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Germination and Dormancy in Annual Halophyte *Juncus ranarius* Song & Perr.

Jeremi KOŁODZIEJEK[1]*, Jacek PATYKOWSKI[2]

[1]*University of Lodz, Faculty of Biology and Environmental Protection, Department of Geobotany and Plant Ecology, Banacha 12/16, 90-237 Lodz, Poland; kolo@biol.uni.lodz.pl (* corresponding author)*
[2]*University of Lodz, Faculty of Biology and Environmental Protection, 2Department of Plant Physiology and Biochemistry, Banacha 12/16, 90-237 Lodz, Poland; jacpat@biol.uni.lodz.pl*

Abstract

The effects of cold stratification and gibberellic acid (GA_3) on dormancy breaking for seeds of the annual halophyte species *Juncus ranarius* were tested. Germination percentage and recovery responses of salt stressed seeds were also tested. Freshly collected seeds germinated slowly under all incubation conditions. Thus, the seeds of *J. ranarius* have physiological dormancy, e.g. they are water permeable, have a fully developed embryo and require cold stratification to come out of dormancy. Furthermore, promotion of germination by GA_3 after-ripening in dry storage also indicated that these seeds have non-deep physiological dormancy. In general, the higher the GA_3 concentration, the more germination occurred within the studied range. *Juncus ranarius* demonstrated a germination preference for light. The highest germination percentage and rate of germination were recorded under constant light conditions at 22 °C after 24 weeks of cold stratification. In saline solutions, the highest percentage of germination was obtained at 25 mM L^{-1} NaCl, and further increase in salinity resulted in a gradual decrease in germination. However, ungerminated seeds were not damaged by salt, showing a high level of recovery. The greater the reduction in salinity, the better the germination rate became. It was concluded that dormancy could be completely broken by cold stratification, indicating spring germination. *Juncus ranarius* can grow well at lower NaCl concentrations under constant light conditions at 22 °C.

Keywords: GA_3, recovery of seed germination, salinity, seed dormancy break, seed germination

Introduction

Halophytes, plants capable of growing and reproducing under saline conditions, vary in their upper limits of salt tolerance, while an increase in salinity usually delays seed germination (Gul *et al.*, 2013; Khan and Ungar, 1996; Khan and Ungar, 1998; Ungar and Riehl, 1980; Ungar, 1995); other effects include reduction of the percentage of germinating seeds, delay in the initiation of the germination process and seeds' dormancy. Therefore, the seeds of halophytes germinate only when soil salinity levels are reduced, condition which usually occurs in saline environments in spring or during a season with high precipitation. The low soil salinity levels will befit seedling survival. Moreover, salinities beyond the tolerance limits of a given species can cause complete inhibition of the germination process and lead to loss of seed viability (Ungar, 1978, 1982).

The seeds of halophytes under natural conditions are subjected to saline stress, which is dominated usually by NaCl. The effect of salinity on seed germination can be attributed to hyperosmotic stress resulting from more negative soil water potential and/or a specific ion toxicity (sodium, chloride), depending on a plant species (Huang and Redmann, 1995; Petruzzelli *et al.*, 1992; Poljakoff-Mayber *et al.*, 1994; Ungar and Hogan, 1970; Poljakoff-Mayber *et al.*, 1994; Verslues *et al.*, 2006; Zekri, 1993). Seeds of many halophytic species are reported to germinate best under fresh water conditions or at salinities below 100 mM L^{-1} NaCl (Ungar, 1982, 1991), although storage conditions may also influence the germination response to salt (Katembe *et al.*, 1998; Li *et al.*, 2002; Rozema, 1975; Wetson *et al.*, 2008). The highest salinity concentration at which a seed was reported to germinate was 1.7 M NaCl (Khan and Gul, 2006).

Germination can only occur when dormancy is lost and specific environmental conditions are present (Baskin and Baskin, 1998). Five classes of seed dormancy are now recognized: morphological (MD), physiological (PD), morpho-physiological (MPD), physical (PY) and combinational dormancy (PY + PD) (Baskin and Baskin, 1998, 2004). There are different mechanisms that prevent seed germination until after the right environmental cues have occurred. In morphological dormancy, an embryo is underdeveloped and needs to reach a specific size or

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developmental stage to germinate. The embryo needs a long period of favourable conditions to grow and then germinate, not just dormancy-breaking treatment. When a seed experiences physiological dormancy, germination is prevented by physiological inhibition radical emergence. Morpho-physiological dormancy is due to an underdeveloped embryo that is physiologically dormant. Physical dormancy is caused by a water-impermeable palisade cell layer(s) in seed or fruit coats. Prior to germination, the seed or fruit coat of species with PY must become permeable in order to imbibe water. Physiological and physical dormancy together is called combinational dormancy (PY + PD). Seeds with (PY + PD) have a water impermeable seed or fruit coat (as in PY) and a physiologically dormant embryo (Baskin and Baskin, 2004; Bewley, 1997).

The available information on germination of halophytic seeds is far from complete (Khan and Ungar, 1999). From a total of about 2,400 species of halophytes reported by Lieth *et al.* (1999), germination data are available for a few hundred species (Ungar, 1995). Some studies have evaluated the germination characteristics of all halophytic *Juncus* species (Greenwood and MacFarlane, 2006; Jones and Richards, 1954). Seeds of *Juncus acutus* germinated well without salt (95% in the control treatment), while high salt concentrations prevented them from germination (Vicente *et al*., 2007). Boscaiu *et al.* (2011) demonstrated that in two related species of *Juncus, J. acutus* and *J. maritimus,* germination was optimal under non-saline conditions, while it was reduced by about 50% in the presence of 1.2% (200 mM L^{-1}) NaCl, and completely inhibited by NaCl concentrations above 1.7% (300 mM L^{-1}). Other authors (Dghim *et al*., 2012) reported that both *Juncus* species had high tolerance to NaCl at concentrations, up to 0.6% (100 mM L^{-1}). Variations of light and temperature under saline conditions also affected germination of *Juncus* species. Absence of light almost completely inhibited seed germination of *J. acutus* (Jones and Richards, 1954). However, in freshwater at 15-25 °C, Martinez-Sanchez *et al.* (2006) reported < 75% germination under dark conditions. Clark and Hannon (1970) and Zedler *et al.* (1990) documented *J. kraussii* germination success at NaCl concentrations of ≤ 10 ppt, but not ≥ 20 ppt.

It is important to know the kind of seed dormancy for successful propagation of plants (Baskin and Baskin 1998), but there is little information about ecological and physiological indices of seeds of *J. ranarius* Song and Perr. (also known as *J. ambiguus* Guss.), especially in relation to seed dormancy and germination. Although *J. ranarius* has been described as a halophyte (Jakubowska-Gabara *et al*., 2011; Piernik, 2012; Szafer *et al*., 1988), the species tolerance to salinity has yet to be estimated. According to Ellenberg's salinity indicator values, *J. ranarius* had the value of 4, which indicates that a species is encountered mainly in saline areas (Ellenberg *et al*., 1992). Therefore, the objective of the study was to better understand the seed dormancy and germination characteristics of *J. ranarius* by: (1) determining whether the seeds are water-permeable or water-impermeable via measurements of imbibitions; (2) testing the effects of cold stratification pre-treatment on dormancy break in the seeds; and (3) evaluating the effect of GA_3, salinity, light and temperature on seed germination.

Materials and Methods

Study species

Juncus ranarius is a small, dark green, annual tufted up to 17 cm high. The leaves are linear with full margins, arranged opposite one another. White flowers are in a compound cyme. The plants bloom from June to August. They are wind-pollinated, as most of *Juncaceae*. The seeds (nuts) are 0.33-0.44 × 0.25-0.35 mm, ovoid in shape, almost smooth, indistinctly reticulated (Cope and Stace, 1978, 1983), they weigh approx. 0.017 mg each (our measurements, five replications of 100 seeds each), they ripen between mid-August and early September (our field observations in 2013). The seeds have relatively small embryos, with starchy endosperm, which is soon used up (Crocker and Barton, 1953).

J. ranarius is a typical halophyte, occurring on the coast on mud- and sand- flats above the high-water mark and on the margins of saline and brackish lakes. It is also found on bare mud and waste-ground associated with inland salt-flashes and salt-workings. It is distributed in Europe, parts of North Africa, Asia and North America (Cope and Stace, 1978).

Seed collection and field site description

Mature inflorescences were collected on 1 September 2013 from at least 30 plants of *J. ranarius* growing at inland saline meadow near Pełczyska village (latitude/longitude 52°00'N; 19°11'E) in central Poland. Immediately after collection, the seeds

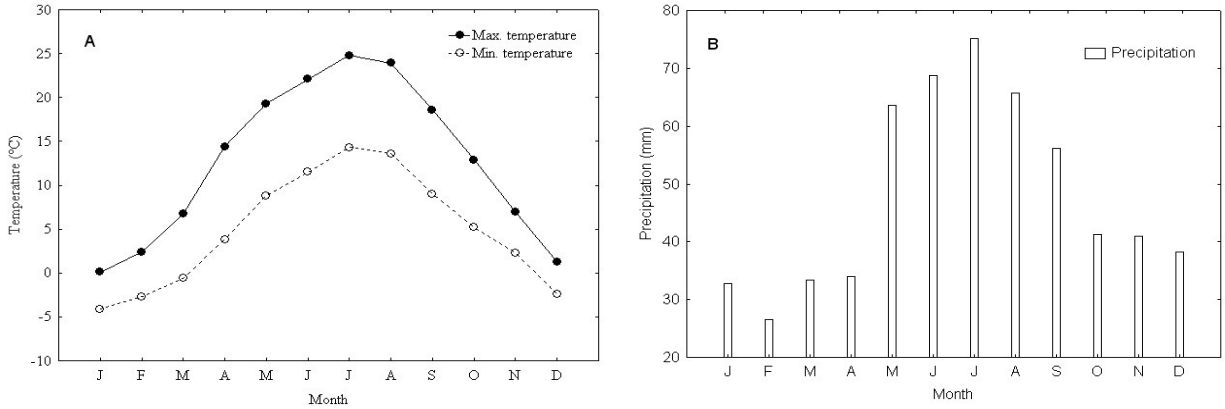

Fig. 1. Mean (2000-2010) minimum and maximum temperatures (A) and precipitation (B) at the nearest meteorological station Lodz

were separated from inflorescences, hand sorted to eliminate broken, small and infected ones. Seeds were sterilized in 1% (v/v) sodium hypochlorite solution for 10 min and washed three times with sterile distilled water and then dry stored in paper bags at room temperature.

The local climate is temperate, the seasons are clearly differentiated. Meteorological data based on 10-year measurements (2000-2010) indicated that the mean annual temperature was 8.8 °C. The average low temperature during winter was -2.5 °C and the average high temperature during summer was 22.4 °C. Annual precipitation (rain and snow) was 587.2 mm, and frost-free period 271 days (Fig. 1).

According to the observations in the field during the current study, *J. ranarius* occurs in almost pure patches or co-occurs with other halophyte species such as *Glaux maritima* and *Spergularia salina* along a gradient of reduced salinity. Minimum and maximum soil salinities (in the 0-2 cm soil layers) in the studied site were 0.1% (22 mM L^{-1}) and 0.6% (100 mM L^{-1}) NaCl respectively, varying markedly through the year.

Experiment 1: seeds imbibition

This experiment was done to determine whether seeds were non-dormant or dormant (due to a water-impermeable seed coat). This test was conducted at room temperature (~24 °C). Four replicates of 150 seeds each were weighed (to the nearest 0.001 mg), moistened for 5 min in individual Petri dishes of 5 cm in diameter, lined with moist filter papers, removed from the dishes, blotted dry and reweighed (time 0). The seeds were reweighed again after 1, 6, 12, 24, 48, 72 and 96 h of water absorption. Percentage increase in fresh weight of the seeds was calculated as follows: $[(W_i - W_d)/W_d] \times 100$, where W_i and W_d = weight of imbibed and dry seeds, respectively (Turner *et al.*, 2006).

Experiment 2: light and temperature effects on seed germination

Freshly matured seeds (14 days after harvest) were placed in individual Petri dishes of 5 cm in diameter with four filter paper discs moistened with distilled water until saturated. Twenty-five seeds were placed in each dish. To assess the effect of light and temperature on germination, four Petri dishes were covered with double layers of aluminium foil to ensure no light penetration (dark treatment) and four dishes were sealed with parafilm (light treatment). Light treated dishes were placed on top of dark treated ones and placed in a room with one of 3 constant temperature regimes: 5, 10, or 22 °C under a cycle of 14 h of light (about 30 µmol m^{-2} s^{-1} provided by fluorescent lamps) and 10 h of darkness. This temperature regimen was chosen to replicate the mean monthly temperatures recorded close-by at a climatological station during the growing season: 4.4 °C (early April), 10.3 °C (early May), 21.6 °C (July), when most seeds germinate in the natural habitat (Fig. 1A). Moisture was maintained with distilled water. The dishes were inspected daily and germinated seeds were counted during 20 days from the start of the test. Germinated seeds were counted in the light treatments, whereas counts in the dark treatments were made under dim green safe light. Seed germination was accepted when the radical appeared. The rate of germination was estimated using a modified Timson's index of germination velocity G/t, where G is seed germination percentage each day and t the total germination period (Khan and Ungar, 1997). Therefore if all of the seeds germinated in one day, the Timson's index would be 100 (e.g. 2000/20), and a higher value indicates a more rapid germination.

Breaking dormancy of seeds

The low germination percentage (< 25%) of freshly matured seeds of *J. ranarius* under both light and dark condition in all temperatures indicated that some seeds were dormant. However, further investigations need to be done in order to find out which dormancy-breaking factor is needed to trigger germination. *Juncus ranarius* seeds are dispersed in autumn, and thus they probably receive cold stratification after dispersion; 5 °C is near-optimal for many seeds with PD, requiring low moisture and low temperature to break dormancy (Baskin and Baskin, 1998; Stokes, 1965). Gibberellic acid (GA_3) is an important endogenous plant growth regulator that can break dormancy in seeds with non-deep PD (Baskin and Baskin 1998; Nikolaeva *et al.*, 1985). Hence, the influence of cold stratification and gibberellic acid (GA_3) on seed dormancy breaking was tested.

Experiment 3: cold stratification treatment

For this experiment, freshly matured seeds (2 weeks after collection) were placed in each of four Petri dishes, wrapped in aluminium foil and stored at 5 °C in a refrigerator, for 24 weeks. Twenty five seeds were placed in each dish. Next, all Petri dishes were incubated in a growth chamber at constant 5, 10, or 22 °C under a cycle of 14 h of light (about 30 µmol m^{-2} s^{-1} provided by fluorescent lamps) and 10 h of darkness. Seeds were considered to be germinated when a radical emerged from the seed coat. The number of germinated seeds was counted daily during 20 days. Germinated seeds were counted in the light treatments, whereas counts in the dark treatments were made under dim green safe light. Filter papers were kept moist with distilled water and germinated seeds were removed during every inspection.

Experiment 4: gibberellic acid (GA_3) treatments

The GA_3 treatments comprised three concentrations of GA_3: 0.1, 1 and 10 mM L^{-1}. For each treatment, four replications of 25 seeds were incubated in 5 ml of each solution mentioned above, for 48 hours. The seeds treated with GA_3 solutions and those not treated with GA_3 (control) were then placed to germinate in a growth chamber at constant 22 °C under a cycle of 14 h of light (about 30 µmol m^{-2} s^{-1} provided by fluorescent lamps) and 10 h of darkness. Preliminary experiments showed that constant 22 °C were optimal for germination of fresh seeds, under the 14 h photoperiod. During experiment, GA_3 solution was added whenever was necessary to keep moist use. The seeds were checked every day for emergence of a radical, during 20 days, and discarded when the tip of the radicle emerged.

Experiment 5: germination tests under saline conditions

To determinate the effect of salinity on germination and recovery of *J. ranarius* seeds, they were germinated in distilled water and 10, 25, 75 and 100 mM L^{-1} NaCl solutions. Four 25-seed replicates of each treatment were placed on filter paper in 5 cm Petri dishes with 5 mL of the test solution under conditions identical to those described for Experiment 4. The water level was adjusted daily with distilled water to avoid changes in salinity due to evaporation (Mauchamp and Mésleard, 2001; Redondo *et*

al., 2004). Salinity concentrations were chosen to cover variations in inland saline meadow near Pełczyska village. The seeds were counted during 20 days as they germinated and discarded when the tip of a radicle emerged. For the recovery period, ungerminated seeds were removed from the Petri dishes, rinsed three times (10 min each) with sterile deionized water, and put into new 5 cm Petri dishes lined with two sheets of filter paper imbibed with 2.5 mL of sterile deionized water. Recovery percentage was calculated by the following formula: $[(A - B)/(C - B)] \times 100$, where A is the number of seeds that germinated in salt solution plus those that recovered to germinate in distilled water (pH 5.7); B is the number of seeds germinated in salt solution and C is the total number of seeds tested (Gul and Weber, 1999). Final germination was recorded as $(A/C) \times 100$ (Wang *et al.*, 2008). The remaining ungerminated seeds were tested for viability by staining with tetrazolium chloride (Moore, 1962). The rate of germination was determined as described for Experiment 2.

Data analysis

The effects of light, salinity and temperature on the germination and rate of germination were examined using analysis of variance (ANOVA). Prior to analysis, percentage of germination and germination velocity were arcsine-transformed to stabilize the variance. The transformed data were subjected to factorial analysis of variance followed by Tukey's test 'a posteriori' multiple rage test and significances are indicated in the form of letters. All statistical tests were performed using a software package STATISTICA (Statsoft Inc., 2011).

Results

Imbibition tests

The seeds imbibed water, and increased in mass with prolongation of the imbibition period. The mass of seeds increased by 12.42 ± 2% (mean ± S.D.) after 24 h, 59.23 ± 4% after 48 h and 73.41 ± 6% after 72 h (Fig. 2).

Effect of temperature and light on germination of freshly harvested seeds

Germination of the freshly matured seeds was poor in all constant temperature regime. Significant difference was observed ($P < 0.05$) in germination percentage between the incubation in light and in dark at all temperature regime. Germination percentage reached 26% at the highest temperature in light (22 °C), but at 10 °C and 5 °C it was only 18% and 3% respectively. In dark, few seeds of *J. ranarius* germinated at 22 °C, while at 10 °C and 5 °C, germination was completely inhibited (Table 1).

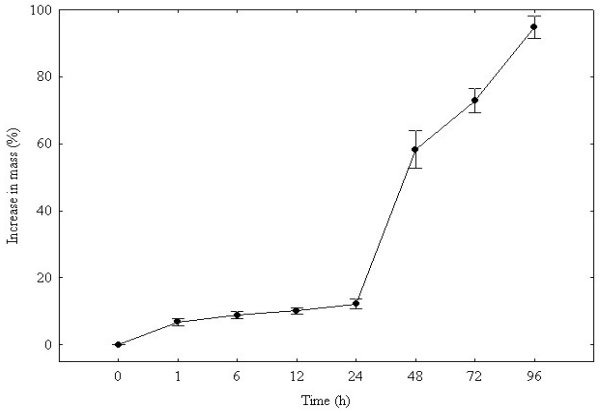

Fig. 2. Imbibition curve for seeds of *J. ranarius* in distilled water

Table 1. Germination percentage (mean ± S.D.) of the non-treated (freshly matured) and 24-week-cold-stratified seeds of *J. ranarius* at three thermo-periods in light and dark

Light condition	Temperature	Non-treated seeds	24-week-cold-stratified
Light	5 °C	3 ± 2 Aa	7 ± 2 Aa
	10 °C	18 ± 2 Ab	33 ± 5 Bb
	22 °C	26 ± 5 Ab	85 ± 6 Bc
Dark	5 °C	0 ± 0 Aa	2 ± 0 Aa
	10 °C	0 ± 0 Aa	13 ± 2 Bb
	22 °C	7 ± 2 Aa	25 ± 3 Bc

Note: Different upper-case letters indicate significant differences by Tukey's test in the germination percentages between the non-treated and 24-week-cold-stratified seeds at the same temperature and different lower-case letters in each column with the same light conditions indicate significant differences in the germination percentages of seeds among different temperatures

Table 2. Index of germination velocity of the non-treated and 24-week-cold-stratified seeds of *J. ranarius* at three thermo-periods under different light conditions. Each value is a mean ± S.D. of four replicates of 25 seeds

Light condition	Temperature	Non-treated seeds	24-week-cold-stratified
Light	5 °C	1 ± 1 Aa	1 ± 1 Aa
	10 °C	5 ± 1 Ab	25 ± 1 Bb
	22 °C	8 ± 2 Ac	58 ± 3 Bc
Dark	5 °C	0 ± 0 Aa	0 ± 0 Aa
	10 °C	1 ± 1 Aa	4 ± 1 Aa
	22 °C	2 ± 1 Aa	11 ± 2 Ba

Note: Different upper-case letters indicate significant differences by Tukey's test between germination rate of the non-treated and 24-week-cold-stratified seeds at the same temperature and different lower-case letters in each column with the same light conditions indicate significant differences in germination rate of the seeds among different temperatures

Seed dormancy-break and germination

The difference in the rate and percentage of germination between the fresh seeds and cold stratified ones was significant ($P < 0.05$). The rate of germination grew after cold stratification, with an increase in the germination index under light from 1 to 58 and under dark from 2 to 11, at 22 °C. The germination rate was slower as the temperature was reduced. The results showed that after cold stratification treatment, germination percentage ($P < 0.01$) and germination velocity ($P < 0.05$) of the seeds under light and dark conditions were significantly higher than of those that had not been stratified. After the period of 24 weeks of cold stratification, the highest germination percentage (85%) was observed at 22 °C in light followed by 10 °C and 5 °C, which caused germination percentages of 37% and 7% respectively (Table 2). Light conditions ($P < 0.001$), temperature ($P < 0.001$), cold stratification ($P < 0.001$) and their interaction ($P < 0.05$) were all of significant importance to both percentage and rate of germination (Table 3).

Effect of GA_3

The treatment with GA_3 was of significant importance for germination. The seed germination percentage was increasing with growing GA_3 concentration. An addition of 0.1 mg L^{-1} GA_3 increased germination, while 10 mg L^{-1} GA_3 was optimum (>

Table 3. Three-way ANOVA of the effects of temperature (T), light conditions (L, light vs. darkness), cold stratification (CS, non-treated vs. 24-week-cold-stratified) seeds of *J. ranarius* and their interactions on seed germination and rate of germination

Dependent variable	Factor	d.f.	SS	MS	F-value	P-value
Germination (%)	Light (L)	1	39.46	39.46	21.09	< 0.001
	Temperature (T)	2	60.62	30.31	21.01	< 0.001
	Cold stratification (CS)	1	10.26	10.26	4.09	< 0.001
	L x T	2	2.05	1.03	5.82	0.006
	L x CS	1	0.88	0.88	4.96	0.01
	T x CS	2	4.25	2.12	12.03	< 0.001
	L x T x CS	2	1.66	0.83	4.71	0.01
Rate of germination	Light (L)	1	26.99	26.99	237.81	< 0.001
	Temperature (T)	2	33.46	16.73	147.36	< 0.001
	Cold stratification (CS)	1	7.31	7.31	64.34	< 0.001
	L x T	2	0.95	0.83	0.93	0.01
	L x CS	1	1.42	1.42	12.48	0.001
	T x CS	2	13.59	6.79	59.87	< 0.001
	L x T x CS	2	0.77	0.38	3.41	0.04

Table 4. Germination percentage and index of germination velocity in *J. ranarius* seeds at different GA_3 concentrations

mM L^{-1}	Germination percentage	Index of germination velocity
0	27 ± 3.0 b	13.5 ± 1.3 c
0.1	77 ± 1.7 c	58.3 ± 1.1 a
1	93 ± 2.5 a	62.5 ± 1.5 ab
10	100 ± 4.0 a	70 ± 2.0 b

Note: Each value is a mean ± S.D. of four replicates of 25 seeds. Data sharing the same letter in each column are not significantly different (Tukey's test, P < 0.05)

Table 5. Seed germination and rate of germination in *J. ranarius* seeds at different NaCl concentrations

mM L^{-1}	Germination percentage	Index of germination velocity
0	78 ± 2 a	16.5 ± 3.8 a
10	86 ± 2 b	17.0 ± 1.7 ab
25	95 ± 6 c	25.8 ± 2.9 b
50	70 ± 2 d	11.8 ± 5.4 ac
75	54 ± 3 e	7.0 ± 2.3 c
100	38 ± 6 f	1.0 ± 0 d

Note: Each value is a mean ± S.D. of four replicates of 25 seeds. Data sharing the same letter in each column are not significantly different (Tukey's test, P < 0.05)

Table 6. Germination of the seeds of *J. ranarius* in relation to salinity and germination recovery in distilled water

Salinity treatment (mM L^{-1})	Germination characteristics	
	Recovery percentage (%)	Final germination (%)
0	19 ± 2 d	82 ± 5 a
10	29 ± 4 e	90 ± 2 abc
25	51 ± 1 a	92 ± 6 ac
50	60 ± 3 ab	95 ± 3 abc
75	66 ± 7 bc	89 ± 2 ab
100	81 ± 5 c	86 ± 4 a

Note: Each value is a mean ± S.D. of four replicates of 25 seeds. Data sharing the same letter in each column are not significantly different (Tukey's test, P < 0.05)

90% germination). The same trend was also visible for seed germination velocity: the Timson's index of the germination velocity was significantly higher after GA_3 substrate supplementations compared to the control. In general, the higher the GA_3 concentration, the more germination occurred within the studied range ($F_{2,21} = 21.331$, $P < 0.001$) (Table 4).

Effect of salinity on germination and recovery of germination

Under salinity conditions, the seed germination percentage of *J. ranarius* was significantly higher for 10 mM L^{-1} and 25 mM L^{-1} NaCl, while significantly lower for 50, 75 and 100 mM L^{-1} NaCl, then the corresponding ones in H_2O. The highest percentage of germination was obtained at 25 mM L^{-1} NaCl, and further increase in salinity resulted in a gradual decrease of germination. Less than 5% of seeds germinated at 100 mM L^{-1} NaCl (Table 5).

The seed germination velocity at the low NaCl concentration (10 mM L^{-1}) was the same as for the water control, while at 25 mM L^{-1} it was significantly higher, and with 50, 75 and 100 mM L^{-1} NaCl it was significantly lower than the corresponding velocity in H_2O. It suggests that *J. ranarius* seed germination time was shortened at the 25 mM L^{-1} NaCl, but higher levels of NaCl (> 25 mM L^{-1}) prolonged it (Table 5).

When the seeds were transferred to distilled water after twenty-days exposure to salinity, recovery of germination was depended on NaCl concentration. There was little recovery in the lowest (10 mM L^{-1}) NaCl variant, but in the highest (100 mM L^{-1}) it reached up to 81% (Table 6).

A tetrazolium test was performed on the non-germinated seeds and revealed that these seeds were viable, showing absolute dormancy.

Discussions

Prior to this study, the types of dormancy and methods to release it in *J. ranarius* seeds were not known. In the present study, it was noticed that the seeds produced by *J. ranarius* are dormant on maturity and require pre-treatment to stimulate germination. The embryo of *J. ranarius* is fully developed, suggesting that the seeds have neither morphological, nor morphophysiological dormancy. Since the seed coat of *J. ranarius* is water permeable, neither physical dormancy, nor combinatorial dormancy appears likely, and thus, these seeds exhibit non-deep PY (sensu Baskin and Baskin, 2004). The result is consistent with the report of Polyakoff-Mayber *et al.* (1992), who suggested that seeds of some species of *Juncus* are dormant at harvest. In contrast to this finding, in other halophyte *Juncus* species, for example *J. balticus,* seeds were considered to be non-dormant (Necajeva and Levinsh, 2008).

GA_3 application and cold stratification substantially increased germination of *J. ranarius* seeds. Thus, the seeds of

this species have non-deep physiological dormancy. Similar results were obtained by Rozema (1975) studying several species of *Juncus*.

Annual *Juncus* species vary in their level of salt tolerance at the germination stage. For example, *J. gerardii* and *J. acutus* are very salt intolerant with germination severely depressed by even moderate salinities (Rozema and Blom, 1977; Shumway and Bertness, 1992). In the present study, germination of *J. ranarius* seeds was considerably reduced at the salinity level of 50 mM L^{-1} NaCl and was completely inhibited by 100 mM L^{-1} NaCl, because it probably exceed individual tolerance limits. Another halophyte from the area, *Spergularia salina* was reported to germinate best under fresh water conditions or at salinities below 100 mM L^{-1} NaCl (Bakker *et al.*, 1985). Keiffer and Ungar (1997) pointed out that halophytes germinate during spring, rather than in summer, in order to avoid the increase in salt concentration in soil solution caused by high evaporation. Some plants that have been classified as salt sensitive can germinate under high concentrations of NaCl, while other tolerant species are more sensitive during germination.

Several investigators reported that the germination process was delayed under salt stress (Keiffer and Ungar, 1997; Khan and Ungar, 1997). However, in some halophytic species, the presence of sodium ions even at low concentrations could have positive effect on seed germination (Ungar 1991), increasing its rate over the distilled water control (Sabahat and Khan, 2004). The results obtained in the present study showing that germination in low salinity (10 and 25 mM L^{-1}) began earlier than in the non-saline control support this finding. It has been suggested that fast germination ensures rapid seedling establishment, which can minimize competition (Rogers *et al.*, 1995). Distilled water has zero osmosis potential and some seeds show lower rate of germination at this potential (Sedghi *et al.*, 2010).

Juncus ranarius seeds, when transferred to fresh water, showed an enhanced rate of germination after pre-treatment with various salinity concentrations. This is in agreement with the results of experiments in which seeds were subjected to salinity and then placed in fresh water (Clarke and Hannon, 1970; Keiffer and Ungar, 1997; Khan and Ungar, 1997; Ungar, 1995; Woodell, 1985). This response suggests that the inhibition of *J. ranarius* seed germination at high salt concentration was mostly due to osmotic effects and reversible, as found by Ungar (1978, 1996). It is likely that after immersion in salt water, seeds will be soon washed by rain, which will stimulate their germination.

The response to salinity and germination recovery patterns allow classification of *J. ranarius* seeds as having intermediate salinity tolerance (Woodell, 1985). However, not only salt concentrations (or osmotic potential), but also nature of the ions in salt solutions and their interactions may have an impact on germination (Sosa *et al.*, 2005). Ungar (1978) pointed out that seed germination in salt-affected soils was influenced by the total concentration of dissolved salt (or the osmotic pressure) as well as by the type of salts involved.

Since NaCl is the major component of most saline soils, it was used to challenge the plants. However, such investigations may not allow to infer germination responses of plants under field conditions, because field soil contains different salts, which collectively influence seed germination in a different way than each one of them separately (Ungar, 1978).

Light (photoperiod) is another important regulatory environmental signal in germination of many halophytes (Ungar, 1978). Light also stimulates germination of *Haloxylon recuvrum* and *Triglochin maritima* (Khan and Ungar, 1997). In the present work, germination was found to be controlled by light. This suggests that successful *J. ranarius* germination and establishment require high-light environment where soils are bare and exposed. This germination response may limit this species colonisation to open locations. A light requirement for seed germination is common, especially in species that have small seeds (Taylorson, 1987). Light was reported as necessary for germination of many *Juncus* species (Burkart *et al.*, 2010; Lazenby, 1955; Martinez-Sanchez *et al.*, 2006; Richards and Clapham, 1941b; Richards, 1943) including *J. effusus* (Richards and Clapham, 1941a).

Temperature is an important environmental signal regulating germination of many herbaceous plants from temperate regions. Some species require cold stratification to initiate germination, or to increase their germination rate in spring (Baskin and Baskin, 1998). Our data clearly indicate that *J. ranarius* had increased germination following cold stratification. Improved germination after short cold stratification is typical of species with non-deep physiological dormancy (Baskin and Baskin, 1998).

Best seed germination of most temperate species occurred at 15-30 °C (Khan and Gul, 2006) with an average of 21 °C (Baskin and Baskin, 1998). It was shown in this study that germination and rate of germination increased with rising temperature and the optimal germination was obtained at a constant temperature of 22 °C. In early spring, lower temperatures can cause high mortality. Field observations suggested that germination only occurred when a certain critical temperature was reached. Seedlings, first seen at the beginning of May, were estimated to have germinated approximately on 20^{-th} April, at which period maximum day temperatures reached 22 °C and night minima was 5 °C (Fig. 1).

Conclusions

The findings of this study indicate that salt stress, light, temperature and cold stratification are critical determinants of the germination of *J. ranarius* seeds. Most of the seeds produced by *J. ranarius* are dormant on maturity and require pre-treatment to stimulate germination. These results suggested that *J. ranarius* becomes established in vegetation gaps during spring through germination of seeds originating from a soil seed bank preserved over winter and brought into light by some local disturbance. Maximum seed germination was obtained in 25 mM L^{-1} NaCl salinity, similar to the minimum soil salinities at the study site. The high salinities (> 50 mM L^{-1} NaCl) typical of locally disturbed microsites, would not allow the seeds to germinate. However, inter-annual variations in climatic conditions may influence substrate salinity during the period of seed germination and, consequently, cause significant inter-annual fluctuations in population size.

References

Bakker JP, Dijkstra M, Russchen PT (1985). Dispersal, germination and early establishment of halophytes and glycophytes on a grazed and abandoned salt-marsh gradient. New Phytologist 101:291-308.

Baskin CC, Baskin JM (1998). Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego, 666 p.

Baskin JM, Baskin CC (2004). A classification system for seed dormancy. Seed Science Research 14:1-16.

Bewley JD (1997). Seed germination and dormancy. The Plant Cell 9:1055-1066.

Boscaiu M, Ballesteros G, Naranjo MA, Vicente O, Boira H (2011). Responses to salt stress in *Juncus acutus* and *J. maritimus* during seed germination and vegetative plant growth. Plant Biosystems 145:770-777.

Burkart M, Alsleben K, Lachmuth S, Schumacher J, Hofmann R, Jeltsch F, Schurr FM (2010). Recruitment requirements of the rare and threatened *Juncus atratus*. Flora 205:583-589.

Clarke LD, Hannon NJ (1970). The mangrove swamp and salt marsh communities of the Sydney district: III. Plant growth in relation to salinity and waterlogging. The Journal of Ecology 58:351-369.

Cope TA, Stace CA (1978). The *Juncus bufonius* L. aggregate in western Europe. Watsonia 12:113-128.

Cope TA, Stace CA (1983). Variation in the *Juncus bufonius* L. aggregate in western Europe. Watsonia 14:263-272.

Crocker W, Barton LV (1953). The physiology of seeds. Waltham, Massachusetts, 257 pp.

Dghim F, Gorai M, Boukhris M, Neffati M (2012). Influence of salinity on germination patterns of two *Juncus* species inhabiting salt marshes in southern Tunisia. Revue des Régions Arides 27:21-34.

Ellenberg H, Weber HE, Dűll R, Wirth V, Werner W, Paulissen D (1992). Zeigerwerte von Pflanzen in Mitteleuropa. Scripta Geobotanica 18.

Greenwood ME, MacFarlane GR (2006). Effects of salinity and temperature on the germination of *Phragmites australis*, *Juncus kraussii*, and *Juncus acutus*: implications for estuarine restoration initiatives. Wetlands 26:854-861.

Gul B, Weber DJ (1999). Effect of salinity, light, and thermo-period on the seed germination of *Allenrolfea occidentalis*. Can J Bot 77:1-7.

Gul B, Ansari R, Flowers TJ, Khan MJ (2013). Germination strategies of halophyte seeds under salinity. Environmental and Experimental Botany 92:4-18.

Huang J, Redmann RE (1995). Salt tolerance of *Hordeum* and *Brassica* species during germination and early seedling growth. Canadian Journal of Plant Science 75:815-819.

Jakubowska-Gabara J, Kucharski L, Zielińska K, Kołodziejek J, Witosławski P, Popkiewicz P (2011). Atlas rozmieszczenia roślin naczyniowych w Polsce Środkowej. [Atlas of vascular plants distribution in central Poland]. Łódź, Wydawnictwo Uniwersytetu Łódzkiego, 283 p (in Polish).

Jones V, Richards PW (1954). *Juncus acutus* L. Journal of Ecology 42:639-650.

Katembe WJ, Ungar IA, Mitchell JP (1998). Effect of salinity on germination and seedling growth of two *Atriplex* species (Chenopodiaceae). Annals of Botany 82:167-175.

Keiffer CH, Ungar IA (1997). The effect of extened exposure to hypersaline conditions on the germination of five inland halophyte species. American Journal of Botany 84:104-111.

Khan MA, Ungar IA (1996). Influence of salinity and temperature on the germination of *Haloxylon recurvum*. Annals of Botany 78:547-551.

Khan MA, Ungar IA (1997). Effects of thermoperiod on recovery of seed germination of halophytes from saline conditions. American Journal of Botany 84:279-283.

Khan MA, Ungar IA (1998). Germination of salt tolerant shrub *Suaeda fruticosa* from Pakistan: Salinity and temperature responses. Seed Science and Technology 26:657-667.

Khan MA, Ungar IA (1999). Seed germination and recovery of *Triglochin maritima* from salt stress under different thermoperiods. Great Basin Naturalist 59:144-150.

Khan MA, Gul B (2006). Halophyte seed germination. In: Khan MA, Weber DJ (Eds). Eco-physiology of high salinity tolerant plants. Springer Publication, Netherlands pp 11-30.

Lazenby A (1955). Germination and establishment of *Juncus effusus* L. The effect of different companion species and variation in soil and fertility conditions. The Journal of Ecology 43:103-119.

Li H, Kefu Z, Xiufeng W (2002). The inhibition of salinity on the germination of halophyte seeds. Journal of Shandong Agricultural University 33:170-173.

Lieth H, Moschenco M, Lohmann M, Koyro HW, Hamdy A (1999). Halophyte uses in different climates. I. Ecological and ecophysiological studies. In: Lieth H (Ed). Progress in biometeriology. Leiden, Backhause, pp 1-258.

Mauchamp A, Mésleard F (2001). Salt tolerance in *Phragmites australis* populations from coastal Mediterranean marshes. Aquatic Botany 70:39-52.

Martínez-Sánchez JJ, Conesa E, Vicente MJ, Jimeneza A, Franco JA (2006). Germination responses of *Juncus acutus* (Juncaceae) and *Schoenus nigricans* (Cyperaceae) to light and temperature. Journal of Arid Environments 66:187-191.

Moore RP (1962). Tetrazolium as a universally acceptable quality test of viable seed. Proceedings of the International Seed Testing Association 27: 795-805.

Necajeva J, Ievinsh G (2008). Seed germination of six coastal plant species of the Baltic region: effect of salinity and dormancy-breaking treatments. Seed Science Research 18:173-177.

Nikolaeva MG, Rasumova MV, Gladkova VN (1985). Reference book on dormant seed seed germination (in Russian). Nauka, Leningrad.

Petruzzelli L, Melillo MT, Zacheo TB, Taranto G (1992). Physiological and ultrastructural changes in isolated wheat embryos during salt and osmotic shock. Annals of Botany 69:25-31.

Piernik A (2012). Ecological pattern of inland salt marsh vegetation in Central Europe. Wydawnictwo Naukowe Uniwersytetu Mikołaja Kopernika, Toruń, 229 p.

Poljakoff-Mayber A, Somers GF, Werker E, Gallagher JL (1992). Seeds of *Kosteletzkya virginica* (Malvaceae): their structure, germination, and salt tolerance. 1. Seed structure and germination. American Journal of Botany 79:249-256.

Poljakoff-Mayber A, Somers GF, Werker E, Gallagher JL (1994). Seeds of *Kosteletzkya virginica* (Malvacae): their structure, germination and salt tolerance. II. Germination and salt tolerance. American Journal of Botany 81:54-59.

Redondo S, Rubio-Casal AE, Castillo JM, Luque CJ, Álvarez AA, Luque T, Figueroa ME (2004). Influences of salinity and light on germination of three *Sarcocornia* taxa with contrasted habitats. Aquatic Botany 78:255-264.

Richards PW (1943). Biological flora of the British Isles: *J. macer, J. filiformis*. Journal of Ecology 31:51.

Richards PW, Clapham AR (1941a). *Juncus effusus* L. Journal of Ecology 29:375-380.

Richards PW, Clapham AR (1941b). Biological flora of the British Isles: *J. inflexus; J. effuses; J. subnodulosus*. Journal of Ecology 29:369, 375, 385.

Rogers ME, Noble CL, Halloran GM, Nicolas ME (1995). The effect of NaCl on germination and early seedling growth of white clover (*Trifolium repens* L.) population selected for high and low salinity tolerance. Seed Science and Technology 23:277-287.

Rozema J (1975). The influence of salinity, inundation and temperature on the germination of some halophytes and non-halophytes. Oecologia Plantarum 10:341-353.

Rozema J, Blom BN (1977). Effects of salinity and inundation on the growth of *Agrostis stolonifera* and *Juncus gerardii*. Journal of Ecology 65:213-222.

Sabahat Z, Khan MA (2004). Effect of light, salinity and temperature on seed germination of *Limonium stocksii*. Canadian Journal of Botany 82:151-157.

Sedghi M, Nemati A, Esmaielpour B (2010). Effect of seed priming on germination and seedling growth of two medicinal plants under salinity. Emirates Journal of Food and Agriculture 22:130-139.

Shumway SW, Bertness MD (1992). Salt stress limitation of seedling recruitment in a salt marsh plant community. Oecologia 92:490-497.

Sosa L, Llanes A, Reinoso H, Reginato M, Luna V (2005). Osmotic and specific ion effects on the germination of *Prosopis strombulifera*. Annals of Botany 96:261-267.

Stat-Soft Inc (2011). STATISTICA for WINDOWS. Stat-soft. Inc. Tulsa.

Stokes P (1965). Temperature and seed dormancy. In: Ruhland W (Ed). Encyclopedia of plant physiology, Vol. 15. Springer-Verlag, Berlin pp 746-803.

Szafer W, Kulczyński S, Pawłowski B (1988). Rośliny polskie [Polish plants]. Wydawnictwo Naukowe PWN, Warszawa (in Polish).

Taylorson RB (1987). Environmental and chemical manipulation of weed seed dormancy. Reviews of Weed Science 3:135-154.

Turner SR, Merritt DJ, Baskin JM, Baskin CC, Dixon KW (2006). Combinational dormancy in seeds of the Western Australian endemic species *Diplopeltis huegelii* (Sapindaceae). Australian Journal of Botany 54:1-6.

Ungar IA (1978). Halophyte seed germination. The Botanical Review 44:233-264.

Ungar IA (1982). Germination ecology of halophytes. In: Sen DN, Rajpurchit KS (Eds). Contributions to the ecology of halophytes. The Hague, Junk pp 143-154.

Ungar IA (1991). Ecophysiology of vascular halophytes. Boca Raton, CRC Press, Louisiana.

Ungar IA (1995). Seed germination and seed bank ecology in halophytes. In: Kigel J, Galili G, Dekker M (Eds). Seed development and seed germination, Marcel Dekker, New York pp 599-628.

Ungar IA (1996). Effects of salinity on seed germination, growth, and ion accumulation of *Atriplex patula* (Chenopodiaceae). Am J Bot 83:604-607.

Ungar IA, Hogan WC (1970). Seed germination in *Iva annua* L. Ecology 51:150-154.

Ungar IA, Riehl TE (1980). The effect of seed reserves on species composition in zonal halophyte communities. Botanical Gazette 14:447-452.

Verslues PE, Agarwal M, Katiyar-Agarwal S, Zhu J, Zhu JK (2006). Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. The Plant Journal 45:523-539.

Vicente MJ, Conesa E, lvarez-Rogel JA, Franco JA, Martinez-Sanchez JJ (2007). Effects of various salts on the germination of three perennial salt marsh species. Aquatic Botany 87:167-170.

Wang L, Huang Z, Baskin CC, Baskin JM, Dong M (2008). Germination of dimorphic seeds of the desert annual halophyte *Suaeda aralocaspica* (Chenopodiaceae) a C4 plant without Kranz anatomy. Annals of Botany 102:757-769.

Wetson AM, Cassaniti C, Flowers TJ (2008). Do conditions during dor-mancy influence germination of *Suaeda maritima*? Annals of Botany 101:1319-1327.

Woodell SRJ (1985). Salinity and seed germination patterns in coastal plants. Vegetatio 61:223-229.

Zedler JB, Paling E, McComb A (1990). Differential responses to salinity help explain the replacement of native *Juncus kraussii* by *Typha orientalis* in Western Australian salt marshes. Australian Journal of Ecology 15:57-72.

Zekri M (1993). Salinity and calcium effects on emergence, growth and mineral composition of seedlngs of eight citrus rootstocks. Journal of Horticultural Science 68:53-62.