Effects of Salinity and Seed Priming on Germination of Sea Fennel (*Crithmum maritimum* L.)

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Summary

Sea fennel (*Crithmum maritimum* L.) is a perennial plant tolerant to the soil salinity and could be used as an alternative culture on salty soils. For successful sea fennel cultivation, it is necessary to test germinability of the seed. In this study, germination tests have been carried out on a population of sea fennel from Lopar, island of Rab, Croatia. The aim was to examine the effect of seed priming on seed germination, radicle length, radicle surface area, average diameter of radicle and radicle volume of sea fennel under different salt concentrations. Seeds were primed with sodium chloride (NaCl) (50 mM) or distilled water (dH_2O) during seven days after which its germinability and early seedling growth was tested on the germination paper treated with different concentrations of NaCl (50, 100 and 150 mM) and dH_2O as control. Seeds that were primed with dH_2O and 50 mM NaCl solution showed the better performance than non-primed seeds; priming alleviated negative effects of low NaCl concentration.

Key words

salinity, germinability, NaCl, radicle, priming

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Introduction

Sea fennel or rock samphire (*Crithmum maritimum* L.) is a wild perennial halophyte that belongs to the family Apiaceae and genus *Crithmum* (Grigoriadou and Maloupa, 2008; Atia et al., 2011). It is most widely spread along the Mediterranean and Black Sea and Atlantic coast of Portugal, South and South-West England, Wales and Southern Ireland (Renna and Gonnella, 2012), but also occurs along the coasts of other countries (e.g. Canada) as a naturalized species (Cornara et al., 2009). Along the Adriatic coast sea fennel usually inhabits maritime rocks and cliffs or grows in shingle or sand by the sea (Maleš et al., 2003; Kovačić et al., 2008).

There has been a significant economic interest for sea fennel due to the high content of the secondary metabolites (Meot-Duros and Magne, 2008). It is an edible plant, and the leaves are rich in several compounds such as vitamin C, phenols, flavonoids, tannins, carotenoids, organic acids, and essential oils (Maleš et al., 2003; Grigoriadou and Maloupa, 2008; Meot-Duros and Magne, 2008; Kulisic-Bilusic et al., 2010). Leaves can be used in salads or as a spice (Renna and Gonnella, 2012).

Temperature and soil salinity are two the most important environmental factors that can affect the germination behaviour of sea fennel seed (Okusanya, 1977). Salinity is a serious threat to agriculture, and it is estimated that salinity affects 7% of the world's land area (Szabolcs, 1994; based on FAO 1989 data). Salinity reduces the ability of plants to uptake water, and this quickly causes reductions in growth rate, along with a suite of metabolic changes like those caused by water stress (Munns, 2002). Subsequent detrimental effects of salinity occur due to ion toxicity, nutritional disorders, and alternation of metabolic processes (Hasegawa *et al.* 2000; Munns 2002).

The fact that sea fennel is a halophyte, makes this species suitable for cultivation on this type of soil. However, Khan et al. (2000) stated that seeds of most halophytes are very sensitive to elevated salinity at the germination and early establishment phases. As in adult stage, salt stress in plant germination stage causes osmotic stress (reduced water absorption) and ion toxicity (Shokohifard et al., 1989).

Seed priming is widely used method not just to enhance seed vigour but also to break seed dormancy and increase tolerance to adverse conditions during seed germination and early seedling growth, including tolerance to higher salinity. The need for increased seed quality has become a priority in agriculture. Most commonly used types of priming treatments are hydropriming, osmopriming, thermopriming, biopriming and chemopriming. Different priming treatments can be applied depending on plant species, seed morphology and physiology (Paparella et al., 2015).

The aim of the present study was to examine the effect of seed priming (hydropriming and osmopriming) on seed germination, radicle length, radicle surface area, average diameter of radicle and volume of the radicle of see fennel under salinity conditions.

Materials and methods

Plant material

The research was carried out on seeds of sea fennel that were collected from different plants on 28th of November 2016 at Javorno bay in Lopar on the island Rab, Croatia. Plants were growing within rocky blocks on sandy soil. Seeds were stored dry in a paper bag in a cold chamber at the University of Zagreb, Faculty of Agriculture, Department of Seed Science and Technology until the beginning of the experiment at 20th of April 2017.

The seeds used in the research were surface sterilised for three minutes with 70% ethanol, then washed under tap water for three minutes, washed in distilled water, and dried between a filter paper.

Seed priming

A total of 700 seeds were used in the experiment. Seed priming treatments were performed for seven days, by soaking 300 seeds in distilled water (dH_2O) and 300 seeds in 50 mM L^{-1} NaCl. The rest of the seeds (100) were not treated and were used as a control.

Germination test

After seed priming (control and primed seeds), seeds were germinated on germination paper in Petri dishes. For seed germination experiment three different NaCl concentrations (50, 100, and 150 mM L^{-1}) and a distilled H_2O (dH_2O) were used. Twenty-five seeds were placed in each Petri dish (treatment) and every treatment was replicated four times. Seed priming treatments and germination treatments resulted in seven different combinations (Table 1).

Table 1. Treatments used in this research

Treatment	Description (seed priming treatment + germination treatment)
Treatment 1	dH_2O + 50 mM NaCl
Treatment 2	dH_2O + 100 mM NaCl
Treatment 3	dH_2O + 150 mM NaCl
Treatment 4	50 mM NaCl + 50 mM NaCl
Treatment 5	50 mM NaCl + 100 mM NaCl
Treatment 6	50 mM NaCl + 150 mM NaCl
Control	no PT + dH_2O

PT - pre-treatment

The seeds were germinated under controlled conditions in a germination chamber. The temperature of the chamber was constant (25°C ± 1°C) with an 18/6 h photoperiod (light/dark).

Measurements

During the germination test, which lasted for 24 days, the number of germinated seeds was checked every two days. A seed was considered germinated when its radicle was ≥ 2 mm.

On the last day of experiment, the radicle traits: length (*L*; cm), surface area (*S*; cm^2), average diameter (*D*; mm), and volume (*V*; mm^3) were measured. The seedlings were scanned by Epson Perfection V700 scanner (Seiko Epson Corporation, Nagano, Japan) and WinRHIZO Pro software (Regent Instruments Inc., Quebec, QC, Canada) was used for the analysis of the variables relevant for this research.

Data analysis

At the end of the experiment, the variables of germination were calculated. Germinability (*G*; %) is the number of germinated seeds in percentages (Ranal et al., 2009).

Mean germination time (*MGT*; day) means the time spent to germinate and was calculated as follows:

$$MGT = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}$$

where t_i is the time from the beginning of the experiment to the time of observation (i^{th}), n_i is a number of seeds germinated in the i^{th} time, and k is the last day of germination (Ranal et al., 2009).

The coefficient of variation of the germination time (CV_t; %) or homogeneity was calculated as follows:

$$CV_t = \frac{s_t}{\bar{t}} 100$$

where s_t is the standard deviation of the germination time and $\bar{t}$ is the mean germination time (Ranal et al., 2009).

The uncertainty of the germination process (U) measures the degree of uncertainty in predicting the informational entropy or the uncertainty associated with the distribution of the relative frequency of germination (Ranal and Santana, 2006). It is calculated by the formula:

$$U = -\sum_{i=1}^{k} f_i \log_2 f_i \ ,$$

being,

$$f_i = \frac{n_i}{\sum_{i=1}^{k} n_i} \ ,$$

where n_i is the number of germinated seeds during t^{th} time, and k is last day of observation (Ranal et al., 2009).

Synchrony of the germination process (Z) is the quotient produced by the division of the sum of the partial combination of germinated seeds during time t_i and the total amount of germinated seeds at the end of the experiment and it is calculated as follows:

$$Z = \frac{\sum_{i=1}^{k} C_{n_i 2}}{C_{\sum n_i, 2}} \ ,$$

being

$$C_{n_i, 2} = \frac{n_i (n_i - 1)}{2}$$

where $C_{ni,\,2}$ is the combination of the seeds germinated in the i^{th} time, two by two, and n_i is a number of seeds germinated in the i^{th} time (Ranal et al., 2009).

The assumption was that germination of the seeds at a certain time was simultaneous so the germination index (GI) was calculated through the formula (Farooq et al., 2005):

$$GI_t = \frac{number\ of\ germinated\ seeds}{number\ of\ days\ since\ the\ first\ count} + \ldots + \frac{number\ of\ germinated\ seeds}{number\ of\ days\ since\ the\ last\ count}$$

The one-way analysis of variance (ANOVA) was conducted in order to determine if there was any significant difference between the treatments. It was calculated using the SAS software PROC GLM (SAS Institute, 2004). The mean difference between the values of the quantitative variables of treatments was determined by the Tukey test ($P < 0.05$). The original variables of G and CV_t that were expressed in percentages were transformed before the analysis.

Results and discussion

Germination

After the 24 days, during which the study was conducted, the highest germination was achieved in control ($G = 80\%$), followed by treatment 1 (dH_2O + 50 mM NaCl) ($G = 68\%$) (Fig. 1). Additionally, the treatment 6 was excluded from the statistical analyses due to poor germination, namely during 24 days only two seeds germinated ($G = 2\%$).

There is a high significant difference ($P < 0.001$) in the percentage of germinability (G) of sea fennel seed between the treatments (Table 2). Higher concentration of salt in germination treatments (soaked filter paper) had a negative effect on germinability. The lowest percentage of germinability was observed in treatment 3, which was primed with dH_2O and was germinated in 150 mM NaCl. More specifically, the higher salt concentration decreased the percentage of germinability, with no significant difference between treatments 1, 4 and control. Furthermore, treatment 5 did not show a significant difference in comparison with treatments 2 and 3. Control demonstrated a lower percentage of germinability compared to treatment 1 and 4 during the first 18 days, but it had the highest percentage at the end of the study (after 24 days) (Figure 1), which indicates positive effect of seed priming on mean germination time. Moreover, the differences in mean germination time (MGT) were significant ($0.05 > P > 0.01$) with the maximum time for germination in treatment 5 and the minimum in treatment 1.

Regarding the coefficient of variation of the germination time (CV_t), no significant difference between the treatments was observed, as well as for the synchrony of the germination process (Z) (Table 2.).

There was a weak significant difference ($0.05 > P > 0.01$) for the uncertainty of the germination process (U), while the highest value was observed in control.

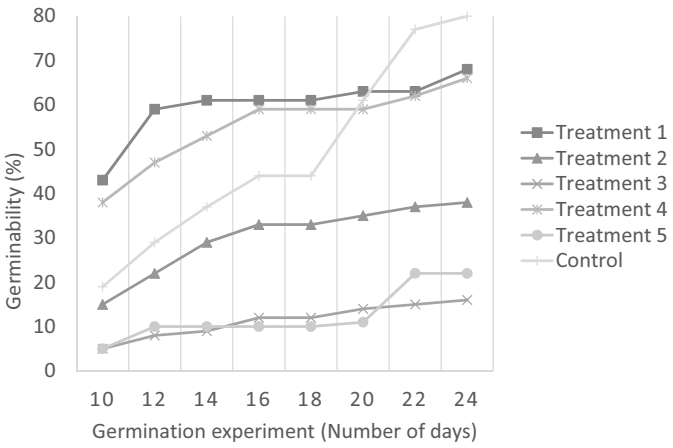

Figure 1. Germinability (G; %) of sea fennel seeds from the day 10th (first germinated seed) to the day 24th (end of experiment)

Table 2. The effects of NaCl salinity on germination of see fennel (mean ± standard deviation)

Treatment	*G* (%)		*MGT* (day)	*CV*$_t$ (%)	*U*		*Z*	*GI*	
Treatment 1	68.00 ± 8.64	a	11.96 ± 0.61	32.79 ± 7.33	1.33 ± 0.07	ab	0.45 ± 0.07	1.52 ± 0.24	a
Treatment 2	38.00 ± 12.44	b	13.13 ± 1.05	28.33 ± 6.72	1.79 ± 0.33	ab	0.24 ± 0.09	0.77 ± 0.22	b
Treatment 3	16.00 ± 7.30	c	13.45 ± 3.46	19.29 ± 13.44	1.05 ± 0.81	b	0.44 ± 0.38	0.30 ± 0.08	c
Treatment 4	66.00 ± 14.79	a	12.45 ± 1.41	27.68 ± 11.15	1.57 ± 0.28	ab	0.39 ± 0.13	1.41 ± 0.29	a
Treatment 5	22.00 ± 4.00	bc	17.33 ± 4.32	24.07 ± 17.16	0.92 ± 0.77	b	0.57 ± 0.35	0.37 ± 0.14	bc
Control	80.00 ± 3.27	a	16.21 ± 1.55	30.07 ± 5.85	2.30 ± 0.34	a	0.20 ± 0.07	1.36 ± 0.16	a
P(F)	***		*	ns	*		ns	***	

G (%) – germinability; *MGT* (day) - mean germination time; *CV*$_t$ (%) - germination time; *U* - uncertainty of the germination process; *Z* - synchrony of the germination process; *GI* - germination index; P(F) - significance of the F-test: ^{ns}P > 0.05, *0.05 > P > 0.01, **0.01 > P > 0.001, ***P < 0.001; Values in the columns were marked by the same letter when there was no observed significant difference in the Tukey test.

Table 3. The effects of NaCl salinity on radicle growth of see fennel (mean ± standard deviation)

Treatment	*L* (cm)		*S* (cm^2)		*D* (mm)	*V* (mm^3)	
Treatment 1	3.65 ± 0.65	a	0.38 ± 0.08	a	0.33 ± 0.01	3.18 ± 0.68	ab
Treatment 2	1.75 ± 0.19	bc	0.19 ± 0.03	bc	0.34 ± 0.02	1.71 ± 0.33	cd
Treatment 3	1.19 ± 0.33	c	0.13 ± 0.03	c	0.39 ± 0.06	1.23 ± 0.25	d
Treatment 4	2.51 ± 0.23	bc	0.28 ± 0.04	bc	0.35 ± 0.02	2.5 ± 0.43	bc
Treatment 5	1.34 ± 0.42	c	0.16 ± 0.04	c	0.39 ± 0.03	1.42 ± 0.29	d
Control	3.63 ± 0.20	a	0.40 ± 0.01	a	0.36 ± 0.02	3.58 ± 0.25	a
P(F)	***		***		ns	***	

L (cm) - length, *S* (cm^2) - surface area, *D* (mm) - average diameter, *V* (mm^3)- radicle volume; P(F) - significance of the F-test: ^{ns} P > 0.05, *0.05 > P > 0.01, **0.01 > P > 0.001, ***P < 0.001; Values in the columns were marked by the same letter when there was no observed significant difference in the Tukey test.

Contrarily, the germination index (*GI*) was highly significant (P < 0.001), although there was no difference among treatments 1, 4, and control, and treatment 5 did not show a significant difference in comparison with treatments 2 and 3. Treatment 1 had the highest germination index, while treatment 3 had the lowest one.

According to Atia et al. (2006) in salt-free medium, germination of *C. maritimum* seeds pre-treated with water (hydropriming) and NaCl (osmopriming) was accelerated. Nevertheless, hydropriming was more effective in alleviating adverse effects of salt stress, salt-induced osmotic stress was a dominant factor reducing seed germination. Our results are in congruence with those obtained by Meot-Duros and Magné (2008), where seed priming with L-ascorbic acid at 40 mM was shown to alleviate the negative effects of low NaCl concentration on germination.

Although, concentrations over 50 mM NaCl were found to delay and partially inhibit germination (Fig. 2), sea fennel seeds have shown possibility to germinate at low salt levels and because of that this species can be used as an alternative culture on slightly salty soils.

Seedling root traits

Seedling radicle traits observed in this research were radicle length (*L*), surface area (*S*), average diameter (*D*), and volume (*V*) (Table 3).

The differences among the treatments were highly significant (P < 0.001) for all the traits, except for the average radicle diameter (*D*). The radicle length (*L*) is considered as a useful criterion to understand the effect of salinity at seedling establishment stage (Ratnakar and Rai, 2013). The highest values for length (*L*) (Figure 2) and surface area (*S*) were observed in treatment 1 and the lowest in treatment 3. Treatments 1 and 7 had the highest values and did not differ significantly. Radicle length decreased with increasing levels of salt. Similar results were reported by Nyagah and Musyimi (2009) who observed a reduction in growth in passion fruit radicle with increasing concentrations of salt in the medium. However, priming with NaCl and GA3 were useful for alleviating salt stress effects and improved germination and seedling establishment of milk thistle (*Silybum marianum* L.) (Sedghi et al., 2010).

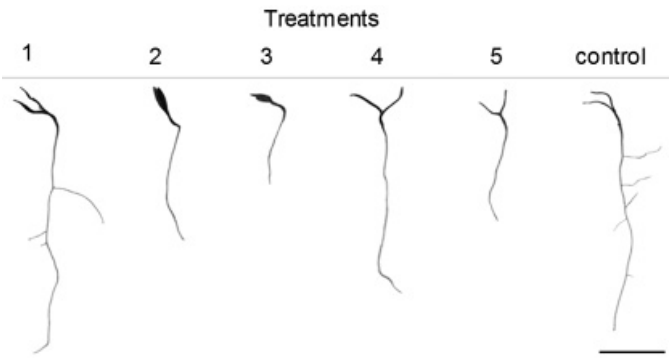

Figure 2. Seedlings length of sea fennel scanned on the last day of experiment (treatments 1, 2, 3, 4, 5 and control respectively; bar 10 mm)

There were also high significant differences for the radicle volume (*V*). The highest value was recorded for treatment 7 which did not differ significantly from treatment 1, while the lowest values were recorded for treatments 3 and 5 and did not differ significantly.

Conclusion

Based on obtained results, it can be concluded that *Crithmum maritimum* is sensitive to salinity i.e. increased NaCl concentrations. Increasing concentration of NaCl in the growth medium adversely affected the percentage of germination and early seedling growth. However, seed which were primed showed the better performance than non-primed seeds especially at the lower salinity levels. This findings indicated that seed priming (with NaCl and dH_2O) may be used to accelerate sea fennel seed germination and improve early seedling growth, which is of great interest for cultivating this plant on slightly salty soils and achieve better crop homogeneity.

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