

## Evaluating seed germination and early seedling growth of wild medicinal plants for saline soil cultivation

Mewuleddeg ZEBRO, Jae-Yun HEO\*

Gangneung-Wonju National University, Department of Plant Science, 25457, Gangneung,  
Republic of Korea; [zmewuleddeg@gwnu.ac.kr](mailto:zmewuleddeg@gwnu.ac.kr); [jyheo@gwnu.ac.kr](mailto:jyheo@gwnu.ac.kr) (\*corresponding author)

### Abstract

The impact of soil salinity on various wild plant species, including *Peucedanum japonicum*, *Astragalus membranaceus*, *Lepidium sativum*, *Acyranthes bidentata*, and *Platycodon grandiflorum*, was investigated in this study. Different concentrations of sodium chloride (NaCl) were used to induce salt stress, and the salt sensitivity index was employed to assess species-specific responses. Among the plants studied, *Lepidium sativum* exhibited the lowest sensitivity to salt stress during germination across all NaCl concentrations. For instance, at 50 mM NaCl, there were decreases of 7.2% in germination percentage, 4.22% in germination energy, 0.35% in germination index, 7.2% in peak value, and 11.99% in germination value. These percentages decreased further, ranging from 15.30% to 30.92%, at 100 mM NaCl, and more substantially at 150 mM NaCl, where reductions ranged from 42.94% to 66.26%, except for the germination rate, which only decreased by 0.05%. Conversely, *Peucedanum japonicum* demonstrated the highest sensitivity, experiencing reductions ranging from 27.46% to 100% at 50 mM NaCl, and complete reductions (100%) at 100 and 150 mM NaCl concentrations across all evaluated parameters. In terms of seedling growth, *Acyranthes bidentata* displayed the lowest sensitivity, with minimal reductions observed in various parameters, while *Lepidium sativum* showed significant reductions in several aspects of seedling growth under salt stress. The study underscored genetic variation in response to salt stress among the evaluated plant species, suggesting *Acyranthes bidentata* as a promising candidate for cultivation under salt-stress conditions. This information holds significance for utilizing unfavorable lands for plant cultivation.

**Keywords:** growth characteristics; salt stress; soil salinity; wild species

### Introduction

Soil salinity is a widespread environmental stress that affects the growth and development of many plant species and poses a significant challenge to global agricultural productivity. Elevated levels of soil salts, particularly sodium chloride (NaCl), can disrupt essential physiological processes within plant cells (Mokrani *et al.*, 2022; Mishra *et al.*, 2023). Excessive salt can interfere with plant water uptake, leading to osmotic stress and reduced water availability for metabolic activity (Misra *et al.* 2021; Yue *et al.*, 2023). Soil salinity can also induce ionic toxicity and disrupt cellular functions through the excessive accumulation of sodium ions (Hailu and Mehari, 2021). Salinity stress disrupts the balance between nutrient uptake and translocation within

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plants, leading to nutrient deficiencies and growth inhibition (El-Ramady *et al.*, 2018; Ondrasek *et al.*, 2021). In addition, it triggers oxidative damage by accumulating reactive oxygen species, which cause cellular injury and compromise plant resistance (Hossain and Dietz, 2016; Singh *et al.*, 2022).

In response to these challenges, there is an increasing need to explore sustainable and eco-friendly approaches to mitigate the adverse effects of salt stress on plants. Medicinal plants have gained attention in this regard because of their ability to thrive in harsh environmental conditions, including saline soils (Miransari *et al.*, 2022; Haider *et al.*, 2023). Korea's mountainous and coastal regions are renowned for their scenic landscapes and their ecological importance. However, these areas face challenges of soil salinization, which limits agricultural practices and reduces productivity. To address this issue, it is important to identify plant species that can thrive in saline soil. Research on medicinal plants may reveal species that are well-suited to adapting to challenging terrains, offering potential for sustainable land use, and developing valuable pharmaceutical resources.

*Peucedanum japonicum*, *Astragalus membranaceus*, *Lepidium sativum*, *Acyranthes bidentate*, and *Platycodon grandiflorum*, native to Korea, are renowned for their functional compounds, which have significant implications for health, industry, and nutrition. These plants are rich in bioactive compounds, including flavonoids, saponins, and polyphenols (He *et al.*, 2022; Su *et al.*, 2023; Lee *et al.*, 2023). These functional compounds exhibit antioxidant properties, which help neutralize harmful free radicals in the body and potentially reduce the risk of chronic diseases (Ke *et al.*, 2020; Ibrahim *et al.*, 2023). In addition, they possess anti-inflammatory effects that support the body's immune response and mitigate inflammatory conditions (Wang *et al.*, 2022; Li *et al.*, 2022). The extracts from these plants are commonly used in the pharmaceutical and nutraceutical industries to formulate supplements and herbal medicines (He *et al.*, 2022; Ibrahim *et al.*, 2023). Moreover, their antioxidant and anti-inflammatory properties make them valuable in the cosmetic industry for skincare products (Lee *et al.*, 2020; Durazzo *et al.*, 2021). Furthermore, these plants offer a range of vitamins, minerals, and essential nutrients that contribute to a well-balanced diet (Zheng *et al.*, 2020; Durazzo *et al.*, 2021). However, their performance and utility under salt stress remains largely unexplored. Therefore, this study aimed to investigate the effects of salinity stress on five medicinal plant species during their germination and early seedling growth stages. The goal was to identify salt-tolerant species that can be cultivated in saline soils to reclaim marginal lands.

## Materials and Methods

The experiment was conducted at the Plant Breeding Laboratory of Gangneung-Wonju National University, located in Gangneung, Korea. Seeds of five plant species were procured from Danong. To assess salt stress tolerance during the seed germination phase of these five species, the impact of salt stress on their seed germination characteristics was examined using petri dishes.

A total of four salt stress levels were applied: 0 mM (control), 50 mM, 100 mM, and 150 mM of NaCl. Thirty seeds were distributed across petri dishes, with each treatment replicated three times. The petri dishes were maintained at a temperature of  $20 \pm 1$  °C, with a relative humidity of  $50 \pm 10\%$ , and a photoperiod of 12 hours. Every two days, 3 mL of each NaCl concentration was applied to the respective petri dishes. The number of germinated seeds was recorded daily from the day following seed sowing until the 20th day. Germination was defined as the emergence of an individual with a rootlet elongated beyond 1 mm. Subsequently, various seed germination-related parameters such as germination percentage, germination energy, germination rate, germination index, peak value, and germination value were calculated using the formulas specified in Table 1. Additionally, the Salt Sensitivity Index was computed.

**Table 1.** Description of formula used to calculate seed germination related parameters

| Traits                      | Formula  | References                    |
|-----------------------------|--|-------------------------------|
| Germination percentage (GP) | $GP = (\text{Number of germinated seeds}) / (\text{Total number of seeds}) \times 100$   | Tarchoun <i>et al.</i> (2022) |
| Germination energy (GE)     | $GE = (\text{Germinated seed total in NaCl concentration in ten days}) / (\text{Total number of seeds}) \times 100$                                    | Kim <i>et al.</i> (2022)      |
| Germination rate (GR)       | $GR = a + (a + b) + (a + b + c) + \dots + (a + b + c + \dots + m) / n$<br>(a + b + c + ... + m)  | Kim <i>et al.</i> (2022)      |
| Germination index (GI)      | $GI = (\text{Number of germinated seeds}) / (\text{Days of first count}) + \dots + (\text{Number of germinated seeds}) / (\text{Days of final count})$ | Turhan <i>et al.</i> (2011)   |
| Peak Value (PV)             | $PV = (\text{germination at peak}) / (\text{number of days to reach that point})$  | Turhan <i>et al.</i> (2011)   |
| Germination Value (GV)      | $GV = (\text{Peak value}) / (\text{Mean daily germination})$   | Turhan <i>et al.</i> (2011)   |

The salt tolerance of five plant species during the seedling stage was evaluated by observing growth characteristics in seedlings cultivated in an intelligent hydroponic planter (Haier, China). The hydroponic planter featured 30 holes spaced at 2.5 cm intervals in both length and width. Salt stress responses were investigated using a selected salt level (50 mM NaCl), determined from a preliminary experiment aimed at ensuring genetic variation expression. In the experiment, 300 seeds were sown in petri dishes, and after 7 days, six uniformly germinated seedlings were transferred to styrofoam medium in six different hydroponic planters containing Moolpoore nutrient solution (Daeyu, Seoul, Korea). They were allowed to grow for 10 days. In the control treatment, the Moolpoore nutrient solution was used without salt addition, while in other treatments, NaCl was added to the nutrient solution at varying concentrations and maintained for 7 days. After 7 days of salt stress treatment, the three largest seedlings from each planter were collected for measurement of salt stress resistance (n=9 per treatment). Subsequently, the length of both above-ground and below-ground parts, the number of leaves developed above-ground, and the fresh and dry weights of both above-ground and below-ground parts were measured. The length of the above-ground and below-ground parts was measured in centimeters using a vernier caliper, while the number of leaves was visually counted. The fresh weight of both above-ground and underground parts was measured using an electronic balance and expressed in grams, whereas the dry weight of both above-ground and underground parts was determined using an electronic balance after the plants were dried in an oven at 70 °C for 3 days. Additionally, the Salt Sensitivity Index was calculated thereafter.

The Salt Sensitivity Index provides a metric for assessing salinity tolerance, evaluating the relative reduction in germination and seedling growth parameters as a percentage of the control, rather than focusing solely on absolute growth rates. This method offers insights into the plant's relative tolerance to salt stress conditions. The index for salt sensitivity was calculated using the following formula, as outlined by Mbarki *et al.* (2020):

$$\text{Salt Sensitivity Index (\% reduction)} = 100 \times ((\text{salt treatment} - \text{control}) / \text{control})$$

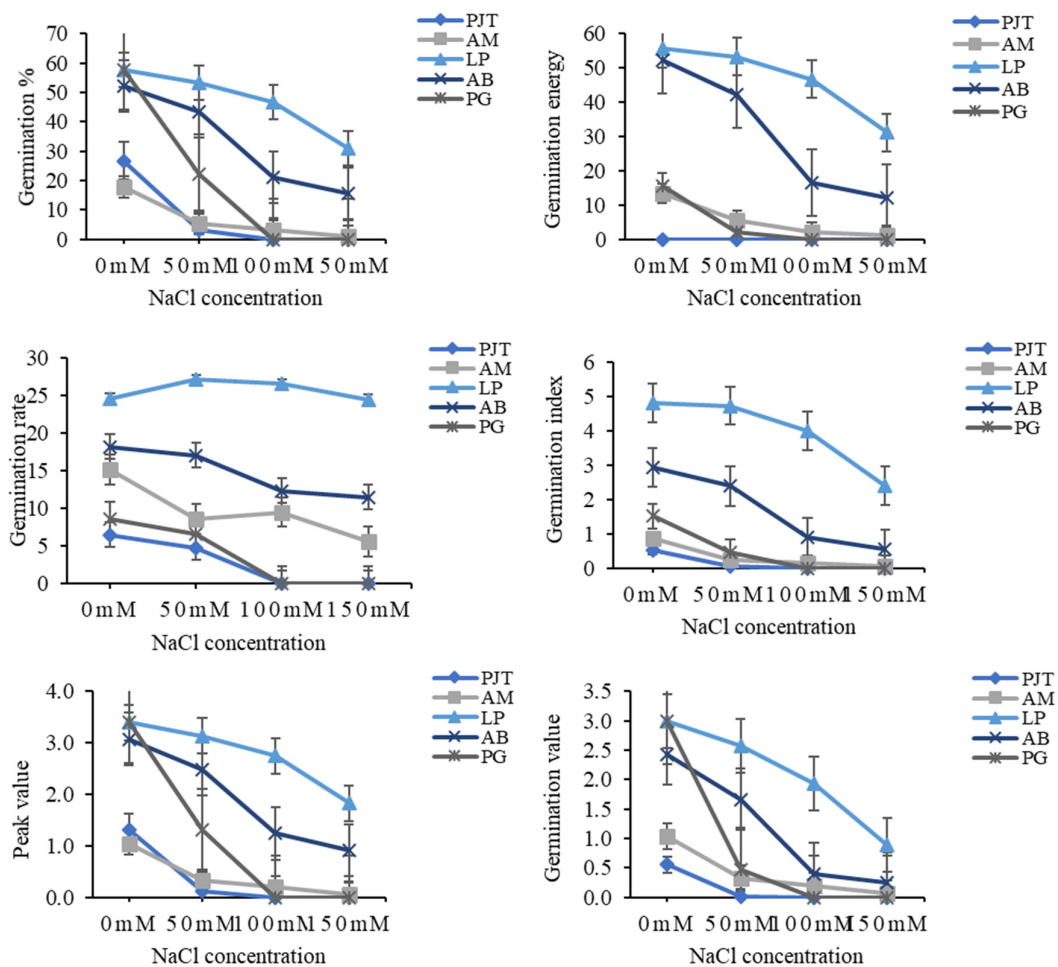
Statistical analysis was performed using SPSS 28.0 software (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was employed to assess treatment impacts. Upon identifying statistically significant treatment effects ( $p < 0.05$ ), means were compared using Duncan's multiple range test. Hierarchical clustering was conducted using SPSS software following Ward's method to categorize species based on their salt tolerance response.

## Results and Discussion

### *Effect of salt stress on seed germination-related characteristics*

The investigation of salt stress on seed germination characteristics is particularly important in medicinal plant species. These plants have been valued for their medicinal properties for centuries and continue to contribute to human health (Akinyemi *et al.*, 2018; Ssenku *et al.*, 2022). The use of medicinal plants as sources of natural medicine and for subsistence through product production and trade has been extensively documented (Aziz *et al.*, 2018; Mofokeng *et al.*, 2022; Manzoor *et al.*, 2023). However, the successful cultivation and long-term viability of medicinal plants face significant challenges due to salt stress (Ghassemi-Golezani and Abdoli, 2022; Haider *et al.*, 2023). Therefore, understanding how medicinal plants respond to salt stress during germination is crucial because it directly affects their growth and seed viability. This study assessed the effects of salt stress on germination characteristics of *Peucedanum japonicum*, *Astragalus membranaceus*, *Lepidium sativum*, *Acyranthes bidentata*, and *Platycodon grandiflorum* medicinal plant species. Seed germination percentage, germination rate, germination energy, germination value, peak value and germination index serve as crucial indicators of a plant's response to salt stress. The germination rate, for example, is a key parameter reflecting a seed's ability to initiate growth under stress, providing insights into a plant's adaptability to adverse conditions (Jisha *et al.*, 2013; Khaeim *et al.*, 2022). Rapid and uniform seedling emergence, indicated by high germination rates, confers a competitive advantage in resource utilization, influencing overall plant fitness (De Ron *et al.*, 2016; Reed *et al.*, 2022). Enhanced seed germination under saline conditions can contribute to biodiversity by promoting the growth of salt-tolerant plant species in challenging environments (Aizaz *et al.*, 2023). In salt stress conditions, high germination energy is essential, directly impacting seedling establishment and overall plant growth. This trait reflects the strength of emerging seedlings, allowing them to withstand adverse effects and establish resilient root systems (Özkan *et al.*, 2022). In addition, germination index, germination value, and peak value offer valuable insights into plant responses to salt stress. The germination index assesses the speed and consistency of germination over time, while the germination value represents overall performance under stress, considering both speed and uniformity (Romano and Stevanato, 2020; Guoying *et al.*, 2021). The highest value signals the optimal time for germination, highlighting the crucial period when seeds exhibit the greatest strength under salt stress. In this investigation, it was observed that salt stress had a negative impact on various seed germination characteristics in all species. As the salt concentrations increased, there was a significant reduction in seed germination characteristics (Figure 1). Particularly, the seed germination characteristics such as germination index, germination value, peak value, and germination energy of *Peucedanum japonicum*, *Astragalus membranaceus*, and *Platycodon grandiflorum* were highly affected compared to other species, with a higher reduction observed at 150 mM NaCl compared to the control group (0 mM) and other treatment groups (Figure 1). Turhan *et al.* (2011), Uçgun *et al.* (2020), and Tarchoun *et al.* (2022) conducted comprehensive studies on the impact of different concentrations of NaCl on Spinach and Squash cultivars. Their research revealed a significant decline in seed germination characteristics, especially at higher salt concentrations. Similarly, in this investigation, the highest level of salt concentration also led to a noticeable reduction in germination characteristics of the evaluated medicinal plant species. For instance, the germination percentage of *Peucedanum japonicum* decreased from 26.66% (control) to 3.33% at 50 mM NaCl, and it was completely inhibited at 100 and 150 mM NaCl concentrations (Table 2). On the other hand, *Lepidium sativum* showed a decrease in germination percentage from 57.78% (control) to 53.33%, 46.66%, and 31.11% at 50, 100, and 150 mM, respectively (Table 2). The decline in seed germination characteristics at high salt concentrations may be due to either osmotic stress or ion toxicity. Previous studies have demonstrated that plants primarily suffer from salt stress caused by excess soil salt, resulting in osmotic pressure, and accumulation of salt ions (Kumar *et al.*, 2021; Lu *et al.*, 2022; Ma *et al.*, 2022). Salt stress alters the osmotic potential of the surrounding environment, thereby affecting seed

water absorption. When the salt concentration is high, the seed's osmotic potential becomes higher than the decreased osmotic pressure outside, preventing water from entering (Farooq *et al.*, 2017; Tebini *et al.*, 2022). Consequently, the processes of seed germination and seedling establishment are significantly impacted. In addition, salt stress disrupts the selective permeability of cell membranes and impairs their functioning due to a lack of integration. The excessive influx of salt ions hinders the regulation of ions both inside and outside the cell, leading to ion toxicity and cell damage (Parihar *et al.*, 2014; Wang *et al.*, 2020). The accumulation of toxic ions, such as sodium, also affects ion-sensitive cell physiology and biochemical processes, including enzyme competency and protein functionality (Negrão *et al.*, 2017; Malakar and Chattopadhyay, 2021). Consequently, the plant's metabolism deteriorates, further compromising its ability to adapt to salt stress (Athar *et al.*, 2022; Behera *et al.*, 2022; Xiao and Zho, 2023). Ultimately, these effects can lead to physiological drought, where the plant experiences water deficiency even when it is present in the soil. Overall, these findings highlight the complex interplay between salt stress and seed germination characteristics, providing valuable insights into plant adaptability in challenging environments.



**Figure 1.** Effects of different salt concentrations (0, 50, 100 and 150 mM) on seed germination characteristics of five plant species  
 Where; PJT: *Peucedanum japonicum* T., AM: *Astragalus membranaceus*, LP: *Lepidium sativum* L., AB: *Acanthopanax bidentata*, and PG: *Platycodon grandiflorum*

**Table 2.** Effects of different levels of salt concentration on the germination related characteristics of five plant species

| NaCl (mM) | Species                        | GP         | GE         | GI        | PV        | GV        | GR         |
|-----------|--------------------------------|------------|------------|-----------|-----------|-----------|------------|
| Control   | <i>Peucedanum japonicum</i>    | 26.66±3.85 | 0.00±0.00  | 0.53±0.09 | 1.04±0.28 | 0.55±0.19 | 6.43±0.19  |
|           | <i>Astragalus membranaceus</i> | 17.77±7.29 | 13.33±5.09 | 0.86±0.35 | 1.31±0.43 | 0.37±0.19 | 15.09±0.80 |
|           | <i>Lepidium sativum</i> L.     | 57.78±4.84 | 55.55±2.94 | 4.83±0.48 | 3.07±0.28 | 2.43±0.50 | 24.61±1.41 |
|           | <i>Acyranthes bidentate</i>    | 52.22±4.01 | 52.22±4.01 | 2.94±0.29 | 3.39±0.24 | 2.98±0.38 | 18.19±0.56 |
|           | <i>Platycodon grandiflorum</i> | 57.78±4.84 | 15.55±2.94 | 1.52±0.08 | 3.39±0.28 | 2.98±0.50 | 8.61±0.31  |
| 50        | <i>Peucedanum japonicum</i> T. | 3.33±1.92  | 0.00±0.00  | 0.07±0.04 | 0.13±0.07 | 0.01±0.01 | 4.74±2.47  |
|           | <i>Astragalus membranaceus</i> | 5.55±2.94  | 5.55±2.94  | 0.24±0.12 | 0.33±0.17 | 0.04±0.03 | 8.61±1.81  |
|           | <i>Lepidium sativum</i> L.     | 53.33±5.77 | 53.33±5.77 | 4.73±0.55 | 3.14±0.34 | 2.56±0.54 | 27.06±2.11 |
|           | <i>Acyranthes bidentate</i>    | 43.33±5.09 | 42.22±5.56 | 2.40±0.31 | 2.48±0.33 | 1.66±0.42 | 17.04±1.03 |
|           | <i>Platycodon grandiflorum</i> | 22.22±4.01 | 2.22±1.11  | 0.47±0.07 | 1.31±0.24 | 0.46±0.17 | 6.55±0.13  |
| 100       | <i>Peucedanum japonicum</i> T. | 0.00±0.00  | 0.00±0.00  | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00  |
|           | <i>Astragalus membranaceus</i> | 3.33±1.92  | 2.22±1.11  | 0.14±0.08 | 0.19±0.11 | 0.02±0.01 | 9.47±1.24  |
|           | <i>Lepidium sativum</i> L.     | 46.66±1.92 | 46.66±1.92 | 3.99±0.04 | 2.74±0.11 | 1.93±0.16 | 26.49±1.36 |
|           | <i>Acyranthes bidentate</i>    | 21.11±2.22 | 16.66±3.85 | 0.89±0.10 | 1.24±0.13 | 0.40±0.08 | 12.31±0.72 |
|           | <i>Platycodon grandiflorum</i> | 0.00±0.00  | 0.00±0.00  | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00  |
| 150       | <i>Peucedanum japonicum</i> T. | 0.00±0.00  | 0.00±0.00  | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00  |
|           | <i>Astragalus membranaceus</i> | 1.11±0.68  | 1.11±0.68  | 0.05±0.03 | 0.06±0.03 | 0.00±0.00 | 5.55±0.56  |
|           | <i>Lepidium sativum</i>        | 31.11±4.84 | 31.11±4.84 | 2.41±0.40 | 1.83±0.28 | 0.89±0.27 | 24.39±0.64 |
|           | <i>Acyranthes bidentate</i>    | 15.55±4.44 | 12.22±4.01 | 0.54±0.15 | 0.92±0.26 | 0.25±0.10 | 11.45±1.04 |
|           | <i>Platycodon grandiflorum</i> | 0.00±0.00  | 0.00±0.00  | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00  |

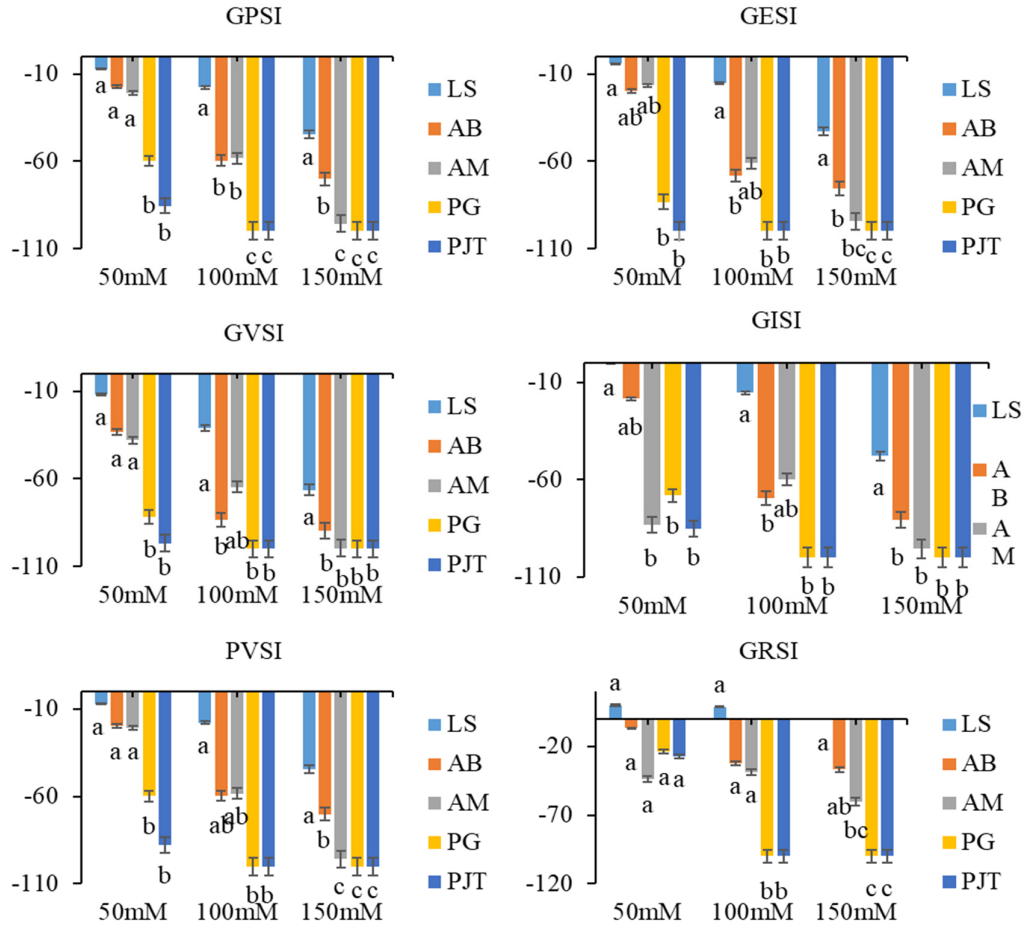
Where; GP: germination percentage, GE: germination energy, GI: germination index, PV: peak Value, GV: germination Value and GR: germination rate.

#### *Salt sensitivity index of seed germination related parameters*

The study used salt sensitivity index to evaluate plant species' response to salt stress during germination. This provided valuable insights into tolerance levels under different salt concentrations. The sensitivity index was calculated based on seed germination characteristics at 50-, 100-, and 150-mM salt concentrations.

Figure 2 shows a consistently negative sensitivity index for all species at different salt concentrations, indicating a decrease in germination characteristics as salt concentration increases. Specifically, *Lepidium sativum* showed a positive sensitivity index at 50 and 100 mM NaCl for germination rate salt sensitivity index. *Peucedanum japonicum* showed a significant decrease in germination characteristics at all concentrations, while *Platycodon grandiflorum* exhibited a significant decrease at 100 and 150 mM NaCl. *Astragalus membranaceus* and *Acyranthes bidentata* displayed a moderate decrease in germination characteristic sensitivity index at 50 and 100 mM NaCl, indicating tolerance to lower salt concentrations. In contrast, *Lepidium sativum* exhibited a less significant decrease at all salt concentrations, indicating potential resilience to salt stress during germination. These results offer valuable insights into the diverse salt stress responses of various plant species during the germination stage. In a study conducted by Mbarki *et al.* (2020), the salt tolerance of various *Medicago* species during seed germination and seedling growth was assessed under different NaCl concentrations, and the sensitive and tolerant species were identified based on their salt sensitivity index. Similarly, in this investigation, we successfully identified plant species that are tolerant and sensitive to salt by using seed germination sensitivity index.

The variations in sensitivity observed among species emphasize that a standardized approach may not effectively address salt stress. Therefore, the development of site-specific strategies is necessary for optimal results. The sensitivity or tolerance of plant species to salt stress is shaped by their physiological and biochemical adaptations. Salt stress disrupts normal cellular functions by posing osmotic and ionic challenges to plants (Lu *et al.*, 2022; Ma *et al.*, 2022). Salt-sensitive plant species lack efficient mechanisms to manage excessive salt, which can lead to water loss and ion toxicity (Guo *et al.*, 2022; Rajkumari *et al.*, 2023).



**Figure 2.** Impact of salt stress (50, 100, and 150 mM) on the salt sensitivity index for seed germination related parameters of five plant species

Where; PJT: *Peucedanum japonicum*, AM: *Astragalus membranaceus*, LS: *Lepidium sativum*, AB: *Acyranthes bidentata*, and PG: *Platycodon grandiflorum*, GPSI: germination percentage sensitivity index, GESI: germination energy sensitivity index, GVSI: germination value sensitivity index, GISI: germination index sensitivity index, PVSI: peak value sensitivity index and GRSI: germination rate sensitivity index.

In contrast, salt-tolerant plant species have evolved intricate strategies involving specialized ion transporters, osmoprotectants, and antioxidant systems (Malakar and Chattopadhyay, 2021; Arzani *et al.*, 2023). These adaptations enable organisms to maintain cellular water balance, regulate ion homeostasis, and mitigate oxidative damage. In addition, genetic diversity plays a crucial role in determining the salt tolerance of plant species. Plant populations have undergone genetic adaptations to cope with saline environments over time, resulting in variations in salt tolerance across species (Fan *et al.*, 2023). The level of salt tolerance of different plant species is explained by the evolutionary aspect of genetic diversity, which is linked to ecological history (Isayenkov, 2019). The case of halophytes provides a practical example of this linkage. This genus of plants has adapted to saline conditions and demonstrates an extraordinary level of salinity due to multiple generations of exposure to saline environments (Mann *et al.*, 2023). Furthermore, Mishra and Tanna (2017) explained the genetic mechanisms that determine salt tolerance, including the significance of metabolic pathways and gene expression involving a combination of several genes. A study conducted by Tasnim *et al.* (2023) has also shown significant differences in salt tolerance between cultivated and halophytic wild rice. By accurately analyzing the genetic structure of salt-resistant mutants, breeders can identify key genetic markers

responsible for the observed trait and develop elite forms that can effectively grow in saline soils (Fang *et al.*, 2023). However, relatively low genetic diversity in species can be a disadvantage. Santo *et al.* (2017) discussed a study involving non-halophyte species inhabiting regions with historically low salinity levels. These plants lack the specialization necessary to survive in this hostile environment due to their evolution in soil conditions with low salt concentrations. These findings provide valuable insights into the ability of different plant species to withstand salt stress, which could be useful in improving agricultural and ecological practices.

#### *Effect of salt stress on seedling growth potential traits*

Salt stress is an important abiotic factor affecting seedling emergence, plant growth and development. In this study, the effects of salt stress on various seedling-related characteristics, including shoot length, shoot number, number of leaves, shoot fresh weight, shoot dry weight, root length, root fresh weight, and root dry weight, were examined in five different plant species under 0 and 50 mM NaCl concentrations (Table 3). Analyzing these parameters can provide insight into how plants respond to and mitigate the effects of salt stress. The results demonstrated a consistent pattern, with the control group consistently exhibiting the highest values for all parameters, while the 50 mM NaCl group consistently showed the lowest values. For example, *Peucedanum japonicum*, *Astragalus membranaceus*, *Lepidium sativum*, *Acyranthes bidentata*, and *Platycodon grandiflorum* demonstrated decreased shoot length from 6.61, 13.41, 29.82, 6.86, and 5.92 cm, respectively, under control conditions to 5.47, 9.16, 12.75, 6.11, and 2.96 cm, respectively, when exposed to 50 mM NaCl concentration. Similarly, the root length of *Peucedanum japonicum*, *Astragalus membranaceus*, *Lepidium sativum*, *Acyranthes bidentata*, and *Platycodon grandiflorum* decreased from 15.92, 13.68, 19.18, 19.34, and 9.57 cm, respectively, under control conditions to 10.87, 10.75, 16.74, 16.58, and 8.62 cm, respectively, at a 50 mM salt concentration. Similarly, number of leaves, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight showed a substantial decrease under a 50 mM salt concentration compared to the control group. These differences in parameters between the control and salt-treated groups for each plant species indicate the varied responses of different plant species to salt stress. The significant reduction across all evaluated parameters under a 50 mM NaCl concentration highlights the overall detrimental impact of salt stress on seedling growth. Shoot dry weight and root dry weight were greatly reduced in all evaluated plant species under a 50 mM NaCl concentration. The substantial decrease in shoot dry weight and root dry weight across all plant species except *Acyranthes bidentata* suggests a common sensitivity to salt stress in terms of biomass accumulation. *Platycodon grandiflorum* and *Lepidium sativum* showed the most pronounced reductions in shoot length, shoot fresh weight, root fresh weight, root dry weight, and shoot dry weight, indicating their high susceptibility to salt stress. On the other hand, *Acyranthes bidentata* exhibited resilience to salt stress, with minimal reductions in most parameters and no change in shoot length, suggesting potential salt tolerance mechanisms in this species. *Acyranthes bidentata* and *Peucedanum japonicum* displayed moderate responses to salt stress, with varying degrees of reduction in different parameters. These observations emphasize the importance of species-specific responses to salt stress and the need for tailored management strategies in agricultural practices. Furthermore, the consistent decrease in shoot length and root length under salt stress provides valuable insights into the underlying physiological mechanisms governing plant responses to environmental stressors.

Under salt stress conditions, root and shoot length are critical traits for evaluating salt tolerance because roots are responsible for absorbing water, and shoots supply water to aboveground tissues (Wu *et al.*, 2019; Shao *et al.*, 2021; Tao *et al.*, 2021). The highest values of root and shoot length indicate the resilience and adaptability of plant species to challenging ecological conditions (Singh *et al.*, 2022; Xiao and Zhou., 2023). This adaptive strategy allows plants to optimize their water and nutrient uptake from the soil, thereby increasing their chances of survival in saline environments (Sayed *et al.*, 2022). In addition, evaluation of shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight provides a comprehensive understanding of how salt stress affects plant biomass allocation and resource use (Ma *et al.*, 2022; Senousy *et al.*, 2023). High



salinity often leads to a decrease in shoot and root biomass, reflecting the inhibitory effects on water and nutrient uptake (Shoukat *et al.*, 2020; Shaheen *et al.*, 2023). The assessment of fresh and dry weights helps quantify these changes and provides a quantitative measure of the plant's overall health and productivity under salt stress conditions (Shahid *et al.*, 2020; Xiao and Zhou., 2023). As a result, understanding how seedling-related characteristics respond to salt stress is essential for improving plant resilience and productivity in saline environments.

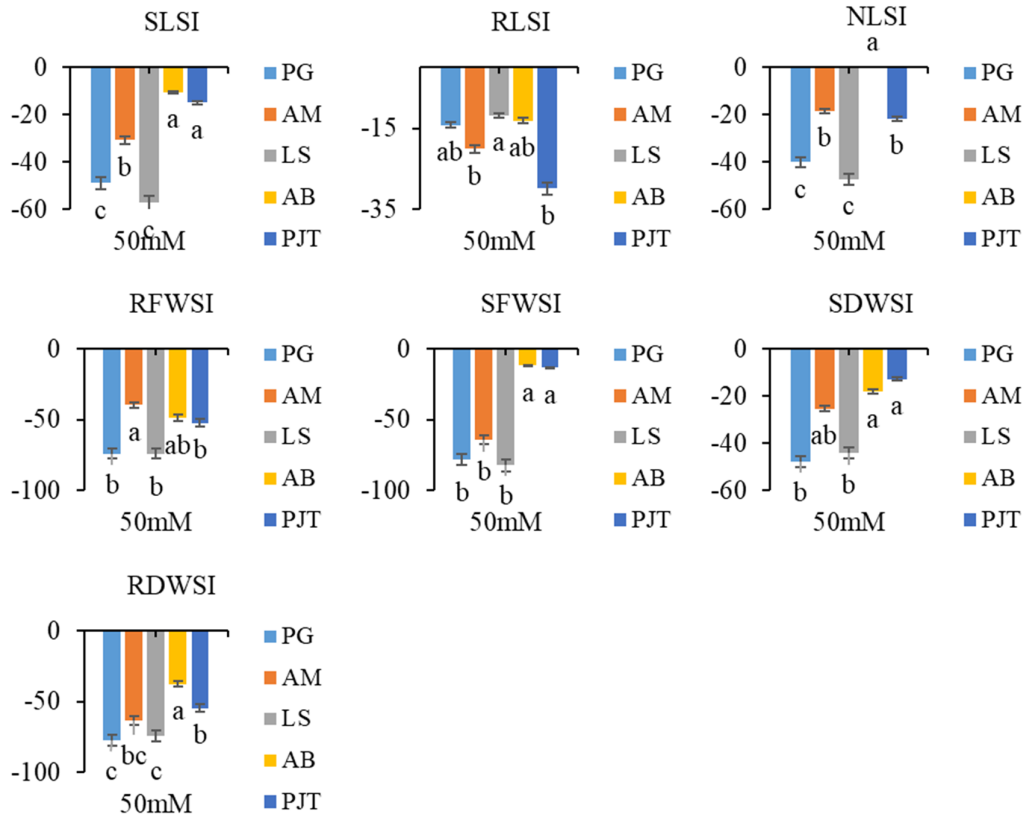
**Table 3.** Effects of different levels of salt concentration on the germination related characteristics of five plant species

| Treatment  | Species                        | SL (cm)        | NS            | NL             | SFW (g)       | RFW (g)       | SDW (g)       | RDW (g)       | RL (cm)        |
|------------|--------------------------------|----------------|---------------|----------------|---------------|---------------|---------------|---------------|----------------|
| Control    | <i>Peucedanum japonicum</i> T. | 6.61±<br>0.37  | 4.03±<br>0.17 | 4.00±<br>0.17  | 0.65±<br>0.06 | 0.41±<br>0.03 | 0.16±<br>0.01 | 0.06±<br>0.00 | 15.92±<br>0.87 |
|            | <i>Astragalus membranaceus</i> | 13.41±<br>0.52 | 1.00±<br>0.00 | 20.00±<br>0.58 | 0.39±<br>0.03 | 0.22±<br>0.03 | 0.10±<br>0.01 | 0.02±<br>0.00 | 13.68±<br>0.53 |
|            | <i>Lepidium sativum</i> L.     | 29.82±<br>0.48 | 1.00±<br>0.00 | 16.00±<br>0.00 | 3.11±<br>0.09 | 0.90±<br>0.05 | 0.49±<br>0.02 | 0.09±<br>0.01 | 19.18±<br>0.40 |
|            | <i>Acyranthes bidentata</i>    | 6.86±<br>0.16  | 1.00±<br>0.00 | 8.00±<br>0.00  | 1.74±<br>0.09 | 0.54±<br>0.05 | 0.38±<br>0.02 | 0.06±<br>0.00 | 19.34±<br>0.86 |
|            | <i>Platycodon grandiflorum</i> | 5.92±<br>0.23  | 1.00±<br>0.00 | 10.00±<br>0.00 | 0.87±<br>0.03 | 0.31±<br>0.01 | 0.22±<br>0.01 | 0.05±<br>0.00 | 9.57±<br>0.23  |
| 50 mM NaCl | <i>Peucedanum japonicum</i> T. | 5.47±<br>0.14  | 3.13±<br>0.20 | 3.11±<br>0.20  | 0.54±<br>0.03 | 0.18±<br>0.03 | 0.13±<br>0.01 | 0.03±<br>0.00 | 10.87±<br>0.38 |
|            | <i>Astragalus membranaceus</i> | 9.16±<br>0.36  | 1.00±<br>0.00 | 16.22±<br>0.91 | 0.12±<br>0.02 | 0.11±<br>0.03 | 0.07±<br>0.01 | 0.01±<br>0.00 | 10.75±<br>0.79 |
|            | <i>Lepidium sativum</i>        | 12.75±<br>0.48 | 1.00±<br>0.00 | 8.44±<br>0.29  | 0.55±<br>0.05 | 0.23±<br>0.02 | 0.27±<br>0.01 | 0.02±<br>0.00 | 16.74±<br>1.06 |
|            | <i>Acyranthes bidentata</i>    | 6.11±<br>0.09  | 1.00±<br>0.00 | 8.00±<br>0.00  | 1.48±<br>0.11 | 0.26±<br>0.03 | 0.30±<br>0.03 | 0.04±<br>0.00 | 16.58±<br>0.47 |
|            | <i>Platycodon grandiflorum</i> | 2.96±<br>0.12  | 1.00±<br>0.00 | 6.00±<br>0.00  | 0.18±<br>0.01 | 0.08±<br>0.01 | 0.11±<br>0.00 | 0.01±<br>0.00 | 8.62±<br>0.32  |

Where; SL: shoot length, NS: number of shoots, NL: Number of leaves, SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight and RL: root length

#### *Salt sensitivity index of seedling growth related parameters*

Similar to seed germination characteristics, we calculated the sensitivity index for seedling growth characteristics to identify species that are resilient to salt stress at the seedling stage. Across all species studied, a consistent negative sensitivity index was observed, except for the shoot length sensitivity index in *Acyranthes bidentata* (Figure 3). This indicates that seedling growth characteristics generally decrease when exposed to salt stress. However, the extent of this reduction varied significantly among different species. Notably, *Lepidium sativum* and *Platycodon grandiflorum* showed significant reductions in multiple growth parameters, including shoot length, number of leaves, shoot fresh weight, and root fresh weight sensitivity indices. This suggests that these species are highly susceptible to salt stress at the seedling stage. On the other hand, *Astragalus membranaceus* exhibited a moderate reduction in certain sensitivity indices, indicating a moderate response to salt stress. *Peucedanum japonicum* showed similar patterns, but with variations in the degree of reduction across different growth parameters. Interestingly, *Acyranthes bidentata* stood out as less sensitive to salt stress, displaying lower sensitivity indices across all seedling growth parameters compared to the other species studied. This suggests a higher level of resilience to salt stress during the early stages of growth.



**Figure 3.** Effects of salt stress (50 mM) on the salt sensitivity index for seedling growth related parameters of five plant species

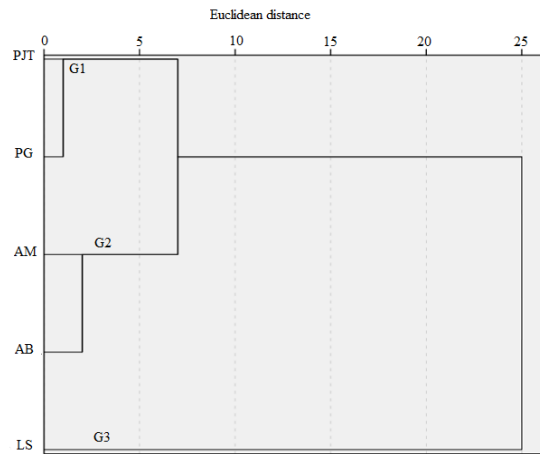
Where; PJT: *Peucedanum japonicum*, AM: *Astragalus membranaceus*, LS: *Lepidium sativum*, AB: *Acyranthes bidentata*, and PG: *Platycodon grandiflorum*, SLSI: shoot length sensitivity index, RLSI: root length sensitivity index, NLSI: number of leaves sensitivity index, RFWSI: root fresh weight sensitivity index, SFWSI: shoot fresh weight sensitivity index, SDWSI: shoot dry weight sensitivity index, RDWSI: root dry weight sensitivity index.

The observed differences in sensitivity index levels among the various species highlight the diverse responses of different plant species to salt stress. These differences may be attributed to inherent genetic variations, adaptive mechanisms, or physiological characteristics specific to each species. In addition, our study revealed that species that were less sensitive during seed germination exhibited different responses during seedling growth. Similarly, Tarchoun *et al.* (2022) conducted a study to examine the impact of salt stress on various landraces of Tunisian squash. Interestingly, they observed that certain evaluated landraces displayed varying responses during both seed germination and seedling stages. Moreover, our findings are consistent with those of Rajabi Dehnavi *et al.* (2020), who also observed diverse responses to salt stress in ten sorghum genotypes during seed germination and seedling growth. This difference in salt tolerance during seed germination and subsequent seedling growth characteristics in the studied plant species may be attributed to the dynamic nature of plant responses to salt stress during different developmental stages. Seed germination and early seedling growth are distinct physiological processes with unique molecular and biochemical mechanisms governing their regulation (Zhang *et al.*, 2013; Song *et al.*, 2023; Szablińska-Piernik and Lahuta, 2023). Although some plant species may tolerate a range of salt concentrations during germination, their sensitivity may increase during later developmental stages due to the activation of stress-responsive pathways or the cumulative effects of prolonged exposure to salt stress (Saisho *et al.*, 2016; Singh *et al.*, 2021). Furthermore, differences in the genetic expression and regulation of salt-responsive genes during various

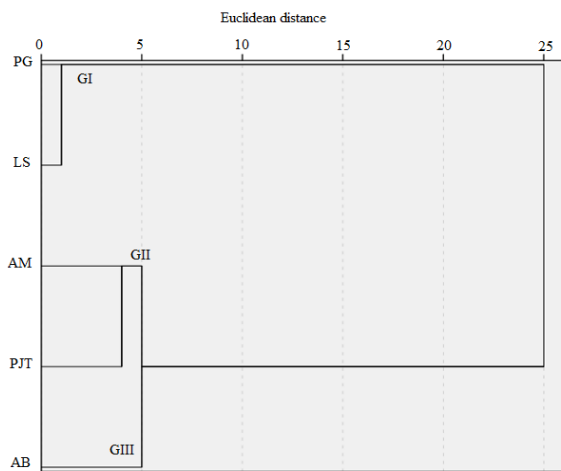
growth phases may contribute to the observed variations in salt sensitivity indices (Hu *et al.*, 2022; Zhang *et al.*, 2022; Afzal *et al.*, 2023). This investigation highlights the complex nature of plant responses to salt stress and underscores the importance of a thorough comprehension of the underlying mechanisms that regulate salt tolerance throughout the entire life cycle. This understanding will inform effective strategies for improving crops in saline environments, especially in regions like South Korea, which are surrounded by seas and covered by mountains.

*Cluster analysis*

Cluster analysis was conducted to categorize species according to their sensitivity to salt. Notably, the response of species to salt stress differed during the germination and seedling growth stages. As illustrated in Figures 4 and 5, two distinct dendrograms were constructed for each stage.



**Figure 4.** Dendrogram of five plant species based on seed germination characteristics Where; G1: group 1, G2: group 2, G3: group 3 PJT: *Peucedanum japonicum*, AM: *Astragalus membranaceus*, LS: *Lepidium sativum*, AB: *Acyranthes bidentata*, and PG: *Platycodon grandiflorum*.



**Figure 5.** Dendrogram of five plant species based on their seedling growth characteristics Where; GI: group I, GII: group II, GIII: group III, PJT: *Peucedanum japonicum*, AM: *Astragalus membranaceus*, LS: *Lepidium sativum*, AB: *Acyranthes bidentata*, and PG: *Platycodon grandiflorum*.

For the germination stage, we employed germination-related characteristics and calculated sensitivity index of germination parameters at 100 mM NaCl. In the case of seedling growth, the calculated sensitivity index parameters were used to elucidate the relationship among species regarding salt sensitivity at a 50 mM salt concentration. Ward's method was applied for species clustering and the number of clusters was determined through an analysis of agglomeration coefficients. For seed germination characteristics, the clustering analysis was divided into three groups. Group 1 (G1) was characterized by species with a low level of germination characteristics and a high reduction in the salt sensitivity index. Due to low germination characteristics and a significant reduction in the salt sensitivity index, the species found in G1 were considered highly sensitive to salt stress. Group 2 (G2) was characterized by species with medium germination characteristics and medium reduction in salt sensitivity index, indicating moderate sensitivity to salt stress. The species in group 3 (G3) was considered less sensitive, given its high germination percentage and low reduction in salt sensitivity index. The clustering results of this analysis aligns with the observed results in seed germination characteristics and seed germination sensitivity index. Species such as *Peucedanum japonicum* and *Platycodon grandiflorum* exhibited lower seed germination characteristics and a significant reduction in sensitivity index during seed germination and salt sensitivity index analysis. These species were grouped together in the cluster analysis. On the other hand, species like *Astragalus membranaceus* and *Acyranthes bidentata* displayed moderate values for these parameters and were grouped in the same cluster. Furthermore, *Lepidium sativum*, which demonstrated high germination characteristics and a smaller reduction in salt sensitivity index during the analysis, formed a separate group in this cluster analysis. Similar to the seed germination stage, the seedling growth stage also formed three groups (GI, GII and GIII). GI was characterized by species with a high reduction in the salt sensitivity index for seedling growth parameters, signifying highly sensitive to salt stress. Meanwhile, GII was characterized by species with a medium reduction in the salt sensitivity index for seedling growth characteristics, indicating comparatively moderately sensitive to salt stress. GIII characterized by a species with a low reduction in the salt sensitivity index for seedling growth parameters, indicating less sensitivity to salt stress. This cluster analysis was consistent with the observed results of salt sensitivity index analysis of seedling growth characteristics. Species like *Platycodon grandiflorum* and *Lepidium sativum* demonstrated a high sensitivity index in seedling growth and were grouped together in the clustering analysis. Similarly, species like *Astragalus membranaceus* and *Peucedanum japonicum* showed a moderate reduction in salt sensitivity index in seedling growth and were also grouped together in the clustering analysis. *Acyranthes bidentata*, which showed a lower reduction in salt sensitivity index during seedling growth, was placed in a separate group during the clustering analysis. Similarly, Pongprayoon *et al.* (2019) and Park *et al.* (2023) successfully employed cluster analysis to distinguish between salt-tolerant and salt-sensitive rice genotypes and Pak Choi, respectively. Seed germination and seedling growth are critical stages in a plant's life cycle, especially under stressful conditions. However, identifying plant species that exhibit salt stress tolerance during seedling growth is crucial for establishing the foundation of a plant's overall growth and development. Therefore, it is essential to focus on salt stress tolerance during seedling growth rather than solely on the germination stage. Although successful seed germination is important, it is only the first step in the transition from a dormant seed to an actively growing seedling. Seedlings face many challenges, particularly from environmental stressors such as salinity, which can impede their growth and survival (Lewis and Weber, 2002; Khan *et al.*, 2022). Plant species that exhibit resilience to salt stress during the seedling stage are likely to show increased adaptability to adverse conditions throughout their life cycle (Xiao and Zhou, 2023). This increased tolerance is crucial for maintaining optimal growth and productivity, making these species more resilient in saline environments (Afzal *et al.*, 2023; Mansour and Hassan, 2022). Therefore, it is crucial to prioritize identifying and breeding plant species with robust salt stress tolerance at the seedling stage. In addition, identifying salt-tolerant species at the seedling stage has significant implications for breeding programs, crop management practices, and ecosystem restoration. In breeding programs, this identification helps select genetic traits that promote salt tolerance, enhancing crop resilience in saline conditions, and improve agricultural

productivity in salt affected regions (Afzal *et al.*, 2023; Zhang *et al.*, 2024). It also aids in developing specific crop management strategies, enabling farmers to allocate resources efficiently and minimize the adverse effects of salinity on crop yield (Truşcă *et al.*, 2023). Moreover, incorporating salt-tolerant species into ecosystem restoration initiatives contributes to establishing robust plant communities, fostering biodiversity, and promoting ecosystem stability in saline environments (Ashraf *et al.*, 2012; Li *et al.*, 2022). This will ensure the long-term success of plants in saline ecosystems. Considering these, *Acyranthes bidentata* was selected for its salt stress tolerance, as demonstrated by its moderate seed germination characteristics and lower sensitivity index for seedling growth.

## Conclusions

This study examined the effects of salt stress on germination and early seedling growth of *Peucedanum japonicum*, *Astragalus membranaceus*, *Lepidium sativum*, *Acyranthes bidentata*, and *Platycodon grandiflorum*. Varying salt concentrations at different developmental stages led to distinct responses among the species. During the germination stage, *Lepidium sativum* was less sensitive to salt concentration, whereas *Peucedanum japonicum* was highly sensitive to salt stress. Conversely, during seedling growth, the species that showed less sensitivity to germination characteristics were highly affected by salt stress, and *Acyranthes bidentata*, which exhibited a moderate reduction in the salt sensitivity index for seed germination characteristics, showed lowest salt sensitivity during this stage. Prioritizing salt stress resistance during seedling growth is crucial for the long-term survival of plants in saline environments. Accordingly, among the medicinal plant species evaluated under salt stress conditions, *Acyranthes bidentata* was found to be the lowest sensitive. This suggests that it could be a practical solution for addressing soil salinity issues, promoting sustainable land use, and utilizing underutilized landscapes for environmental and economic benefits.

## Authors' Contributions

Conceptualization MZ and JYH; Data curation MZ and JYH; Formal analysis MZ; Investigation MZ and JYH; Methodology MZ and JYH; Statical analysis MZ; Supervision JYH; Writing - original draft MZ; Writing - review and editing JYH. Both authors read and approved the final manuscript.

## Ethical approval (for researches involving animals or humans)

Not applicable.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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