EFFECT OF SALINITY ON SEED GERMINATION OF TRIGLOCHIN MARITIMA UNDER VARIOUS TEMPERATURE REGIMES

M. Ajmal Khan1,2 and Irwin A. Ungar1,3

ABSTRACT.—Triglochin maritima L. (arrow grass), an herbaceous perennial in the family Juncaginaceae, is widely distributed in inland and coastal salt marshes of North America. Triglochin maritima seeds from a population growing in a salt marsh at Faust, Utah, were germinated at 4 temperature regimes (12-h night/12-h day, 5–15°C, 5–25°C, 10–20°C, and 15–25°C) and 5 salinities (0, 100, 200, 300, 400, and 500 mol m⁻³ NaCl) to determine optimal conditions for germination and level of salt tolerance. Ungerminated seeds were returned to distilled water after 20 d to determine whether seeds could recover from salinity treatments. Maximum germination occurred in distilled water, and increases in NaCl concentration progressively inhibited seed germination. No seeds germinated at concentrations higher than 400 mol m⁻³ NaCl. A temperature regime of low night (5°C) and high day (25°C) temperature yielded maximum germination; all other temperature regimes significantly inhibited seed germination relative to this optimum. Recovery of germination was highest at 5–25°C and lowest at 5–15°C. Recovery of seed germination when seeds were transferred to distilled water from salt solutions was highest at 5–25°C (72%) for seeds exposed to the 500 mol m⁻³ NaCl pretreatment and significantly reduced at other temperature regimes. The recovery germination response indicates a synergistic inhibitory interaction effect on germination when seeds were exposed to high salinities at suboptimal thermoperiods.

Key words: Triglochin maritima, halophyte, recovery, seed germination, thermoperiod, Utah.

Triglochin maritima L. (Juncaginaceae), commonly known as arrow grass, is a clonal perennial that can form regular clumps up to 2 m across and 60 cm high (Davy and Bishop 1991). Common in saline habitats, particularly coastal marshes on rocky shores in temperate, subarctic, and arctic regions, it also extends southward to the subtropics (Davy and Bishop 1991). Triglochin maritima is distributed in inland and coastal brackish and freshwater marshes and bogs of North America (Shefler and Skog 1978). Ungar (1974) surveyed a Triglochin maritima community located at Park County, Colorado, and reported that T. maritima grew in almost pure stands in a wetter area with soil salinity ranging from 0.5% to 1% (85–170 mol m⁻³). Triglochin maritima was also found growing in salt marshes at the Fish Springs research site, Juab County, Utah, in an Eleocharis meadow community where salinity averaged 0.5% total salts (Bolen 1964).

Germination of halophytes is affected by temperature and soil salinity content, and seeds are characterized by varying types and degrees of dormancy (Binet 1965, 1968, Ungar 1991). Binet (1959) reported that seeds from a French population of T. maritima had a primary morpho-physiological dormancy and that seeds of T. maritima were more dormant than T. palustris, probably because of the more resistant sclerenchyma tissue in the pericarp of the former. The 2 basic types of dormancy that seeds develop are due either to some morphological or biochemical characteristics of the diaspore that produce a primary dormancy (fruit or seed) or to an environmental factor that induces seeds into a secondary dormancy (Bewley and Black 1982). Binet (1961a, 1961b) reported that T. maritima had a secondary dormancy induced by darkness, which in nature is probably triggered by the burial of seeds in the soil.

Germination responses of seeds of T. maritima populations from North America have not been previously investigated, and one of the goals of this investigation was to determine if responses to environmental variables differ from populations studied from Europe and related species from Africa. Naidoo and Naicker (1992) studied the effect of light, temperature, and salinity on the germination of T. bulbosa and T. striata populations from South
Africa and determined that both species have a light requirement for germination and achieve higher germination at a warmer temperature regime (20–30°C). Germination was highest in distilled water and decreased significantly with an increase in salinity up to 500 mol m⁻³. Transfer of ungerminated, salt-treated seeds to distilled water stimulated germination more in T. striata than in T. bulbosa. The interaction between salinity and temperature on germination has been the subject of investigation (Khan and Ungar 1984, Gutterman 1986, Khan and Weber 1986, Khan et al. 1987, Badger and Ungar 1989, Khan 1991, Khan and Rizvi 1994) because it plays a significant role in determining the timing of germination. However, no data are available concerning the effect of the interaction between different temperature regimes and salinity on seed germination of North American populations of T. maritima. These 2 environmental factors play a significant role in determining whether plants can successfully establish in saline habitats because they interact in determining if seeds germinate or remain dormant in the seed bank (Ungar 1985). One of the purposes of this investigation was to determine how the germination response of T. maritima to temperature and salinity may affect its establishment in salt marsh habitats.

Recovery germination of seeds in freshwater after they were exposed to saline conditions has been investigated (Ungar 1962, 1978, Barbour 1970, Parham 1970, Macke and Ungar 1971, Seneca and Cooper 1971, Woodell 1985, Keiffer and Ungar 1989) to determine if seeds can remain viable after being exposed to hypersaline conditions, but no similar data are available for T. maritima seeds. The ability of seeds to germinate after exposure to hypersaline conditions plays a significant role in the establishment of halophyte populations. Seeds of glycophytes cannot germinate after exposure to salt stress, while halophytes show a range of responses from partial to complete germination recovery when salinity stress is alleviated (Woodell 1985, Ungar 1991).

This study was initiated to obtain a better understanding of germination requirements of seeds of a population of T. maritima from the Great Salt Lake region of Utah. Initial establishment of species in salt marsh habitats is related to germination response of seeds to salinity and temperature regime and usually determines if a population will survive to reproductive maturity. Each species has very specific germination requirements, and its response to stress varies from that of other species. For this reason it is important to determine the range of tolerance to salinity and temperature regime effects on germination. The effects of salinity and temperature regime on germination and recovery responses of T. maritima were studied to determine their individual effects and any interaction between these factors on seed germination. We also determined if salinity and temperature regime interact in their effects on recovery germination of seeds initially exposed to saline conditions.

**Materials and Methods**

We collected Triglochin maritima L. seeds during August 1995 from a salt marsh situated 30 mi south of the Great Salt Lake, at Faust, Utah. Seeds were separated from the inflorescence and brought to Ohio University where they were stored at 4°C. Preliminary tests indicated the seeds were viable and germination experiments were initiated in September 1995 in 50 x 9-mm (Celman No. 7232) tight-fitting plastic petri dishes with 5 ml of test solution. Each dish, containing 25 seeds that were surface sterilized with the fungicide Phygon, was placed in a 10-cm-diameter plastic petri dish as an added precaution against water loss by evaporation. Four petri dishes containing 25 seeds each were used as replicates for each salinity and temperature treatment; seeds were considered to be germinated with the emergence of the radicle.

To determine the effect of temperature on germination, we used regimes of 5, 15°C, 5–25°C, 10–20°C, and 15–25°C. We used a 24-h cycle, where the higher temperature (15, 20, or 25°C) coincided with the 12-h light period (Sylvania cool white fluorescent lamps, 25 μmol m⁻² s⁻¹, 400–750 nm) and the lower temperature (5, 10, or 15°C) coincided with the 12-h dark period. Seeds were germinated in distilled water, 100, 200, 300, 400, and 500 mol m⁻³ NaCl solutions in each of the temperature regimes, and germination was recorded every other day for 20 d. After 20 d we transferred ungerminated seeds from the NaCl treatments to distilled water and a temperature regime of 5–25°C to determine the recovery germination, which was also recorded at
TABLE 1. Results of a 2-way ANOVA of final percent germination of *Triglochin maritima* in different salinity and temperature treatments.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>Significance of F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>19237.3</td>
<td>3</td>
<td>3442.4</td>
<td>72.4</td>
<td>0.0001</td>
</tr>
<tr>
<td>Salinity</td>
<td>17729.3</td>
<td>5</td>
<td>3546.8</td>
<td>74.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>Temperature × salinity</td>
<td>7762.7</td>
<td>15</td>
<td>513.5</td>
<td>10.8</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

2-d intervals for 20 d. Rate of germination was estimated by using a modified Timson index of germination velocity (TI = \( \Sigma G/t \)), where \( G \) is the number of seeds germinating at 2-d intervals and \( t \) is the total germination period (Khan and Ungar 1984). The maximum value possible using this index with our data was 50 (i.e., 1000/20), and the higher the value, the more rapid the rate of germination.

Germination data were transformed (arc-sine) before statistical analysis and data were analyzed with a 2-way ANOVA using SPSS for Windows, release 6.1 (SPSS Inc. 1994).

**Results**

Different temperature regimes, salinity, and their interaction significantly \( (P < 0.0001) \) affected the final percent germination of *T. maritima* seeds (Table 1). Germination of *T. maritima* was highest in distilled water and at a regime with low night (5°C) and high day (25°C) temperatures (Fig. 1). Maximum germination percentages were achieved in 12 d in all treatments. Germination of seeds decreased with increases in salinity; few seeds germinated at salt concentrations higher than 300 mol m\(^{-3}\) NaCl (Fig. 1). Variation in temperature regime significantly affected seed germination under both saline and non-saline conditions. In fact, there was less than 10% germination at the 5–15°C temperature regime in the control and all salinity treatments (Fig. 1). At other temperature regimes there was a significant inhibitory interaction between temperature and salinity on final germination percentages (Table 1, Fig. 1).

Different temperature regimes, salinity, and their interaction significantly \( (P < 0.0001) \) affected the rate of germination of *T. maritima* seeds as determined from the Timson index of germination velocity (Tables 2, 3). The rate of germination, calculated using a modified Timson index of germination velocity, was lowest in 5–15°C and highest in 5–25°C (Table 2). At temperature regimes of 10–20°C and 15–25°C, the rate of germination was similar in the controls, but the interaction caused by the addition of NaCl to the medium adversely affected the germination rate at 10–20°C (Tables 2, 3).

After 20 d of NaCl treatment, seeds were transferred to distilled water, where there was less than 25% recovery germination in the 10–20°C temperature regime at all NaCl concentrations (Fig. 2). At 15–25°C and 5–25°C the final germination percentages increased to more than 50% at 500 mol m\(^{-3}\) NaCl. Recovery at the highest salinity concentration (500 mol m\(^{-3}\)) was lower than that in the 400 mol m\(^{-3}\) NaCl treatment, which did not differ significantly from the control at 5–25°C (Fig. 2). Ungerminated seeds from the 5–15°C temperature regime were transferred to 5–25°C after 20 d and germination increased, but germination was significantly lower than those seeds that initially germinated at 5–25°C (Fig. 2).
Recovery germination percentages increased with an increase in salinity concentration (Fig. 3). At a temperature regime of 10–20°C, a maximum of 20% recovery germination was obtained in 500 mol m⁻³ NaCl, but seeds treated with 400 mol m⁻³ NaCl at 5–25°C had 72% recovery (Fig. 3).

**DISCUSSION**

*Triglochin maritima* germination is most probably regulated through variation in soil salinity and temperature regime under natural conditions. When soil salinity is beyond the levels at which seeds can germinate, seeds may die or remain dormant in the soil seed bank. Seed germination can then take place at a later time in the growing season or in another year after salt stress has been alleviated (Ungar 1995). Bolen (1964) and Ungar (1974) reported that *T. maritima* was found growing in communities with moderate salinity, 0.5–1.0% total salts (85–170 mol m⁻³). We determined that seeds from the Utah population required low soil salinity and a temperature regime with low night (5°C) and high day temperatures (25°C) to promote maximum germination. Binet (1959) reported that freshly collected *T. maritima* seeds enclosed by the pericarp had an innate dormancy and poor germination in the dark (Parham 1970). Our results indicate that seeds of this Utah population were not dormant and that germination was inhibited by high salinities and low day temperatures. We determined that *Triglochin maritima* seeds had their highest germination percentages in distilled water and a progressive decline in germination with increases in salinity. Similar results were found in populations from Europe (Binet 1960, 1965, Pigott 1969, Lotscher 1970). Our results agree with those of Binet (1965), who determined that germination in saline media of seeds from a French population of *T. maritima* was greatly facilitated by alternating temperature regimes of 5–25°C, which can substitute substantially for the light requirement. Likewise, germination of the related species *Triglochin bulbosa* and *T. striata* was also highest in non-saline controls and decreased significantly with an increase in salinity up to 500 mol m⁻³ (Naidoo and Naicker 1992). Higher day temperatures were more stimulating for germination compared to lower thermoperiods in all of these species of *Triglochin*. Similar promotive effects of high daytime temperatures on germination also were found in other perennial halophytes such as *Cressa cretica* (Khan 1991), *Atriplex griffithii* (Khan and Rizvi 1994), *Salicornia pacifica* var. *utahensis* (Khan and Weber 1986), *Halopyrum mucronatum*

### TABLE 2. Index of germination velocity, using a modified Timson index (Khan and Ungar 1984) to estimate rate of germination of *Triglochin maritima*.

<table>
<thead>
<tr>
<th>NaCl (mol m⁻³)</th>
<th>Temperature regime (°C)</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>15.7 ± 2.3</td>
<td>17.8 ± 3.4</td>
</tr>
<tr>
<td>100</td>
<td>4.2 ± 2.3</td>
<td>12.4 ± 2.4</td>
</tr>
<tr>
<td>200</td>
<td>2.7 ± 0.8</td>
<td>6.6 ± 1.7</td>
</tr>
<tr>
<td>300</td>
<td>1.2 ± 0.5</td>
<td>5.0 ± 1.2</td>
</tr>
<tr>
<td>400</td>
<td>0</td>
<td>1.7 ± 0.7</td>
</tr>
<tr>
<td>500</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

### TABLE 3. Results of a 2-way ANOVA, using data from the Timson index of germination velocity to estimate rate of germination of *Triglochin maritima* at different salinities and temperatures.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>Significance of F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>1118.4</td>
<td>3</td>
<td>372.8</td>
<td>51.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>Salinity</td>
<td>243.5</td>
<td>5</td>
<td>48.69</td>
<td>66.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>Temperature × salinity</td>
<td>1016.9</td>
<td>15</td>
<td>67.8</td>
<td>9.3</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
(Noor and Khan 1995), and Chrysothamnus nauseosus (Khan et al. 1987).

Seeds of Triglochin maritima from the Utah population, when transferred to distilled water after a 20-d treatment at various salinity concentrations, responded differentially under different temperature regimes. There was little recovery (20%) with the 10–20°C temperature regime in non-saline controls, but at 5–25°C seeds incubated previously at 400 mol m\(^{-3}\) NaCl had about 72% recovery. It seems that recovery germination of T. maritima is temperature dependent. Binet (1961b) determined that seeds of T. maritima from a French coastal population had a stratification and light requirement, and when immersed in seawater at 3°C for 60–80 d, they were capable of germinating subsequently in freshwater upon transfer to 25°C in the light or dark. Seeds from the Utah population did not require stratification and were not dormant. Woodell (1985) included T. maritima in the group of coastal species whose subsequent germination is stimulated by exposure to high salinity, although the germination percentages he recorded were low. Our results indicate the recovery germination response of T. maritima seeds in distilled water after 20 d exposure to 500 mol m\(^{-3}\) NaCl was temperature dependent. Keiffer and Ungar (1995) exposed seeds of 5 halophytes (Atriplex prostrata, Hordeum jubatum, Salicornia europaea, Spargularia marina, and Suaeda calceolariformis) to salinity treatments for 2 yr and determined their recovery responses when transferred to distilled water. They used the Woodell (1985) classification system and placed Atriplex prostrata seeds in Type 1 (recovery inhibited by high salinity), Hordeum jubatum and Spargularia marina in Type 2 (recovery equal to original controls), and Salicornia europaea and Suaeda calceolariformis in Type 3 (salt stimulated, recovery greater than controls). Our data from the 500 mol m\(^{-3}\) NaCl treatment indicate that T. maritima recovery germination could be classified in Type 1 (10–20°C) or Type 2 (15–25°C), depending on the temperature regime used in the recovery germination experiment. Seeds exposed to 5–15°C in all salinity treatments had low recovery germination percentages.

Triglochin maritima seeds had maximum germination at a 5–25°C temperature regime at all NaCl concentrations tested. Few seeds germinated at the 5–15°C in non-saline controls. Inability to germinate at low day temperature in the laboratory indicates that a
threshold of higher day temperatures is necessary to stimulate germination under field conditions. A combination of reduced salinity and high daytime temperatures stimulates germination and determines the sites along salinity gradients where germination and establishment of *T. maritima* can occur. Because soil salinity stress usually increases during the summer months, salinity conditions early in the growing season at the germination stage determine whether halophytes will be able to successfully establish at a site (Ungar 1995). Recovery germination responses were also dependent on temperature, ranging from 0% recovery at 5–15°C to 72% at 5–25°C. Seeds of *Triglochin maritima* will germinate when soil salinity is low and the temperature regime is appropriate under natural conditions, or they will remain dormant in the seed bank until soil salinity is reduced and an appropriate temperature regime occurs. Our laboratory investigations with seeds from this Utah population more precisely define the temperature and salinity conditions necessary for maximum germination and recovery of seeds of *T. maritima* from hypersaline conditions. Seeds were not dormant but did have specific temperature requirements for maximum germination.

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**LITERATURE CITED**


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