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EFFECTS OF MELATONIN TREATMENT ON SEED GERMINATION OF LEAF CHICORY (*CICHORIUM INTYBUS*) UNDER SALT STRESS

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Abstract

This study was aimed to investigate the effects of salt stress and melatonin on the seed germination of leaf chicory. Salt stress significantly reduced various seed germination indexes, while melatonin treatment had a positive effect on seed germination. 250 µmol/l concentration of melatonin was identified as the optimal concentration for improving seed germination. Mixed treatment with melatonin significantly improved all seed germination indexes compared to separate salt stress. These findings suggest that melatonin alleviates the inhibitory effects of salt stress on seed germination and enhances the salt tolerance in leaf chicory.

Introduction

Leaf chicory (*Cichorium intybus*), belongs to Asteraceae family, is a distinctive vegetable crop native to Europe, Central Asia, and northern Africa and is now widely cultivated globally (Street *et al.* 2013). Leaf chicory, valued for its rich nutritional value, bright color, and mildly bitter taste, is recognized as both a culinary and medicinal crop (Barcaccia *et al.* 2016). It contains essential nutrients such as protein, fat, sugar, calcium, phosphorus, and iron and is recognized for its benefits, including liver regulation, promotion of gastrointestinal digestion, diuretic effects, and detoxification (Migliorini *et al.* 2019). Salt stress significantly impacts plant growth and development, inducing physiological drought in crops and affecting water absorption and seed germination. This leads to osmotic damage, ion toxicity, and nutrient deficiencies, all of which negatively affect seed germination, a critical phase that determines crop quality and growth potential. Research has shown that seeds are particularly sensitive to salt stress during the germination stage (Nasrallah *et al.* 2022). Higher salt concentrations suppressed germination rate, germination index, root length, sprout length, and vitality index in spinach (Yuan *et al.* 2023). Seed germination and seedling growth in *Dianthus chinensis* were also inhibited under higher salt stress (Huang *et al.* 2020). However, studies on the seed germination of leaf chicory under salt stress remain limited.

Melatonin plays a vital role in regulating plant growth and physiological processes and alleviating various environmental stresses, including salt stress in alfalfa (Li *et al.* 2024). It enhance photosynthetic performance, promotes growth and development, supports seed germination, and increases stress tolerance in plants (Cheng *et al.* 2023, Wang *et al.* 2023). Melatonin mitigates the effects of salt stress on carrot seedlings, promoting their growth and development (Peng *et al.* 2024). Melatonin improves the germination rate, germination potential, germination index, and vitality index of wheat seeds (Su *et al.* 2023). Similarly, in soybean seedlings subjected to salt stress, exogenous melatonin treatment significantly increased plant height, leaf area, total root length, and dry weight (Shen *et al.* 2024). Despite significant progress in understanding the role of melatonin in improving plant growth and development under salt stress, its effects on seed germination in leaf chicory under salt stress remain unexplored.

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This study aims to investigate the effects of melatonin on seed germination in leaf chicory under salt stress, to mitigate the inhibitory effects of salt stress. This research also aims to elucidate the role of melatonin in the salt stress response of leaf chicory, providing theoretical support for understanding the mechanisms by which melatonin enhances seed germination under salt stress and offering reference and guidance for future studies on salt tolerance in other plant species.

Materials and Methods

Leaf chicory inbred line 'HJ26' was obtained from the Vegetable Research Group, College of Agronomy, Jilin Agricultural Science and Technology University. Sodium chloride (NaCl), melatonin, and sodium hypochlorite (NaClO) were purchased from Shanghai Macklin Biochemical Co., Ltd. (China).

Uniform sized and healthy leaf chicory seeds were sterilized with 5% NaClO. The sterilized seeds were placed in Petri dishes lined with filter paper, with 30 seeds per dish. Different concentrations of NaCl solutions (0, 50, 100, 150, and 200 mmol/l) and melatonin solutions (0, 250, 500, 750, and 1000 µmol/l) were applied to each dish, followed by incubation at 27°C for germination. The optimal melatonin concentration was selected based on individual treatment results for the combined melatonin and salt treatments. Each treatment was conducted in triplicate.

The experimental parameters included germination potential, germination rate, germination index, vitality index, fresh weight, dry weight, and sprout length. Germinated seeds were counted every 24 hours, and germination potential and rate were recorded on days 3 and 7, respectively. On day 7, the fresh weight, dry weight, and sprout length were measured. Determination of dry weight was carried out using air drying method. Germination potential, germination rate, germination index, and vitality index were calculated as described by Liang *et al.* (2016).

Statistical analysis was performed using SPSS v26. Significant differences ($p < 0.05$) were determined by one-way ANOVA and Duncan's multiple range test, with different letters representing significant differences. Data were presented as mean ± standard deviation (SD) for three biological replicates.

Results and Discussion

The germination potential of 'HJ26' showed a progressive decline with increasing salt concentrations (Fig. 1A). At 100-200 mmol/l NaCl, a significant reduction in germination potential was observed ($p < 0.05$), with a sharp decrease compared to the control group. Under 50 mmol/l NaCl, the reduction in germination potential was moderate. The lowest germination potential was recorded at 200 mmol/L NaCl. The germination rate remained stable under 50 mmol/l NaCl but decreased significantly at 100 mmol/l and higher concentrations (Fig. 1B). As salt concentration increased, the germination rate declined, with significant reductions observed between 100 and 200 mmol/l compared to the control.

Salt stress had a significant inhibitory effect on the germination index; as salt concentration increased the germination index decreased, with the lowest value recorded at 200 mmol/l (Fig. 1C). Similarly, the vitality index declined with increasing salt concentrations, with the most significant decrease observed at 200 mmol/L (Fig. 1D).

Fresh sprout weight showed an initial increase at 50 mmol/l NaCl compared to the control but declined at higher concentrations (Fig. 1E). Dry weight followed a similar trend, with a slight increase at 50 mmol/l, a moderate decrease at 100 and 150 mmol/l, and a more significant decrease at 200 mmol/l (Fig. 1F). Sprout length exhibited a gradual reduction with increasing salt concentrations, with a significant decline observed at 200 mmol/l (Fig. 1G). Overall, all the seed germination indices of leaf chicory were significantly affected by salt stress, showing a consistent decrease as salt concentration increased (Fig. 1H).

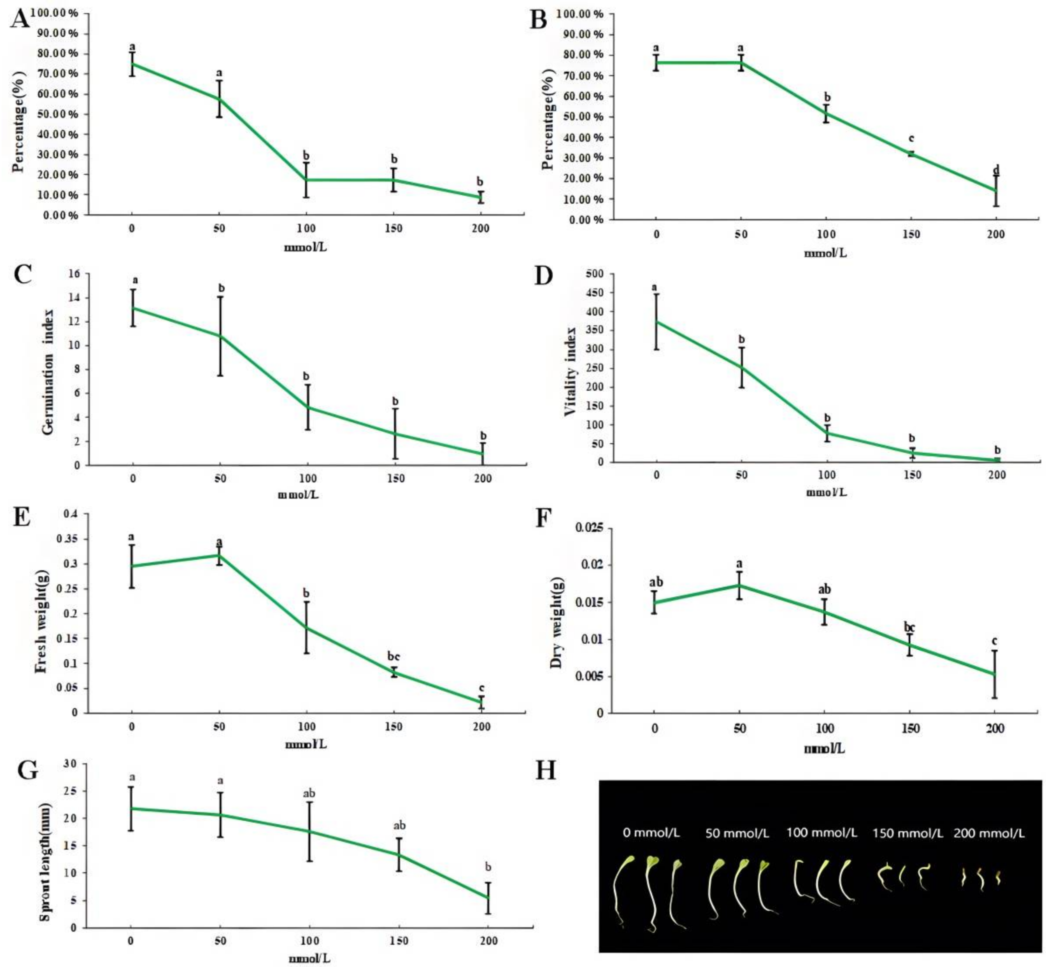

Fig. 1. Effects of salt stress on the germination of leaf chicory seeds. A: Germination potential, B: Germination rate, C: Germination index, D: Vitality index, E: Fresh weight, F: Dry weight, G: Sprout length and H: Germination phenotypes of leaf chicory under salt stress. Black letters on the top of bar charts indicated significant differences between different concentrations (one-way ANOVA, $p < 0.05$).

Low concentrations of melatonin improved germination potential, with the highest value at 250 µmol/L compared to the control group, followed by a gradual decline. No significant differences were observed between the experimental and control groups across all concentrations (Fig. 2A). Although a slight increase in germination potential was observed at 1000 µmol/l, it was not significantly different from the control ($p < 0.05$). The lowest germination potential was recorded at 750 µmol/l. The germination rate followed a similar trend, maximum at 250 µmol/l and slightly decreasing thereafter, with no significant differences between the experimental groups and the control (Fig. 2B).

As melatonin concentration increased, the germination index initially increased, maximum at 250 µmol/l (Fig. 2C). The lowest germination index was observed at 750 µmol/L. The vitality index showed minimal fluctuation at low melatonin concentrations, with a significant decrease only at 750 µmol/l (p < 0.05), indicating that high-concentration melatonin had a negative effect on vitality (Fig. 2D).

No significant differences in fresh and dry sprout weights were observed across melatonin concentrations (Figs 2E and 2F). A slight increase in both fresh and dry weight was observed at 250 µmol/L compared to the control group, followed by a slight decline, suggesting minimal impact of melatonin on sprout weight. Sprout length increased significantly at 250 µmol/l, with the shortest sprout length recorded at 500 µmol/l and the longest at 250 µmol/l (Fig. 2G). Based on phenotypic analysis, 250 µmol/l melatonin was identified as the optimal concentration for enhancing all germination indices of leaf chicory and was selected for mixed treatments with salt (Fig. 2H).

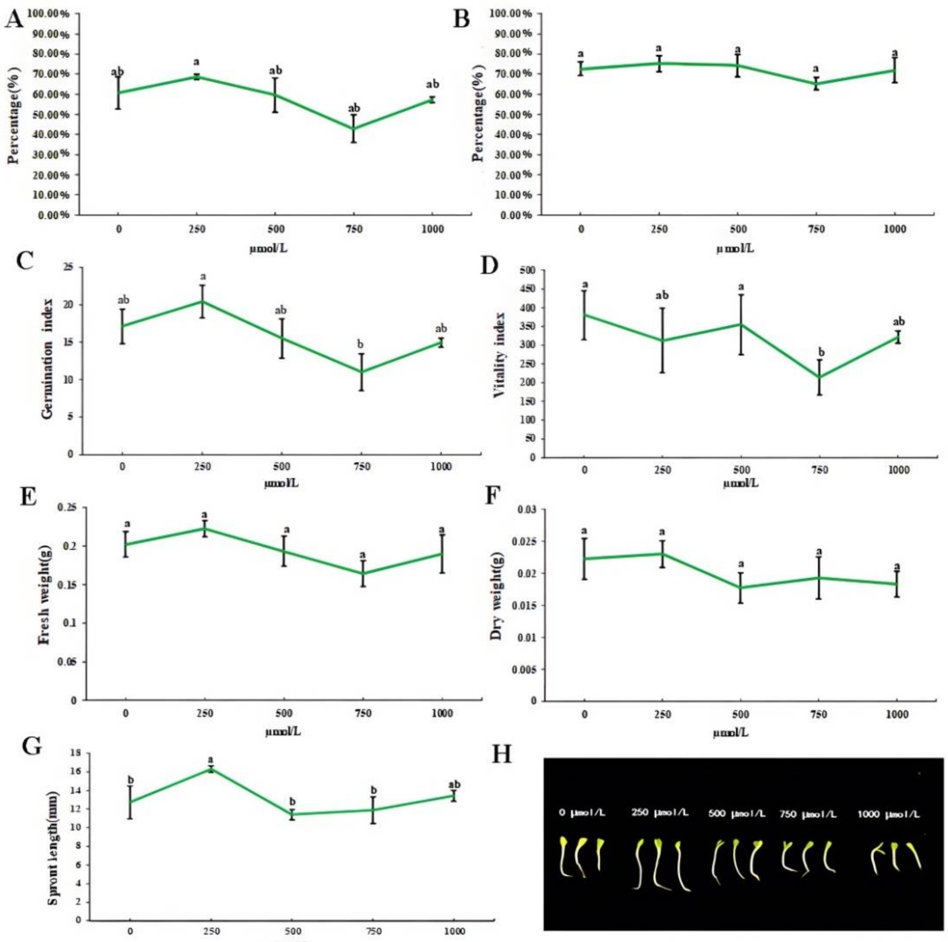

Fig. 2. Effects of melatonin on the germination of leaf chicory seeds. A: Germination potential, B: Germination rate, C: Germination index, D: Vitality index, E: Fresh weight, F: Dry weight, G: Sprout length and H: Germination phenotypes of leaf chicory under melatonin treatment.

Under salt stress treatments, the germination potential showed a general decline, remaining significantly lower than in the control group ($p < 0.05$). However, compared to salt stress alone, adding 250 µmol/l melatonin improved the germination potential under all salt stress conditions. At 200 mmol/l salt concentration, the germination potential increased by approximately 26%. At 100 and 150 mmol/l salt stress, germination potential increased by 20 and 16%, respectively (Fig. 3A). Melatonin increased germination rates by approximately 12, 28, and 55% under 100, 150, and 200 mmol/L salt stress, respectively, compared to the salt stress alone treatment (Fig. 3B).

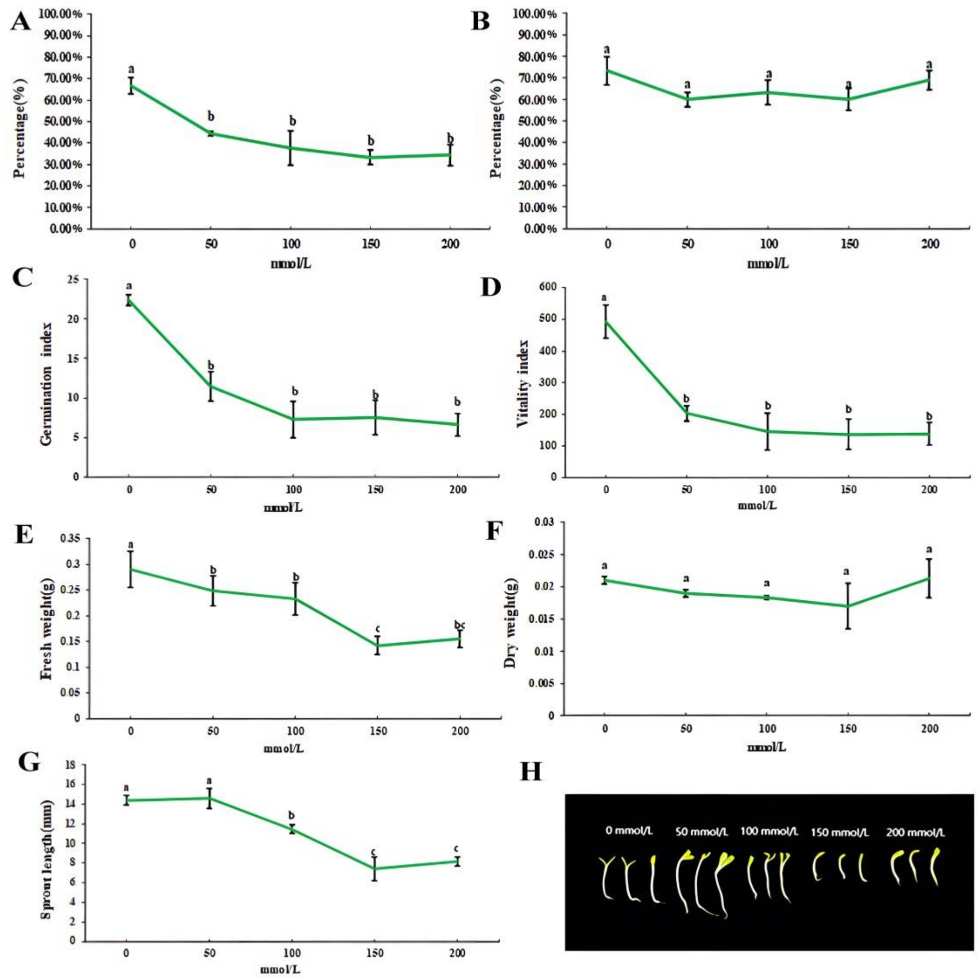

Fig. 3. Effects of melatonin on the germination of leaf chicory seeds under salt stress. A: Germination potential, B: Germination rate, C: Germination index, D: Vitality index, E: Fresh weight, F: Dry weight, G: Sprout length, and G: Germination phenotypes of leaf chicory treated with melatonin under salt stress.

The germination index decreased with increasing salt concentration. However, melatonin improved the germination index across all salt concentrations by approximately 0.1, 0.5, 1.9, and 6 times compared to salt stress alone, with the greatest improvement observed at 200 mmol/l salt stress (Fig. 3C). The vitality index followed a similar pattern, decreasing significantly with increasing salt concentration. However, adding melatonin (250 µmol/L) significantly increased the vitality index at 100, 150, and 200 mmol/l salt stress by 0.9, 4.4, and 20.2 times, respectively, compared to salt stress alone (Fig. 3D). Melatonin effectively alleviated the inhibitory effect of salt stress on fresh weight compared with the salt stress alone treatment; under 100, 150 and 200 mmol/l salt concentration, fresh weight increased about 0.4, 0.7 and 6 times, respectively (Fig. 3E). Melatonin treatment significantly improved the dry weight of leaf chicory under salt stress; compared with salt stress alone treatment, the dry weight increased about 1, 0.3, 0.8 and 3 times, respectively (Fig. 3F), suggesting that the melatonin effectively increased dry weight.

The sprout length under 50 mmol/l salt stress was comparable to the control, but at higher salt concentrations, sprout length decreased significantly. Compared to salt stress alone, melatonin significantly improved sprout length (Fig. 3G). Overall, melatonin treatment significantly improved all germination indices of leaf chicory under salt stress, indicating that melatonin has positive effects on seed germination under salt-stress conditions.

Studying the effect of melatonin on regulating the germination of chicory seeds under salt stress is of great practical significance. This will help stabilize the germination and growth of chicory seeds under salt stress and lay the foundation for future research on the mechanism of melatonin regulating seed germination under salt stress. Salt stress significantly inhibited seed germination in leaf chicory. Except for the fresh and dry weight of sprouts under 50 mmol/l salt concentration, all growth parameters were significantly reduced. The inhibitory effect was most pronounced at 100, 150 and 200 mmol/l salt concentrations. The germination rate under 200 mmol/l salt stress was reduced to 20%, compared to 75% in the control group, highlighting the strong inhibitory effect of high salt concentrations. A study on Chinese cabbage varieties reported germination rates below 50% at 0.4 mmol/l salt concentration and 0% at 0.8 mmol/l (Wu *et al.* 2021). Similar result was also observed by Fu and Zhang (2017) in lettuce. These results align with the present study, where seed germination inhibition increased with increasing salt concentrations in leaf chicory.

In this study, low concentrations of melatonin positively affected multiple germination parameters in leaf chicory, with 250 µmol/l delivering optimal results. This is consistent with previous research on rice and *Toona sinensis*, which demonstrated similar enhancements in seed germination following melatonin treatment (Zhang *et al.* 2014, Liu *et al.* 2020). Melatonin treatment improve the germination rate and potential of cotton seeds under drought conditions while also promote cucumber seed growth and increase dry weight at low temperatures (Posmyk *et al.* 2009, Bai *et al.* 2020). However, higher melatonin concentrations suppressed or caused fluctuations in germination parameters, likely due to the inhibitory effects of excessive hormone levels on seed germination.

The seed germination parameters of leaf chicory treated with exogenous melatonin under salt stress significantly improved compared to those under salt stress alone. Previous studies demonstrated that salt stress reduced the physiological indexes of sunflower seeds, but melatonin treatment significantly increased germination rate and germination potential, compared to the control group (Li and Li 2024). This finding aligns with the present study, further demonstrating that melatonin promote seed germination. In subsequent research, clarifying the gene regulatory network of melatonin promoting the germination of leaf chicory seeds, identifying key regulatory genes, and clarifying the mechanism of action will be the focus of exploration. The findings of this study indicate that melatonin positively impacts seed germination under salt stress, highlighting its potential to mitigate the inhibitory effects of salt stress on seed germination.

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