ABS ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2016 Vol. 59, Št. 1: 5–11

Germination rate of stinkwort (*Dittrichia graveolens)* and false yellowhead *(D. viscosa)* in relation to salinity

Kaljivost smrdljive (*Dittrichia graveolens*) in lepljive ditrihovke (*D. viscosa*) v odvisnosti od slanosti

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Abstract: This study was conducted to investigate the effect of salinity on germination rate of stinkwort (*Dittrichia graveolens* (L.) Greuter) and false yellowhead (*D. viscosa* (L.) Greuter). Lettuce (*Lactuca sativa* L.) was used as a positive control. Seeds of all three test species were sown on agar plates with three different NaCl treatments (2.5 g NaCl/L ≈ 42 mM NaCl, 5 g NaCl/L ≈ 85 mM NaCl and 10 g NaCl/L ≈ 171 mM NaCl) and control treatment without NaCl. The three tested species germinated under all salinity conditions. However, they thrived best under control conditions and their germination rate was gradually declining with increasing salinity. Additionally, the start of their germination was delayed with increasing NaCl concentration. According to our findings, we can conclude that both *Dittrichia* species are very tolerant to salinity.

Keywords: salinity, germination rate, tolerance, *Dittrichia graveolens*, stinkwort, *Dittrichia viscosa*, false yellowhead, *Lactuca sativa*, lettuce

Izvleček: V raziskavi smo ugotavljali, kakšen je učinek slanosti na kaljivost smrdljive ditrihovke (*Dittrichia graveolens* (L.) Greuter) in lepljive ditrihovke (*D. viscosa* (L.) Greuter). Kot pozitivno kontrolo smo uporabili solato (*Lactuca sativa* L.). Semena vseh treh testnih vrst smo posejali na agarne plošče s tremi različnimi koncentracijami NaCl (2,5 g NaCl/L ≈ 42 mM NaCl, 5 g NaCl/L ≈ 85 mM NaCl and 10 g NaCl/L ≈ 171 mM NaCl) ter kontrolnim tretmajem brez NaCl. Vse tri testne vrste so kalile pri vseh tretmajih, vendar je bilo njihovo uspevanje najboljše v kontrolnih razmerah, kaljivost pa je z naraščajočo slanostjo postopoma upadala. Poleg tega se je z naraščajočo slanostjo zamaknil tudi pričetek procesa kalitve. Na podlagi rezultatov lahko zaključimo, da sta obe vrsti ditrihovk zelo tolerantni na slanost.

Ključne besede: slanost, stopnja kaljivosti, toleranca, *Dittrichia graveolens*, smrdljiva ditrihovka, *Dittrichia viscosa*, lepljiva ditrihovka, *Lactuca sativa*, solata

Introduction

Stinkwort (*Dittrichia graveolens* (L.) Greuter) and false yellowhead (*D. viscosa* (L.) Greuter) are the only known species belonging to the genus *Dittrichia*, which is classified under the *Asteraceae* family (Wraber 2010). They both originate in the Mediterranean region and can be found in Slovenia. However, only false yellowhead is considered to be indigenous in Slovenia, whereas stinkwort was only first discovered in 2008 and is regarded as an invasive alien species in this area (Frajman and Kaligarič 2009). The occurrence of false yellowhead in Slovenia is limited solely to its coastal part, where it is quite common in ruderal habitats (Wraber 2010, Jogan et al. 2001). Stinkwort has an even more intriguing distribution pattern, spreading only in ruderal habitats along highway (Frajman and Kaligarič 2009) together with some regional roads. The coastal region is greatly affected by the sea salt, whereas sites along main roads are frequently exposed to excessive application of ice-melting salts in the winter. Thus, we hypothesized that the occurrence of both *Dittrichia* species in Slovenia could be limited to sites with seemingly elevated salinity level.

There exist various types of salts among which many are essential in terms of plant survival. Nonetheless, they quickly become detrimental in excessive amounts. One of the most fundamental and ubiquitous salts is NaCl. Soil salinity can be either primary, i.e. caused by natural processes, or secondary, that is human-induced by different human activities such as irrigation, fertilization and application of de-icing salts (Kotuby-Amacher et al. 2000, Parihar et al. 2015). Most plants cannot bear high salinity level as it causes water stress, ion toxicity, oxidative stress, nutritional disorders, alteration of metabolic processes, membrane disorganization, etc. (Hasegawa et al. 2000, Carillo et al. 2011). All these small-scale consequences reflect in their most vital processes, e.g. germination, growth and photosynthesis (Parihar et al. 2015) and can sometimes even lead to early senescence and death (Zhu 2007).

Seed germination is believed to be the most critical phase of the plant life cycle. Therefore, species that are able to germinate under elevated salinity level have a substantial competitive advantage over species whose germination is entirely suppressed or at least delayed (DiTommaso 2004).

Throughout evolution, some plant species have evolved different mechanisms to combat high salinity, for instance restriction of salt uptake, control of long distance transport of salt, extrusion of salt from the plants, compartmentalization of salt (Carillo et al. 2011, Parihar et al. 2015) and production of compatible solutes otherwise known as osmoprotectants (McNeil et al. 1999).

So far, not many studies have discussed the relationship between either of the two *Dittrichia* species and salinity. Only a handful of studies have already reported about stinkwort being more sensitive to elevated salinity level (Ghorbanali et al. 2013) and the ability of false yellowhead to thrive in such conditions (Curadi et al. 2005). False yellowhead had already been proven to be tolerant to elevated salinity level (Flowers et al. 2012–2016) and to be able to survive in drastic salinity conditions with electrical conductivity values reaching up to 52 000 µS/cm (Curadi et al. 2005). Two other studies have focused more on the capability of stinkwort and false yellowhead to cope with dry conditions, where similarly false yellowhead did better than stinkwort (Öztürk and Mert 1983, Pérez-Fernández et al. 2006).

Our major goal was to find out how increased salinity level affects germination of both *Dittrichia* species, as the existing distribution patterns of stinkwort and false yellowhead in Slovenia indicate to a potential competitive advantage of both species in habitats with elevated salinity level.

Materials and methods

We collected plant material from the following locations:

- Flowering shoots of *Dittrichia graveolens*: Slovenia, Ljubljana, Roje, Obvozna cesta, gravel road bank, 46°6'17.17" N 14°28'54.36" E. Leg. *&* det.: S. Strgulc Krajšek & S. Anžlovar, 24. 9. 2013.
- Flowering shoots of *Dittrichia viscosa*: Slovenia, Primorska, Koper, near the road exit from Istrska road to Ljubljanska street, along the fence enclosing a warehouse next to Planet Tuš commercial complex, ruderal site, 45°32'16.51" N 13°44'7.38" E. Leg. *&* det.: S. Strgulc Krajšek & S. Anžlovar, 10. 10. 2014.

Lettuce seeds were bought from a local seed producer Agrina.

Harvested plant material of both *Dittrichia* species was air-dried and stored separately in a dark and dry room until use. Only mature seeds were selected for this experiment.

Before sterilising the seeds, we removed all seed appendices excluding pappus hairs. Seeds of all three test species were then surface sterilised by 15 min immersion in aqueous solution of sodium hypochlorite (16.5 g/l, Arekina, Šampionka Renče) and afterwards rinsed three times in distilled water for 5 min each time.

To set the germination test, we first prepared 2% water-agar solutions, to which we added appropriate quantity of NaCl to get the desired NaCl concentrations (0, 2.5, 5 and 10 g NaCl/L). All test solutions were thereafter autoclaved and poured into 9-cm sterile Petri dishes. Seeds were placed on agar plates in a 5 × 5 cm array the following day. We used 3 or 4 replicates with 25 seeds for each tested combination. Agar plates were wrapped with transparent foil to limit evaporation and were stored at room temperature in good light conditions until the end of the experiment on its 25th day.

Seeds were examined every day at roughly 24-hour intervals. A seed was considered germinated on the day of emergence of its radicle. We also monitored cotyledon opening as a marker of further seedling development.

Data analysis was done using survival analysis in programme GraphPad Prism 5.01, which automatically compared curves representing different data sets (treatments) using Log-rank (Mantel-Cox) test.

Along with germination tests, we also measured electrical conductivity of the soil from sampling locations of both *Dittrichia* species and electrical conductivity of the two test solutions with the highest NaCl concentrations. To avoid potential influence of any impurities while measuring electrical conductivity, ultrapure water (Milli-Q Plus 185 system) was used to prepare samples instead of distilled water. We repeated each measurement three times in 10-min intervals and ultimately expressed it as a mean value.

Results

Seeds of all three test species germinated under all salinity conditions (Figure 1: A, C, E). However, their germination rate was decreasing correspondingly with increasing NaCl concentration. Minor differences in this general trend could be due to numerous fungal infections in some Petri dishes. In addition to lowering final germination rate, increasing concentration of NaCl also delayed the sole process of germination in both *Dittrichia* species.

As expected, lettuce reached the highest final germination rate of the three tested species (Figure 1: E). With the exception of the highest NaCl concentration, increasing salinity seemed to have a very negligible negative effect on its final germination rate. Nevertheless, the differences between 2,5 g NaCl/L and 5 g NaCl/L treatments and the control were statistically significant ($P < 0.001$).

Final germination rate of stinkwort (Figure 1: A) and false yellowhead (Figure 1: C) in control treatment was very comparable. In general, ontogenesis was slow in both *Dittrichia* species, but considerably faster in false yellowhead compared to stinkwort. Although differences between treatments were a little less evident in false yellowhead than in stinkwort, they were still statistically significant in most cases. The alternation of dynamics of germination with increasing salinity was more pronounced in stinkwort than in false yellowhead.

Cotyledon opening revealed similar findings as the process of germination (Figure 1: B, D, F), as the increasing NaCl concentration was slowing down the process of seedling development. In the case of *D. graveolens* in 10 g NaCl/L treatment, none of the tested seedlings developed to the phase with opened cotyledons (Figure 1: B). The percentage of *D. viscosa* seedlings with opened cotyledons in 5 and 10 g NaCl/L treatments was very low as well (Figure 1: D).

Electrical conductivity of the soil from both sampling locations was relatively low and differed only slightly between both sampling locations (63.9 µS/cm and 80.5 µS/cm in Ljubljana and Koper, respectively). In contrast, high values of electrical conductivity were measured in our test NaCl solutions (9 000 and 18 000 µS/cm in 5 and 10 g NaCl/L solutions, respectively), with the exception of control treatment (1 µS/cm in 0 g NaCl/L solution).

Discussion

Overall, elevated salinity had a restraining effect on both germination of the seeds as well as cotyledon opening in seedlings, and was getting more and more intense with increasing NaCl

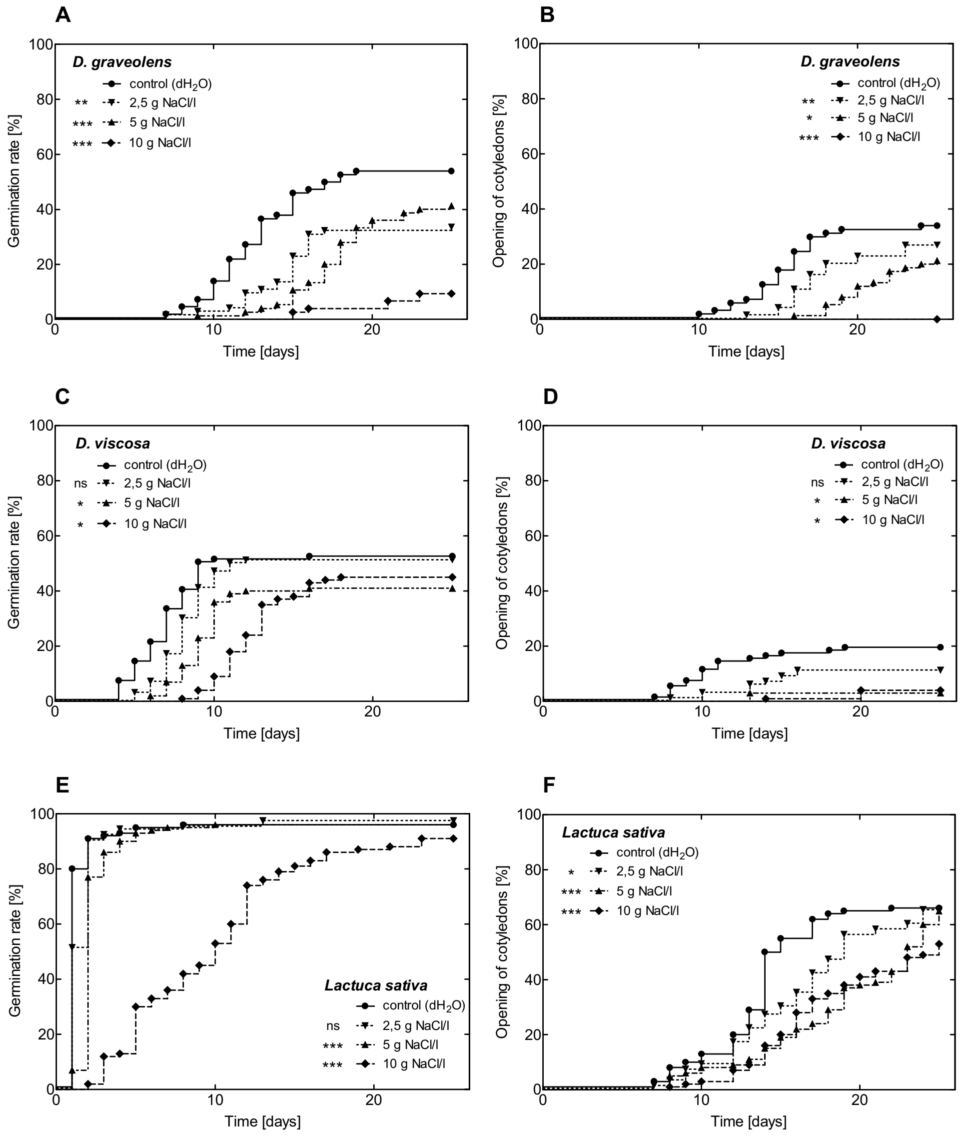

Figure 1: Germination rates and percentage of seedlings with opened cotyledons of *Dittrichia graveolens* (A, B), *Dittrichia viscosa* (C, D) and *Lactuca sativa* (E, F) in relation to salinity. The results of survival analysis done using Log-rank (Mantel-Cox) test, that compared each curve with corresponding control treatment, are shown in the legends of all graphs (***: $P < 0.001$, **: $0.001 < P < 0.01$, * $0.01 < P < 0.05$, ns: $P > 0.05$).

Slika 1: Kaljivost semen in odstotek kalic z razprtimi kličnimi listi vrst *Dittrichia graveolens* (A, B), *D. viscosa* (C, D) in *Lactuca sativa* (E, F) v odvisnosti od slanosti. Rezultati preživetvenih testov, narejenih z uporabo Log-rank (Mantel-Cox) testa, s katerim smo primerjali vsako od krivulj s kontrolo, so prikazani v legendah ob vsakem grafu (***: $P < 0,001$, **: $0,001 < P < 0,01$, * $0,01 < P < 0,05$, ns: $P > 0,05$).

concentration in all three test species, especially in stinkwort and false yellowhead. It was gradually slowing down their development and lowering the final percentage of germinated seeds and seedlings with opened cotyledons. Similar changes in patterns of germination dynamics under increasing salinity levels have also been observed by Läuchli and Grattan (2007). Along with having a direct negative impact on young plants, increasing NaCl concentration also hindered their development indirectly by affecting their roots. The roots in different NaCl treatments had dark root tips and less root hairs than the control seedlings. Many root tips have also turned upwards, as was already observed in a study by Levizou et al. (2002), where *Lactuca sativa* seeds were treated with *Dittrichia viscosa* extracts.

Considering smaller differences in final germination rates between treatments and less variable developmental dynamics in false yellowhead compared to stinkwort, false yellowhead appeared to be more successful in dealing with elevated salinity level than stinkwort, which is in accordance with related preceding studies (Öztürk and Mert 1983, Curadi et al. 2005, Pérez-Fernández et al. 2006, Ghorbanali et al. 2013).

In our case, final germination rate is not the best indicator of the sensibility of the tested plant species to increased salinity, as final germination rates of seeds treated with different NaCl concentrations did not differ much from the results of the control. The differences were much more visible in the following phases of seedling development, so the phase of cotyledon opening was more informative. It would be interesting to prolong the experiment to see further development, but for such purpose the experiment design should be different.

According to USDA Agricultural Research Service, the threshold value that defines saline soils is 4 dS/m (Criteria for Diagnosing Saline and Sodic Soils 2006), which equals 4 000 µS/cm. Therefore, soil salinity of samples from both sampling locations was well below values that delineate non-saline soils from saline soils, whereas salinity of all of the tested NaCl solutions, even our lowest NaCl concentration (2.5 g NaCl/L), turned out to be above the aforementioned limit (except distilled water as control treatment).

Regardless of the negative effect of elevated salinity level on the two *Dittrichia* species, our results have proven that both species, false yellowhead as well as stinkwort, are very tolerant regarding salinity. Their seeds were able to germinate and also mostly normally further develop in a remarkably wide range of NaCl concentrations, spanning from as low as 0 to the extreme 10 g NaCl/L ($\approx$ 171 mM NaCl), while the majority of known plant species hardly grow or even cannot survive in such high salinity level as 100–200 mM NaCl (Carillo et al. 2011). Hence, according to our measurements of electrical conductivity, both *Dittrichia* species managed to handle even more than astonishing 200 times higher values of electrical conductivity compared to those that occur on their usual growing sites.

Conclusions

Apparently, elevated salinity level cannot be considered as the main factor in determining the occurrence of stinkwort and false yellowhead. It is presumably only one of many different factors that can also act in a hostile manner towards most plant species on growing sites with higher salinity level. For this reason, the distribution pattern of both *Dittrichia* species in Slovenia could be due to little competition on their growing sites and also their plasticity, which enables them to thrive on generally less favourable sites with many limitations.

Povzetek

Smrdljiva ditrihovka (*Dittrichia graveolens*) in lepljiva ditrihovka (*D. viscosa*) sta vrsti iz družine nebinovk (*Asteraceae*), ki imata v slovenskem prostoru različen status glede izvora. Lepljivo ditrihovko obravnavamo kot domorodno vrsto, ki pri nas uspeva le v primorski regiji (Jogan et al. 2001, Wraber 2010) v bližini morske obale oz., kjer je vpliv morja še zaznaven, medtem ko so pojavljanje smrdljive ditrihovke v Sloveniji prvič zabeležili šele leta 2008. Vse od takrat se hitro razrašča le vzdolž avtocestnega križa (Frajman in Kaligarič 2009) ter ob nekaterih večjih prometnicah. Glede na opisana vzorca razširjenosti obeh

vrst smo domnevali, da obravnavani vrsti zelo dobro prenašata povišano slanost. K povišani slanosti na rastiščih lepljive ditrihovke najverjetneje prispeva morje, v primeru smrdljive ditrihovke pa se slanost tal poviša predvsem na račun zimskega soljenja avtocest. S to raziskavo smo skušali ugotoviti, kako povišana slanost vpliva na kalijovst in zgodnji razvoj kalic obeh vrst ditrihovk.

Izvedli smo kalitvene teste na agarnih ploščah. Kot testno vrsto smo poleg obeh vrst ditrihovk uporabili še solato kot pozitivno kontrolo. Pripravili smo štiri različne koncentracije NaCl vključno s kontrolo, kamor smo dodali le destilirano vodo (0, 2,5, 5 in 10 g NaCl/L). Izmerili smo tudi elektroprevodnost vzorcev tal z nahajališč obeh vrst ditrihovk.

Obe vrsti sta najbolje kalili v kontrolnem tretmaju (0 g NaCl/L). Višanje koncentracije NaCl je vse bolj zaviralo kalitev ter nadaljnji razvoj kalic. Določena semena do konca poskusa sploh niso vzklila, preostala pa so glede na kontrolo vzklila bistveno kasneje. Zaviralen učinek soli je bil nekoliko bolj opazen na smrdljivi ditrihovki. Kljub vsemu se je izkazalo, da sta obe vrsti ditrihovk zmožni kaliti in uspevati pri povišanih koncentracijah soli, ki so tudi dvestokrat višje kot na njunih rastiščih v naravi. Prav zaradi tako širokega razpona uspevanja pri različnih koncentracijah soli ju v odnosu do slanosti lahko obravnavamo kot zelo tolerantni vrsti. Iz rezultatov lahko razberemo, da na vzorec razširjenosti smrdljive in lepljive ditrihovke v Sloveniji lahko vpliva tudi povišana slanost tal, saj prisotnost višje koncentracije soli na rastišču zmanjša konkurenčnost drugih vrst, ki nimajo tolikšne tolerantnosti na prisotnost soli kot obe vrsti ditrihovk.

Acknowledgements

This work was supported by Slovenian Research Agency grant no. P1-0212.

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