Interactive effects of salinity, light and temperature on seed germination of *Zygophyllum simplex* L. (Zygophyllaceae)

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**Abstract**

A number of abiotic factors are known to affect seed germination and distribution patterns of plants in natural habitats. In this study, the interactive effects of temperature, light/darkness and different salts of different concentrations on seed germination of the Jordanian succulent herb *Zygophyllum simplex* were investigated. Seeds showed 60% germination percentage in non-saline controls at 25°C. Cooler temperatures diminished germination to less than 10% at 5°C. Seed germination of *Z. simplex* was significantly affected by salt type, concentration, temperature and their interactions. Similar germination percentage was found for seeds incubated under light or dark conditions. *Z. simplex* germinated best at intermediate temperature regime (15 and 25°C), and at this temperature, salinity co-acts and affects seed germination. Results of this study indicate that the interactive effect between temperature and salinity could be an adaptive strategy that allows *Z. simplex* to survive and reproduce in harsh desert habitats of Jordan.

**Keywords:** Germination, light, salinity, temperature, *Zygophyllum simplex*.

**INTRODUCTION**

Seed germination is a critical developmental stage governing the ecological success and distribution patterns of plants, especially in arid and saline environments (Bewely, 1997; Song et al., 2005). A number of abiotic stresses (light, temperature, salinity and water) are known to interfere with the process of seed germination. The effect of salinity on seed germination has been the most extensively studied (Khan et al., 2000; Khan et al., 2001; Zia and Khan, 2002; Song et al., 2005; Ochoa-Alfaro et al., 2008). Plants adapted to dry and saline habitats have been leading study systems, due to the sustainable soil salinization and aridization plants experience in these habitats. Generally, it has been found that an increase in salinity beyond a threshold tolerance value diminishes germination, although responses to salinity vary among species (Khan et al., 2000; Tobe et al., 2004).

The succulent forb, *Zygophyllum simplex* L. is widely distributed in the southern region of Jordan, which characterized by sandy, high salt levels and dry soil. Studies to investigate plants responses to salinity have focused on the use of sodium chloride (NaCl) as it contributes the most to soil salinity (Yazici et al., 2007); however, saline soils contain a variety of other salts (Mg\(^2\)+, Ca\(^{2+}\), Cl\(^-\), SO\(_4\)\(^{2-}\)), which may exert different effects on plants (Tobe et al., 2000, 2002, 2003, 2004).

In the literature, only a few studies addressed the individual and co-active effect of multiple types of salts on plants (Tobe et al., 2004). The inhibitory effect of salinity on seed germination has been shown to be ascribed to an osmotic effect and/or ion toxicity, depending on the species (Song et al., 2005 and references therein). For instance, enhanced soil salinity (due to loss of moisture, soil infiltration with seawater or accumulation of rocks deposits in soils) lowers water potential of soil solution, thereby alleviating the pressure gradient required for water absorption and consequential germination. This explains why seeds of many desert plants germinate only when enough water is available, which reduces salinity stress and ensures water uptake by seeds (Khan et al., 2001; Zia and Khan, 2002; Song et al., 2005). In addition to salinity, other environmental variables (temperature and light) have been shown to play roles and regulate the process of seed germination. The
interaction of various factors in the soil surface was found to be a key determinant of time and sites of germination in the field. Khan and Ungar (1996) showed that germination of *Haloxylon recurvum* (Chenopodiaceae) decreases as temperature increases (> 25ºC) while cooler temperatures promoted its germination at all NaCl concentrations tested. Similar findings were reported with studies on closely related species (*H. ammodendron* and *H. persicum*) (Tobe et al., 2000) and on other species of the same family such as *Salicornia rubra* (Khan et al., 2000), and *Kochia scoparia* (Khan et al., 2001). Different species were found to demonstrate a contrasting behavior in response to temperature. For example, in a study with *Zygophyllum simplex* (Zygophyllaceae) (Khan and Ungar, 1997) found that lower temperatures inhibited germination considerably, whereas higher temperatures caused some inhibition at all NaCl treatments. The effect of light on seed germination was also studied and it was found to have insignificant impact on seed germination under different thermoperiods (Tobe et al., 2000).

In this study, we examined and compared the effects of different salts (NaCl, KNO₃, CaCl₂, MgCl₂, and MgSO₄), light and temperature on germination of *Zygophyllum simplex* (Zygophyllaceae), an annual succulent herb distributed in desert areas of Africa, Asia and Australia. In Jordan, *Z. simplex* is a primary vegetative component of hot arid regions and grows successfully in inland and coastal sandy habitats south of Aqaba city. It also occurs in waste places in association with other species of the family Chenopodiaceae. These species are considered a great model system for studying stress physiology. In a previous study, Khan and Ungar (1996) reported that *Z. simplex* is moderately-salt tolerant to NaCl during germination; however, we are unaware of studies that address germination responses of *Z. simplex* to different types of salts and to their interactive effects with other environmental variables, which we carried out in this study.

**MATERIALS AND METHODS**

**Plant material**

Seeds of *Z. simplex* were collected during March 2009 from plants growing in dry and sandy sites along the main shore south of Aqaba city, Jordan (Fig. 1, identified by Professors Jamil Laham and Ahmad El-Oqlah (Zohary, 1972). Seeds were collected from several separate plants selected randomly throughout the whole region, cleaned from floral debris and used for various germination experiments after surface sterilization with 10% Sodium hypochlorite solution for five minutes and then washed three times with sterile distilled water. Voucher specimens were prepared and preserved at the herbarium of Department of biological sciences at Yarmouk University.

**Effects of individual salts on seed germination**

Germination of *Z. simplex* in response to salinity was investigated by placing 20 seeds in a 9-cm-diameter Petri dish containing two layers of Whatman No. 1 filter papers, moistened with 10 ml of distilled water or the treatment solution (50, 100, 150 and 200 mM of NaCl, MgCl₂, MgSO₄, KNO₃ and CaCl₂). The petri dishes were tightly sealed using parafilm to prevent evaporation and subsequently placed in a growth chamber set at 25ºC under 16 hr/8 hr photoperiod. Fluorescent lamps were used to produce a photosynthetic photon flux density of 80 µmol m⁻² s⁻¹.

**Effects of temperature and light on germination**

To determine the effect of temperature and light on germination, seeds were incubated at 5, 15, 25 and 35°C. Seeds were germinated in distilled water and in constant fluorescent light (80 mmol m⁻² s⁻¹) or in complete darkness. Germination in the light was monitored every 2 days for 10 days. For those seeds incubated in darkness, they were checked first and only at this time (after 10 days of incubation).

**Effects of temperature and salt on germination**

Seeds were incubated in a 9-cm-diam Petri dish containing two layers of Whatman No. 1 filter paper. Ten ml of distilled water or the treatment solution (50, 100 and 150 mM of KNO₃ and MgSO₄) was added to each petri dish. Petri dishes were incubated at 15, 25 and 35ºC±1°C of target levels. Seed germination was determined 10 days after the beginning of the germination period.
Interactive effects of salinity, light and temperature on seed germination were conducted at 15, 25 and 35°C in combination with the desired concentrations of NaCl, KNO$_3$, CaCl$_2$, MgSO$_4$ and MgCl$_2$ to Z. simplex seeds and incubate them under 25°C and 16/8 photoperiod. Results showed that Z. simplex seeds have 60% germination percentage when distilled water was used as control (Fig. 2 [Supplementary data]). Seed germination decreased with increase in the concentration of NaCl, KNO$_3$, CaCl$_2$, MgSO$_4$ and MgCl$_2$. MgSO$_4$ showed the strongest effect on seed germination among other salts and showed complete inhibition at concentration of 150 mM and above. On the other hand, KNO$_3$ showed a moderate effect on seed germination inhibition. Seeds treated with high concentrations of KNO$_3$ (up to 150 mM) still showed more than 50% germination percentage of the control plants. Treating seeds with 50 mM MgSO$_4$ or MgCl$_2$ reduced germination percentage significantly compared with other salt types at the same concentration (Fig. 2).

To test the effect of light and temperature on germination percentage of Z. simplex, seeds were incubated at 5, 15, 25 and 35°C under complete darkness or 16 hr/8 hr photoperiod. Germination percentages in the light and in darkness increased significantly (P <0.001) with the increase in temperature up to 25°C, then a significant reduction in germination percentage was observed when seeds were incubated at 35°C (Fig. 3 [Supplementary data]). Incubation of seeds at 5°C significantly reduced germination percentages for more than 10 fold compared to seeds incubated at 25°C. Results show that there is no significant difference in germination percentage between light and dark incubated seeds. Similarly, two-way ANOVA showed that there is no interaction between light and temperature.

Finally, an experiment was conducted to test if there is an interaction between temperature and salinity (Fig. 3 [Supplementary data]). Two salts were used in this experiment (KNO$_3$ and MgSO$_4$). Seeds were incubated under 15, 25 and 35°C in combination with the desired salt type and level. A significant interaction was found between temperature and salinity. Germination percentage of Z. simplex was highest at 25°C regardless of salt type and level. On the other hand, incubating seeds at 35°C in combination with any salt used, resulted in a significant reduction in germination percentage (Fig. 3). Results showed that as salt concentration increase the germination percentage of Z. simplex seeds decrease when seeds were incubated at 15 or 25°C. In contrast, incubating Z. simplex seeds at 35°C did not affect germination percentage at any salt type or level used. This indicates that the high temperature is the limiting factor of Z. simplex seeds germination even in the presence of high levels of salt.

**DISCUSSION**

Seed germination is a fundamental trait at both individual and population levels. It marks the transition from the seed stage that is most tolerant to environmental conditions to the seedling stage that is most vulnerable in plant life (Harper, 1977; Khan and Sheith, 1996). In addition, seed germination is a delicate process because the time and location of germination determine the fate of individuals and consequently population dynamics (Bewley and Black, 1994; Silvertown and Charlesworth, 2001). In this study, the influence of various types of salts on germination of the Jordanian desert herb Z. simplex was investigated. Germination percentage of Z. simplex decreased significantly with increased salinity level, regardless of the salt type. This result is in agreement with previously published reports examined on different plant species (Duan et al. 2003; Khan and Weber 1986; Katembe et al. 1998; Gulzar and Khan 2001; Li et al. 2002). An experiment was conducted by Mahmood et al. (1996) to examine the effect of salinity on germination percentage of Suaeda fruticosa, Kochia indica, Atriplex crassifolia, Sporobolus arabicus, Cynodon dactylon, Polypogon monspeliensis and Desmostachya bipinmata. Results showed that as salinity concentration increase, a gradual decrease in seed germination was observed for each species.

Previous studies have examined the effect of salinity stress on germination percentage of Z. simplex using only NaCl (Khan and Ungar, 1997). Though, saline soils include several types of salt, each of which has a different effect on plant growth pattern (Toke et al., 2002, 2003). Moreover, it has been shown that the composition of soluble salts in saline areas diverge significantly among different sites. In this study, germination of Z. simplex was also affected differently using various types of salts, and this is likely related to the ionic composition of different types of salts (Na$^+$, Mg$^{2+}$, Cl$^-$, and SO$_4^{2-}$) (Ungar 1978; Wang 1993; Zhou et al. 2003) (Fig. 1). The effect of salt stress on germination of Z. simplex was found to be in the order...
Interactive effects of salinity, light and temperature on seed germination of Z. simplex: MgSO$_4$ > MgCl$_2$ > CaCl$_2$ > NaCl > KNO$_3$. This difference in response of Z. simplex seeds to various salt solutions could be due to the varied impact of salt components on membrane permeability, toxicity and/or the cell wall structure (Tobe et al. 2004).

In nature, seed germination is largely influenced by the interactive effect of different environmental factors including temperature and light (Bewley and Black, 1994; Baskin and Baskin, 1998). In this study, germination percentage of Z. simplex was found to be significantly influenced by temperature under both light and dark conditions. However, germination percentage did not differ whether the seeds were exposed to light or dark. These results reflect a negligible interaction between temperature and light on seed germination (Fig. 2). It has been previously shown that light requirement for seed germination varied from no effect, as reported in this study, to an obligate requirement, depending on the species (Garcia et al., 1995; Khan and Ungar, 1998; Khan and Ungar, 1997; Khan and Ungar, 1999; Thanos et al., 1991). Germination percentage of the Halophyte, Kallidium capsicum was shown to be significantly lower under light conditions than in the dark conditions (Tobe et al., 2000). In contrast, an adverse effect of darkness on Salicornia pacifica germination percentage were observed where only 50% of the seeds germinate under dark conditions compared to 100% under light conditions (Khan and Weber, 1986). Additionally, this paper shows that seeds of Z. simplex germinate best at intermediate temperature regime (15, 25°C), and interestingly at this temperature we found that salinity co-act on seed germination (Fig. 3). The interactive effect of temperature and salinity on Z. simplex seed germination is in agreement with other studies on Z. simplex from other areas (Khan and Ungar, 1996). However, at high temperature seed germination is independent of salinity.

Finally, results of this study indicate that the interactive effect between an intermediate temperature regime and salinity are necessary for optimal seed germination of Z. simplex and this could be part of the adaptive strategy of this species that allows it to tolerate saline conditions of Jordan desert. Other information is available with the cell wall structure (Tobe et al. 2004).

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