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Research Article

Functional Roles of Seed Mucilage of Capsella Bursa-Pastoris L. on Seed Germination and Dispersal

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Abstract

The seed mucilage of some plant species is suitable for use in dry regions because of its water-retaining properties. However, the role of mucilage in plants growing in humid regions has not yet been fully elucidated. In the present study, we investigated the effect of seed mucilage in Capsella bursa-pastoris (L), which grows in temperate humid regions, on seed germination and dispersal. Seeds with mucilage had higher germination rates than those without mucilage. This suggests that seed mucilage may be related to the dormancy of C. bursa-pastoris seeds. In addition, our study revealed that under conditions where dormancy was broken by temperature alteration, seeds with mucilage germinate 1–2 days later than seeds without mucilage. In a walking experiment in which seeds with vesicles were attached to shoes, 61.4% of the seeds were carried a distance of 500 m. From these results, it is suggested that the seed mucilage of Capsella bursa-pastoris functions in adhesion and dispersal and also may delay germination during the period when the seeds are moving.

Keywords: *Capsella bursa-pastoris, mucilage, germination, asynchronous emergence, seed dispersal.*

Introduction

Some plant species produce mucilage in their seeds (Western, 2012; Yang *et al*., 2012). Seed mucilage is produced by 110 angiosperm families and at least 230 genera (Yang *et al*., 2012). The adaptive value of mucilage has attracted the attention of plant ecologists, and numerous studies have been conducted on its functions (Yang *et al*., 2012). Seed mucilage has ecological roles such as 1) seed maturation within fruits, 2) seed dispersal, 3) seed bank maintenance, 4) seed dormancy, 5) seed germination, and 6) seedling growth (Yang *et al*, 2012). One of the most important functions of seed mucilage is the retention of moisture under dry conditions (Korobkov, 1973; Evenari *et al*.,1982; Young and Martens, 1991; Huang and Gutterman, 1999a; Huang *et al*. 2000). The water-retaining capacity of seed mucilage is advantageous for seed survival, anchorage to soil, germination, and seedling establishment in arid regions. Many plants with mucilage in their seeds are found in dry regions (Mott, 1974; Gutterman, 1990, 1993; Huang and Gutterman, 1999b; Gorai *et al*., 2014; Zhao *et al*., 2021). Although some plants with mucilage in their seeds are distributed outside arid regions, few studies have been conducted on the role of seed mucilage in such plants. Therefore, to further elucidate the ecological role of seed mucilage, it is necessary to investigate plants in habitats other than arid regions (Yang *et al*., 2012).

In the arid state of Nevada, USA, many plants that produce mucilage have been reported to be important weed species (Young and Evans, 1973). These findings suggest that seed mucilage confers considerable ecological advantage to species that colonize disturbed habitats (Yang *et al*., 2012). In contrast, in Japan, a humid region of monsoon Asia with heavy rainfall, few weed species that have a mucilaginous seed found in agricultural environments are fertile. Typical Japanese weed species that produce seed mucilage include *Plantago asiatica* L. (Plantaginaceae) and *Capsella bursa-pastoris* L. (Brassicaceae).

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Although the primary role of the seed mucilage of *P. asiatica* has not been fully elucidated, *P. asiatica* is a weed that grows on trails, adheres to the soles of shoes and car tires, and plays a secondary role in seed dispersal (Abe *et al*., 2022; Inagaki and Kishiyama, 2022). Thus, although some knowledge has been gained regarding the seed mucilage of *P. asiatica*, little research has been conducted on the seed mucilage of *C. bursa-pastoris*.

C. bursa-pastoris (shepherd's purse) seeds exhibit deep dormancy and unequal germination times. These factors make it difficult to control *C. bursa-pastoris* (Ishikawa and Abe, 1995). It has been reported that the factors of asymmetric emergence in *C. bursa-pastoris* are due to intra-individual and genetic variations (Salisbury, 1963; Hurka, 1990). Environmental factors that affect the breaking of dormancy in shepherd purses include low temperature, humidity, light, temperature changes, and nitrogen. These complex environmental requirements are thought to be the cause of their asynchronous emergence (Popay and Roberts, 1970a, b). However, seed mucilage has been reported to both promote and inhibit seed germination (Garwood, 1985; Figueiredo, 1986).

Therefore, we hypothesized that seed-coating mucilage may be related to the asymmetry of germination in *C. bursa-pastoris*. In this study, we investigated the effects on seed mucilage on germination of *C. bursa-pastoris*. Moreover, the effect of seed mucilage on seed dispersal in *C. bursa-pastoris* was compared to that in *P. asiatica* which is known to disperse seeds via mucilage.

Material and Methods

Study species

The seeds of *Capsella bursa-pastoris* (collected in the Aichi Prefecture in 2022) were supplied by ESPEC MIC Corp. The seeds were stored at 5°C. For comparison, both seeds with mucilage removed and untreated seeds (with mucilage), were prepared. Seeds without mucilage were obtained by repeatedly wiping the mucilage exuded from the seeds with a pulp paper until no further mucilage exudation was observed.

I: Germination test

The filter paper was laid on a 90 mm diameter plastic Petri dish. Thirty seeds were placed in each dish and 2 mL of water was added to each petri dish. Five replicates were used in each plot. Germinated seeds were counted every twice per week in one month.

Exp. 1. Comparison of germination rates of seeds with and without mucilage

The Petri dished were maintained under temperature conditions of 20 °C and a 12 h photoperiod. The first germination test started on March 31, 2022, and the second germination test was started on May 31, 2024. In the second test, seeds stored at 0°C and -30°C for 7 d were also tested.

Exp. 2. Effect of mucilage thickness on germination time

One hundred seeds were placed individually in a 40 mm plastic Petri dish, and distilled water was added. The thickness of the seed mucilage was calculated as the difference between the area of the vertical diameter × horizontal diameter of the seed before the mucilage oozed, and the area of the vertical diameter × horizontal diameter of the area including the oozed mucilage. The number of days until each seed germinated was investigated under 20°C and a photoperiod of 12 h.

Exp.3. Comparison of germination rates of seeds between seeds with and without mucilage under alternating temperature conditions

The Petri dishes were kept under temperature conditions of 5/20 °C, 10/20°C, and 20/20°C as per Popay and Roberts (1970a). Under diurnal temperature alternations, the seeds were held at the lower temperature and in the dark for 19 h and at the higher temperature and light for 5 h each day. Germination tests began on September 19, 2024.

The number of days until 50% of final germination rate was reached (G50) was calculated. The average germination period was calculated using the following equation (Bewley and Bradford, 1986; Yamasue, 2001):

Average germination time (days) = Σ (t × n) / Σn
where n is the number of germinated seeds and t is the number of days after placement.

II: Walking Experiment
This study was conducted on an asphalt road at the Center for Education and Research in Field Science at Shizuoka University. The walking experiments were performed on November 12, 2024.

The same experimental trials were repeated by all three researchers. One hundred seeds were trampled by stepping on them 20 times. The researchers walked a distance of 25m back and forth at a constant normal speed, and all seeds attached to the shoes were counted at each observation point (1, 2, 5, 10, 15, 25, 50, 100, 200, 300, 400, and 500m from the starting point). The test was administered three times to each person for seeds both with and without mucilage.

For comparison, the same test was also performed on seeds of *P. asiatica* (collected in Aichi Prefecture, 2017) stored at 5°C.

Data analysis
The data obtained in this study were analyzed using Bell Curve for Excel 5.0 (Social Survey Research Information Co., Ltd.). After performing analysis of variance, T-test after aricsin transform was used for the comparison of seeds between seeds with and without mucilage.

Tukey's multiple range test was conducted to detect significant differences among the treatments with a confidence interval of 95% (α = 0.05) for the difference of thickness of the mucilage of seeds.

Results

I: Germination test

Exp.1 Comparison of germination rates of seeds with and without mucilage
Fig. 1 shows the germination rates of seeds of with and without mucilage. When seeds were stored in a refrigerator at 5°C, the germination rate was higher in the seeds without mucilage than in the seeds with mucilage. On the other hand, when seeds were stored in a freezer at 0°C or -30°C for 7 d, the germination rate was increased to the same extent in the seeds with and without mucilage as in the seeds with and without mucilage.

Exp.2 Effect of mucilage thickness on germination time
Fig. 2 shows the relationship between the seed size and seed mucilage thickness. Seed size varied from 0.4 mm^2 to 0.8 mm^2. The seed thickness varied from 0 mm^2 to 0.6 mm^2. No significant correlation was observed between seed size and seed mucilage thickness (p=0.56). Fig. 3 shows the difference in the mucilage thickness of seeds with different germination dates. The thickness of the seed mucilage was similar in ungerminated seeds, seeds that took 20 days to germinate, and seeds that germinated within 1 d. In contrast, seeds that germinated within 1–7 d had a significantly thicker seed mucilage.

Exp.3. Comparison of germination rates of seeds between with and without mucilage in alternating temperature conditions

Table 1 shows the final germination rate, number of days to reach 50% of the final germination rate (G50), and average number of days to germination at the end of the test. At 20°C–20°C, where no temperature change was performed, the germination rate was slightly higher without mucilage, but both with and without mucilage were low. At 5–20°C and 10–20°C, where temperature change treatment was performed, the germination rate increased (Table 1). No clear difference was observed in the final germination rate between the non-mucilage and mucilage groups (Table 1). G50 was approximately 1–2 days slower in the plots with mucilage seeds than in those without mucilage seeds in all temperature-change test plots (Table 1). The average number of days to germination was also approximately 1–2 days slower in the mucilage-treated seeds than in the seeds without mucilage in all temperature change test groups (Table 1).

Table 1. Effect of the presence or absence of mucilage on germination characteristics.

	5–20°C		10–20°C		20–20°C区	
	With	Without	With	Without	With	Without
Final germination rate (%)	35.1	35.4	31.8	35.3	5.3	7.1
G50 (days)	8.3	6.3 **	5.9	4.4 **	5.3	2.7
Average germination days (days)	10.5	8.6 **	8.8	7.1 **	8.9	6.7

G50：Number of days until 50% of final germination rate is reached.

** indicate significant difference at 1% level in T-test after aricsin transform.

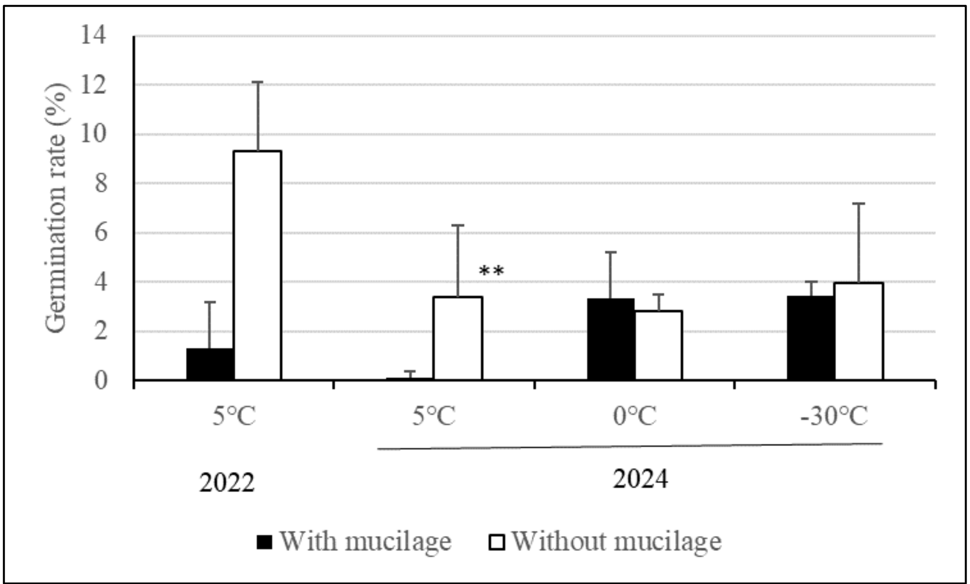

Fig. 1 Comparison of germination rates of seeds with and without mucilage. Error bars indicate standard deviation.

** indicates significant difference at 1% level in t-test after aricsin transform.

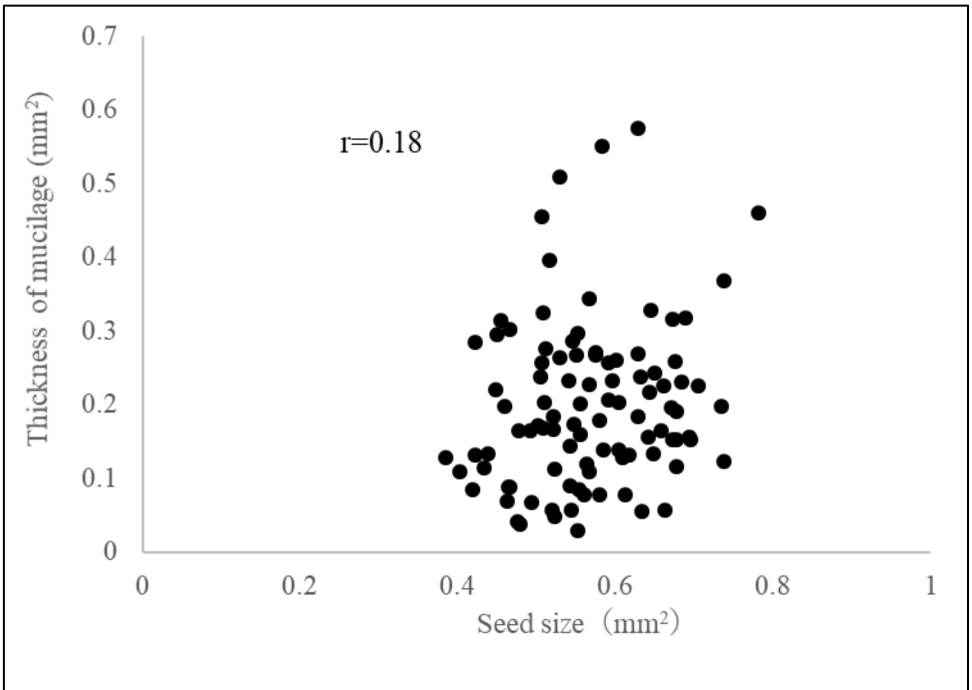

Fig. 2 Correlation between seed size and thickness of mucilage.

Seed size: Seed length x width
Thickness of mucilage: Length x width of area including insect storage

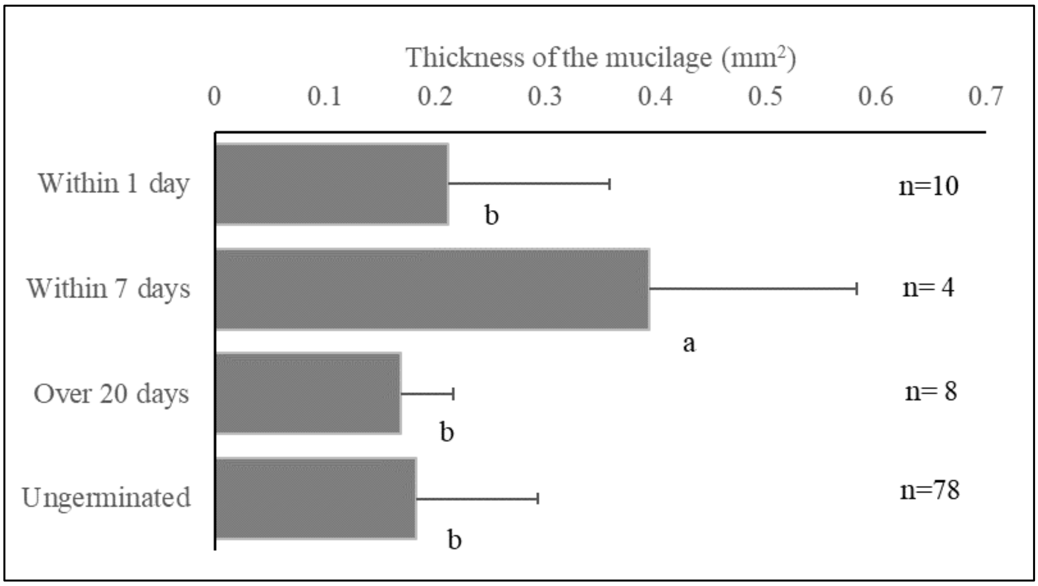

Fig. 3 Difference in the thickness of seed mucilage with different germination rates. Error bars indicate standard deviation.

Different letters indicate significant differences based on Tukey's multiple test at the 5% significance level.

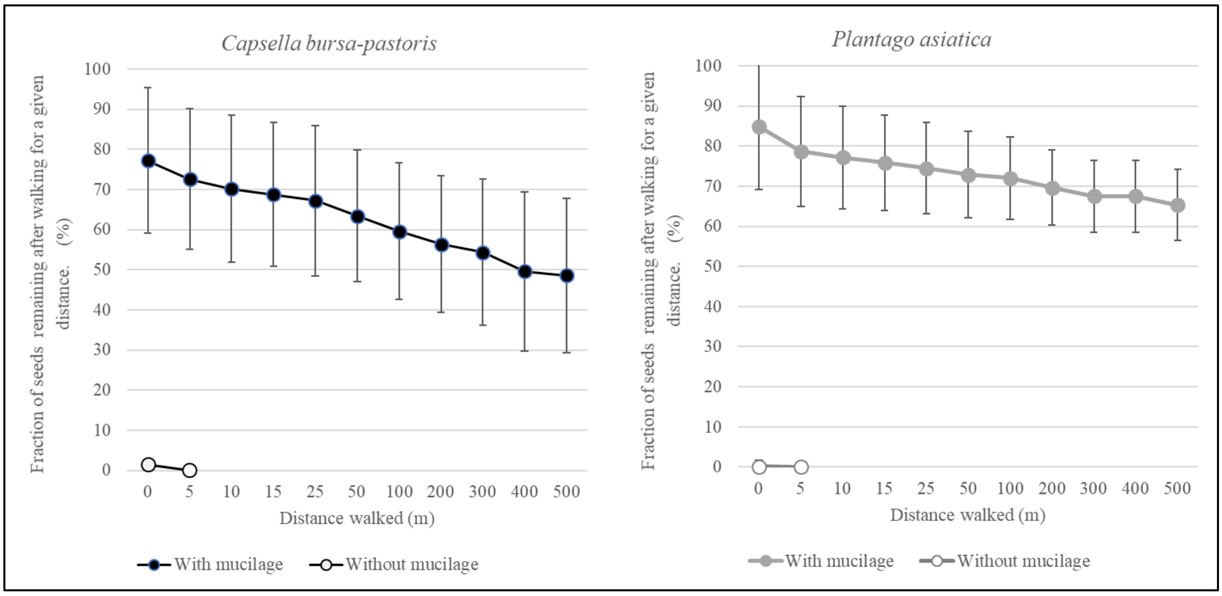

Fig. 4 Adhesion of *Capsella bursa-pastoris* and *Plantago asiatica* seeds to shoes.

II: Walking Experiment

Fig. 4 shows the adhesion of *C. bursa-pastoris* and *P. asiatica* seeds to shoes. For *C. bursa-pastoris*, the adhesion rate of seeds with mucilage to the soles of shoe i was 77.2% at the 0 m point, and it was 61.4% at the 500 m walking point (Fig. 4). In contrast, in the area without mucilage, almost no adhesion was observed on the soles of the shoes. In *P. asiatica*, the adhesion rate of seeds with mucilage was 84.8% at the 0 m point and 77.3% at the 500 m walking point (Fig. 4). In the absence of mucilage, almost no adhesion was observed on the soles of the shoes.

Discussion

In the present study, we found that the germination rate of *C. bursa-pastoris* seeds increased when seed mucilage was removed (Fig. 1). This suggests that seed mucilage may be related to seed dormancy. Although many studies have been conducted on the relationship between seed mucilage and seed germination, most have reported its effects on promoting germination in dry areas while we report on the effects in humid areas. It has also been reported that seed mucilage can function as a physical barrier to regulate the diffusion of water and oxygen to the internal tissues of seeds, which inhibits germination under unsuitable conditions (Gutterman and Shem-Tov, 1996; Werker, 1997). In addition, chemicals present in seed mucilage may directly regulate germination (Yang *et al*., 2012). The inhibition of germination of *C. bursa-pastoris* seeds by mucilage observed in this study may be due to mucilage blocking oxygen or the presence of substances that inhibit germination. The mechanism by which mucilage inhibits germination remains a topic for future research. Furthermore, when seeds were frozen or exposed to temperatures below freezing, no inhibition of germination by mucilage was observed. This suggests that temperatures below freezing temperatures may destroy the physical mechanisms or chemical composition of seed mucilage, thereby inhibiting germination. *C. bursa-pastoris* flowers and fruits from spring to autumn. Exposure to low temperatures during winter may eliminate the inhibitory effect of mucilage on germination. This suggests that mucilage in *C. bursa-pastoris* seeds may affect the asymmetry of germination of seeds in spring to autumn.

The seed size of *C. bursa-pastoris* varied from 0.4 mm^2 to 0.8 mm^2 (Fig. 2). Such variations in seed size may be related to the asymmetry of germination in *C. bursa-pastoris* as smaller seeds have deeper dormancy and larger seeds have shallower dormancy (Gray and Thomas, 1982; Yoshioka *et al*., 1985). Furthermore, this study revealed a large variation in the thickness of the exuded mucilage. It is assumed that variations in the amount of seed mucilage also affect dormancy. Furthermore, no significant correlation was observed between seed size and seed mucilage thickness. Therefore, the relationship between random seed size variation and variation in the amount of mucilage may complicate the mechanism of dormancy in *C. bursa-pastoris*. However, the amount of seed mucilage was greater in seeds that germinated within 2–7 days than in seeds that germinated within 1 d (Fig. 3). This suggests that a more of seed mucilage delays germination. However, because *C. bursa-pastoris* seeds have deep dormancy and a low germination rate, it is difficult to fully consider the effects of seed mucilage on germination rate (Tajima and Kudo, 2022). Therefore, in this study, we performed a temperature change treatment, which was reported to be effective in breaking dormancy in *C. bursa-pastoris* by Popay and Roberts (1970a), to increase the germination rate and then investigate the effect of the presence or absence of seed storage on germination. No difference was observed in the final germination rate between seeds with and without mucilage. However, the number of days to reach 50% of the final germination rate (G50) and the average number of days to germination were delayed in seeds with mucilage, and a tendency for germination to be delayed by 1–2 days was observed. It has been reported that the seed mucilage of *P. asiatica* delays germination, which is known to inhibit germination when seeds are attached and dispersed, thereby aiding seed dispersal (Inagaki and Kishiyama, 2022). The results of this study suggest that the seed mucilage of *C. bursa-pastoris* may play a similar role in increasing seed dispersal.

Although *P. asiatica* is known to be an adhesive seed-dispersal plant, where seeds are dispersed by adhering to mobile animals, the dispersal type of *C. bursa-pastoris* is unclear. Therefore, in the present study, we quantitatively investigated whether the seed mucilage of *C. bursa-pastoris* functions in adhesive seed dispersal by comparing it with *P. asiatica*, an adhesive seed dispersal plant. No adhesion was observed in the seeds without mucilage of *C. bursa-pastoris*, whereas the adhesion rate of the seeds with the mucilage was 61.4% at the 500 m walking point. This was slightly lower than the 77.3% of the seeds of *P. asiatica* but was considered to have sufficient dispersal ability.

Conclusion

These results suggest that the seed mucilage of *C. bursa-pastori* may affect seed dormancy. It also delays germination by approximately 1–2 days while the seeds are transported, and is effective in seed attachment and dispersal mechanism. However, while *P. asiatica* grows naturally in footprints, this tendency in *C. bursa-pastoris* is unclear. Therefore, the role of seed mucilage in *C. bursa-pastoris* in the wild requires further study.

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