Promotion of hard-seed germination in *Lotus corniculatus* var. *japonicus* for use in amenity grasslands

T. KONDO

Fukui Agricultural Experiment Station, 52-22 Ryo-cho Henguri, Fukui 910, Japan

(Accepted July 1993)

Summary

The purpose of this study was to find methods to promote the germination of hard seeds of *Lotus corniculatus* var. *japonicus*, a species used to create amenity grasslands. Seeds of *L. corniculatus* var. *japonicus* were round or wrinkled, and many of the round seeds were hard. Scarification with emery paper helped to make round seeds permeable, but it increased the numbers of rotted seeds or abnormal seedlings and had little effect on germination. Dry storage in moisture-proof plastic containers with silica gel at 3°C increased the percentage of round seeds that germinated, but dry storage at higher temperatures and non-dry storage at room temperature increased the percentage of hard seeds. Chilling at 3°C with seeds put between moist gauze did not increase the percentage of round seeds that germinated. Of all methods tried, soaking in sulphuric acid (36 N) for 10 min increased the germination percentage of round seeds most. Seeds soaked in sulphuric acid still had a high germination percentage 12 months later. Scanning electron micrographs showed that wrinkled seeds had cracked surfaces, but that round seeds were uncracked. Soaking in sulphuric acid caused cracking of the coat of round seeds.

Introduction

In Japan, seeds are sown in dry riverbeds, on roadside slopes, etc., to create amenity grasslands. However, seeds of foreign origin or of horticultural cultivars produce plants which are not always suitable for the climate and ecosystem. They tend to lose in competition with native weeds. Colourful wild flower populations that are adapted to the environment are often observed in rural districts, and methods are needed to introduce such native species to green areas being constructed artificially. The ecology and the germination characteristics of Japanese wild flowers are being studied.

In one such study, we reported on the hard seeds of *Lotus corniculatus* var. *japonicus* Regel, a Japanese native wild flower (Kondo and Takahashi, 1988). Hard seeds increase adaptability to an environment because they germinate at a different time from soft seeds. Thus, hard seeds are desirable for regeneration and maintenance of communities by the natural dispersal of seeds. However, when we raise seedlings by sowing, germination needs to be uniform and high. The purpose of this study was to find methods to promote the germination of hard seeds of *L. corniculatus* var. *japonicus* and to identify the features of the coat of hard seeds.
Materials and methods

Seed source
Seeds were collected by hand from populations grown at Fukui Prefectural College near a school building (plot A), near a greenhouse (plot B), and in a field for experiments (plot C) from 1986 to 1991. Seeds were dried indoors for one week and selected by being winnowed and classified under a stereoscopic microscope as being wrinkled (seeds were flat and had wrinkled seed coats) or round (seeds were round and had smooth seed coats). Moldy seeds were discarded.

Expt 1
Round and wrinkled seeds were collected from plots A and C in July 1991 and immediately used in tests. Round and wrinkled seeds were tested separately. Seeds were placed on two layers of moistened filter paper (Whatman No. 1) with three replicates of 50 seeds for each treatment. The dishes were kept moist with distilled water. Tests were done in an incubator at 20°C with a 12-hour light photoperiod. The light source was 20-W cool-white fluorescent tubes, and irradiance at the seed level was about 3 klx. Germination tests lasted for 40 days when most of germination finished. Seeds showing radicles with geotropism were scored as having germinated normally. Seeds that produced damaged radicles or from which cotyledons emerged earlier than the radicles were recorded as having germinated abnormally. Forty days after the day of sowing, the test was ended, and the number of rotted seeds and hard seeds were counted.

Expt 2
Seeds were collected from plots A, B, or C yearly from 1986 to 1991 and were placed in dry storage in moisture-proof plastic containers with silica gel at 3°C. After about 10 days of storage, 1 ml of seeds (about 800) was examined with two to four replicates and the percentages of round, wrinkled, and moldy seeds were calculated.

Expt 3
Seeds were collected from plot A in July 1991 and were tested immediately. Only round seeds were used. Seeds were scarified with #180 (the Japanese industrial standard) emery paper by hand till slight injury of the seed coat was observed by eye. The seeds were put in dishes for germination. Conditions of the germination tests were as described above.

Expt 4
Seeds collected from plot C in July 1991 were used. Germination tests were done immediately after seed collection and also after 3, 6, and 12 months of storage. Seeds were put in dry storage at constant temperatures of 3, 10, 15, 20, 25, 30, or 35°C; in dry storage at fluctuating room temperature; and in non-dry storage in beaker without cap at room temperature. Round and wrinkled seeds were tested separately. Conditions of germination tests were as described above.
**HARD SEEDS OF LOTUS CORNICULATUS VAR. JAPONICUS**

*Expt 5*
Round seeds collected from plot C in July 1991 were used. The seeds were put between sheets of moist gauze and placed in the dark at 3°C. Germination tests were done immediately after seed collection and also after 1, 3, and 6 months of chilling. After 3 months of chilling, a few seeds had germinated, but only non-germinated seeds were used in germination tests. Conditions of germination tests were as described above.

*Expt 6*
Seeds collected from plot A in July 1991 were used. Round and wrinkled seeds were treated with sulphuric acid as follows. First, 200 seeds were put into a tea strainer made of stainless steel and soaked without being agitated in 5 ml of 36 N sulphuric acid in a ceramic dish for 1, 2, 5, 10, 15, 20, 25, or 30 min. The starting temperature of the sulphuric acid was about 32°C. Then the seeds were rinsed for 2 min with running water. Three replicates of 50 seeds were used. In a separate experiment, round seeds were soaked in 18 N sulphuric acid for 10 min or for 0.5, 1, 2, 4, or 8 hour and rinsed as before. The starting temperature of this sulphuric acid was about 30°C. Conditions of germination tests were as described above.

Round seeds were treated with 36 N sulphuric acid for 10 min and dried with a fan for 20 hours. Then they were put in dry storage at 3°C or at room temperature. After 1, 3, 6, or 12 months, germination tests were done as described above.

*Expt 7*
Seeds collected from plot A in July 1991 were used. Untreated round seed, untreated wrinkled seed, scarified round seed, and round seed treated with 36 N sulphuric acid for 10 or 30 min were sputter-coated about 30 nm, thick with platinum. The surfaces of the seeds were then observed under a scanning electron microscope (Hitachi S-450) at 15 kV.

**Results**

*Germination of round and wrinkled seeds*
By day 15, about 70% of the wrinkled seeds from both plots had germinated. Only 4% and 19% of round seeds from plots A and C, respectively, germinated within 40 days. By day 40, 14–30% of the wrinkled seeds had rotted, as had 1–4% of the round seeds (Figure 1). The round seeds that had neither germinated nor rotted had not absorbed water and were not swollen, when judged by eye.

*Percentage of collected seeds that were round, etc.*
The percentage of collected seeds that were round differed depending on the year and the collection area (Table 1). For example, 76% of seeds from plot A were round in 1991, but only 9% of seeds from plot B were. The percentage of seeds from plot A that were round was between 9% and 83% in different years.
Figure 1. Germination and other changes in round and wrinkled seeds by 40 days after they were sown. Germination of treatments with the same letter was not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 1. Percentages of different kinds of collected seeds.

<table>
<thead>
<tr>
<th>Plot</th>
<th>Percent seeds collected (day/month/year) on</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>19/7/86</td>
</tr>
<tr>
<td>A</td>
<td>Round</td>
</tr>
<tr>
<td></td>
<td>Wrinkled</td>
</tr>
<tr>
<td></td>
<td>Mouldy</td>
</tr>
<tr>
<td>B</td>
<td>Round</td>
</tr>
<tr>
<td></td>
<td>Wrinkled</td>
</tr>
<tr>
<td></td>
<td>Mouldy</td>
</tr>
<tr>
<td>C</td>
<td>Round</td>
</tr>
<tr>
<td></td>
<td>Wrinkled</td>
</tr>
<tr>
<td></td>
<td>Mouldy</td>
</tr>
</tbody>
</table>

Results are given as means ± standard deviation.
Two for four replicates were done.
–: Not collected. Plot A, near college building; B, near greenhouse; C, in field for experiments.

**Effects of scarification**
Eleven percent of scarified seeds germinated, compared with 4% of unscarified seeds (Figure 2). Scarification made seeds permeable to water, and reduced the percentage of hard seed to zero. The percentages of rotted seeds and abnormal seedlings were increased by scarification.

**Effects of storage**
When round seeds were sown immediately after being collected, 19% germinated. Twelve months of dry storage at 3°C increased the germination of round seeds to
Figure 2. Effects of scarification on germination and other changes in round seeds by 40 days after they were sown. Germination of treatments with the same letter was not significantly different ($P = 0.05$) according to Duncan’s multiple range test.

65% and increased the germination speed (Figure 3). Dry storage at other temperatures and non-dry storage at room temperature somewhat reduced the germination of round seeds, and increased the number of hard seeds. Dry storage of wrinkled seeds did not reduce the percentage that germinated even after 12 months. Non-dry storage at room temperature reduced the germination, increased the numbers of abnormal seedlings and rotted seeds, and decreased the germination speed.

Effects of chilling
The germination of round seeds immediately after seed collection was higher than that after chilling for 6 months (data not shown).

Effects of sulphuric acid
The germination of untreated round seeds was 4% at 40 days. Treatment with 36 N sulphuric acid increased the germination percentage and increased the germination speed. Most seeds soaked for 10 min in 36 N sulphuric acid germinated within 5 days, and the germination was 89% after 40 days (Figure 4). Soaking for 20 min or more reduced this percentage and increased the numbers of abnormal seedlings and rotted seeds. The germination of untreated wrinkled seeds was 63%. When soaking was for 5 min or more, this percentage decreased, and the numbers of abnormal seedlings and rotted seeds increased. Treatment of round seeds with 18 N sulphuric acid for 18 hours had no effect on the germination percentage and germination speed; most seeds remained hard.

Seeds treated with 36 N sulphuric acid for 10 min and then kept in dry storage at 3°C or at room temperature had a germination of about 90% by 5 days after they were sown, even after 12 months of storage.

Scanning electron microscopy of seed surfaces
Scanning electron micrographs showed that untreated round seeds had smooth sur-
faces without scratches (Figure 5A). Untreated wrinkled seeds had rough surfaces and many cracks (Figure 5B). Figure 5C shows these cracks at higher magnification.

Scarification removed the seed coat in places, causing deep injury (Figure 5D). Sulfuric acid (36 N) treatment for 10 min caused cracks and small holes in the surface (Figure 5E). The interior of the seed was dissolved by 30 min of treatment with sulfuric acid (Figure 5F).

**Discussion**

The results showed that wrinkled seeds could absorb water through cracks on their surfaces and then germinate, but that round seeds without cracks on their surfaces did not germinate.

616
Scarification reduced the proportion of hard seed to zero but increased the numbers of abnormal seedlings and rotted seeds because of excessive injury. Scarification by hand with emery paper in this experiment was difficult. It is difficult to scarify seed uniformly with an abrasive disc, as well. To make a break in the seed coat with a file or needle is impractical if many seeds are to be treated (Brant, McKee and Cleveland, 1971). Scarification, therefore, was not useful for this purpose.

Dry storage at 10°C or higher and in non-dry storage at room temperature decreased the germination of round seeds by increasing the number of hard seeds. Dry storage at 3°C increased the germination percentage because the number of hard seeds decreased. However, scanning electron micrographs did not show cracks or other changes on the surface of round seeds stored at 3°C (Figure is not shown). These results suggest that the site of water conduction into the seeds might be a particular tissue such as the strophiole (Quinlivan, 1968; Hagon, 1971).

In dry storage of wrinkled seeds, the initial germination percentage was unchanged for one year. Non-dry storage at room temperature decreased the percentage of germination and increased the production of abnormal seedlings and rotted seeds. Wrinkled
Figure 5. Scanning electron micrographs of surfaces of *Lotus corniculatus* var. *japonicus* seeds.  
(A) Round seed.  
(B) Wrinkled seed.  
(C) Magnified view of wrinkled seed.  
(D) Round seed after scarification.  
(E) Round seed treated with sulphuric acid (36 N) for 10 min.  
(F) Round seed treated with sulphuric acid (36 N) for 30 min.

Seeds may be affected by moisture in the air because their seed coat is permeable; some wrinkled seeds were partly covered with mould. Wrinkled seeds, therefore, should be stored under dry conditions if storage is to be long-term.

Chilling did not increase permeability to water.

The most effective treatment to lower the number of hard seeds was soaking in sulphuric acid (36 N) for 10 min. The cracks and holes that formed made the seeds permeable to water so that they could germinate. Sulphuric acid treatment etches the seed coat of *Coronilla varia* L. and dissolves the caps of the macrosclereid cells in places (Brant *et al.*, 1971). Seeds soaked in sulphuric acid had a high germination percentage after dry storage at 3°C or at room temperature. Soaking for 20 min or more hindered normal germination of round seeds, and soaking for 5 min or more reduced the germination of wrinkled seeds. Thus, wrinkled seeds do not require treatment; round seeds should be selected and treated with 36 N sulphuric acid for 10
HARD SEEDS OF LOTUS CORNICULATUS VAR. JAPONICUS

Round seeds store better than wrinkled seeds, so if seed selection is improved, round seeds would be more useful than wrinkled seeds.

Acknowledgements

I thank Dr. Ken-ichi Hatano for advice, and Dr. Shizuka Ohki and Dr. Hiroshi Okamoto for their guidance concerning the scanning electron microscopy. I am grateful to Miss Sanae Kusaka for help in carrying out the experiment.

References


