

ORIGINAL ARTICLE

Effects of Salt Treatment on Seed Germination and Plant Growth of Korean Native *Apocynum lancifolium* Russanov

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Abstract

This research was carried out to investigate seed germination and growth of the perennial plant *Apocynum lancifolium* under different NaCl concentrations, with a view for future cultivation on reclaimed land. Initial characterization revealed that the average length and weight of *A. lancifolium* seed pods was 133.6 mm and 0.23 g, respectively, and the thousand-grain weight was 0.59 g. Upon examining the effects of light on seed germination, we found germination to be 1.7% higher under light conditions (90%) than under dark conditions (88.3%). In terms of the response to salt stress, we found that 90% of seeds germinated in the 0.00%, 0.25%, and 0.50% salt treatment groups. Although salt treatment up to a concentration of 0.5% was found to have little effect on seed germination, the rate of germination decreased at higher concentrations and was completely inhibited in the 2% treatment. We also established that germination rates were higher in seeds sown in horticultural topsoil than in the coarse sandy soil found in the plant's natural habitats. Although the growth of *A. lancifolium* tends to decrease with an increase in salt concentration, we found that the stem thickness, fresh weight, and dry weight of *A. lancifolium* seedlings subjected to 0.25%–1.0% salt were comparable to those of the control seedling that were not exposed to salt. Furthermore, in contrast to those plants subjected to 2.0% salt, these plants continued to grow and remained viable.

Key words : *Apocynum lancifolium*, Salt concentration, Seed pod, Thousand-seed weight, Halophyte

1. Introduction

Unlike common land, reclaimed land and coastal areas are unique areas, in which soil physical, chemical, and biological factors are complexly intertwined. Moreover, the environment undergoes frequent temporal and spatial changes as a consequence of tidal wave action, and the distribution of plants is limited by the high salinity of the soil (Yang, 1999). In Korea,

most of the coastal salt marshes have disappeared in the wake of large-scale reclamation projects. Nevertheless, reclaimed land has recently been the focus of restoration projects, although these areas are still in need of further reforestation. However, given the generally poor growth base and soil conditions of reclaimed land, there are few plants that are suitable for future landscaping initiatives (Ihm and Lee, 1998).

In Korea, reclaimed land development has been

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promoted in several regions and, to date, the total area of land reclaimed amounts to 135,100 hectares, which is equivalent to approximately 14% of the country's paddy area (Choi et al., 2011). At approximately 28,300 hectares, the Saemangeum reclaimed land area is the largest among the recent land reclamation developments, 30% (8,570 hectares) of which, is destined for agricultural use, such as sites devoted to high-quality high-tech agriculture, export agriculture, and agroecological space (Kang et al., 2014). However, in saline soils, crop growth generally tends to be limited to soils with salt concentrations of less than 0.3% (Kim and Son, 2013), whereas most soils in the Saemangeum reclaimed land area are characterized by concentrations ranging from 0.1% to 2.5%, even within comparatively small areas. Consequently, cultivating plants with weak salt resistance would realistically be incredibly difficult. Therefore, it is important to select plants that can grow stably in reclaimed soil with high salt concentrations.

Apocynum lancifolium (*Apocynum venetum*) Russanov is a perennial herb distributed in Korea, China, Russia, Mongolia, Central Asia, Tibet, and the Himalayas. In Korea, it is designated as a Vulnerable (VU) B2b(iii)c(iii) endangered plant in the National Red List. Currently, it is found sporadically in certain areas along the west coast of Korea and also grows at inland sites in the Chungbuk area, although these populations are threatened with extinction, owing to adverse environmental conditions (Korea Forest Service, 2008; Son et al., 2011).

The plants of this species that grow wild in Xinjiang, Inner Mongolia, and Mt. Baekdu in China, where it is referred to as Napoma (羅布麻), is rich in fiber that is used similarly to the fiber obtained from hemp (麻). Moreover, the leaves of *A. lancifolium* have long been used as a substitute for tea, and thus the plant is also referred to “multi-leaf flower” or “yacha.” Furthermore, clinical trials in China have revealed that Napoma is beneficial in the treatment of high blood

pressure and hyperlipidemia, and it is accordingly cultivated as a medical crop, mainly for use in the treatment of hypertension. The species native to Korea typically has stems of 100 cm to 200 cm in height, and thin and white branches, with the leaves and stems lacking distinct hairs. The rhizome has a woody rootstock, and the lanceolate or oval leaves are dislocated from the main stem and arranged oppositely on the branches. Furthermore, there is a protrusion that is an extension of the leaf line at the round end. The leaves are approximately 2.5–5.5 cm in length and 5–17 mm wide, the lower parts of which are dull or round, and the edges are smooth. The purple flowers bloom in June, and the panicle at the end of the stem is in flower for more than 2 months. Compared to *Amsonia elliptica*, the anthers are appendages at the base the stigma, the corolla is bell-shaped, the fruits ripen into golden stones, and the seeds have hairs.

Areas along the west and south coasts of Korea with wide tidal flats are inhabited by a range of annual and perennial halophytes that are adapted for growth in the saline soils of these tidal flats, reclaimed land, and salt marshes (Kim, 1983 Flowers et al., 1986 ; Shim et al., 2002). Recently, interest in halophytes has increased owing to the associated pharmacological properties of some species and the capacity to purify pollutants, and with the currently higher levels of cultivation, the availability of these plants is rapidly increasing (Min, 1998, Lee et al., 2004; Min,). Given these properties, a number of studies have been conducted to examine the adaptation of certain halophytes to growth in saline soils, including glassworts and herbaceous seepweed. Studies have also been launched on the germination and growth of the halophyte sandspurry (*Spergularia marina*) in response to exposure to salt stress (Lee et al., 1999 , Nam et al., 2007). By determining the salt and general environmental resistance of these plants, such studies are not only important with respect to the development of medicinal crops, but can also contribute to identifying those plants that could be



Fig. 1. The decomposed granite soil (left) and horticultural medium (right) used for seed germination studies.

utilized in the greening of reclaimed land and their development as tourism resource plants. The present study was carried out to provide basic data for the development of a halophyte distribution area as a coastal ecology learning site, by identifying the seed germination characteristics and plant growth of *A. lancifolium* in response to different salt concentrations and soil conditions. We also sought to prepare basic data for increasing the diversity of plant species by creating a halophyte hall in a botanical garden and using this as a coastal ecology education center.

2. Material and Methods

2.1. Plant Materials

The seeds used in this study were harvested in October 2019 from plants growing wild on reclaimed land along the west coast of Korea. The harvested seeds were visually identified as *A. lancifolium*, and following drying at room temperature to a moisture content of 10% to 12%, they were subsequently refrigerated at 4°C until used in the experiments.

To establish seed characteristics, we determined seed pod length, seed weight, the number of seeds per pod, and the thousand-grain weight. Seed germination was examined in Petri dishes that were 90 mm in diameter, by placing 100 seeds on two sheets of filter paper (Quantitative filter paper No. 2; Advantec, Japan) soaked with 5 mL of distilled water. The seeds were then germinated for 10 days in a 25°C incubator, during which time, germination and early growth were

monitored. The effect of salt concentration on germination was determined by treating seeds with salt (NaCl) solutions of the following five concentrations: 0%, 0.25%, 0.5%, 1.0%, and 2.0%.

Furthermore, to examine the effects of light on the germination of seeds, we subjected seeds sown in Petri dishes to light (90 $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and dark treatments. For dark treatments, the Petri dishes were wrapped in aluminum foil and then placed in a paper box to completely block exposure to light.

Furthermore, to examine germination under soil conditions similar to those of the general habitat environment (soil bulk density greater than 1.6 g/m^3 , pH 7.4, EC 0.3), we sowed seeds (four seeds per hole in 72-hole trays) in a horticultural medium (volume density 0.3 mg/m^3 , pH 5.5–7.7, EC 0.6) used as soil and in comparative trays of decomposed granite soil. The trays were placed in a greenhouse and watered once daily over a 2-week period in parallel with soil germination assessments (Fig. 1).

2.2. The effects of salt concentration on plant growth

To examine the effects of salt concentration on plant growth, in May 2021, we sowed seeds of *A. lancifolium* in 6-cm-diameter pots (3 to 5 seeds per pot) containing horticultural soil (moisture content $40\% \pm 10\%$, soil bulk density 0.3 mg/m^3 , pH 5.5–7.7, EC 0.6). The pots were placed in a vinyl greenhouse and the plants were grown until they reached a height of over 5 cm. Selected plants were then treated with



Fig. 2. The capsules and seeds of *A. lancifolium*.

one of five concentrations of NaCl (0%, 0.25%, 0.50%, 1.00%, and 2.00%), with each treatment applied to plants in 10 pots. Pots were irrigated twice weekly with the respective NaCl solutions for 2 months, after which, growth was compared with respect dry weight, plant height, stem thickness, and number of leaves.

For statistical processing, the significance of data was assessed by applying a one-way analysis of variance implemented in Excel.

3. Results and Discussion

3.1. Seed germination

The seeds of *A. lancifolium*, which are characterized

by hairs, are borne within elongated capsules that develop as the fruit matures (Fig. 2). On the basis of an examination of 10 seed pods, we established that on average, each pod contains approximately 103 seeds.

The average length and weight of seed pods were 13.36 mm and 0.23 g, respectively, whereas the average thousand-grain weight was 0.59 g (Table 1). Compared to small pods, larger pods were found to be relatively heavier and contain a larger number of seeds.

The seeds of *A. lancifolium* were considered to have germinated when cotyledons penetrated the ruptured seed coat and, as shown in Figure 3 indicates that 90.0% and 88.3% of seeds germinated under the light

Table 1. The length and weight of *A. lancifolium* seed pods

Fruit pod No	Weight (g)	Length (mm)	Number of seeds in a pod (ea)
1	0.26	15.0	120
2	0.22	11.0	80
3	0.23	12.0	90
4	0.23	12.5	87
5	0.22	13.2	120
6	0.32	15.1	150
7	0.21	16.1	140
8	0.22	12.5	70
9	0.20	13.0	80
10	0.22	13.2	90
Average±Standard deviation	0.23±0.034	13.36±1.574	102.7±27.680

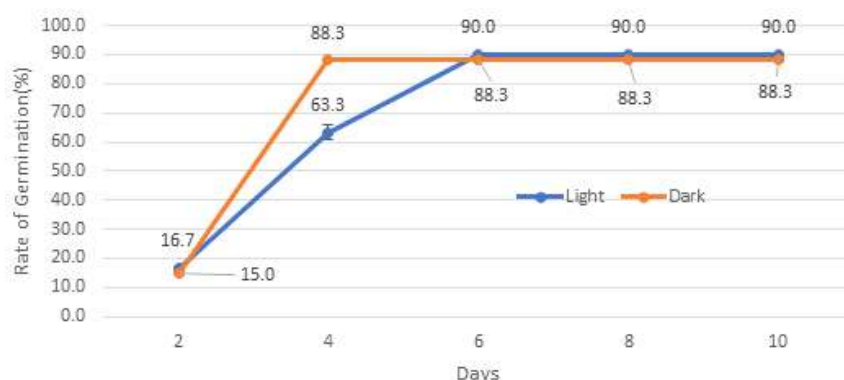


Fig. 3. Changes in the rate of seed germination under light and dark conditions. Data are presented as the means \pm standard deviation of triplicate treatments. Bars indicate the standard deviation.

and dark condition, respectively. Our investigation of the rate and duration of seed germination revealed that seedlings initially appeared 2 days after sowing, and after 3 days, cotyledons began to appear above the ground as the seedlings elongated. After 4 days, 63.3% and 88.3% of seeds had germinated under light and dark conditions, respectively, indicating a high initial germination rate. After 6 days, respective germination rates of 90% and 88.3% were recorded, although these showed no further increases at the final assessment after 10 days (Fig. 3).

Our examination of the effects of salt concentration on seed germination revealed that 90.0% of seeds germinated at salt concentrations of 0.00%, 0.25%, and 0.50%, indicating that salt concentrations up to 0.5% have little in the way of adverse effects on the germination of *A. lancifolium* seeds. In contrast, however, exposure of seeds to a 1.0% saline solution reduced germination to 55%, whereas germination was completely inhibited by exposure to a 2.0% saline solution (Fig. 4, 5). Notably, however, our observation that *A. lancifolium* seeds failed to germinate when exposed to a salt concentration of 2.0% is consistent with the response observed in another halophyte, salt sandspurry, thus indicating that even the growth of halophytes is limited at NaCl concentrations of 2% and

above (Jeong et al., 2014).

Although our observations differ slightly from those on the seed germination of common-seepweed (*Suaeda glauca*) adapted to coastal salt marshes, in which the germination rate is higher in distilled water than in brine, high germination rates in the absence of salt have similarly been reported for most other halophytes (Ungar, 1995; Khan et al., 2002; Wei et al., 2008; Lee et al., 2016). This can be seen as a general germination characteristic of halophyte seeds, and can be interpreted as an ecological adaptation that contributes to broadening the variability of the germination period in salt marshes.

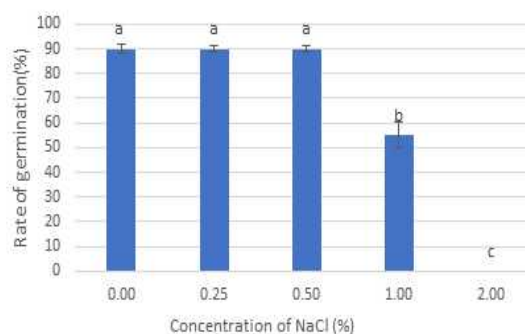


Fig. 4. The effect of salt concentration on the *A. lancifolium* seed germination rate (%). Data are presented as the means \pm standard deviation of triplicate treatments. Bars indicate the standard deviation.



Fig. 5. The effect of salt concentration on *A. lancifolium* seed germination [from left to right: control (0%), 0.25%, 0.5%, 1.0%, and 2.0% saline].

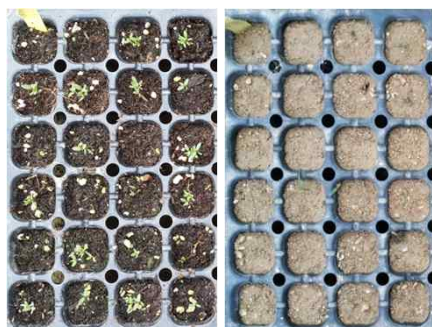


Fig. 6. The germination of *A. lancifolium* in horticultural soil (left) and habitat soil (right).

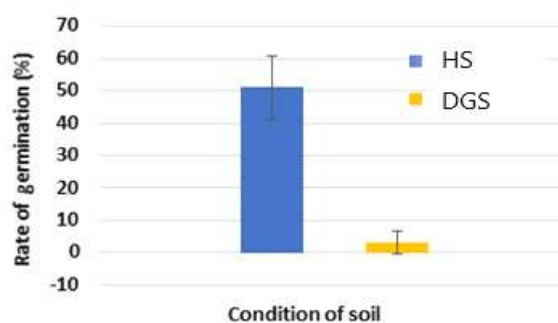


Fig. 7. The effect of soil type on the germination of *A. lancifolium* (%). Data are presented as the means \pm standard deviation of triplicate treatments. Bars indicate the standard deviation. HG: horticultural soil, DGS: decomposed granite soil.

To examine the effect of soil medium type on the germination of *A. lancifolium* seeds, we assessed germination status in two soil types, namely, a horticultural soil and decomposed granite soil, the latter of which is the soil found in the natural habitat of the *A. lancifolium* plants which we collected the seeds used in the present study. The results are presented in Fig. 6, 7.

We found that seed germination was higher in horticultural topsoil than in soil collected from the plants' natural habitat, and suspect that the lower rate of germination in the natural soil could be attributable to the low water-holding capacity of this soil under the same once-daily irrigation conditions.

3.2. The effects of salt treatment on plant growth

Our examination of the effects of salt (NaCl) treatment (0%, 0.25%, 0.50%, 1.00%, and 2.00%) on

seedling growth revealed that the growth of seedlings exposed to salt concentrations of 0.25% and 0.50% was similar to that of the control seedlings (0% NaCl) when viewed with the naked eye. However, exposure to salt concentrations of 1.00% or 2.00% was found to cause a visible reduction in plant in height, although leaf and overall plant growth appeared little affected by these treatments (Fig. 8). Notably, we also detected differences in the growth of seedlings subjected to the same salt treatment. Given that the seedlings used in this experiment were selected from among those plants that had initially grown to a height of more than 5 cm in pots sown with 3 to 5 seeds, we assume that the observed difference in growth were not influenced by possible differences in pot conditions. We also observed that although the initial stems of *A. lancifolium* often died when exposed to a salt

concentration of 2.00%, new shoots were generated from the below-ground rhizomes and continued to grow at this concentration. This reflects the fact that *A. lancifolium* grows as a perennial herb or small shrub that develops rhizomes after germination from seeds and this attribute is considered to facilitate strong vitality even when plants are exposed to high salt concentrations.



Fig. 8. The effects of salt concentration on *Apocynum lancifolium* growth. From left to right: seedlings treated with 0%, 0.25%, 0.50%, 1.00%, and 2.00% saline (NaCl) solutions.

The total fresh and dry weights of plants (leaves, stems, and roots) exposed to salt concentrations 0.00%, 0.25%, 0.50%, 1.00%, and 2.00% were 0.63 (0.28), 0.67 (0.17), 0.67 (0.13), 0.85 (0.14), and 0.28 (0.13) g, respectively. We also observed that plant

height, stem thickness, and leaf number decreased with an increase in salt concentration. However, compared to the control group, there were no significant differences in the stem thickness or number of leaves in plants exposed to salt concentrations of 0.25%, and 0.50% (fresh weight of leaves: $p = 0.91$, fresh weight of stems: $p = 0.46$, dry weight of leaves: $p = 0.7691$, and dry weight of roots: $p = 0.11$) (Table 2). The growth of the top and root parts of plants showed a tendency to decrease with increasing salt concentration. However, stem thickness was found to be higher in plants subjected to the 0.25%, 0.5%, and 1.0% salt treatments, and these plants continued to grow and remained viable. A similar non-significant effect of salinity has been reported for the growth of *Tetragonia tetragonoides* (Kim et al., 2011). However, in contrast to the findings of the present study, these authors observed a continuous reduction in growth in response to treatment with 50 mM (approx. 0.3%) of salt (Kim et al., 2011; Yousif et al., 2010). In terms of the growth and physiological characteristics of halophytes at different salt concentrations, Kim et al. (2019) observed optimal growth at a concentration of 50 mM, whereas Flowers (1977) found that the accumulation of salt in halophytes is closely related to the absorption of water via osmotic control. Sodium (Na^+) is an essential element in halophytes and these plants have the ability to accumulate large amounts of salt. It was said that salt has differential effects on different aspects of plant

Table 2. The effects of salt (NaCl) concentration (%) on the growth of *A. lancifolium* plants

NaCl (%)	L (g)		S (g)		R (g)		H (cm)	SD (cm)	LN (ea)	FN (ea)
	FW	DW	FW	DW	FW	DW				
0.00	0.13	0.03	0.27	0.11	0.23	0.14	36.44	1.10	11.00	2.00
0.25	0.10	0.02	0.34	0.06	0.23	0.09	32.44	1.00	11.25	1.71
0.50	0.07	0.02	0.38	0.03	0.22	0.08	29.44	1.00	7.40	2.42
1.00	0.09	0.03	0.62	0.05	0.14	0.06	24.33	0.93	8.60	2.42
2.00	0.08	0.03	0.10	0.02	0.10	0.05	11.38	0.99	8.90	2.00

FW (Fresh weight), DW (Dry weight), L (Leaf), S (Stem), R (Root), H (Height), SD (Stem diameter), LN (Leaf number), FN (Flower number).

growth. For example, while plant height tends to decrease with increasing salt concentration, what tends to remain constant are the amount of water, the fresh and dry weight of leaves, and the fresh and dry weights of stems and roots; no differences in sensitivity are observed. In the present study, we found that the dry matter of leaves increases, the yield of seeds decreases, and the dry matter of stems and roots remains constant with increasing salt concentration. These findings tend to differ from those reported for *Tetragonia tetragonoides* by Kim et al. (2011), in which different organs tended to be characterized by differing extents of salt storage, and the reproductive system was more sensitive to salt than the vegetative system.

In this study, we sought to assess the tolerance of *A. lancifolium* to salt stress. On the basis of our findings for growth in response to exposure to different NaCl concentrations, we found that this herbaceous species can tolerate relatively high concentrations (2.0%) of NaCl. However, compared to above-ground growth, we found that the growth of below-ground parts decreased in plants exposed to high salt concentrations, which was similar to the results obtained for *Carex lenta* and *Typha angustifolia* (Shim et al., 2012). A similar trend to that observed in *A. lancifolium* has also been reported for *Salicornia herbacea*, which can adapt to a wide range of salinity from 0% to 40% NaCl and grows when treated with 200 mM (1.16%) NaCl (Baik et al., 2011; Kim et al., 2019). Furthermore, an examination of the response of *Dianthus japonicus* to salt stress revealed that seed germination was little affected by treatment with 100 mM NaCl, whereas germination tended to be poor when seeds were exposed to salt at 200 mM, and severe stress symptoms were observed at the seedling stage in response to treatment with 100 mM NaCl (Heo et al., 2007). In addition, it has been reported that germination rates as high as 90% have been obtained for *Spergularia marina* when exposed salt concentration up to 1.0%, whereas no germination was

observed at 2.0% NaCl. Among *S. marina* plants cultured for 8 weeks in the presence of different salt concentrations (0 mM, 50 mM, 100 mM, 200 mM, 300 mM, and 400 mM), it was found that those subjected to 50 mM NaCl showed the best growth in terms of plant height and dry matter content (Jeong et al., 2014).

Collectively, the findings of the present study indicate that the seed germination and plant growth of *A. lancifolium* are not significantly affected by salt in the concentration range of 100 mM to 200 mM (0.58% to 1.16%) and, consequently, this species is considered to have potential use as a salt-resistant plant in landscaping.

With respect to the vulnerability of *A. lancifolium* in its natural habitats, we suspect that the composition and structure of the moisture-deficient soils in these areas tend to be unfavorable for germination and growth. Moreover, these remaining natural habitats are rapidly disappearing as a consequence of rampant urbanization and insensitive development. Given the drought-prone nature of soils in these habitats, it is considered that seed germination would be markedly suppressed, and that it would be difficult to maintain or increase population sizes naturally. However, by optimizing the germination temperature and soil conditions artificially, we believe that *A. lancifolium* can be cultivated not only as a specific income crop for reclaimed land reforestation and agriculture, but also be utilized as a tourism resource, such as in ecological parks, nature learning centers, and halophyte gardens.

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