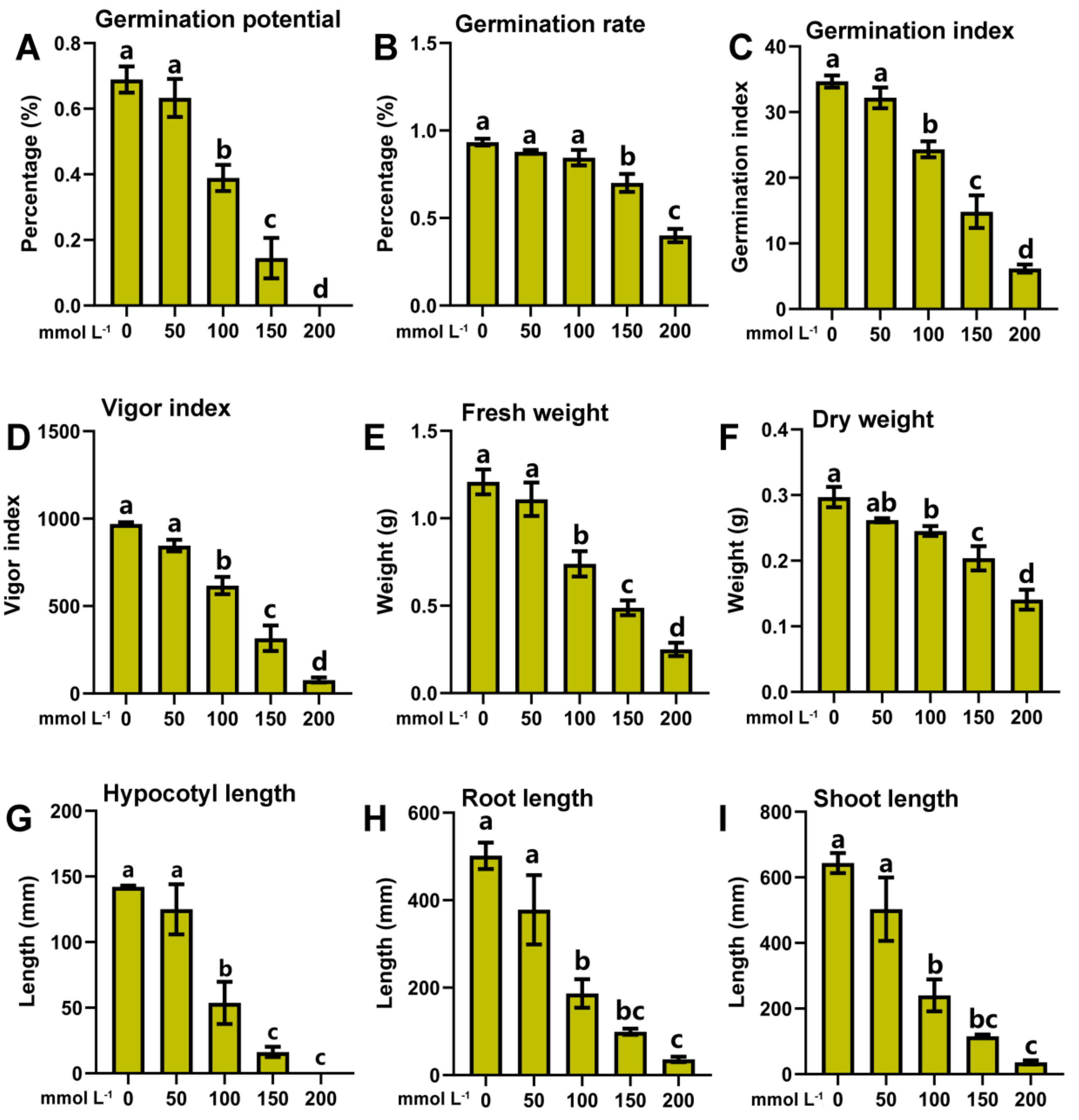

Fig. 1. Concentration-dependent effects of NaCl on radish seed germination and seedling growth parameters. Data show (A) germination potential, (B) germination rate, (C) germination index, (D) vigor index, (E) fresh weight, (F) dry weight, (G) hypocotyl length, (H) root length, and (I) shoot length. Values represent mean ± SE (n=3). Different lowercase letters indicate significant differences (p<0.05, Duncan's test).

The germination index displayed a consistent upward trend with melatonin application, showing increases of 9%, 44%, and 83% under 50–150 mmol L^{-1} salt stress, and a remarkable 338% increase under 200 mmol L^{-1} salt stress relative to the group subjected to salt stress alone (Fig. 2C). This finding reveals a positive correlation between salt stress severity and the promotive effect of melatonin on germination. Regarding the vigor index, melatonin treatment induced a pronounced upward trend compared to single salt stress, with increases of 55%, 113%, and 817% observed under 100–200 mmol L^{-1} salt stress, particularly a dramatic surge under 200 mmol L^{-1} (Fig. 2D). These results highlight the robust enhancement of seed vigor by melatonin under high salt stress, underscoring its protective role in maintaining seed germination performance under salt stress.

Melatonin treatment exhibited differential effects on fresh weight depending on salt concentration. Under mild salt stress (50 mmol L^{-1}), a slight reduction of 0.1 g was observed. However, at higher salt concentrations (100–200 mmol L^{-1}), melatonin application significantly increased fresh weight by 0.22 g, 0.29 g, and 0.45 g compared to the single salt stress group, demonstrating its beneficial role in mitigating moderate to severe salt stress (Fig. 2E). For dry weight, a trend of initial decrease followed by increase was observed with rising salt concentration: melatonin-treated seeds exhibited 21%, 27%, and 34% increases under 50–150 mmol L^{-1} salt stress, and a twofold increase under 200 mmol L^{-1} when contrasted with single salt stress, reflecting progressively stronger promotion as salt stress intensified (Fig. 2F).

Melatonin significantly increased hypocotyl length across salt concentrations: a 20.33 mm elongation was recorded under 50 mmol L^{-1} salt stress, while under 100–150 mmol L^{-1}, hypocotyl length increased by 122% and 306% relative to the single salt stress group (Fig. 2G). Root length displayed a stress-dependent response: a decrease was observed under 50 mmol L^{-1}, but marked increases of 60%, 85%, and 316% were evident under 100–200 mmol L^{-1} salt stress, with the most pronounced promotion occurring under high salt conditions (Fig. 2H). Shoot length displayed contrasting responses to salt stress depending on concentration. While a modest 9 mm decrease occurred at 50 mmol L^{-1} salt stress, significant growth stimulation was observed at higher concentrations, with increases of 74%, 116%, and 464% compared to the single salt treatment. These results demonstrate melatonin's remarkably enhanced protective effects under moderate to high salt stress conditions (Fig. 2I). These findings collectively indicate that melatonin modulates seed generation, preferentially promoting growth under moderate to severe salt stress while mitigating minor inhibitory effects at lower salt concentrations.

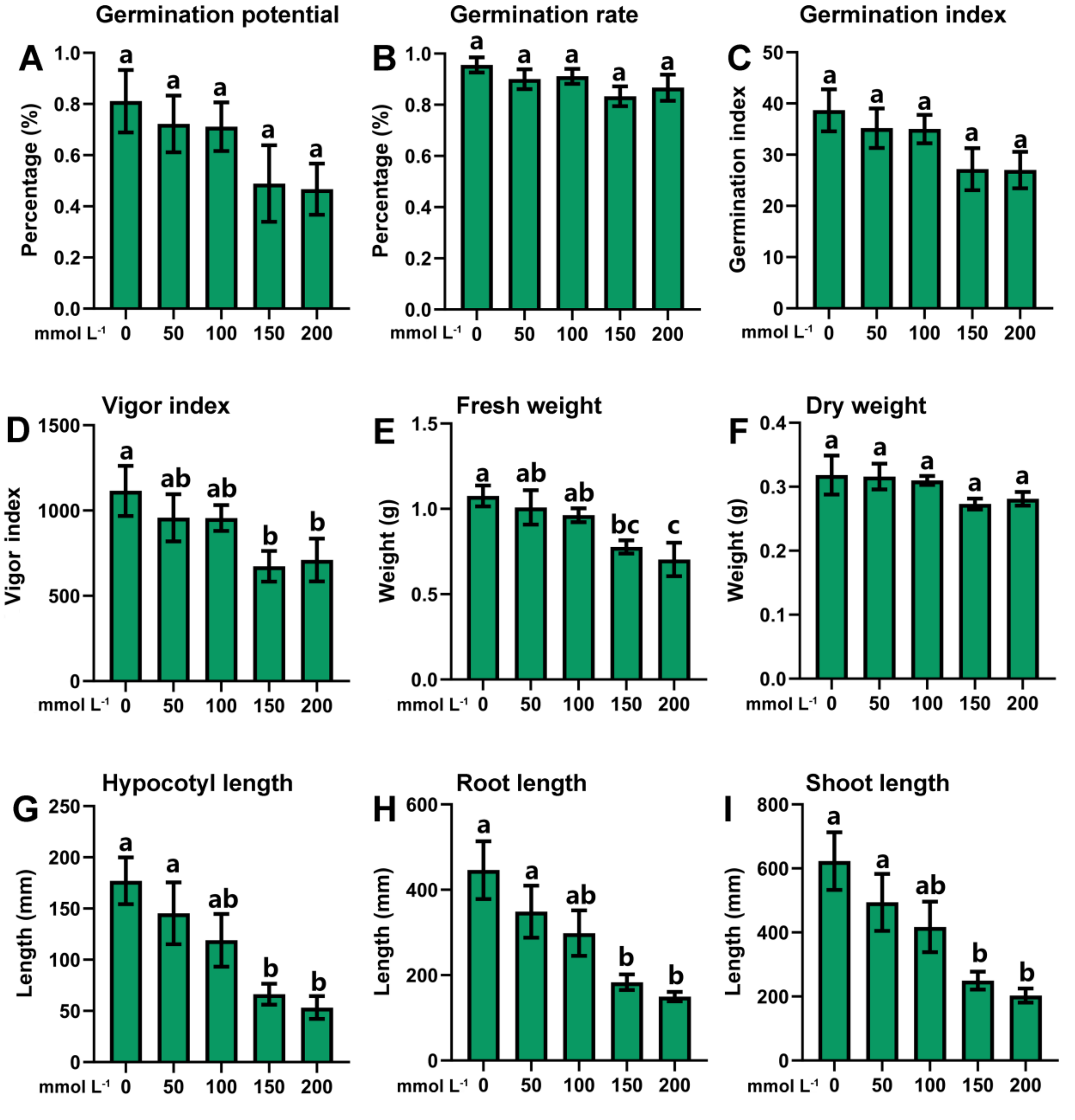

Fig. 2. Effects of melatonin on radish seed germination under salt stress. Data show (A) germination potential, (B) germination rate, (C) germination index, (D) vigor index, (E) fresh weight, (F) dry weight, (G) hypocotyl length, (H) root length, and (I) shoot length. Values represent mean ± SE (n=3). Different lowercase letters indicate significant differences (p<0.05, Duncan's test).

Discussion

This study showed that different salt concentrations significantly affected the germination of radish seeds. At salt concentrations ranging from 100 to 200 mmol L^{-1}, all measured indices of radish seeds showed significant reductions, and the most severe inhibition occurred at 200 mmol L^{-1}, where the germination rate decreased by 57 percent relative to the control group. In contrast, seed germination at 50 mmol L^{-1} showed only a slight decrease but overall performed better than high-salt treatments. Studies have shown that melon seeds exhibited a reduction in germination rate under salt stress (Xie *et al*., 2023), which is consistent with the results of this experiment. Similar trends were observed in asparagus seeds (Cao *et al*., 2012), with overall inhibitory effects intensifying with increasing salt concentration. Collectively, radish seed germination was progressively inhibited by higher salt concentrations.

Unlike cereal or leafy vegetable crops, radish undergoes concurrent shoot and taproot primordium development during early germination, which may increase its sensitivity to salt stress (Sun *et al*., 2016), yet radish-specific studies on melatonin's protective effects have been notably lacking until our findings. Our results showed that melatonin treatment significantly promoted radish seed germination under salt stress, consistent with findings that exogenous melatonin enhances germination rate in *Sorghum bicolor* (Zou *et al*., 2024). Under salt stress, melatonin application to tartary buckwheat seeds enhanced vigor index, fresh weight, root length, dry weight, and antioxidant enzyme activity, reducing damage (Shi *et al*., 2021). Consistent with these findings, this experiment further confirms the promoting effect of melatonin on seed germination. Similarly, exogenous melatonin also alleviated salt stress during germination in other Brassicaceae crops, such as cabbage (An *et al*., 2025) and rapeseed (Zeng *et al*., 2018). These results indicate that melatonin exerts significant positive effects on growth indices and stress resistance of plants under salt stress.

Further studies are needed to elucidate the molecular pathways through which melatonin enhances salt tolerance in radish. It should be noted that only one melatonin concentration (250 µmol L^{-1}) was tested in this study, which was selected based on documented efficacy in other horticultural crops (Cao *et al*., 2025; Lang *et al*., 2025), while our results confirm its promotive effect in radish, future studies examining a gradient of melatonin concentrations are needed to determine the optimal concentration and establish comprehensive dose-response relationships under salt stress. Furthermore, given that radish is cultivated for its fleshy taproot, future research should extend observations beyond germination to later developmental stages, including taproot formation, biomass accumulation at harvest, and yield-related traits, to better assess the practical agricultural value of melatonin application under salt stress.

Conclusions

Salt stress negatively impacts radish seed germination, with inhibitory effects intensifying as NaCl concentration increases. High salt stress ($\geq$ 100 mmol L^{-1}) severely reduces germination-related indices and seedling biomass, whereas low salt stress (50 mmol L^{-1}) causes milder inhibition. Melatonin effectively alleviates salt-induced stress, particularly at moderate to high salt concentrations, by promoting germination potential, rate, index, and vigor index in a concentration-dependent manner. Additionally, melatonin enhances seedling fresh and dry weight and promotes hypocotyl, root, and shoot growth under moderate to high salt stress, while mitigating minor inhibitory effects at lower salt levels. These results suggest that melatonin plays a crucial role in modulating seed germination, offering a potential approach to improve radish tolerance to salt stress.

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References

An, T.H., N.J. Methela, M.S. Islam, D.S. Lee, M.W. Msarie, Y.J. Woo, B.G. Mun, M.S. Lee and B.W. Yun. 2025. Mitigating salt stress in cabbage: synergistic effects of nitric oxide donor GSNO and melatonin. *Plant Biotechnol. Rep.*, 19(5): 465-479.

Cao, W., N. Wang, J. Qin, S. Lu, Y. Wang and H. Mou. 2025. Effects of exogenous melatonin on growth and physiological characteristics of *Xanthoceras sorbifolium* Bunge seedlings under flooding stress. *Jiangsu Agric. Sci*., 53(1): 209-217.

Cao, Y., P. Dai, J. Tian and S. Dai. 2012. Effect of salt stress on seed germination of asparagus. *Hebei Agric. Sci*., 2012(4): 39-41.

Chele, K.H., M.M. Tinte, L.A. Piater, I.A. Dubery and F. Tugizimana. 2021. Soil salinity, a serious environmental issue and plant responses: a metabolomics perspective. *Metabolites*, 11(11): 724.

Cheng, G., T. Ma, Z. Deng, G. Gutiérrez-Gamboa, Q. Ge, P. Xu, Q. Zhang, J. Zhang, J. Meng, R.J. Reiter, Y. Fang and X. Sun. 2021. Plant-derived melatonin from food: A gift of nature. *Food Funct*., 12(7): 2829-2849.

Huang, F. 2021. Molecular mechanism of melatonin in promoting rice seed germination under salt stress, and in regulating leaf senescence and yield related traits. Dissertation, Yangzhou University.

Jia, X., Y. Yang, G. Cao, Y. Xing, R. Qiao, Y. Li and Y. Gao. 2023. Effect of salt stress on seed generation of *Sarcozygium xanthoxylon*. *J. Inner Mongolia Forest. Sci. Technol*., 49(4): 21-25.

Jiang, J. 2023. Effect of exogenous melatonin on seed germination and seedling growth of cauliflower under low temperature stress. Dissertation, Shandong Agricultural University.

Kim, N., Y.M. Jeong, S. Jeong, G.B. Kim, S. Baek, Y.E. Kwon, A. Cho, S.B. Choi, J. Kim, W.J. Lim, K.H. Kim, W. Park, J.Y. Kim, J.H. Kim, B. Yim, Y.J. Lee, B.M. Chun, Y.P. Lee, B.S. Park, H.J. Yu and J.H. Mun. 2016. Identification of candidate domestication regions in the radish genome based on high-depth resequencing analysis of 17 genotypes. *Theor. Appl. Genet*., 129(9): 1797-1814.

Knez, E., K. Kadac-Czapska, K. Dmochowska-Ślęzak and M. Grembecka. 2022. Root vegetables-composition, health effects, and contaminants. *Int. J. Environ. Res. Public Health*, 19(23): 15531.

Lang, H., H. Liu and M.L. Jiang. 2025. Effects of melatonin treatment on seed germination of leaf chicory (*Cichorium intybus*) under salt stress. *Bangladesh J. Bot*., 54(2): 245-252.

Li, L. 2022. Effects of exogenous melatonin on seed germination and seeding growth of alfalfa under salt stress. Dissertation, Xinjiang Agricultural University.

Li, P., S. Xie, L. Fang, H. Luo and G. Lin. 2022. Rsearch on the impacts of melatonin at different concentrations on the germination of foxtail millet seeds under drought-stress conditions. *J. Shanxi Agric. Uni*., 42(5): 76-83.

Liu, S., H. Li, Y. Zhang, H. Sun, J. Shen and Q. Zhu. 2024a. Effects of exogenous MT on seed germination and seedling growth of *Cucumis sativus* under paclobutrazol stress. *Seed*, 43(3): 110-117.

Liu, X., S. Wang, H. Zhang and J. Zhang. 2024b. Effect of anti-salt agent on pumpkin seed germination and seedling growth under salt stress. *J. Fujian Agric. Forest. Uni*., 53(5): 577-586.

Mannino, G., C. Pernici, G. Serio, C. Gentile and C.M. Bertea. 2021. Melatonin and phytomelatonin: chemistry, biosynthesis, metabolism, distribution and bioactivity in plants and animals—an overview. *Int. J. Mol. Sci*., 22(18): 9996.

Shi, T., L. Zhu, H. Wang, J. Huang and Q. Chen. 2021. Effects of melatonin on germination and seedling growth of tartary buckwheat under salt stress. *Hubei Agric. Sci*., 2021(15): 25-28.

Sun, C., L. Liu, L. Wang, B. Li, C. Jin and X. Lin. 2021. Melatonin: a master regulator of plant development and stress responses. *J. Integr. Plant Biol*., 63(1): 126-145.

Sun, X.C., L. Xu, Y. Wang, X.B. Luo, X.W. Zhu, K.B. Kinuthia, S.S. Nie, H.Y. Feng, C. Li and L.W. Liu. 2016. Transcriptome-based gene expression profiling identifies differentially expressed genes critical for salt stress response in radish (*Raphanus sativus* L.). *Plant Cell Rep*., 35(2): 329-346.

Xie, Z., M. Wusiman, Z. Qin and Z. Fang. 2023. Effects of salt stress on seed germination and physiological characteristics of four melon species. *Jiangsu Agric. Sci*., 51(11): 146-152.

Xu, L., Y. Wang, J. Dong, W. Zhang, M. Tang, W. Zhang, K. Wang, Y. Chen, X. Zhang, Q. He, X. Zhang, K. Wang, L. Wang, Y. Ma, K. Xia and L. Liu. 2023. A chromosome-level genome assembly of radish (*Raphanus sativus* L.) reveals insights into genome adaptation and differential bolting regulation. *Plant Biotechnol. J*., 21(5): 990-1004.

Zeng, L., J.S. Cai, J.J. Li, G.Y. Lu, C.S. Li, G.P. Fu, X.K. Zhang, H.Q. Ma, Q.Y. Liu, X.L. Zou and Y. Cheng. 2018. Exogenous application of a low concentration of melatonin enhances salt tolerance in rapeseed (*Brassica napus* L.) seedlings. *J. Integr. Agr*., 17(2): 328-335.

Zhao, S., Q. Zhang, M. Liu, H. Zhou, C. Ma and P. Wang. 2021. Regulation of plant responses to salt stress. *Int. J. Mol. Sci*., 22(9): 4609.

Zou, X., Y. Zhou, G. Li, J. Wang, Q. Bu, J. Wang, L. Yang and F. Zhang. 2024. Study on the regulatory effect of exogenous melatonin on the physiological metabolism of sorghum under drought stress. *J. Shanxi Agric. Uni*., 44(4): 24-31.