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Effect of Salinity on *Orobanche cernua* Seed Germination

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Seeds of broomrape (*Orobanche cernua*) were exposed to 0, 25, 50, 75, and 100 mM NaCl solutions during their preconditioning period (14 days of moisture) under laboratory conditions and induced to germinate by synthetic germination stimulant (GR24). There was significant reduction in seed germination with increased salt concentration as shown in 35.2, 32.5, 23.6, 14.3, and 9.2% germination, respectively. Exposure of *Orobanche cernua* seeds to 0.0, 1.0, 1.25, and 1.5 M levels of NaCl for 9 hours resulted in 29.4, 21.3, 20.5, and 17.4% germination, respectively. Water preconditioned seeds showed heavier protein profile bands of 6.5-14.2 KDa than those of dry seeds. Seeds treated with 0.75 M NaCl showed profile similar with that of water preconditioned ones, plus an extra band at 29-36 KDa. The protein profiles of 1.0 and 1.5 M NaCl treated seeds showed weaker bands with the absence of 29-36 KDa band.

Keywords **:** *Orobanche cernua*, germination, salinity, PEG, SDS page

Broomrapes (*Orobanche* spp.) are phanerogamic holoparasites that subsist on the roots of a variety of commercial crops (Jain and Foy, 1997). *Orobanche* grows at the expense of its host for water, mineral and organic compounds lowering the biomass and productivity of that host (Hibberd et al., 1996; Morozov et al., 2000). There are about seven *Orobanche* spp. namely, *Orobanche aegyptiaca* Pers., *O. crenata* Forsk., *O. cumana* Walfr., *O. foetida* Poiret., *O. minor* Sm., *O. cernua* and *O. ramosa* L. parasitizing economically important crops in different parts of the world (Hassan, 2000). These parasitic seed plants are characterized by their capability to produce large number of seeds (5.000-100.000 seeds/plant) with a seed weight ranging from 4-9× 10^{-3} mg (Miller, 1994). These seeds need to undergo a preconditioning period (14 days) in moisture and an external germination stimulant molecule in order to break their dormancy and germinate. Bar Nun and Mayer (1993) reported the synthesis of certain new proteins during preconditioning and subsequent germination of *Orobanche* seeds.

Maps of the general distribution and existence of *Orobanche* spp. around the world indicate the absence of these parasitic seed plants in regions characterized by saline soil, as confirmed by Abu-Irmaleh (1998) in his observation of the absence of these parasites in the high salt soils of the region south to the dead sea in Jordan.

In case of green vascular plants, salt stress is probably more critical during their seed germination (Al Karaki, 2000), through induced plasmolysis and/or permeation of toxic salt ions into their embryos (Tobe et al., 1999). However, salt treatment (osmopriming) of certain plant seeds was advocated for enhancing and increasing seed germination (Biniek, 1994). The effect of salinity on the seeds of *O. cernua* during their preconditioning period and later on after being exposed to the germination stimulant exuded by their host and some non-host root systems remains not clearly understood. Therefore, this study was undertaken to explore the changes in protein fingerprinting of *O. cernua* seeds during germination under different salt concentrations.

Materials and Methods

O. cernua seeds were first surface sterilized by immersion in 70% ethanol for 10 seconds, followed by 10 minutes in 2% NaOCl containing 0.1% v/v Tween 20, and then rinsed with sterile distilled water. These seeds were preconditioned in moisture carried on filter paper disks (1 cm diameter), about 50 seeds/disk on filter paper inside Petri dishes and incubated for 14 days at 24°C temperature. During this preconditioning period, seeds were moisted with the following concentrations of NaCl solution: 25, 50, 75, and 100 mM NaCl (-0.11, -0.23, -0.33, and -0.45 MPa). Five solutions of polyethylene glycol (PEG) (-0.2, -0.4, -0.6 -0.8, and -1.0 MPa) were used to test the effect of various water potentials upon the seeds. In the control treatment, seeds were exposed to distilled water only. Solutions were replaced by fresh ones every 2 days in order to maintain precise salt concentrations and osmotic potential during 14 days of incubation. By the end of the preconditioning period in salt or PEG solutions, seeds were spread on water agar plates and induced to germinate by the addition of 2 ml of GR24 solution.

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The effect of salt exposure time upon *O. cernua* seeds during their preconditioning period was explored by exposing them to 0.75, 1.0, 1.25, and 1.5 M NaCl solutions for 3, 6, and 9 hours and induced to germinate in the same manner as above. Seeds were considered to be germinated once their radicle could be seen protruding through their microbile under magnification with the aid of a dissecting microscope.

Protein profile analysis of *Orobanche* seeds. Fifty (50) mg of dry and preconditioned *Orobanche* seeds in 0.0, 0.75, 1.0, and 1.5 M NaCl were separately grind in mortar and pestle into thick homogenous slurry and transferred into microfuge tubes. One (1) ml of 10% sodium dodecyl sulfate (SDS) buffer containing 30% sucrose, 0.005% bromophenol blue, and 50 µL of beta-mercaptoethanol were added to each tube and placed inside a water bath for 5 minutes. The reaction mixture was subjected to centrifugation at 13,000 g for 2.5 minutes. Fifteen (15) µL aliquots of the supernatant from each preparation were used for protein profile analysis. One-dimensional 12% polyacrylamide gradient gel electrophoresis was used to separate proteins present in the *Orobanche* seed preparation according to the protocol described by Laemmli (1970). Flooding the gel with Coomassie blue R solution, documented by photography developed protein profiles.

Results and Discussion

A negative linear relationship was observed between salt levels and germination percentage of *O. cernua* seeds (Fig. 1). As salt concentration increased to 75 and 100 mM, germination percentage was significantly decreased to 14.3 and 9.2%, respectively. However, seed germination at zero (control) and 25 mM NaCl were not significantly different from each other. The lowest seed germination (9.2%) was observed in the 100 mM NaCl treatment. Abu Irmaileh (1998) reported that *O. ramosa* seeds rarely germinated when incubated in 77 mM NaCl solution. Therefore, it was not merely the increasing water potential of the surronding medium around the seeds that influence germination This was further substantiated by the nonsignificant decrease in seed germination within the range of 0-0.4 MPa of PEG which corresponded with 0-75 mM NaCl solution applied in this study. The adverse effect of decreasing water potential on *Orobanche* seed germination was only evident at values approching -0.8 MPa, allowing only 9.2% seed germination as compared with -0.2 MPa which gave 31.3% germination. On the other hand, NaCl solution of 50 mM with osmotic potential equal to -0.22 MPa, gave 23.6% seed germination. Similarly, Nandula et al. (1996) found that germination percentage of *O. aegyptiaca* and *O. ramosa* was not adversly affected when osmotic potential was changed from 0 to -0.2 MPa. However, it drastically declined as osmotic potential approched to -0.8 MPa.

The effect of salinity on seed germination could be due to the toxic effect of NaCl on seeds, or to the osmotic effect that prevents the seeds from imbibition (Tobe et al., 1999). In this study, results showed that using a solution with -0.2 MPa osmotic potential gave 31.3% germination. Meanwhile, a solution with 50 mM NaCl (-0.22 MPa) resulted in 23.6% germination. Therefore, it can be concluded that the effect of salinity on the germination of *O. cernua* seed was not due to osmotic potential effect under these conditions, but to some biochemical changes occurring within the seeds.

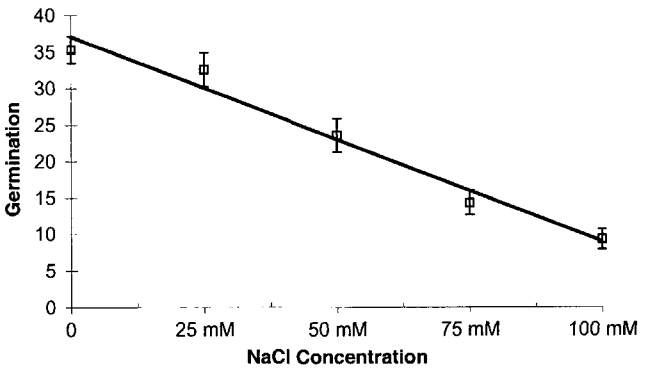

Fig. 1. Effect of NaCl concentration of the preconditioning medium on the germination percentage of *Orobanche cernua* seeds. Y=-7.03X+44.09, R^2=0.974. Bars represent standard deviation.

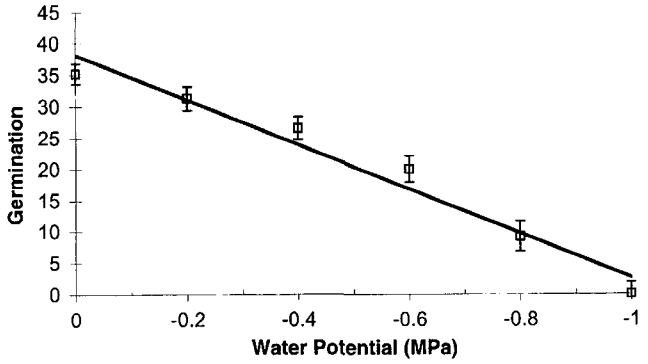

Fig. 2. Effect of water potential of the preconditioning medium on the germination percentage of *Orobanche cernua* seeds. Y=-7.11 X+45.27, R^2=0.963. Bars represent standard deviation.

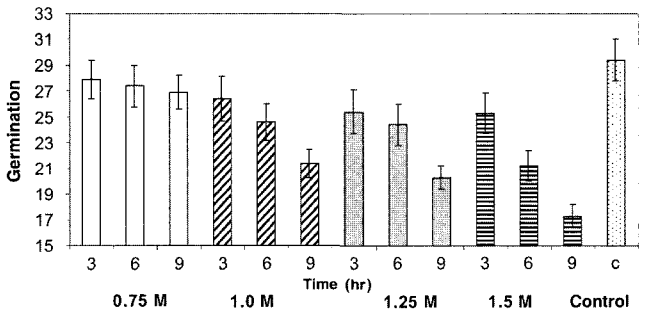

Fig. 3. Effect of four levels of NaCl (0.75, 1.0, 1.25, and 1.5 M) on the percent germination of *Orobanche cernua* seeds at three different periods (3, 6, and 9 hours) of exposure. Bars represent standard deviation.

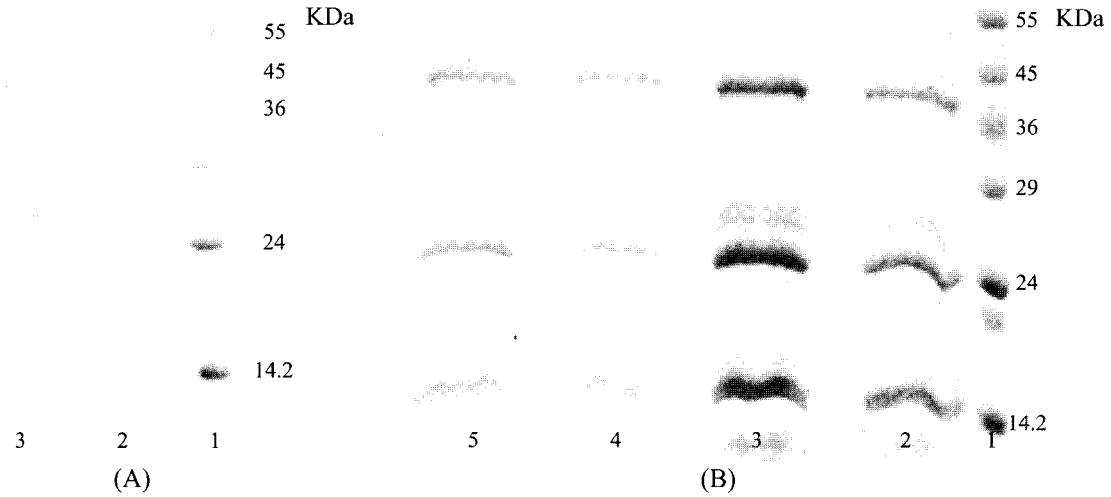

Fig. 4. Electrophoretic pattern for extracts of *Orobanche cernua* seeds. (**A**) lane 1=marker, lane 2=dry seeds, and lane 3= preconditioned seeds for 9 hours in water. (**B**) lane 1=marker, lane 2=preconditioned seeds for 9 hours in water, lane 3= preconditioned seeds for 9 hours in 0.75 M NaCl, lane 4= preconditioned seeds for 9 hours in 1 M NaCl, lane 5= preconditioned seeds for 9 hours in 1.5 M NaCl.

Such biochemical changes lead to decreased seed germination and were pastulated upon as a specific ion toxicity of the NaCl rather than osmotic potential on the seeds. Ghoulam and Fares (2001), in their study on the effect of salinity on the germination of sugar beet, found that at the same osmotic potential, the germination was different in NaCl compared with that in mannitol. They suggested that NaCl affects seed germination not only because it is osmo active, but also via specific ion toxicity.

Preconditioning *O. cernua* seeds in 0.75 M NaCl solution for 3, 6, and 9 hours had no significant difference in germination percentage. However, treatment of higher levels of NaCl (1, 1.25 M) for 6 and 9 hours significantly decreased germination, and treatment with 1.5 M for 9 hours severely inhibited germination (Fig. 3). Prado et al. (2000) found that germination percentage of quinoa (*Chenopodium quinoa* Wild.) decreased markedly under saline conditions; at 0.4 M NaCl, the percentage of germination was only 14% after 14 hours, whereas the control at the same time reached maximum germination (87%). On the contrary, Cuartero and Fernandez (1999) found that priming tomato seeds with 1 M NaCl for 36 hours improved germination and seedling growth. *Orobanche* seeds seem to be a special case in their interaction with NaCl treatment at much lower concentrations and much shorter time of exposure.

The protein finger printing of *O. cernua* seeds (Fig. 4) revealed the presence of a new protein band (24-36 KDa) in the protein profile of water preconditioned seeds which was not present in the profile of the dry non-preconditioned seeds. Preconditioned *O. cernua* seeds in 1.0 and 1.5 M NaCl solutions, on the other hand, showed weaker protein bands (Fig. 4B), similar to those observed by Bar Nan and Mayer (1993). Furthermore, these protein bands were missing in the profile of seeds preconditioned in 1.0 and 1.5 M NaCl solutions for 9 hours. Therefore, NaCl treatments might have affected the protein from being synthesized. Hence, it may act against the expression of a relevant gene for that protein biosynthesis, in analogy of anticipated ion specificity. Findings in this study shed some light on the distribution of *Orobanche* in different regions of the world.

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