Effects of Salinity and Temperature on Seed Germination in a Japanese Endangered Halophyte *Triglochin maritimum* (Juncaginaceae)

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Effects of salinity and temperature were examined on the seed germination of an endangered halophyte in Japan, *Triglochin maritimum* (Juncaginaceae). Germination tests were carried out at three salinity levels (0, 200, 400 mM NaCl) in which seeds were exposed to increasing- or decreasing-temperatures. Effects of moist-chilling pretreatment (stratification) in 0, 200 and 400 mM NaCl on seed germination at 0, 200 and 400 mM NaCl, respectively, were also examined. Under the highest salinity condition (400 mM NaCl), no germination was observed. The seeds germinated very well (88%) in fresh-water after 5-month moist-chilling pretreatment. Longer moist-chilling pretreatment resulted in higher germination percentages. Moist-chilling pretreatment in 0, 200 and 400 mM NaCl for 5 month enhanced germination percentages to 10% (in 400 mM NaCl) - 88% (in 0 mM NaCl).

Key words: Dormancy — Endangered species — Halophyte — Salinity — Seed germination — *Triglochin maritimum*

*Triglochin maritimum* L. (Juncaginaceae) is a perennial halophyte of salt marshes. It is widely distributed in the northern hemisphere (Davy and Bishop 1991), including Japan (Yamashita 1981). It flowers from late spring to early autumn and disperses small seeds around parent plants. The seeds germinate in spring (Davy and Bishop 1991). Because plants propagate vegetatively in a round shape (20-50 cm in diameter), it is not easy to identify genets growing adjacentlly.

The red list of Japanese wild plants (Anonymous 1997), adopting the IUCN categories (IUCN 1994) listed the species as 'vulnerable' to extinction. Because the habitat of the species in Japan has been destroyed through the construction of harbor facilities and industrial areas, the species is seriously threatened (Anonymous, 1977). For example construction of industrial areas at the mouth of Wajiro River is planned, and therefore the population of the species in that area will become extinct in 2000.

For ex situ conservation, sampling and stocking of seeds from natural populations are important for restoring populations with genetic variations. Genetic variation is regarded as an important resource, allowing for short-term adaptation to environmental fluctuation as well as for long-term evolutionary change (Wright 1948, Frankel and Soule 1981, Beardmore 1983, Harris 1984). Regeneration by seeds is effective to maintain genetic variations (Menges, 1994). When a population confronts extinction, we can tentatively evacuate the population as seed stock, and restore it elsewhere. It is therefore important to clarify the experimental conditions required for seed germination of endangered plants.

Seed germination of halophytes, which grow under saline conditions such as salt marshes and salt deserts, has long been of great interests to plant ecologists (Ungar 1978, 1991, Adam 1990), because the ion-specific toxicity on germination is expected to cause low rates of germination (Ungar 1991). In general, the percentage and rate of seed germination decrease with increasing salinity, while the extent of the decline in germination varies among different species (Yokoishi and Tanimoto 1994, Ungar 1995). Only a few species germinate in 3.0% NaCl (nearly equivalent to 500 mM NaCl) (Ungar 1995). In 5.0% NaCl (nearly equivalent to 850 mM NaCl), only six species *Salicornia bigelovii* (Rivers and Weber 1971), *S. europaea* (Ungar 1962, 1967), *S. herbacea* (Khan and Weber 1986), *Cressa cretica* (Khan 1991), *Zygophyllum dumosum* (Agami 1986) and *Tamarix pentandra* (in Ungar 1967) have been reported to germinate. In contrast, the seeds of most halophytes germinate very well in fresh-water (Bakker et al. 1985, Ungar 1991, 1995, Mariko et al. 1992, Yokoishi and Tanimoto 1994, Baskin and Baskin 1998).

The germination of halophyte seeds is also affected by an interaction of salinity and temperature (Ungar 1978, 1995). Under higher saline conditions, the temperature ranges permitting seed germination tends to become narrow or to shift down (Ungar 1995, Baskin and Baskin 1998). Moist-chilling breaks dormancy in most halophyte seeds (Baskin and Baskin 1998). If seeds of a halophyte germinate in spring, moist-chilling is probably a part of the dormancy-
breaking mechanisms. If seeds germinated in autumn, they would require only warm stratification. Usually, seeds have been stratified in cold fresh water, therefore we have little information on the possible interacting effects of moist-chilling and salinity on seed germination. Thus seed germination experiments and moist-chilling treatment at various salinity levels and temperature regimes are indispensable to understand the germination ecology of halophytes. The purpose of our study is to clarify the effects of salinity and temperature on seed germination of the endangered halophyte T. maritimum.

Materials and Methods

Field census
To determine the season of seedling emergence of the species in the field, we conducted observation on seedling emergence in the marsh at a mouth of the Wajiro river on 25 November 1997 and 20 March 1998. We also sampled ca. 5,000 cm² of surface soil (0-10 cm) from the marsh on 20 March 1998.

Sampling and thermal pretreatment of seeds
We collected over 1,200 seeds from a population in a salt marsh near the mouth of the Wajiro river in Fukuoaka, Japan, on 25 August and 11 September 1997. Due to the difficulty of genet identification, seeds were mixed and have been originated from more than 10 different genets.

After air-drying in paper bags for 1 month at room temperature to facilitate after-ripening (not to harm the seed coat), the seeds were soaked out from fruit clusters and rinsed with distilled water, air-dried again, and subjected to a screening test at 0, 200 and 400 mM NaCl concentrations. These salinity conditions were adopted to include a range of salinity levels in the field. The salinity of the soil-water where the population grew was 104 mM NaCl at ebb tide and 450 mM NaCl at rising tide on 1 and 2 October 1997. The conductivity meter (HORIBA ES14) was used for the measurements of water conductivity. These salinities were calculated by the ratio of the conductivity between sample water and standard seawater.

The moist-chilling pretreatments at 0, 200 and 400 mM NaCl concentrations were conducted. Thermal germination responses of these seeds were examined at 0, 200 and 400 mM NaCl concentrations after moist-chilling pretreatments for 1, 3 and 5 month. During the moist-chilling at 4°C, 50 seeds were wrapped in a sheet of paper for wrapping individual doses of powdered medicine and placed in a Petri dish containing 7 ml of 0, 200 and 400 mM NaCl solutions.

Seed germination experiments
Because no information on seed germination of the species was available, we adopted a screening test system, a modification proposed by Washitani (1987), using a pair of temperature-treatment regimes. This method is efficient for screening the conditions required for seed germination, even if the number of seeds available for experiments is relatively small and the facilities for the experiment are limited. To interpret germination patterns observed in this test system, a model describing germination behavior in a given thermal environment has been proposed on the basis of thermal germination responses of non-dormant seeds (Washitani 1987). In one thermal regime (IT), soaked seeds were exposed successively to 4°C for eight days, 8, 12, 16°C for four days, 20, 24, 28, 32, 36°C for two days; in the other thermal regime (DT), they were exposed to decreasing temperatures in the same series from 36 to 4°C. In general, the rate of germination of non-dormant seeds is higher at higher temperatures, thus a shorter period of exposure was adopted for higher temperatures. The percentage of germination at the end of each thermal regime was defined as the final percentage of germination. Then, the seeds were incubated under a constant temperature of 20°C for 10 days. The percentage of germination on the 10th day after transferred to 20°C was defined as the maximum percentage of germination. Fifty seeds were placed on filter paper with freshwater, 200 or 400 mM NaCl in a Petri dish and subjected to the thermal treatment. Three dishes were used for each treatment. Seeds were exposed to diffuse laboratory light in an incubator (1 to 3 μmol m⁻² s⁻¹). The number of germinated seeds was recorded immediately before every temperature change and at the end of the thermal treatment. Effects of NaCl concentrations and lengths of moist chilling pretreatment on germination were examined by the logistic regression. Statistics were calculated with LogXact-Turbo (Mehta and Patel 1992) using asymptotic inference. The results of the germination experiments provided information on the thermal germination characteristics of the seed population, whether induction or breakage of dormancy occurred under particular thermal regimes, the permissible and optimal temperature range for the germination of non-dormant seeds, and the time required for germination under particular thermal regimes.

Ungerminated seeds, which had been soaked in 0, 200 or 400 mM NaCl at increasing (IT) and decreasing temperature (DT) regimes, were exposed successively to 4°C for 3 months at respective NaCl concentrations and then transferred to 20°C for two weeks in 0 mM NaCl to investigate the viability of the ungerminated seeds.

Terminology
The terminology for 'dormancy' and its categories follows that of Bewley and Black (1982). In this paper 'enforced dormancy' (Harper 1977) was not included in the dormant state. Only primary (in most cases 'innate'), relative and secondary (identical to 'induced') dormancy were distinguished.

Results

Field census
At the time of seed dispersal the seeds of T. maritimum were expected to be in a dormant state, because any seedlings were not observed from August to November in 1997 and more than 30 viable seeds were detected in the soil sampled on 20 March 1998.
Effects of moist-chilling

Moist chilling pretreatment greatly influenced seed germination in *T. maritimum*. Longer pretreatment led to higher germination percentages. Without moist-chilling pretreatment, only small percentages germinated at any salinity levels, while approximately 88% of the seeds germinated at 0 mM NaCl after 5-month moist-chilling in fresh-water. The maximum germination percentages were shown in Table 1. There were little differences between the final and maximum percent germination in all treatments. Most seeds were dormant, because little germination occurred in either IT or DT regimes in the germination test without moist chilling pretreatment at any NaCl concentrations (Table 1). Moist chilling at 4°C for 1 month in fresh-water was effective in releasing the seeds from primary dormancy, because germination in the IT regime was observed after the moist chilling (Table 1). The final and maximum percent germination recorded in the seeds treated with 5-month moist chilling in 0 mM NaCl was higher than that recorded after 1- or 3-month moist-chilling in 0 mM NaCl (Table 1). There were 1.5 times differences between IT and DT regimes in the maximum percent germination in seeds treated with 5-month moist-chilling in fresh water (Table 1).

In the IT regime after 3 and 5 months of moist-chilling pretreatments, the lowest temperature for germination decreased from 24°C to 4°C and the percentage of non-dormant seeds in fresh water rose to 88%. In the IT regime the maximum rate of germination was observed at 20°C after 1-month moist chilling, whereas after 5-month moist chilling it was recorded at 16°C.

Most seeds pretreated with 1- and 3-month moist-chilling in 200 or 400 mM NaCl were dormant, because few seeds germinated in either IT or DT regimes. However, 5-month moist-chilling in 200 or 400 mM NaCl released seeds from primary dormancy, although the effect was smaller than that in 0 mM NaCl (Table 1). Results of the logistic regression of seed germination against the duration of moist-chilling pretreatment and NaCl concentrations were shown in Table 2. Significant effects of NaCl concentrations and duration of moist chilling pretreatment on seed germination were detected.

Ungar (1995) reported that tolerance of seeds to salinity should be interpreted at two levels: (1) the ability to germinate at saline conditions and (2) the ability to germinate at a non-saline condition after exposure to high salinity. In this study the two levels of tolerance of seeds to salinity were observed. Moist-chilling pretreatment in 200 and 400 mM NaCl for 5 month promoted germination in 200 and 400 mM NaCl. During winter seeds of *T. maritimum* are in saline conditions (104 mM to 500 mM NaCl). Thus it is expected that moist-chilling during winter breaks dormancy and increases seed tolerance to germinate in saline conditions. Seeds of *T. maritimum* would therefore germinate in early spring when temperatures are still quite low.

Seeds cannot come out of dormancy during moist-chilling unless they have tolerance to the saline condition in the field. An ability to germinate in freshwater after exposure to high

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**Table 1.** The final percent germination and maximum percent germination (in parentheses) at two temperature regimes (IT and DT) after 0-, 1-, 3-, and 5-month moist-chilling pretreatment in 0, 200 and 400 mM NaCl

<table>
<thead>
<tr>
<th>Duration of Moist chiling</th>
<th>Temperature regime*</th>
<th>Salinity level during moist chiling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 mM</td>
</tr>
<tr>
<td>0 month</td>
<td>IT</td>
<td>2 (2)</td>
</tr>
<tr>
<td></td>
<td>DT</td>
<td>0 (2)</td>
</tr>
<tr>
<td>1 month</td>
<td>IT</td>
<td>24 (24)</td>
</tr>
<tr>
<td></td>
<td>DT</td>
<td>4 (4)</td>
</tr>
<tr>
<td>3 months</td>
<td>IT</td>
<td>26 (26)</td>
</tr>
<tr>
<td></td>
<td>DT</td>
<td>8 (8)</td>
</tr>
<tr>
<td>5 months</td>
<td>IT</td>
<td>88 (88)</td>
</tr>
<tr>
<td></td>
<td>DT</td>
<td>54 (54)</td>
</tr>
</tbody>
</table>

* For details of the temperature regimes, see the text.

**Table 2.** Effects of NaCl concentrations and duration of moist-chilling pretreatments on seed germination at decreasing (DT) and increasing temperature (IT) regimes

<table>
<thead>
<tr>
<th>Temperature regime</th>
<th>Independent variable</th>
<th>β*</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT</td>
<td>NaCl treatment</td>
<td>-0.003</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Stratification</td>
<td>0.876</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>IT</td>
<td>NaCl treatment</td>
<td>-0.009</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td></td>
<td>Stratification</td>
<td>0.862</td>
<td>&lt;0.00001</td>
</tr>
</tbody>
</table>

* β indicates the natural logarithm of the odds ratio.
salinity has been reported in some halophytes (Khan and Ungar 1984, 1996, Ungar 1996, Baskin and Baskin 1998). In addition, halophyte seeds are more tolerant to high salinity than those of non-halophyte species (Mariko et al. 1992). The degree of tolerance of seeds of T. maritimum to salinity was in a range which other halophytes exhibited (Baskin and Baskin 1998).

Little is known about the changes in temperature requirements for germination of halophyte seeds as affected by mosit-chilling pretreatments. Primary dormancy of many halophytes is probably broken during moist-chilling (Baskin and Baskin 1998), resulting in a decrease in the minimum and optimum temperature for germination. Relatively long periods of moist-chilling (more than 3 month) were required for breaking primary dormancy in the seeds of T. maritimum. For plants that germinate in spring, requirement of moist-chilling for breaking seed dormancy is an effective mechanism to avoid germination in autumn. In the field, seed dispersal of T. maritimum begins in late summer. If seeds germinated in autumn, seedlings of the species would experience cold winter at the weakest stage through their life cycle. Indeed many Japanese plant species, which disperse their seeds before winter, require a long moist-chilling period for germination (Masuda and Washitani 1990, Washitani and Masuda 1990).

In addition to primary dormancy, secondary dormancy caused by burial of seeds in the dark may play a role in preventing seed germination before winter and thus assuring seedling survival (Baskin and Baskin 1998). Exposure to a high temperature might induce secondary dormancy, since only 54 percent of seeds could germinate at the end of the DT regime (in contrast to 88% at IT regime) after 5-month moist chilling pretreatment in freshwater (Table 1). If seeds of the species cannot germinate in spring (due to high salinity or being deeply buried), secondary dormancy would play an important role for reestablishment of a population of T. maritimum.

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References


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