



Effect of Seed Mucilage on the Germination of *Plantago Asiatica* and *Plantago Lanceolata* under Salt Stress Conditions

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Abstract

Although the role of seed mucilage in plants in arid areas has been well studied, there have been few investigations on the role of mucilage in plants in non-arid areas. In this study, we hypothesized that seed mucilage of *P. asiatica*, which is usually found in temperate, humid areas, may play a role in protecting seeds from external stress. This study analyzed the effect of seed mucilage on germination under salt stress in *P. asiatica* and *P. lanceolata*, which are commonly found in humid and arid areas, respectively. Under salt stress conditions, the seed germination rate of *P. asiatica* did not decrease, with or without mucilage. This suggests that the seeds of *P. asiatica* are originally salt-tolerant, regardless of seed mucilage. In contrast, in *P. lanceolata*, the germination rates of seeds without mucilage decreased compared to that of seeds with mucilage under salty conditions (NaCl 200 mM). From this, it is supposed that seed mucilage of *P. lanceolata* plays a role in protecting the germinating seed from external stresses, such as salt stress. In addition, the average germination time of seeds with mucilage tended to be shorter than that of seeds without mucilage in *P. lanceolata*; therefore, seed mucilage may promote its germination. In direct contrast, in *P. asiatica*, the average germination time of seeds with mucilage was approximately 1 to 2 days longer than that of seeds without mucilage. It could be considered that this delay in germination aids in the safe transport of seeds of *P. asiatica* across greater distances.

Keywords: Mucilage, Germination, *Plantago Asiatica*, *Plantago Lanceolata*, Stress condition

Introduction

Some plant species have a sticky substance on their seeds referred to as 'mucilage' (Western. 2012; Yang. *et al.*, 2012). There are numerous studies on the role of seed mucilage in plant growth and development (Yang. *et al.*, 2012). One of the key roles of seed mucilage is the retention of water under dry conditions (Korobkov. 1973; Young and Martens. 1991; Huang and Gutterman. 1999a; Huang. *et al.*, 2000). It also acts as an anchor that rapidly absorbs water, and fixes the seeds in soil (Fahn and Werker. 1972; Evenari. *et al.*, 1982; Gutterman. 1990. 1993; Huang. *et al.*, 2000). In addition, mucilage of *Artemisia sphaerocephala* has been reported to aid seed germination under salt stress conditions in deserts where

salt accumulation occurs (Yang. *et al.*, 2010), and the biodegraded polysaccharides of mucilage provide nutrients for the growth of seedlings after seed germination under poor nutrient conditions (Yang. *et al.*, 2012). It has also been reported that the mucilage of the Acanthaceae plant species, found in the Negev Desert, acts as a physical barrier to oxygen and suppresses germination during the irrigation period in the rainy season (Witztum. *et al.*, 1969).

These roles of mucilage are advantageous for seed survival, germination, and colonization of arid areas. Since many plants with mucilage in seeds are distributed in arid areas (Mott. 1974; Huang and Gutterman. 1999b;

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Gorai. *et al.*, 2014; Zhao. *et al.*, 2021), much of the research on the effect of mucilage in seeds have been conducted on the plants in these areas. However, although some plants with mucilage in seeds are distributed in humid areas, there is little research on its role in such plants. Therefore, to further elucidate the role of plant mucilage, it is necessary to conduct surveys and investigations on plants in habitats other than arid areas (Yang. *et al.*, 2012).

Plantago asiatica is a typical plant from Japan that has seed mucilage in a humid subtropical climate with heavy rainfall throughout the year. *P. asiatica* is a major constituent species of trampling plants (Miyawaki. 1964). When seeds of *P. asiatica* get wet in the rain, mucilage is exuded from the seed that adheres to the soles of shoes and tires of automobiles. Therefore, it is generally considered that the seed mucilage of *P. asiatica* is useful for seed dispersal. However, the ecological significance of mucilage in *P. asiatica* has not been fully studied. Although *P. asiatica* is not a halophyte, it is well known to adapt and grow even in highly saline soils (Hayasaka and Fujiwara. 2006). Based on this, we hypothesized that mucilage of *P. asiatica* may play a role in protecting seeds from external stresses, such as salinity. In this study, we compared the effect of mucilage on seed germination under salt stress conditions between *P. asiatica*, which grows in the humid regions of Asia and the ribwort plantain *P. lanceolata*, which is native to Europe, which has less rainfall.

Materials and Methods

Plant Material

Tested seeds of *P. asiatica* (collected in Niwagun, Aichi Prefecture, December 11, 2017) and *P. lanceolata* (collected in Noda City, Chiba Prefecture, July 12, 2017) were supplied by ESPEC MIC Corp. For each type, "seeds without mucilage" (from which mucilage was removed) and "seeds with mucilage" (which were untreated), were prepared. The seeds without mucilage were obtained by repeatedly wiping the mucilage exuded from the seeds with pulp paper until no further exudation of mucilage was observed. The

seeds with mucilage were supplied with distilled water in advance, and seeds with mucilage were used for the test.

Germination Test

Three test plots of NaCl solutions—an NaCl 100mM plot, an NaCl 200Mm plot, and a distilled water control—were set up in 90 mm diameter plastic petri dishes (SANSEI MEDICAL CO.,LTD, Kyoto) for the germination test with aqueous NaCl solution (Wako Pure Chemical Industries, Tokyo). Filter paper was laid on the 90 mm diameter plastic petri dish. Twenty-five seeds were placed in each dish, and 2 mL of the respective solutions was added to each petri dish. Three replicates were used for each plot. The plates were kept under temperature conditions of 25 °C and a 12 h photoperiod. The germination test was started on May 28, 2021, and the number of germinated seeds was counted every 0.5 weeks.

Calculation of Average Germination Period

The average germination period was calculated using the following equation (Bewley and Bradford. 1986; Yamasue, 2001):

$$\text{Average germination time (in days)} = \frac{\sum (t \times n)}{\sum n}$$

(n: number of germinated seeds; t: days after placement).

Data Analysis

Data obtained in this study were analyzed using the BellCurve for Excel 5.0 (Social Survey Research Information Co., Ltd.) software. After performing analysis of variance, Tukey's multiple range test was conducted to detect significant differences among the treatments with a confidence interval of 95% ($\alpha = 0.05$).

Results

Table 1 shows the seed areas of *P. asiatica* and *P. lanceolata* both with and without mucilage. The seed areas of *P. asiatica* and *P. lanceolata* were similar to each other. Seeds of *P. lanceolata* exuded a large amount of mucilage, and the ratio of exudate area of mucilage relative to the seed area was extremely large, approximately 7.14, compared with that of *P. asiatica*, 4.60 (Table 1).

Table 1: Areas of *P. asiatica* and *P. lanceolata* seeds with and without mucilage

species	Seed area without mucilage (a) (mm ²)	Seed area with mucilage (b) (mm ²)	b/a
<i>Plantago asiatica</i>	2.84 ± 0.41	4.60 ± 0.39	1.62
<i>Plantago lanceolata</i>	2.63 ± 0.74	7.14 ± 3.24	2.72

In the control plot of *P. asiatica*, the germination rate at 0.5 weeks was higher in the seeds without mucilage than in seeds with mucilage (Fig.1-a). However, after a period of 1 week, there was no difference in the germination rate of seeds without mucilage and those with mucilage (Fig.1-a). In contrast, in the control plot of *P. lanceolata*, the germination rate at 0.5 weeks was lower in the seeds without mucilage than in the seeds with mucilage (Fig.1-d). There was no difference in germination rate between the seeds without mucilage and the seeds with mucilage after a period of 1 week (Fig.1-d).

In the NaCl 100 mM (Fig.1-b) and NaCl 200 mM plots (Fig.1-c) of *P. asiatica*, a decrease in the final germination rate due to NaCl stress was not observed in either the seeds without or with mucilage. The germination rate of *P. asiatica* in both the plots was similar to that in the control plot. Although there was no

difference in the final germination rate in the seeds without and with mucilage, the seeds without mucilage germinated earlier than those with mucilage (Fig.1-c, Fig.1-d).

Germination rate of *P. lanceolata* in the NaCl 100 mM plot was similar to that of the control plot. Though there was no difference between the final germination rates of the seeds without and with mucilage, the germination rate at 0.5 weeks was lower in the seeds without mucilage than in those with mucilage (Fig.1-e). On the other hand, in *P. lanceolata*, the final germination rate in the NaCl 200 mM plot was lower than that in the control plot (Fig.1-f). At higher concentrations (200 mM) of NaCl, the suppression of germination was more pronounced in the seeds without mucilage, and the germination rate remained lower in the seeds without mucilage than in those with mucilage (Fig.1-f).

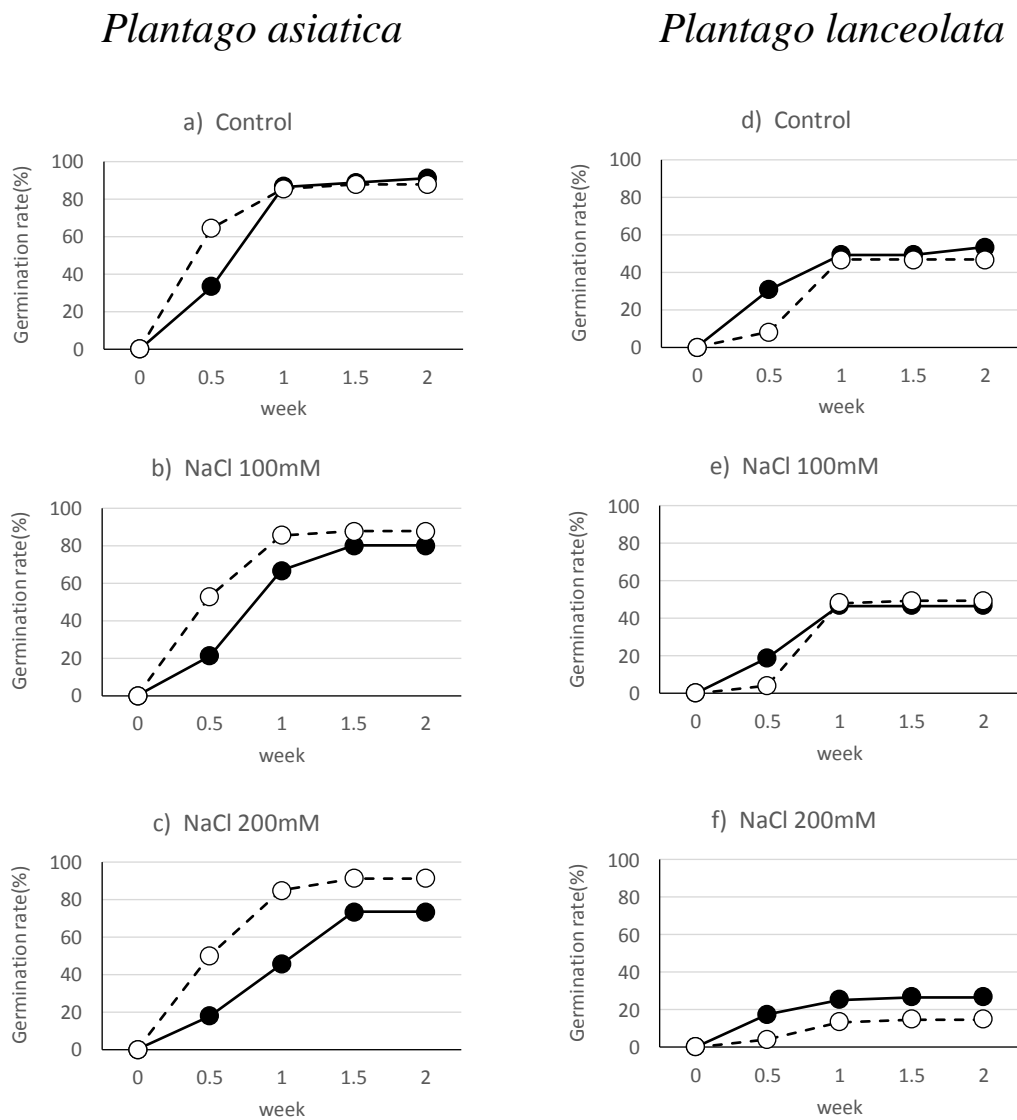


Fig 1: Effect of mucilage on the germination of *P. asiatica* and *P. lanceolata* seeds under salt stress
 ●: seeds with mucilage, ○: seeds without mucilage.

The average germination time of *P. asiatica* seeds was statistically longer in the seeds with mucilage than in those without mucilage, regardless of the concentration of NaCl, and the germination of the seeds with mucilage tended to be delayed by approximately 1 to 2 days in comparison to that of the seeds

without mucilage (Fig.2). In contrast, the average germination time in the 100mM and 200mM plots of *P. lanceolata* seeds was significantly shorter in the seeds with mucilage than in those without mucilage (Fig.2).

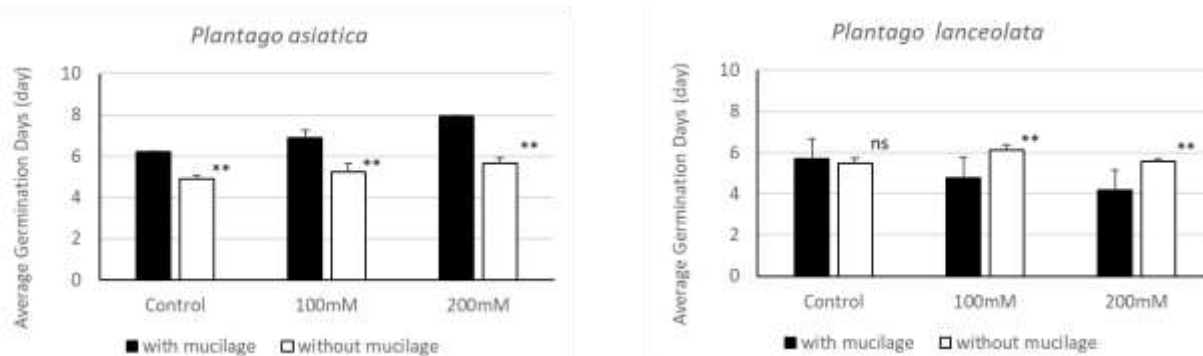


Fig 2: The average germination days of *P. asiatica* and *P. lanceolata* seeds with and without mucilage under salt stress

Error bars signify standard deviation (n=3)

** Significant difference at 1 % level by the t test. ns, no significant difference was observed.

Discussion

Plantago is a genus of plants that consists of over 200 different species, and occurs mainly in the temperate zones around the world. There are many cosmopolitan species within *Plantago* that are widely distributed and naturalized. Four species of *Plantago* are endemic to Japan, including *P. asiatica*, whereas others such as *P. lanceolata*, are naturalized. Many species of *Plantago* bear seed mucilage but some members such as *P. virginica*, do not show this characteristic. It has been reported that the mucilage of *P. lanceolata*, *P. coronopus*, *P. albicans*, and *P. lagopus*, all of which are native to arid areas, improves seed-soil adhesion, facilitates water absorption, and aids in germination under drought stress (Gutterman and Shem-Tov. 1994; Teixeira. *et al.*, 2020). Although members of *Plantago*, which produce abundant mucilage are often distributed in arid areas, *P. asiatica* is endemic to relatively humid areas (Teixeira. *et al.*, 2020). As a result, the function of mucilage in retaining water in these plants would likely have little evolutionary advantage. We have hypothesized that mucilage of *P. asiatica* may play a role in protecting against external stress that are not caused by arid conditions.

Contrastingly, *P. lanceolata* is another *Plantago* species that is native to Europe and is a naturalized plant that was introduced to Japan at the end of the Edo period. *P. lanceolata* plantain grows naturally near beaches, and is said to be is salt-tolerant

(Mira. *et al.*, 2018). However, in the present study, we reported that the germination rate of *P. lanceolata* seeds decreased under salt stress conditions. In addition, the germination rate of seeds without mucilage was lower than that of seeds with mucilage in NaCl 200 mM. From this, we hypothesized that seed mucilage of *P. lanceolata* plays a role in protecting the germinating seed from external stresses such as salt stress. In contrast, the germination rate in *P. asiatica* did not decrease under conditions of increased salinity regardless of mucilage. This suggests that the seeds of *P. asiatica* are originally salt-tolerant, and independent of seed mucilage. There was no difference between the final germination rates of *P. asiatica* seeds with or without mucilage. However, the germination rate of seeds with mucilage after 0.5 week was lower than that of seeds without mucilage, and tended to be delayed compared to that of seeds without mucilage. The average germination time of seeds with mucilage was approximately 1 to 2 days longer than that of seeds without mucilage. Therefore, we presumed that mucilage of *P. asiatica* has a slight delaying effect on seed germination. The mucilage bearing seeds of *P. asiatica* adhere to the soles of shoes and tires of automobiles (Miyawaki. 1964). A delay of approximately 1 to 2 days in germination may contribute to the safe transport of *P. asiatica* seeds across greater distances. A similar phenomenon in which seeds with mucilage exhibit delayed germination compared to seeds without mucilage has been reported in

Artemisia sphaerocephala, which is an Asteraceae plant. It has been postulated that this delay in germination is caused by the absorption of water by the mucilage and the delayed arrival of water in the seeds (Huang and Gutterman, 1999b). On the other hand, the present study revealed that the average germination time of seeds with mucilage tended to be shorter than that of seeds without mucilage in *P. lanceolata*. Therefore, seed mucilage of *P. lanceolata* may promote germination.

Conclusion

We reported that it has a delaying effect on seed germination, and that seed mucilage of *P. asiatica* may promote seed dispersal over longer distances. *P. asiatica* grows on roadsides, and seed dispersal occurs through adhesion of the seed mucilage to shoes and tires. However, it would be natural to consider that the mucilage of *P. asiatica* was originally produced for some additional role other than only seed dispersal. Thus, it may be possible that the delaying effect of mucilage on germination enhances the advantages conferred by mucilage dependent seed dispersal in *P. asiatica*. However, we did not confirm the seed-protective effect of seed mucilage in *P. asiatica* in this study. Further studies are required to determine the potential original functions of seed mucilage in *P. asiatica*.

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