Effect of NaCl on *Orobanche* spp and *Striga hermonthica* seeds germination during and after conditioning.

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The parasitic plants *Orobanche* and *Striga* spp. are holo- and hemi-parasites respectively, which largely depend on a host plant to obtain their nutrients and water. The objective of this study was to investigate the effect of NaCl on *Orobanche* and *Striga* seed germination under laboratory condition. Seeds of *Orobanche minor* were exposed to 50, 75, 100 and 150 mM NaCl solutions during or after conditioning period (for 7 days) and induced to germinate by a synthetic germination stimulant GR24. As a result, seed germination was decreased significantly with the increase in salt solution concentration during conditioning. Significant reduction in *O. minor* seed germination was observed at highest NaCl level (150 mM). It reduced germination by 92% as compared to the corresponding control. On the other hands, germination response of *O. minor* seeds conditioned in water and then treated with mixture of GR24 and NaCl 1:1 (v/v) was studied, and results displayed that germination of *O. minor* seeds was inhibited with increasing NaCl concentrations. Seeds treated with 150 mM, exhibited reduced germination by 77% as compared with control. With respect to *O. crenata* and *Striga*, results showed that all concentrations of NaCl decrease seeds germination. Of all NaCl concentration tested, 100 mM displayed the most inhibitory effect. Osmotic potential may significantly affect germination and radicle elongation of parasitic weeds.

Key words: parasitic weeds, NaCl, germination, suppression

Broomrapes (*Orobanche* spp.) are obligate, chlorophyll-lacking root parasites that parasitize many dicotyledonous species and cause damage to vegetable and field crops worldwide (Parker and Riches 1993; Foy et al. 1989). Broomrape plant lives directly on their hosts by attaching strong haustoria to their roots, penetrating the tissues, and absorbing the food gathered by the host plants for their own development (Manschadi et al. 2001 and Alkhateeb et al., 2005). However, the seeds need to be exposed to a moist environment (called preconditioning) for several days at a suitable temperature (optimum 15 to 20 °C) before seeds respond to germination stimulants (Kebreab and Murdoch 1999; Morozov et al., 2000). Bar Nun and Mayer (1993) reported the synthesis of certain new proteins during preconditioning and subsequent germination of *Orobanche* seeds. Seeds upon germination, the small broomrape seed develop a tube-like radicle that attaches to the host root surface. After attachment to the host root, the radicle develops a haustorium, which penetrates the root and forms connections to the vascular system of the host plant (Parker and Riches 1993; Hassan, 2000; Kubo et al. 2009). The portion of the parasite remains outside of the root tissue then develops into a tubercle that initiates crown roots and a floral meristem that produces a floral spike (Foy et al. 1989;
Parker and Riches 1993). The small broomrape flower stalks emergence from the tubercle about 4 to 5 months after initial parasitic attachment to red clover (Lins et al. 2005). Small broomrape seeds require the presence of germination stimulant, a chemical signal for germination (Foy et al. 1989). Germination stimulants include alectrol and orobanchol, which are analogues of strigolactones and have been isolated from the root exudates of the host red clover (Yokota et al. 1998).

Maps of the general distribution and existence of Orobanche spp. around the world indicate the absence of these parasitic plants in regions characterized by saline soil, as confirmed by Abu-Irmaileh (1998) who observed 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). The effect of salinity on the seeds of Orobanche spp. 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. In Jordan, Abu Irmaileh (1998) observed that no Orobanche infections were found on tomato in the area around the Dead Sea and the southern Jordan valley where soil salinity is reported to reach 16.4 ds/m. Therefore, in this study, the effect of different levels of NaCl on O. crenata, O. minor and Striga hermonthica germination will be investigated.

MATERIALS AND METHODS

Plant Materials: Three set of laboratory experiments were conducted to study the effects of NaCl on parasitic weeds Striga hermonthica, O. minor and O. crenata germination in response to GR24 and root macerate of sorghum and faba bean. O. minor seeds were collected in Japan. S. hermonthica and O crenata seeds were collected in Suda. Striga and O. crenata seeds were surface sterilized as described by Hsiao et al. (1981). Briefly, the seeds were soaked in 70% for 2 min in 70% ethanol and rinsed three times with distilled water. Subsequently the seeds were immersed in 1% NaOCl solution for 3 min with continuous agitation, thoroughly washed with sterilized distilled water; air dried and kept in sterilized vials, at ambient temperature till used. Parasitic seeds germination stimulant GR24 was provided by Professor B. Zwaneberg, the University of Nimijhen, the Netherlands. NaCl at 0, 25, 50, 75, 100, and 150 mM was applied to Orobanche seeds during and after conditioning.

Preparation of host root extracts: Seeds of sorghum (cultivar, Tabat) and faba bean (cultivars, Hudiba and Solium) were surface disinfected by immersing in aqueous solution of 1% sodium hypochlorite for 5 min. Seeds were washed three times with sterilized distilled water then planted in sand in plastic pot (19 cm-diameter) root harvested 10 days after sowing were thoroughly washed with sterilized distilled water. Root sampling (1 g each) were crushed in 10 ml sterilized distilled water in a mortar. The root macerate was filtered, then diluted 2-times with distilled water prior to use.

Effect of NaCl on germination of O. minor seeds, during conditioning, in response to GR24: O. minor seeds were conditioned as described by Hassan et al. (2009). Briefly glass fiber filter papers (GF/C) discs (8 mm diameter) were cut, wetted thoroughly with water and placed in an oven at 100 ºC for 1 h to be sterilized and ready for further use. The discs, placed in 9 cm Petri dishes lined with glass fiber filter papers (GF/C), were moistened with 5 ml distilled water, or different concentrations of NaCl viz. 25, 50, 75, 100, and 150 mM. About 25-50 surface disinfected Orobanche seeds were sprinkled on each of the glass fiber discs in each petri dish. The dishes, sealed with parafilm were placed in black polythene bags and incubated at 23ºC in the dark for 7 days. Orobanche seeds were treated with GR24 at 0, 0.034, 0.34 and 3.4 µM, then re-incubated and determined the germination rate after 24 h of GR24 treatment.

Effect of the combination of NaCl and GR24 on germination of O. minor seeds after conditioning: O. minor seeds were conditioned in water as described above. The sterilized discs, placed in 9 cm Petri dishes lined with glass fiber filter papers (GF/C), were moistened with 5 ml distilled water.
About 25-50 surface disinfected O. minor seeds were sprinkled on each of the glass fiber discs in each petri dish. The dishes, sealed with parafilm were placed in black polythene bags and incubated at 23°C in the dark for 7 days. O. minor seeds were treated with mixture of different level of NaCl and GR24 (1:1 v/v). NaCl viz. 25, 50, 75, 100, and 150 mM were mixed with GR24 at 1µM re-incubated and determined the germination rate after 24 h of GR24 treatment.

Effect of NaCl on germination of S. hermonthica and O. crenata seeds in response to root macerates: Parasitic seeds were conditioned as described above. The discs placed in 9 cm Petri dishes lined with glass paper (GF/C) were moistened with 5ml distilled water, or different concentrations of NaCl viz 5, 10, 25, 40, 55 mM. About 25 – 50 seeds were sprinkled on discs in each Petri dish. The dishes, sealed with parafilm were placed in black polythene bags and incubated at 30ºC in the dark for 10 days. Striga and O. crenata seeds were treated with different root macerate (Tabat, Hudiba and Solium) reincubated and determined the germination rate after 24 h of GR24 treatment.

In all experiments, treatments were arranged in a randomized complete design with 4-5 replicates. Data on percentage germination was calculated for each disc, (Gomez and Gomez, 1984) and subjected to analysis of variance (ANOVA). Means were compared with the Least Significance Difference (LSD) at 5% level.

RESULTS

Effect of NaCl treatment during conditioning on germination of O. minor seeds in response to GR24: O. minor seeds, previously conditioned in presence of NaCl, showed variable response to GR24. Results revealed that O. minor seeds treated with distilled water displayed negligible germination in all experiments. GR24 at 0.034 - 3.4 µM effectively induced germination of water-conditioned seed in a dose dependent manner. GR24 applied to seeds conditioned in water induced the highest germination (59 - 85%) (Table 1). All NaCl treatments decreased O. minor germination in response to GR24 in comparison with the corresponding aqueous controls. Seed conditioned in the presence of 50 mM NaCl and treated with GR24 at 0.034, 0.34 and 3.4 µM displayed 17.4, 31.8 and 48.8% germination, respectively. However, seed conditioned in the presence of 150 mM NaCl and treated with GR24 at 0.034, 0.34 and 3.4 µM displayed 5.22, 17.9 and 25% germination, respectively. Of all NaCl levels, the highest concentration of NaCl (150 mM) was the least effective to GR24 treatments. (Alternative: most inhibitory to germination induced by GR24). It reduced germination between 71 and 92% as compared to corresponding control. However, at 50 mM NaCl reduced germination between 43-71% (Table 1).

Effect of the combination of NaCl and GR24 (1:1 v/v) on germination of O. minor seeds after conditioning: Orobanche seeds conditioned in water were induced at the highest germination rate (82 %) in response to GR24 (Fig. 1), whereas all concentrations level of coexisting NaCl—decreased Orobanche seeds germination in response to GR24. NaCl concentrations increased to 100 and 150 mM, germination percentage was significantly decreased to 53 and 90%, respectively in response to GR24. Generally a combination between NaCl with GR24 was more suppressive with increasing NaCl concentrations. Seed conditioned in the 50 and 75 mM NaCl and similarly treated with GR24 displayed comparable germination. The higher concentration of NaCl at 100µM reduced seeds germination by 43% as compared to their control, while the highest concentration of NaCl, 150 mM was the most inhibitory. It reduced the germination significantly by 77% as compared to the control (Fig.1).

Effect of NaCl on radical elongation: Seeds of Orobanche minor were induced to germinate with the germination stimulant GR24 in the presence or absence of test NaCl (Plate 1). Radicle lengths were measured microscopically after 5 days of incubation at 23°C. NaCl applied to Orobanche seeds, during conditioning, inhibited the germination and radicle growth of conditioned seeds of Orobanche minor, in response to the germination stimulant GR24. The results indicated that higher concentration of NaCl (150 mM) significantly inhibited radicle elongation relative to control radicles.
Table 1: Effect of NaCl on *O. minor* seeds germination in response to GR24 (during conditioning)

<table>
<thead>
<tr>
<th>GR24 (µM)</th>
<th>NaCl (mM)</th>
<th>Water</th>
<th>50</th>
<th>75</th>
<th>100</th>
<th>150</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4 µM</td>
<td>84.6</td>
<td>48.8</td>
<td>44.2</td>
<td>25.4</td>
<td>24.0</td>
<td>45.30</td>
<td></td>
</tr>
<tr>
<td>0.34 µM</td>
<td>62.9</td>
<td>31.8</td>
<td>26.7</td>
<td>30.0</td>
<td>18.0</td>
<td>33.86</td>
<td></td>
</tr>
<tr>
<td>0.034 µM</td>
<td>58.9</td>
<td>17.4</td>
<td>16.4</td>
<td>18.2</td>
<td>5.2</td>
<td>23.23</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>68.79</td>
<td>32.66</td>
<td>29.13</td>
<td>24.52</td>
<td>18.73</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD interaction=6.516, LSD GR24=13.033, LSD NaCl=9.216

Figure 1: Effect of different level of NaCl mixed with GR24 on *O. minor* germination (after conditioning). Vertical bar indicates LSD

Plate 1: Effect of NaCl on radical elongation

whereas the lowest concentration (50 mM) showed less inhibitory effects (Plate 1).

Effect of NaCl on *O. crenata* seeds germination: Faba bean root extracts Hudiba and Solium applied to Orobanche seed conditioned in water induced 26 and 30% germination, respectively (Table 2). All concentrations of NaCl decreased Orobanche seeds germination as compared with control.
Table 2: Effect of NaCl on *O. crenata* seeds germination

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Root macerate of faba bean</th>
<th>Water</th>
<th>25</th>
<th>50</th>
<th>100</th>
<th>means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soiliun</td>
<td></td>
<td>30</td>
<td>23</td>
<td>18</td>
<td>12</td>
<td>20.75</td>
</tr>
<tr>
<td>Hudibia</td>
<td></td>
<td>26</td>
<td>12</td>
<td>17</td>
<td>18</td>
<td>18.25</td>
</tr>
<tr>
<td>Means</td>
<td></td>
<td>28</td>
<td>17.4</td>
<td>17.2</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>LSD ± 8.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Effect of NaCl on *S. hermonthica* seeds germination

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Treatment</th>
<th>Water</th>
<th>25</th>
<th>50</th>
<th>100</th>
<th>means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soiliun</td>
<td>Sorghum root macerate</td>
<td>21</td>
<td>12.8</td>
<td>13.7</td>
<td>10.6</td>
<td>14.5</td>
</tr>
<tr>
<td>Hudibia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Means</td>
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</tbody>
</table>

Of all NaCl treatments, 100 mM displayed the most inhibitory. Generally, *Soiliun* root extracts induced the highest germination of *O. crenata* seeds as compared with Hudiba, irrespective of NaCl concentrations. Seeds conditioned on NaCl at 100 mM displayed 12% germination in response *Soiliun* root extracts. However, seeds conditioned in NaCl at 25 mM displayed the lowest germination in response to Hudiba root extracts as compared to control (Table 2).

**Effect of NaCl on ** *S. hermonthica* seeds germination:** *Striga* seed, previously conditioned in presence of water induced the highest germination (21%) in response to root extracts of sorghum Tabat (Table 3). All concentrations of NaCl decreased *S. hermonthica* seed germination in response to sorghum stimulant in comparison with the corresponding aqueous control. However, the highest concentrations of NaCl (100 mM) reduced germination significantly. It reduced germination by 52% as compared to their control (Table 2).

**DISCUSSION**

To control germination of parasitic weeds by salt treatment, this study focused on inhibition and/or perturbation of early growth stages of the *Orobanche* and *Striga* parasite. Several factors influence germination of broomrapes in the soil including temperature, moisture, pH, nutrients, soil type, and stimulants produced by host plants. A negative relationship was observed between salt levels and germination percentage of *Orobanche* spp. and *Striga* seeds during or after conditioning (Table 1, 2 and 3). As salt concentrations increased to 100 and 150 mM, *O. minor* seeds germination percentage was significantly reduced to 54 and 92%, respectively, irrespective of synthetic stimulant concentrations. The lowest seed germination (5%) was observed in 150 mM salt level. With respect to *O. crenata*, the highest concentration (100 mM) was significantly reduced by 46%, irrespective to stimulants, while in *Striga* the highest concentration (100 mM) reduced germination to 52% (Table 2 and 3). Abu- Irmaileh (1998) reported that *Orobanche ramosa* seeds rarely germinated when incubated in 77 mM NaCl solution. 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 imbibitions (Tobe et al., 1999). Therefore, it can be concluded that the effect of salinity on the germination of *Orobanche* seed may be due to some biochemical changes occurring within the seeds. Such biochemical changes lead to decreased seed germination and were postulated upon as a specific ion toxicity of the NaCl rather than osmotic potential on the seeds. Furthermore, this result was consistent with Al-Khateeb et al. (2005), who displayed that tomato pot experiment irrigated with 75 mM NaCl resulted in complete absence of *Orobanche* emergence and attachment. From these studies, salinity may prevent seed germination and/or inhibit establishment of infections on tomato roots. In addition, salinity also might affect root exudation, of chemicals required for *Orobanche* seed germination.

With respect to radicle elongation, results shown in plate 1 displayed that NaCl inhibited radicle growth significantly. The inhibition of germination and radicle growth may be due to the osmotic potential. Higher concentration of NaCl (150 mM) significantly inhibited radical elongation relative to control radicals, whereas the lowest concentration (50 mM) showed less inhibitory effects. Nitrogen-
containing nutrients, \( \text{NH}_4\text{NO}_3 \) and \( \text{NH}_4\text{Cl} \) inhibited the germination and radicle elongation of broomrapes such as \( O. \text{aegyptiaca} \) and \( O. \text{ramosa} \) (Westwood and Fey, 1999). The above results are consistent with Linke (1987) who observed that osmotic stress reduced broomrape germination. However, even at 25 mM concentrations of \( \text{NH}_4\text{NO}_3 \) and \( \text{NH}_4\text{Cl} \) were highly inhibitory to radicle development of broomrapes (Westwood 1995).

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