*Acta Universitatis Latviensis, 2007, Vol. 723, Biology, pp. 115–122*

# Interacting influence of cold stratification treatment and osmotic potential on seed germination of *Triglochin maritima* L.

**Jevgenija Ņečajeva*, Gederts Ievinsh**

Department of Plant Physiology, Faculty of Biology, University of Latvia, Kronvalda Bulv. 4, Rīga LV-1586, Latvia

*Corresponding author, E-mail: sb20025@lanet.lv

**Abstract**

The effect of cold stratification treatment and isoosmotic NaCl and polyethyleneglycol (PEG) concentrations on germination of *Triglochin maritima* seeds was assessed. The highest (400 mM) NaCl concentration caused a significant decrease in germination percentage and no germination was observed at the two highest (64 and 128 mM) PEG concentrations. The large difference in germination among experiment replications suggests that time of collection and length of storage may have influenced germination. Stratification treatment had a significant positive effect on seed germination and there was a significant interaction between PEG, but not NaCl, concentration and duration of cold stratification during germination. Further research is required to determine the importance of the degree of seed ripeness as well as the possible effect of after-ripening or induction of secondary dormancy of undispersed seeds on seed germination.

**Key words:** cold stratification, dormancy, NaCl, polyethyleneglycol, seed germination, *Triglochin maritima*.

## Introduction

Seeds of most of the plants growing in the temperate climate zone possess dormancy mechanisms, the main function of which is to prevent seed germination in an inappropriate season (Vleeshouwers et al. 1995; Baskin, Baskin 1998). Apart from the innate mechanisms, various external factors such as water availability, temperature, light and other determine the ability of seeds to germinate. Endogenous seed dormancy mechanisms interact with external factors throughout the dormancy period (Finch-Savage, Leubner-Metzger 2006).

In regions with cold winter seeds generally germinate in spring, therefore seed dormancy is often broken during a certain period of low temperature, provided that water is available (Baskin, Baskin 1998). Such conditions can be imposed artificially, by storing imbibed seeds at low temperatures, a treatment known as a cold stratification. It has been noted that cold stratification widens the range of temperature at which seeds can germinate, and that the same effect takes place under other environmental factors, including salinity (Baskin, Baskin 1998).

Salinity of the soil solution is an important environmental factor affecting seed

germination of plants growing in the coastal zone. However, comparatively few studies have investigated the interaction of salinity and low temperature treatments. Salinity affects plants in at least two ways: by lowering the osmotic potential in the ambient solution and by ion toxicity (Munns 2002). Low osmotic potential hinders water intake by seeds, thus slowing or altogether inhibiting germination. However, temporary exposure to a substance that lowers osmotic potential to a certain value and subsequent rehydration is known to produce a germination enhancing, or priming, effect (Obroucheva 1999). Treatment with neutral osmotica, such as polyethyleneglycol (PEG) is most often used in promoting germination, however in natural conditions seeds are more likely to be exposed to NaCl and other mineral salts that are toxic at high concentrations. Woodell (1985) classified seed germination that is enhanced after a period of exposure to high salinity as a type three, that is, most salinity-tolerant, seeds. Apart from a priming effect, it has been noted that low salinity levels as such can sometimes enhance seed germination (Baskin, Baskin 1998).

*Triglochin maritima* L. is a halophytic species distributed throughout the Northern hemisphere and restricted to coastal areas and inland saline habitats (Davy, Bishop 1991). Although certain characteristics of seed germination of this species have been described before (Masuda et al. 1999; Khan, Ungar 2001), there is no information available on aspects of the biology of *T. maritima* in the Baltic region. The habitats where *T. maritima* occurs on the coast of the Baltic sea may differ considerably from habitats elsewhere in respect to salinity levels. In addition, *T. maritima* is not a homogeneous species but a species complex (Davy, Bishop 1991). Therefore, considerable differences may exist between different populations. *Triglochin maritima* is listed in the Red Data Book of Latvian flora and is a protected species, which makes it important to understand aspects of reproduction and ecophysiological demands of this species. Seeds of *T. maritima* are characterized by innate dormancy, which can be broken by cold stratification treatment (Masuda et al. 1999).

The aim of this study was to investigate the combined effects of cold stratification and osmotica, in order to determine whether an interaction takes place between them. The use of two different osmotically active substances – potentially toxic NaCl and neutral PEG – was aimed at discerning the effect of lowering the osmotic potential of the solution and that of ion toxicity.

## Materials and methods

Seeds of *Triglochin maritima* were collected in August 2005 and July 2007 near lake Liepaja, Latvia. Seeds were stored dry at 2 - 3 °C before germination experiments were carried out in January 2006 - January 2007 with seeds collected in 2005 and in August 2007 with seeds collected in 2007; germination was pre-assessed shortly after the collection. Seeds were germinated in Petri dishes on top of a double layer of filter paper moistened with distilled water or NaCl or PEG-4000 solution. Concentrations of solutions used were 25, 50, 100, 200 or 400 mM NaCl, and 8, 16, 32, 64 and 128 mM isoosmotic PEG. To test germination recovery after exposure to NaCl solution, at the end of the germination period (14 days) ungerminated seeds were rinsed with distilled water, re-imbibed during 0.5 h and germinated on filter paper moistened with distilled water for another 14 days. Germination was carried out at 20 - 25 °C with a 16 h photoperiod. Germinated seeds were counted three times a week. The effect of cold stratification on seed dormancy was tested

**Table 1.** Germination (%) of *Triglochin maritima* seeds collected in August 2005 and July 2007 imbibed for either 0.5 or 5 h in distilled water. n.d., not determined

| Time of collection | Time of analysis | Imbibition time (h) | |
|---|---|---|---|
| | | 0.5 | 5 |
| August 2005 | January 2006 | 49 ± 6 | n.d. |
| August 2005 | January 2007 | n.d. | 76 ± 4 |
| July 2007 | August 2007 | 78 ± 3 | 81 ± 1 |

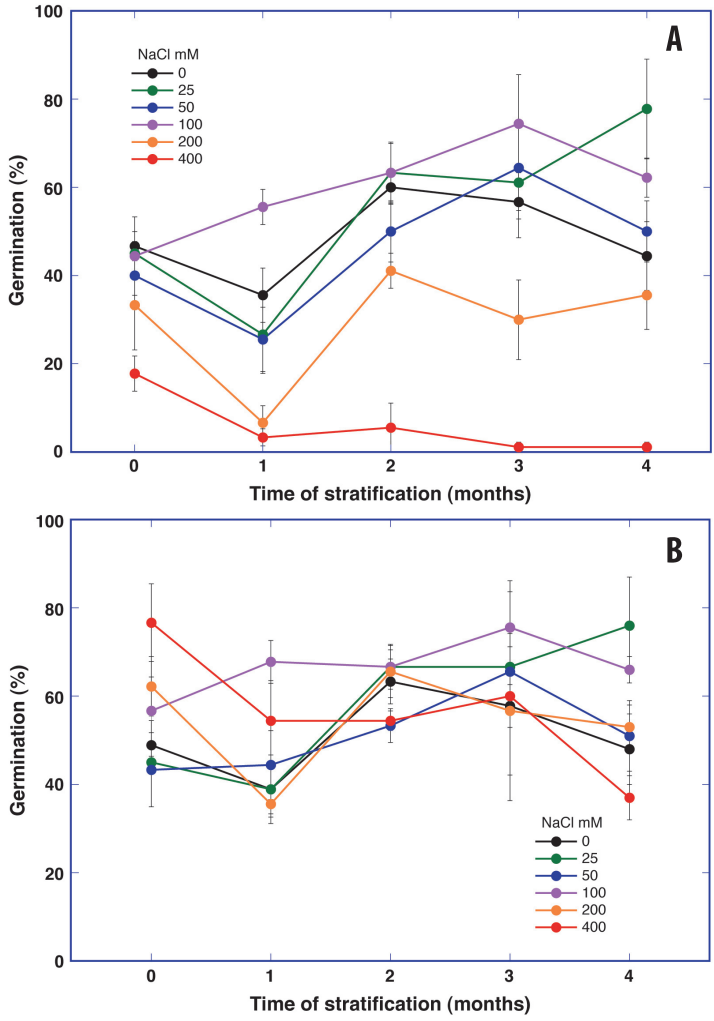


**Fig. 1.** Effect of cold stratification on seed germination of *Triglochin maritima* at different NaCl concentrations before (A) and after (B) seed rinsing with distilled water. Germination percentage after rinsing is a sum of the number of seeds germinated within 14 days before and within 14 days after the rinsing. Seed imbibition time before germination: 0.5 h. Data are means of three replications (30 seeds per replication) for each NaCl concentration, vertical bars denote standard error.

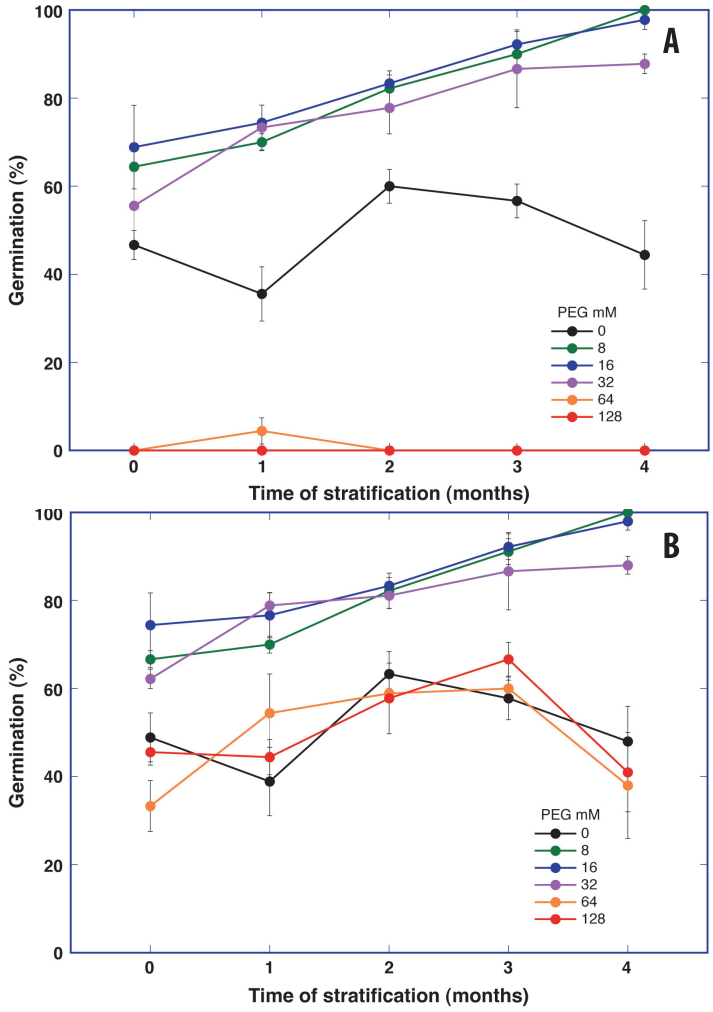


**Fig. 2.** Effect of cold stratification on seed germination of *Triglochin maritima* at different PEG concentrations before (A) and after (B) seed rinsing with distilled water. Germination percentage after rinsing is a sum of the number of seeds germinated within 14 days before and within 14 days after the rinsing). Seed imbibition time before germination: 0.5 h. Data are means of three replications (30 seeds per replication) for each NaCl concentration, vertical bars denote standard error.

by storing fully imbibed seeds at 2 - 3 °C. Originally seeds were imbibed in distilled water or a respective solution for 0.5 h and stratified for one to four months. This experiment was replicated with seeds colleted in 2005, imbibed for 5 h using both NaCl and PEG in variants without a one and two-month-long stratification treatment, but only NaCl in the variants with stratification treatment, as well as with seeds collected in 2007, imbibed for either 0.5 or 5 h and germinated without a stratification pre-treatment (both NaCl and PEG were used). There were three replications of 30 seeds or four replications of 20 seeds (August 2007). Seeds tested for germination immediately after imbibing were used as a control.

Statistical analysis (ANOVA and multiple range tests using 95 % Scheffe interval) was performed using Statgraphics Plus for Windows 4.1.

## Results

The maximum germination percentage observed varied widely among the control variants (Table 1). Nevertheless, the impact of NaCl and PEG was similar in all replications. The number of germinated seeds was significantly reduced when NaCl concentration reached 400 mM; no germination was observed at 64 and 128 mM PEG. There was an interaction between length of cold stratification and PEG concentration ($P < 0.0001$): germination percentage at 8 and 16 mM PEG increased up to 100 ± 0 % and 98 ± 2 %, respectively, after four months of cold stratification (Fig. 2). In contrast, no interaction between cold stratification and NaCl concentration was observed neither in the first, nor in the second replication, although the main effect of cold stratification in the second replication (seeds imbibed for 5 h) was significant ($P < 0.01$; Fig. 1). After re-imbibing seeds in distilled water germination recovered up to the level of the control variant (Fig. 1), except in variants where seeds collected in 2007 were used.

There was a significant difference between variants in which NaCl and PEG were used. In the variants with a higher osmotic potential (25 - 50 mM NaCl and 8 - 16 mM PEG), seed germination was higher if PEG was used as an osmoticum for seeds collected in 2005, but not for seeds collected in 2007, when similar germination percentages were obtained (results not shown). However, in the variants with low osmotic potential (200 - 400 mM NaCl and 64 - 128 mM PEG) germination was always higher if NaCl was used (Fig. 1, 2). It is noteworthy that while the germination percentage was low in the control variant without stratification treatment, a priming effect was observed when seeds germinated at 200 and 400 mM NaCl were re-imbibed in distilled water (Fig. 1).

## Discussion

The difference in final percentage of germinated seeds in the two experiments may have been caused by different time period of storage as well as difference in time when seeds were collected. Storage of dry seeds at low temperature cannot guarantee that no changes occur in the physiological state of the seeds (Baskin et al. 2006). Seeds collected earlier or later in the vegetation season may differ in the degree of ripeness. Moreover, environmental conditions faced by the mother plant were shown to influence dormancy and germination patterns of the ripening seeds (Donohue et al. 2007). Seeds of *T. maritima* ripen at different times during vegetation season, mainly in August and September (Davy, Bishop 1991). Factors such as photoperiod and temperature experienced by mother plants during seed ripening, which obviously vary during vegetation season, can influence the degree of dormancy in mature seeds, creating a heterogeneous seed population (Finch-Savage, Leubner-Metzger 2006). In addition, ripe seeds tend to persist in the spikes and can be dispersed as late as in February (Davy, Bishop 1991), thus seeds may undergo after-ripening or enter secondary dormancy before dispersal. It is also possibile that seeds collected in late July were not fully ripe, which may account for the differences in germination responses. On the one hand, unripe seeds may have not entered dormancy period, which would explain the high germination percentage. On the other hand, unripe seeds may

be more vulnerable when subjected to treatment with osmotica. When seeds collected in July 2007 were germinated at 128 mM PEG, a high proportion of seeds were infected by fungi (Ņečajeva, unpublished results). Fungal infection can serve as an indicator of seed mortality (Baskin, Baskin 1998), and high mortality suggests that the seeds were strongly affected by a low osmotic potential.

It is important to know at which time the seeds had ripened, in addition to the collection time, in order to determine what kind of processes could have taken place before collection. Testing germination of seeds which ripen at different times during several years may shed light on the impact of environmental factors on resulting seeds dormancy, as well as help to reveal the stage at which innate or secondary dormancy is imposed.

It is supposed that seed coats of *T. maritima* contain germination inhibitors (Davy, Bishop 1991). Therefore, lengthy imbibition could lead to enhanced germination by leaching of such substances. However, the imbibition time appears to be an insignificant factor for *T. maritima* seeds, despite a slightly higher germination percentage observed in variants imbibed for 5 h (Table 1). During stratification treatment, the negative effect of the inhibitors, if there was any, may have been overpowered by the mechanisms promoting germination.

In the present experiments, there was a significant difference between the variants where NaCl or PEG were used as an osmoticum (Fig. 1, 2). It is possible that even at high concentrations of NaCl seeds of *T. maritima* are able to imbibe more solution that at isoosmotic concentrations of PEG, which can explain the higher germination percentage at the lowest osmotic potentials. Possibly, this effect accounts for the significant interaction with stratification treatment observed in variants where PEG was used as an osmoticum. Almansouri et al. (2001) found that PEG solution inhibits germination of durum wheat more strongly than NaCl or mannitol, suggesting that intake of NaCl and mannitol occurs to a certain degree, whereas PEG cannot penetrate seeds which as a result do not imbibe. Apparently, NaCl was not toxic to *T. martima* seeds at concentrations used in the experiments, as complete recovery of germination was observed after rinsing (Fig. 1, 2). The priming effect observed when seeds germinated at 400 mM NaCl were re-imbibed in distilled water further supports that osmotic potential in general and ambient concentration of NaCl in particular is among those environmental factors which interact with internal factors in the process of germination. Duan et al. (2004) reported that the effect of priming was greater in *Chenopodium glaucum* seeds when using NaCl, rather than PEG. Enhanced germination after alleviation of salinity effect can be explained as an adaptation to germinate in the rainy season, in the temperate climate – in spring, when rain and thawing of snow and ice reduce salinity (Baskin, Baskin 1998). Seeds of certain halophytes were shown to be able to intake water at elevated salinity levels owing to specfic adaptations, such as high NaCl content in the testa (Song et al. 2005). However, it is not as yet clear what inner physiological mechanisms take part in promoting germination after alleviation of salinity and whether other environmental factors or physiological state of the seed determine the strength of the priming effect.

**Acknowledgements**

This research was funded by the University of Latvia in terms of the project "Biological basis of conservation of rare and endangered species of Latvia: coastal zone" (LU 23).

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## Aukstās stratifikācijas un osmotiskā potenciāla mijietekme uz *Triglochin maritima* L. sēklu dīgšanu

**Jevgēnija Ņečajeva*, Ģederts Ieviņš**

Augu fizioloģijas katedra, Latvijas Universitātes Bioloģijas fakultāte, Kronvalda bulv. 4, Rīga, LV-1586, Latvija
*Korespondējošais autors, E-pasts: sb20025@lanet.lv

**Kopsavilkums**

Pētīja aukstās stratifikācijas un izoosmotisku NaCl un polietilēnglikola (PEG) koncentrāciju ietekmi uz *Triglochin maritima* sēklu dīgšanu. Augstākā (400 mM) NaCl koncentrācija izsauca būtisku dīgšanas procenta samazināšanos, un dīgšana nenotika divu augstāko PEG koncentrāciju klātbūtnē (64 un 120 mM). Ievērojamās dīgšanas atšķirības eksperimentālo atkārtojumu starpā liecina, ka sēklu ievākšanas laiks un to uzglabāšanas ilgums iespējami ietekmējuši dīgšanu. Stratifikācijai bija būtiska pozitīvā ietekme uz sēklu dīgšanu un varēja novērot mijiedarbību starp PEG (bet ne NaCl) koncentrāciju un aukstuma stratifikācijas ilgumu dīgšanas laikā. Nepieciešami tālāki pētījumi, lai noteiktu sēklu gatavības pakāpes un iespējamā pēcgatavības vai sekundārā miera perioda indukcijas ietekmi neizbirušām sēklām uz sēklu dīgšanu.