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# EFFECT OF NaCl ON SEED GERMINATION IN SOME *CENTAURIUM* HILL. SPECIES (GENTIANACEAE)

[1]S. ŽIVKOVIĆ, [1]M. DEVIĆ, [1]B. FILIPOVIĆ, [2]Z. GIBA, and [1,2] D. GRUBIŠIĆ

[1]*Siniša Stanković Institute for Biological Research,* 11060 Belgrade, Serbia
[2]*Institute of Botany, Faculty of Biology, University of Belgrade,* 11000 Belgrade, Serbia

*Abstract* – The influence of high NaCl concentrations on seed germination in both light and darkness was examined in the species *Centaurium pulchellum*, *C. erythraea*, *C. littorale*, *C. spicatum*, and *C. tenuiflorum*. Salt tolerance was found to depend on the life history of the seeds. To be specific, seeds of all five species failed to complete germination when exposed to continuous white light if kept all the time in the presence of 100-200 mM and greater NaCl concentrations. However, when after two weeks NaCl was rinsed from the seeds and the seeds were left in distilled water under white light for an additional two weeks, all species completed germination to a certain extent. The percent of germination not only depended on NaCl concentration in the prior medium, but was also species specific. Thus, seeds of *C. pulchellum*, *C. erythraea*, and *C. littorale* completed germination well almost irrespective of the salt concentration previously experienced. On the other hand, seeds of *C. tenuiflorum* completed germination poorly if NaCl concentrations in the prior media were greater than 200 mM. When seeds after washing were transferred to darkness for an additional 14 days, they failed to complete germination if previously imbibed on media containing NaCl concentrations greater than 400 mM. However, the seeds of all species, even if previously imbibed at 800 mM NaCl, could be induced to complete germination in darkness by 1 mM gibberellic acid.

*Key words*: Germination, NaCl, light, gibberellin, Gentianaceae, *Centaurium*

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## INTRODUCTION

The genus *Centaurium* Hill. (Gentianaceae) comprises herbaceous plants, mostly annuals and biennials. The two main regions of its distribution are the Mediterranean area with Asia and the western part of North America and Mexico. Natural habitats of *C. spicatum* are damp grassy or sandy soils. In former Yugoslavia, it is widespread along the Adriatic Coast. *Centaurium erythraea* is a common species distributed in forests, in light thickets, and on field margins. The species *C. tenuiflorum* grows on humid soils of marsh margins, on grasslands, and in meadows, mostly in the Mediterranean area of the Balkan Peninsula. In Serbia, this species is distributed only in Vojvodina (Deliblato Sands). It is also found along the Montenegrin Coast. *Centaurium pulchellum* is widespread in Europe, but in Serbia its natural habitat is restricted to a few regions on the territory of Vojvodina. The species *C. littorale* grows on maritime grassland and in sandy places near the sea. In Europe, it is distributed southwards from Ireland to NW France, Austria, and SE Russia. Thus some species of the genus inhabit coastal regions and saline terrains.

Germination is a critical part of plant life histories. The ability of their seeds to germinate at high salt concentration in the soil is therefore of crucial importance for the survival and perpetuation of these species. In saline habitats, seed germination takes place after high precipitation, i.e., under conditions of reduced soil salinity (K h a n and R i z v i, 1994). The ability of the soil seed bank to remain quiescent at a high salt level and to germinate immediately after salinity reduction (B a j j i et al., 2002) is very significant not only to halophytes, but also to other species in colonizing their environment. Although salinity stress mostly reduces the germination percentage and delays the onset of germination, its effects are modified by interactions with other environmen-

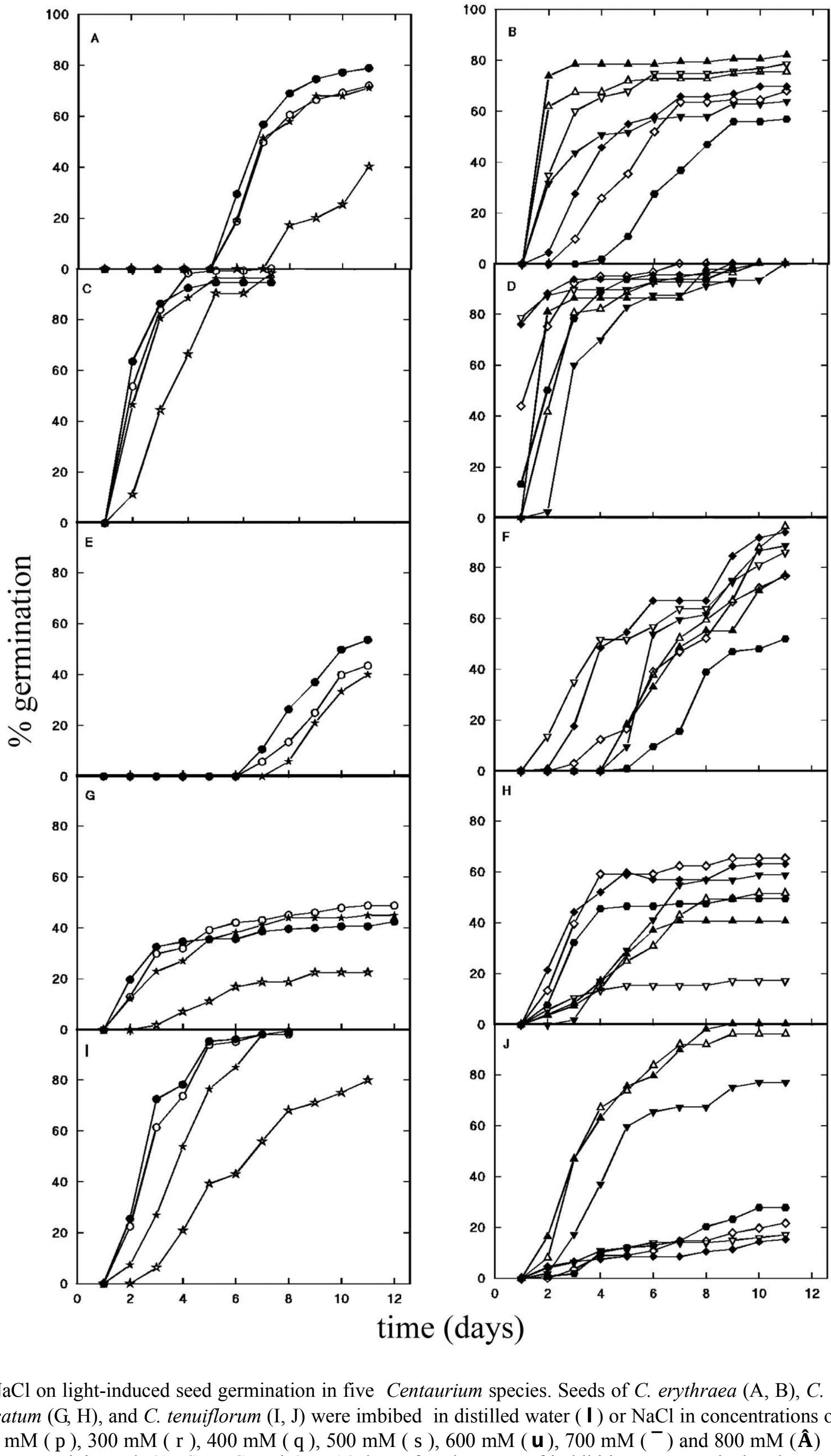


Fig. 1. Effect of NaCl on light-induced seed germination in five *Centaurium* species. Seeds of *C. erythraea* (A, B), *C. littorale* (C, D), *C. pulchellum* (E, F), *C. spicatum* (G, H), and *C. tenuiflorum* (I, J) were imbibed in distilled water ( l ) or NaCl in concentrations of 25 mM (m), 50 mM ( ê ), 100 mM ( ¶ ), 200 mM ( p ), 300 mM ( r ), 400 mM ( q ), 500 mM ( s ), 600 mM ( u ), 700 mM ( ¯ ) and 800 mM (Â) and kept all a time in white light on the medium containing salt (A, C, E, G, and I) or 14 days after the onset of imbibition were washed and transferred to distilled water and incubated for an additional 12 days in white light (B, D, F, H, and J). Germination was scored each day either from the onset of imbibition (A, C, E, G, and I) or from the transfer to distilled water (B, D, F, H, and J).

tal factors such as temperature (M a l c o l m, 1964) and light (K a h n, 1960). Salinity can affect germination by affecting the osmotic component, which influences water uptake, and by interfering with the ionic component, i.e., Na and Cl accumulation. Data on centaury seed germination in relation to light requirements are rather scarce. To our best knowledge, apart from data on the germination of seeds of *C. erythraea* (M i j a j l o v i ć et al., 2005) and the species *C. spicatum* and *C. tenuiflorum* (Ž i v - k o v i ć et al., 2002) [reported in the survey of N i k o - l a e v a et al. (1985)] and information about ecological aspects of germination (S i l v e r t o w n, 1980), no other data on centaury seed germination are available in the literature. The present study deals with germination of NaCl-stressed seeds in five species from this genus.

## MATERIALS AND METHODS

Seeds of *C. erythraea* were collected around Lake Vlasina (Serbia) in 2001 and those of *C. spicatum* in 2004 in the vicinity of Herceg Novi (Montenegro). The seeds of other species (*C.littorale*, *C. pulchellum*, and *C. tenuiflorum*) were harvested from plants grown in the greenhouse of the Siniša Stanković Institute for Biological Research (Belgrade) in 2004. Lots of 30 seeds each were placed in Petri dishes 6 cm in diameter with 2 ml of distilled water or NaCl solution. The fungicide Nistatin was supplied at a concentration of 500 mg $L^{-1}$ in order to prevent fungal infections. Seeds were germinated at 25 ± $2^{0}$C in continuous white light or induced to germinate by white light for 14 days and then transferred to darkness. White light was provided by white fluorescent tubes (65 W, 4500 K, "Tesla", Pančevo, Serbia) giving a fluence rate of 17.46 μmol $m^{-2}$ $s^{-1}$ at seed level. Each solution change was done by washing the seeds with copious amounts of distilled water using a Buchner funnel and side-arm flask attached to a vacuum pump (Gilford Instrument, vacuum receiver 3021). Gibberellic acid ($GA_3$) was purchased from Sigma (St. Louis, USA), and 1 mM were solutions prepared. Germination was scored for 12 days after the onset of imbibition or four weeks after seed pretreatment. All experiments were repeated three times. Standard errors are not shown because they never exceeded 5 %.

## RESULTS

Figure 1 shows the effect of NaCl in the medium on the germination of all five *Centaurium* species. As evident, seeds kept all the time in a medium containing 200 mM and higher concentrations of NaCl did not germinate, even if kept in continuous white light. The extent of germination at lower NaCl concentrations varied for different species (A, C, E, G, and I). However, if after two weeks NaCl was washed away and the seeds were left in distilled water in white light for an additional two weeks,

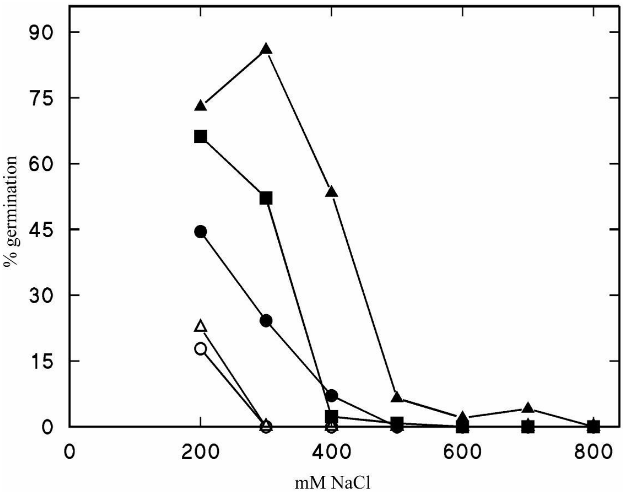


Fig. 2. Effect of NaCl on *Centaurium* seed germination in darkness. Seeds of *C. erythraea* ( l ), *C. littorale* ( p ), *C. pulchellum* (m), *C. spicatum* ( r ), and *C. tenuiflorum* (n) were incubated at different NaCl concentrations (indicated on the abscissas) in white light for 14 days, washed and transferred to distilled water and kept in darkness for an additional 14 days. Germination was scored at the end of the experimental period.

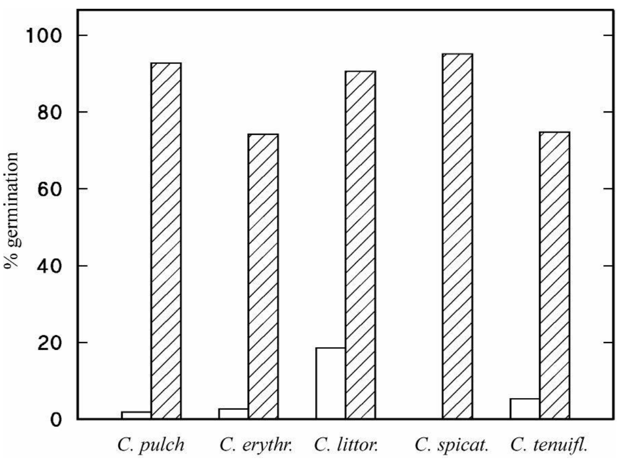


Fig. 3. Effect of gibberellic acid on *Centaurium* seed germination in darkness. *Centaurium* seeds were incubated in 800 mM NaCl in white light for 14 days, washed, and transferred to distilled water (open bars) or $10^{-3}$ M $GA_3$ (hatched bars) and kept in darkness for an additional 14 days. Germination was scored at the end of the experimental period.

all species germinated to a certain extent (Fig. 1, B, D, F, H, and J). Seeds of some of them germinated up to 100% even if pre-treated with the highest NaCl concentration. It seems that *C. littorale* is better adapted to higher salt concentrations in the medium than the other four species (Fig. 1, C and D). Their germination could be ensured only if the washed seeds were kept in white light. If after rinsing they were incubated in distilled water in darkness for an additional 14 days, they either did not germinate (*C. pulchellum* and *C. spicatum*) or germinated poorly, depending on the NaCl concentration that they experienced. However, seeds of none of the species germinated in darkness if previously kept at 500 mM of NaCl (Fig. 2). On the other hand, when seeds were rinsed and transferred to darkness in the presence of 1 mM $GA_3$, more than 70% seeds of all species germinated (Fig. 3).

## DISCUSSION

The group of plants that are well adapted to saline habitats are called halophytes. Their seeds germinate well in freshwater and the germination is similar to that of seeds of nonadapted species (glycophytes). However, they differ from glycophytes in their ability to germinate at higher salt concentrations in the soil (Ungar, 1995). Salinity tolerance of many perennial halophytes differs greatly (Khan, 2002) and is frequently dependant on a variety of abiotic factors such as light and temperature (Baskin and Baskin, 1998). Generally, plants display great diversity with regard to soil salinity tolerance. Species distribution and survival mainly depend on the seed ability to complete germination and the seedling ability to develop successfully under unfavorable conditions. Most seeds are located near the soil surface. Salt concentration in the soil surface changes over time. Continuous water evaporation causes surface salt deposition (Ungar, 1991) while rain dissolves and washes away salt deposits and provides enough water for germination. In the course of evolution, seeds have adapted to such changes, staying viable at high soil salinity and being able to germinate under appropriate external conditions (Khan and Ungar, 1997).

Our previous and preliminary results showed that seeds of all five *Centaurium* species examined by us are light-requiring. *Centaurium erythraea*, *C. tenuiflorum*, and *C. spicatum* seeds are induced to germinate by continous red light. The germination of *C. spicatum* seeds can also be induced, apart from red light, by continuous far red light (Živković et al., 2002). Although *Centaurium* species are not typical halophytes, their seeds germinated well at a moderately elevated salt concentration. However, none of the seeds germinated if kept all the time at relatively high concentrations of NaCl in the medium. Yet they remained viable and germinated in light when washed and transferred to a medium free of NaCl (Fig. 1 B, D, F, H, and J). Published data show that the effect of NaCl on seed germination is mostly osmotic (Kahn, 1960; Loercher, 1974; Reynolds, 1975), but the nature of its effect is unknown. It is improbable that high NaCl concentrations affect the photoconversion of Pr to Pfr (Scorer et al., 1985). It is more likely that low water potentials in seeds affect phytochrome transduction chain (Grubišić and Konjević, 1990) because *Centaurium* seeds incubated at high NaCl concentrations could not germinate when transferred to darkness, even if previously washed. This fact indicates that phytochrome did not complete its action in seeds at a low seed water potential. Germination requires the embryonic axis to develop a growth potential high enough to counteract mechanical restraints imposed by the surrounding tissues. It has been shown that phytochrome induces a decrease of water potential in excised lettuce embryos, thus increasing their growth potential (Scheibe and Lang, 1965; Carpita et al., 1979). Germination of NaCl-stressed *Centaurium* seeds under continous white light may therefore be attributable to the fact that phytochrome is needed for a longer period of time for the embryo to develop of the appropriate growth potential. This view seems to be corroborated by the effect of gibberellic acid, which under the same conditions was able to stimulate germination in darkness (Fig. 3).

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## REFERENCES

*Bajji, M., Kinet, J-M.,* and *S. Lutts* (2002). Osmotic and ionic effects of NaCl on germination, early seedling growth and ion content of *Atriplex halimus*. *Can. J. Bot.* **80**, 297-304.

*Baskin, J. M.,* and *C. C. Baskin* (1998). Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego, Calif., U.S.A.

*Carpita, N. C., Nabors, M. W., Ross, C. W.,* and *N. I. Petretic* (1979). The growth physics and water relation of red-light induced in lettuce seeds. III Changes in the osmotic and pressure potential in the embryonic axes of red- and far-red-treated seeds. *Planta* **144**, 217-224.

*Grubišić, D.,* and *R. Konjević* (1990). Germination of *Paulownia tomentosa* Steud. seeds under reduced water potential. *Arch. Biol. Sci. (Belgrade)* **42,** 207-212.

*Kahn, A.* (1960). An analysis of "dark-osmotic inhibition" of germination of lettuce seeds. *Plant Physiol*. **35**, 1-7.

*Khan, M. A.* (2002). Halophyte seed germination: success and pitfalls. In: *Proceedings of the International Symposium on Optimum Resource Utilization in Salt-Affected Ecosystems in Arid and Semi-arid Regions, Cairo, 8-11 April 2002*. (Eds. A. M. Hegazi, H. M. El-Shaer, S. El-Demerdashe, R. A. Guirgis, A. A. Metwally, F. A. Hassan, and H. E. Khashaba). 346-358. Desert Research Institute, Cairo, Egypt.

*Khan, A. M.,* and *Y. Rizvi* (1994). Effect of salinity, temperature, and growth regulators on the germination and early seedling growth of *Atriplex griffithii* var. *stocksii*. *Can. J. Bot*. **72**, 475-479.

*Khan, M. A.,* and *I. A. Ungar* (1997). Effect of thermoperiod on recovery of seed germination of halophytes from saline conditions. *Am. J. Bot*. **84,** 279-283.

*Loercher, L.* (1974). Persistence of red light induction in lettuce seeds of varying hydration. *Plant Physiol*. **53**, 503-506.

*Malcolm, C. V.* (1964). Effect of salt, temperature and seed scarification on germination of two varieties of *Arthrocnemum halocnemoides*. *J. Roy. Soc. West Aust.* **47,** 72-74.

*Mijajlović, N., Grubišić, D., Giba, Z.,* and *R. Konjević* (2005).The effect of plant growth regulators on centaury (*Centaurium erythraea* Rafn.) seed germination. *Arch. Biol. Sci*. (Belgrade) **51,** 25-28.

*Nikolaeva, M.G., Rasumova, M. V.,* and *V. N. Gladkova* (1985). *Reference Book on Dormant Seed Germination*. (Eds. M. F. Danilova). "Nauka" Publishers, Leningrad Branch, Leningrad.

*Reynolds, T.* (1975). Characterization of osmotic restraints on lettuce from germination. *Ann. Bot*. **39,** 791-796.

*Scheibe, J.,* and *A. Lang* (1965). Lettuce seed germination: evidence for a reversible light induced increase in growth potential and for phytochrome mediation of the low temperature effect. *Plant Physiol*. **40,** 485-492.

*Scorer, K. N., Epel, B. I.,* and *Y. Waisel* (1985). Interactions between mild NaCl stress and red light during lettuce (*Lactuca sativa* L. Cv Grand Rapids) seed germination. *Plant Physiol*. **79,** 149-152.

*Silvertown, J.* (1980). Leaf-canopy-induced seed dormancy in a grassland flora. *New Phytol*. **85,** 109-118.

*Ungar, I. A.* (1995). Seed germination and seed-bank ecology in halophytes. In: *Seed Development and Germination* (Eds. J. Kigel and G. Galili) 529-544. Arcel Dekker, New York.

*Ungar, I. A.* (1991). *Ecophysiology of Vascular Halophytes*. CRC Press, Boca Raton, U.S.A.

*Živković, S., Dević, M., Radičević, N., Todorović, S.,* and *D. Grubišić* (2002). Seed germination of some species from genus *Centaurium*. *Abstracts of Second Conference on Medicinal and Aromatic Plants of Southeast European Countries*, Chalkidiki, Greece, 167.

## ЕФЕКАТ НАТРИЈУМ-ХЛОРИДА НА КЛИЈАЊЕ СЕМЕНА КОД НЕКИХ ВРСТА РОДА *CENTAURIUM* HILL. (GENTIANACEAE)

[1]С. ЖИВКОВИЋ, [1]М. ДЕВИЋ, [2]З. ГИБА и [1,2]Д. ГРУБИШИЋ

[1]*Институт за биолошка истраживања "Синиша Станковић",* 11060 Београд, Србија
[2]*Институт за ботанику, Биолошки факултет, Универзитет у Београду,* 11000 Београд, Србија

Утицај високих концентрација NaCl на клијање семена, како на светлости тако и у тами, испитивано је код врста *Centaurium pulchellum, C. erythraea, C. littorale, C. spicatum* и *C. tenuiflorum*. Толерантност ових биљака на натријум-хлорид зависи од животног циклуса семена. Заправо, семена свих пет испитиваних врста нису завршавала клијање када су била изложена континуелној белој светлости у присуству 100-200 mM и при већим концентрацијама. Поред тога, семена свих ових врста, изложена имбибицији на 800 mM NaCl, могла су бити индукована да заврше клијање у мраку уз додатак 1 mM гиберелинске киселине.