Research Article

©*ISISnet Publishers*

Bioscience Research, 7(1): 26-31, 2010
Available online at **www.isisn.org**
Print ISSN: 1811-9506
Online ISSN: 2218-3973

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

M. M. Hassan[1*], Y. Sugmuto[2], A. G. E. Babiker[3], Y. Yamauchi[2], M. G. E. Osman[4], S. O. Yagoub[3]

[1]Environment and Natural Resources Research Institute, Khartoum, **Sudan.**
[2]Laboratory of Functional Phytochemistry, Graduate School of Agricultural Science, Kobe University **Japan**.
[3]Sudan University of Science and Technology, College of Agricultural Studies, Khartoum, **Sudan.**
[4]Commission for Biotechnology and Genetic Engineering, Khartoum, **Sudan.**
***Corresponding author**

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 anda floral meristem that produces a floral spike (Foy et al. 1989;

Parker and Riches 1993). The small broomrape flower stalks emergences 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. Zwanenberg, 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 23ºC 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 100µmM 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)

Germination (%)						
	NaCl (mM)					
GR24 (µM)	Water	50	75	100	150	Average
3.4 µM	84.6	48.8	44.2	25.4	24.0	45.30
0.34 µM	62.9	31.8	26.7	30.0	18.0	33.86
0.034 µM	58.9	17.4	16.4	18.2	5.2	23.23
Average	68.79	32.66	29.13	24.52	18.73	

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

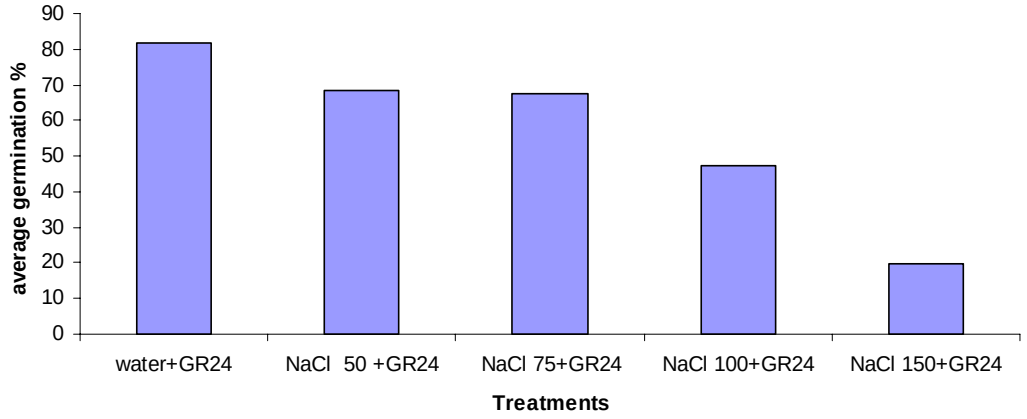
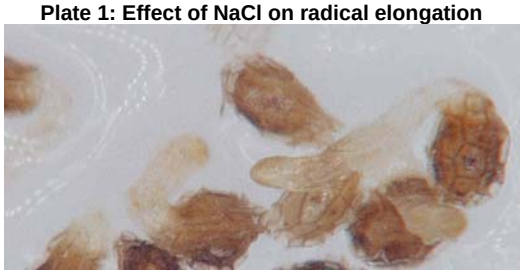
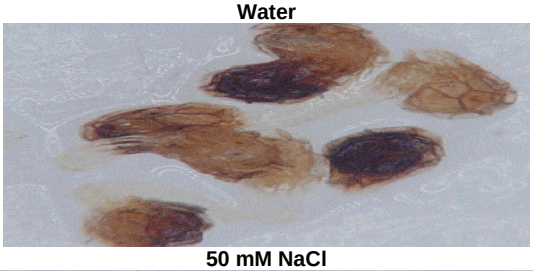


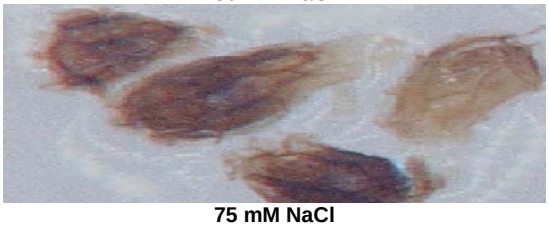
Plate 1: Effect of NaCl on radical elongation



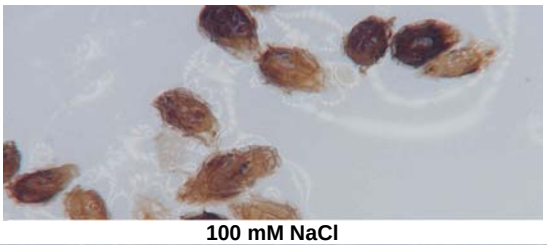
Water



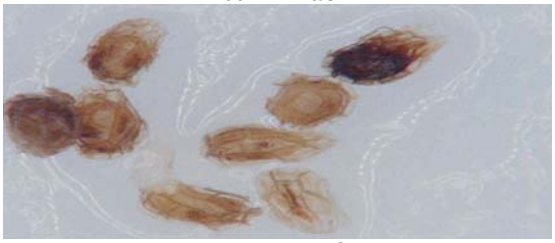
50 mM NaCl



75 mM NaCl



100 mM NaCl



150 mM NaCl

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

Root macerate of faba bean	Germination (%) NaCl (mM)				
	Water	**25**	**50**	**100**	**means**
Solium	30	23	18	12	20.75
Hudibia	26	12	17	18	18.25
Means	28	17.4	17.2	15	

LSD ± 8.9

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

Treatment	Germination (%) NaCl (mM)				
	Water	**25**	**50**	**100**	**means**
Sorghum root macerate	21	12.8	13.7	10.6	14.5

SE ± 3.1

Of all NaCl treatments, 100 mM displayed the most inhibitory. Generally, Solium 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 Solium 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 3).

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 *Orobnache* emergence and attachment. From these studies, salinity may prevent seed germination and/ or inhibite 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, NH_4NO_3 and NH_4Cl inhibited the germination and radicle elongation of broomrapes such as *O. aegyptiaca* and *O. 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 NH_4NO_3 and NH_4Cl were highly inhibitory to radicle development of broomrapes (Westwood 1995).

REFERANCES

Abu Irmaileh B E 1998. Effect of salinity on *Orobanche* germination and establishment. In: *Current Problem of Orobanche researches, proceeding of the fourth international workshop on Orobanche*, ed. by K. Wegmann, Musselman, L. and D. Joel. Bolgaria.

Al Karaki G 2000. Germination of tomato cultivars as influenced by salinity. *Crop Res.* 19:225-229.

Al-Khateeb WM, Hameed KM and Shibli RA 2005.Effect of Salinity on *Orobanche cernua* Seed Germination. *Plant Pathol. J*. 21:391-394.

Bar Nun N and Mayer A 1993. Preconditioning and germination of *Orobanche* seeds: respiration and protein synthesis. *Phytochemistry* 34:39-45.

Foy CL, R. Jain, and Jacobsohn R 1989. Recent approaches for chemical control of broomrape (*Orobanche* spp.). *Rev. Weed Sci*. 4:123–152.

Gomez K.A. and Gomez AA 1984. Statistical Procedures for Agricultural Research. 2nd Edn., A Wiley-Interscience Publication. John Wiley & Sons, Inc., Singapore, pp: 734.

Hassan E 2000. *Efficacy of broomrape (Orobnche ramosa L.) control methods on faba bean (Vicia faba L) growth and development*. M.Sc. Thesis, Jordan University of Science and Technology, Irbid, Jordan.

Hassan M M, Abdel gain, ME and Babiker, AGT 2009. Selection of Soil Borne Bacteria for Suppression of *Striga hermonthica* (Del.) Benth. Advances in Natural and Applied Sciences 3 (2) 35 - 42.

Hsiao A, Worsham AD and Moreland DE 1981. Regulation of witcheed (*Striga asiatica*) conditioning and germination by dl-strigol. *Weed Sci*., 29: 101-104.

Kebreab E and Murdoch AJ. 1999. A quantitative model for loss of primary dormancy and induction of secondary dormancy in imbibed seeds of *Orobanche spp*. *Exp. Bot.* 50:211-219.

Kubo M, Ueda H, Park P Y, Kawaguchi M and Sugimoto Y 2009. Reactions of *Lotus japonicus* ecotypes and mutants to root parasitic plants. *Journal of Plant Physiology* 166(4): 353-362.

Lins RD, Colquhoun J. BC., Cole M and Mallory-Smith CA. 2005. Post-emergence small broomrape (*Orobanche minor*) control in red clover. *Weed Technol*. 19: 411–415.

Manschadi A M, Sauerborn J and Stutzel H 2001. Quantitative aspects of *Orobanche crenata* infestation in faba beans as affected by a biotic factors and parasite soil seed bank. *Weed Res.* 41:311-324.

Morozov I V, Foy, C L and Westwood J H 2000. Small broomrape (*Orobanche minor*) and aegyptian broomrape (*Orobanche aegyptiaca*) parasitization of red clover (*Trifolium pratense*). *Weed Tech.* 14:312-320.

Parker C and Riches C R 1993. Parasitic Weeds of the World: Biology and Control: Wallingford, Great Britain: CAB International. Pp 111–164.

Tobe K, Zhang, L and Omasa K 1999. Effect of NaCl on seed germination of five nonhalophytic species from a Chinese desert environment. *Seed Sci.& Technol.* 27:851-863.

Westwood, J.H. a Fey, C.L 1999. Influence of nitrogen and early development of broomrape (*Orobanche* spp.). Weed 47:2-7.

Yokota TH, Sakai K, Okuno K, Yoneyama K and Takeuchi Y 1998. Alectrol and orobanchol, germination stimulants for *Orobanche minor,* from its host red clover: *Phytochem*. 49:1967–1973.