Jiang, Tanveer, Han, Tian and Wang (2020).
Seed Science and Technology, **48**, 2, 231-239.
https://doi.org/10.15258/sst.2020.48.2.10

High and differential strontium tolerance in germinating dimorphic seeds of *Salicornia europaea*

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(Submitted December 2019; Accepted April 2020, Published online April 2020)

Abstract

Salicornia europaea, a highly salt-tolerant halophyte, is potentially resistant to other metals because plant stress tolerance partly relies on common physiochemical mechanisms. Large median seeds and small lateral seeds of *S. europaea* have high salt tolerance and display contrasting germination responses. Thus, we hypothesised that dimorphic seeds of *S. europaea* might also have high and differential strontium (Sr) tolerance during germination. Both types of seeds were incubated in different $SrCl_2$ concentrations at 25°C. 0-300 mmol L^{-1} $SrCl_2$ did not significantly affect germination of median seeds. However, for lateral seeds, relatively high concentrations (≥ 200 mmol L^{-1}) dramatically inhibited germination. The simulated critical value (when germination percentage is 50%) was 502 mmol L^{-1} for median seeds and 224 mmol L^{-1} for lateral seeds. Dimorphic seeds of *S. europaea* are highly tolerant to Sr stress and large median seeds display higher tolerance. The results suggest that direct seeding of large seeds of *S. europaea* might be an effective method to remediate heavy Sr-polluted soils.

Keywords: dimorphic seeds, germination, *Salicornia europaea*, Sr, phytoremediation

Introduction

Strontium (Sr) is a Group II alkaline earth metal and is present at about 340 ppm in the earth's crust (Burger and Lichtscheidl, 2019). Sr behaves in a similar way as the other alkaline earth metals in its group. For example, Sr and Ca are chemically closely related and they behave as chemical analogues in a variety of plants (Burger and Lichtscheidl, 2019). High concentrations of Sr are found in strontianite and celestite. In mining places, Sr might cause environmental toxicity. Sr is released to the environment from routine and accidental discharge, and causes threat to environment and human health (Sachse *et al.*, 2011; Burger and Lichtscheidl, 2019). Thus, there are many experiments concerning uptake and distribution of Sr by plants (Chen *et al.*, 2012; Bengtsson *et al.*, 2013).

Measures should be taken for stabilising or removing Sr contamination (Moyen and Roblin, 2010; Long *et al.*, 2017; Burger and Lichtscheidl, 2019). Traditional methods to reduce Sr in soil include leaching with $CaCl_2$, electrokinetic methods and addition of organic fertilizers (Gupta *et al.*, 2018). There are also several methods to decrease Sr concentration in water (Anamika *et al.*, 2009). Using plants to remove Sr from soil and solutions is considered a clean, cost-effective and eco-friendly technology. Appropriate plants that are highly capable of accumulating Sr are required for the success in phyto-remediation of Sr-contaminated soil or water (Anamika *et al.*, 2009; Bengtsson *et al.*, 2013). According to previous studies, several plants species have high potential as Sr bioaccumulators, such as *Andropogon gayanus* Kunth (Long *et al.*, 2017), *Cyperus iria* and *Parthenocissus quinquefolia* (Li *et al.*, 2011). Euhalophytes exhibit more potential for Sr remediation because plant stress tolerance partly relies on common physiochemical mechanisms. There is evidence that some halophytes, such as *Suaeda salsa*, are resistant to several metals (Song and Sun, 2014). Because concentrations of Sr in non-eudicots were significantly less than in eudicots, halophytic eudicots should be screened for Sr accumulation.

Seed germination is the first stage of the plant life cycle and is a major factor restricting successful plant establishment (Liu *et al.*, 2018). Many studies have shown that seed germination is extremely sensitive to environmental stresses, such as salinity (Gul *et al.*, 2013). Therefore, evaluation of Sr tolerance at the germination stage is very important for potential Sr phytoremediation plants. While some authors reported the tolerance of different plants to Sr stress, there is little information about seed germination responses to Sr (Burger and Lichtscheidl, 2019).

Salicornia europaea, a highly salt-tolerant halophyte, is widely distributed on alkaline and saline soils, salt-lake shores and beaches in southwestern Asia, Europe and North America (Editorial Committee of Flora of China, 2003). This plant species is a herbaceous annual, 100-350 mm in height, has erect stems and fleshy branches. The inflorescence of *S. europaea* is shortly pedunculate and spicate. Flowers are axillary with three per bract. The median flower is larger than both lateral flowers. Large median flowers produce large seeds (1.8 mm) with wings and small lateral flowers produce small seeds (1.1 mm) without wings (Gul *et al.*, 2013; Orlovsky *et al.*, 2016). Ungar (1979) reported that dimorphic seeds of *S. europaea* are highly salt tolerant and large seeds have higher salt tolerance than the small. Thus, we hypothesised that dimorphic seeds of *S. europaea* might have high and differential Sr tolerance during germination. Specifically, we addressed the following questions: (1) what is the limitation of Sr tolerance for germinating dimorphic seeds of *S. europaea*? (2) do dimorphic seeds of *S. europaea* display different germination responses to Sr stress?

Materials and methods

Seeds

Terminal infructescences of *S. europaea* were collected on 12 October 2018, from hypersaline soils in the southern part of the Junggar Basin of Xinjiang Province, China. The infructescences were taken from at least 50 plants and allowed to dry naturally for two weeks in the laboratory. Seeds were separated from the dried plant material and sorted into large seeds and small seeds. Each type of seeds was pooled and stored in plastic bags at room temperature.

Germination tests

The germination experiment was started on 10 November 2018 under laboratory conditions. Twenty-five seeds of *S. europaea* were subjected to nine $SrCl_2$ concentrations: 0 (distilled water control), 100, 200, 300, 400, 500, 600, 700 and 800 mmol L^{-1}. Seeds were placed in 50 mm-diameter Petri dishes containing two layers of filter paper, to which 2.5 ml of the respective $SrCl_2$ solution was added. Petri dishes were sealed with parafilm and incubated at 25°C with a 10-hour night/14-hour day photoperiod for 14 days. A seed was regarded as germinated when its radicle length reached 1 mm. The number of germinated seeds was recorded each day and the seedlings were removed from the Petri dishes at each counting.

Data analysis

The velocity of germination was estimated using a modified Timson's index (hereafter "index") of germination velocity (Khan and Ungar, 1984). The simulated critical value is defined as the $SrCl_2$ concentration when germination is 50% and the simulated limit value is the $SrCl_2$ concentration when germination is 0%.

Data for germination percentage and index were analysed by linear regression. The multiple linear regression model included seed type and Sr concentration. One-way ANOVA and Tukey's test were used to determine statistically significant differences among Sr treatments in germination percentage or index for each seed type of *S. europaea*. Independent-samples T-tests were used to determine whether there were significant differences in germination percentage or index of dimorphic seeds at the same Sr concentrations.

Results

Germination percentage and germination index of *S. europaea* was significantly affected by seed type ($P < 0.001$) and $SrCl_2$ ($P < 0.001$). Median seeds showed higher germination percentage and germination index than lateral seeds in response to Sr treatments (table 1). Comparing the germination of median and lateral seeds, there was 13% germination of lateral seeds when germinated with 300 mmol L^{-1} $SrCl_2$ while median seeds showed 94% germination. Increasing $SrCl_2$ from 300 mmol L^{-1} $SrCl_2$, lateral seeds did not germinate while median seeds were able to germinate at 600 mmol L^{-1} $SrCl_2$.

Table 1. Effects of $SrCl_2$ on germination percentage, germination index and time to 50% germination of dimorphic seeds of *Salicornia europaea*. Different upper-case letters indicate significant difference in germination trait at the same $SrCl_2$ concentration; different lower-case letters indicate significant difference among different concentrations of $SrCl_2$ at $P < 0.05$.

Concentration (mmol L^{-1})	Germination (%)		Germination index		Time to 50% germination (days)	
	Median	Lateral	Median	Lateral	Median	Lateral
0	97.0aA	97.0aA	86.8aA	83.6aA	2aA	3aB
100	98.0aA	95.0aA	85.7aA	79.6aB	3bA	3aB
200	96.0aA	66.0bB	79.0aA	51.8bB	3bA	4bB
300	94.0aA	13.0cB	73.4aA	8.9cB	3b	–
400	84.0bA	0.0dB	63.6bA	0.0dB	3b	–
500	49.0cA	0.0dB	32.4cA	0.0dB	–	–
600	20.0dA	0.0dB	11.0dA	0.0dB	–	–
700	3.0eA	0.0dA	1.6eA	0.0dA	–	–
800	0.0eA	0.0dA	0.0eA	0.0dA	–	–

Moreover, germination percentages of median seeds decreased to ≥ 20% when $SrCl_2$ increased to 600 mmol L^{-1} (table 1; figure 1A). The germination index of both seed types was significantly decreased by $SrCl_2$ and was more pronounced in lateral seeds (table 1). Under 200 mmol L^{-1} $SrCl_2$, time to 50% germination for median and lateral seeds was 3 and 4 days, respectively.

The equation of the relationship between non-germination percentages of median seeds of *S. europaea* and $SrCl_2$ concentration was: $y = 101.7023 / (1 + \exp(-(x-504.3476) / 69.0262))$ (figure 2A). $R^2 = 98\%$, the simulated critical value was 502 mmol L^{-1} and the simulated limit value was 784 mmol L^{-1}. The equation of the relationship between non-germination percentage of lateral seeds and $SrCl_2$ concentration was: $y = 100.3009 / (1 + \exp(-(x-225.9632) / 39.8990))$ (figure 2B). $R^2 = 99\%$, the simulated critical value was 224 mmol L^{-1} and the simulated limit value was 399 mmol L^{-1}.

Discussion

Salicornia europaea has attracted much attention due to its high salt tolerance, reclamation potential and pharmaceutical value (Ungar, 1979; Singh *et al.*, 2014; Orlovsky *et al.*, 2016). Although seed germination and growth of *Salicornia europaea* have been studied extensively, these data are the first to report germination responses of *S. europaea* seeds to different Sr concentrations. In addition, our data indicate that *S. europaea* seeds have high Sr tolerance during germination and that the dimorphic seeds displayed differential Sr tolerance.

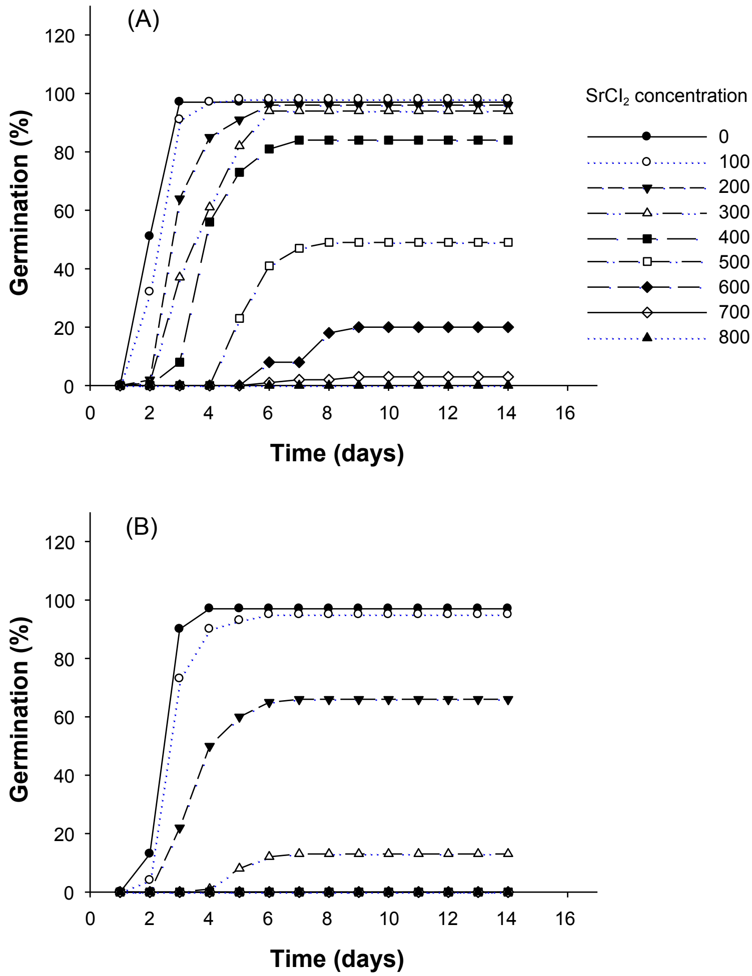

Figure 1. Cumulative germination of large median seeds (**A**) and small lateral seeds (**B**) of *Salicornia europaea* exposed to $SrCl_2$ solution.

Germination percentage and velocity of *S. europaea* seeds was not affected by 100 mmol L^{-1} $SrCl_2$. Even at 200 mmol L^{-1} $SrCl_2$, germination percentages of both types of seeds were higher than 65% (table 1). However, there are only a few studies about seed germination responses to Sr stress. Compared with the distilled water control, germination percentage of *Vicia faba* was reduced by 36.32% at 10 mmol L^{-1} $SrCl_2$ (Qian *et al.*, 2015). Similarly, germination of maize caryopses declined under 10 mmol L^{-1} $Sr(NO_3)_2$ as compared with the control (Seregin and Kozhevnikova, 2005).

Since seeds at the germination stage are usually more sensitive to metal stresses than adult plants (Tanveer and Shah, 2017), we suspect that the germination critical value for most species is less than 100 mmol L^{-1} Sr. Several studies showed numerous plant growth

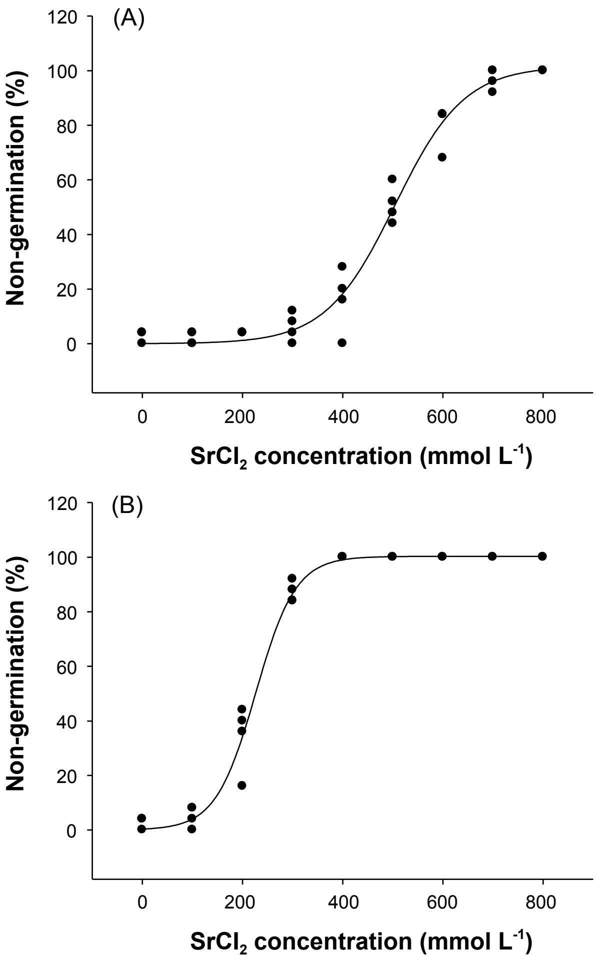

Figure 2. Relationship between germination percentages of large median seeds (**A**) or small lateral seeds (**B**) of *Salicornia europaea* and concentrations of $SrCl_2$ solution.

responses under different $SrCl_2$ concentrations (Chen *et al.*, 2012; Sowa *et al.*, 2014; Burger and Lichtscheidl, 2019). For instance, in soybean, treatment with 2 mmol L^{-1} Sr inhibited fresh and dry weights, and in *Brassica napus* application of 40 mmol L^{-1} Sr significantly decreased chlorophyll content and rubisco activity (Chen *et al.*, 2012; Sowa *et al.*, 2014). For the Cd-hyperaccumulator *Brassica juncea*, growth decreased dramatically when Sr content in soil was $\geq$ 3360 ppm (Walsh, 1944/1945). Wheat seedlings grown in 66 mmol L^{-1} $SrCl_2$ solutions showed retarded growth and finally died (Spinkes *et al.*, 1948). Experimental studies confirmed the phytoremediation potential of several plant families: Fabaceae, Brassicaceae, Amaranthaceae and Asteraceae (Burger and Lichtscheidl, 2019). Thus, it might be a fast method to screen Sr tolerant plants from halophytes of these families at the germination stage.

Dimorphic seeds of *S. europaea* displayed differential germination responses to $SrCl_2$. Germination percentage of median seeds reached 84% at 400 mmol L^{-1} $SrCl_2$. However, germination of lateral seeds was inhibited completely at this concentration (figure 1). The difference of dimorphic seeds in Sr tolerance may be related to their differential salt tolerance. Large median seeds of *S. europaea* are more salt tolerant than small lateral seeds (Ungar, 1979; Orlovsky *et al*., 2016). The contrasting salt tolerance of dimorphic seeds are also found in other plant species, such as *Suaeda salsa* (Wang *et al*., 2015), *Salicornia ramosissima* (Ameixa *et al*., 2016) and *Atriplex canescens* (Bhatt and Santo, 2016). This may due to the difference of antioxidant mechanism between dimorphic seeds (Xu *et al*., 2017; Nisar *et al*., 2019). Furthermore, the difference in stress tolerance exists even in plants grown from dimorphic seeds (Xu *et al*., 2011; Ameixa *et al*., 2016).

The findings of this study show that *S. europaea* has high Sr tolerance and direct seeding of this species might be an effective method for phytoremediation of Sr contaminated soil. Because the germination difference between dimorphic seeds increased when Sr concentration increased, to a certain high level, large median seeds should be selected in order to optimise seedling emergence in heavily Sr-polluted soils. Further studies will be required to test seed germination and growth characteristics of *S. europaea* in Sr contaminated soils.

Acknowledgements

This study was supported by the Key Research and Development Program of Xinjiang province (2018B01006-4).

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