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Effect of Salinity on *Striga hermonthica* Seed Germination and Incidence on Infested Sorghum

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Abstract: The parasitic weed *Striga hermonthica* poses a serious threat to cereal production in Sudan. Seeds of *Striga hermonthica* were exposed to 25, 50, 75, 100 and 150 mM NaCl solutions during their preconditioning period (for 10 days) under laboratory conditions and induced to germinate by synthetic germination stimulant (GR24). Seed germination was decreased significantly with the increase in salt solution concentration. *Striga* germination was reduced by 79% at salinity level of 150 mM. Haustorium initiation in response to sorghum root macerate showed differential response to salinity. Significant reduction in haustorium initiation was observed only at salinity level of 150 mM. It reduced haustorium initiation by 66% than the corresponding control. In the pot experiment, the effects of salt stress on *Striga* incidence were investigated. Soil saturated with 75 mMNaClresulted in complete absence of *Striga* emergence. While sorghum treated with 50 mM NaCl sustained the least *Striga* infestation, it reduced *Striga* infestation by 74 and 55% after 45 and 60 days, respectively.

Key words: Germination, haustorium initiation, salinity, *Striga hermonthica*

INTRODUCTION

Striga hermonthica (Del.) Benth. Is a widespread hemi – parasitic weed in sub-Saharan Africa, where an estimated 26 million hectares of cereal fields (maize, sorghum and millet) are infested with *S. hermonthica* and *Striga asiatica*, leading to an estimated loss in production of about 10.7 million tons (Gressel *et al.*, 2004). Of all *Striga* species, *S. hermonthica* (Del.) Benth is the most economically important parasitic weed in Africa, causing losses in many cereal crops. The growth of its host is retarded, and crop yields are lowered or reduced to zero under severe infestation. The life cycle of *Striga* is intimately associated with that of its host; *Striga* seeds will only germinate in response to specific chemical cues (sesquiterpene lactones) that are present in root exudates (Yoder, 1999). Following germination, a sticky radicle attaches to the root of the host and following perception of host-derived haustorial initiation factors; parasite cells invade the host cortex, reaching the host vasculature within a period of approx. 5 day (Albrecht *et al.*, 1999). At this stage they form direct contact with host xylem vessels. (Abu-Irmaileh, 1998) in his studies on *Orobanche* spp. Confirmed that maps of the general distribution and existence of this parasite around the world indicated the absence of these parasitic seed plants in regions characterized by saline soil. 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 objectives of this study were to evaluate the effect of salinity (NaCl) on the growth and development of *Striga* seeds.

MATERIALS AND METHODS

S. hermonthica seeds were collected in 2004 from infected sorghum fields at the Gezira Research Station Farm, Sudan. 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.

Striga 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 *Striga* seeds during conditioning.

Sorghum root macerate preparation: Seeds of sorghum cvs. Tabat (*Striga* susceptible) and Ahmed (*Striga* tolerant) were surface disinfected by immersing in an

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aqueous solution of 1% sodium hypochlorite for 10 min. The seeds, thoroughly washed with sterilized distilled water were planted in sand, in plastic pots (19 cm diameter), perforated at the bottom. Roots, harvested 21 days after sowing, were thoroughly washed with sterilized distilled water. Root samples (1 g each) were crushed in 10 ml sterilized distilled water in a mortar. The root macerate, filtered through Whatman No. 1 filter paper, was kept in a refrigerator at 5ºC for 2 days. The filtrate was diluted 3- times with distilled water prior to use.

Effect of salinity on germination of *S. hermonthica* seeds: *Striga* seeds were conditioned as described by (Babiker *et al*., 1993). 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 *S. hermonthica* 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 30ºC in the dark for 10 days. *Striga* seeds were treated with GR24 at 0. 00, 0.034, o.34 and 3.4 µM, re-incubated and examined for germination 24 h later.

Effects of salinity on haustorial initiation in *S. hermonthica*: Surface sterilized *Striga* seeds, placed on 8 mm glass fibre discs conditioned in the presence of different salt, were dapped on filter papers (Whatman No. 1) and transferred to sterile Petri dishes. The discs containing *Striga* seeds were treated, each, with 20 µl GR24 solution (0.1 ppm) to induce germination. The Petri dishes sealed with parafilm and placed in black ploythene bags, were incubated in the dark at 30ºC for 48 h. The discs containing the germinated *Striga* seeds dapped on a filter paper, were placed, and inverted top-down on similar discs without *Striga* seeds. The Pairs of discs were treated with 40 µl solution of two sorghum (Wad Ahmed and Tabat) root macerate. Germinated *Striga* seeds resulting from seeds conditioned in distilled water similarly treated with sorghum root macerate were included as controls for comparison. The Petri dishes, sealed with Para- film and placed in polyethylene bags, were incubated in the dark at 30ºC for an additional 24 h and then examined for haustorium initiation using a binocular stereomicroscope.

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

Pot experiment: In this experiment, soil mix made of river silt and sand (2:1 v/v), in a polyethylene bag contains 5 Kg soil. Surface seed sterilized of sorghum Abu sabeen (7/bag) was planted and immediately irrigated. The soil was saturated with the following NaCl concentrations: 0, 50, 75 and 100 mM to give the following ECe values 1.63, 1.89, 3.47, 4.72 dSm^{-1}, respectively. Plant was irrigated when required to filed capacity, with no leaching. *Striga* infestation was accomplished by mixing 16 mg of sterilized *Striga* seeds in the top 6 cm soil in each bag. *Striga* infested and un-infested sorghum controls were included in each experiment for comparison. Emergent *Striga* plants (*Striga* incidence) were counted at 30, 45 and 60 days. Each treatments have four replicates.

RESULTS

Effects of NaCL on GR24-induced germination of *S. hermothica* seeds: S. hermonthica seeds, previously conditioned in presence of NaCl, showed variable response to GR24. Results revealed that *Striga* 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 seed conditioned in water induced the highest germination (74-91%) (Table 1). All Nacl decreased *S. hermonthica* germination in response to GR24 in comparison with the corresponding aqueous controls. In among all salt level the highest concentration of salt (150 mM) was the least effective to GR24 concentrations. It reduced germination to between 73 and 84% as compared to corresponding control. However, at the lowest concentration of Nacl reduced germination between 26-29%. In among all Nacl level 150 mM was consistently the inhibitoriest.

Effects of salinity on haustorial initiation in *S. hermonthica*: Sorghum root macerate Wad Ahmed and Tabat applied to *Striga* germilings resulting from seeds previously conditioned in water and treated with GR24 induced 58 and 43% haustoria, respectively (Table 2). The effects of NaCl level on haustorial initiation varied from non- significant to significant when compared with the control. Salt at 150 mM caused a significant reduction in haustorial initiation, irrespective of the haustorium-inducing factor. It reduced haustorium initiation between 65-66%, as compared to corresponding control. However, other NaCl concentrations used, showed no affect on haustorium initiation in response to root macerate (Table 2).

Pot experiment: The experiment was carried out in the period 7 July to 21 October 2009. Through the experiment period, results showed that *Striga* was not detected until three month at 75 mMNaCl (Table 3). However, at the

Table 1: Effects of different salt level, on *S. hermonthica* seeds germination

	Germination (%) Salt level (mM)						
GR24 (µM)	150	100	75	50	25	Water	Mean
	30.24	39.66	50.29	52.72	54.14	72.63	49.95
3.4	(25.40)	(40.86)	(59.16)	(63.09)	(64.55)	(90.94)	(57.33)
	23	35.37	49.25	47.02	55.71	64.60	45.83
0.34	(15.60)	(33.76)	(57.35)	(53.47)	(67.94)	(81.15)	(51.54)
	19.31	42.32	48.26	44.80	47.59	60.32	43.76
0.034	(11.91)	(45.48)	(55.68)	(49.70)	(54.46)	(73.99)	(48.53)
	24.18	39.13	49.27	48.18	52.49	65.85	
means	(17.63)	(40.03)	(57.39)	(55.42)	(62.32)	(82.02)	
LSD interaction	(±8.98)						
LSD concentration	(±3.67)						
LSD salt	(±5.19)						

Data out of ( ) indicates arcsine transformed data, and in bracket are origin data

Table 2: Effects of different salt level on haustorium initiation in *S. hermonthica*

	Haustoria (%) Salt level (mM)						
Root macerate		25 50		75	100	150	Watermean
	36.63	39.24	43.83	38.93	25.83	50.08	38.99
Wad Ahmed	(35.98)	(39.24)	(48.23)	(39.52)	(19.91)	(58.04)	(40.15)
	48.75	46.83	45.48	43.79	22.86	41.49	41.53
Tabat	(56.47)	(53.11)	(50.90)	(47.59)	(15.34)	(43.39)	(44.46)
	42.69	42.75	44.65	41.36	24.35	45.78	
mean	(46.23)	(46.17)	(49.56)	(43.56)	(17.63)	(50.72)	
LSD interaction	±11.18						
LSD root macerate	±4.56						
LSD salt	±7.89						

Data out of ( ) indicates arcsine transformed data, and in bracket are origin data

Table 3: Effect of salinity on *Striga* incidence on sorghum at 30, 45 and 60 days

	Days after sowing					
Salinity	30		45		60	
concentrations (mM)	Population	Reduction %	Population	Reduction%	Population	Reduction%
0	18.5		28		29	
50	0	100	10	74	13	55
75	0	100	0	100	0	100
100	0	100	0	100	0	100

higher salinity 100 mM NaCl, sorghum seeds failed to germinate at this level and therefore *Striga* was not observed. At 30 day after sowing, *Striga* emergence was only observed on the uninoculated sorghum cv. Tabat (19 *Striga* plants/ bag). While at 45 and 60 days after sowing, *Striga* uninoculated Tabat, sustained the highest infestation (28 and 29 *Striga* plants/ bag, respectively). Pot treated with 50 mM NaCl significantly reduced the number of *Striga* emergence. Saturation of the soil with 50 mM reduced *Striga* infestation by 74 and 55% after 45 and 60 days, respectively.

DISCUSSION

The study focused on inhibition and/or perturbation of early growth stages of the parasite in an endeavour to develop an integrated control strategy. A negative relationship was observed between salt levels and germination percentage of *Striga* seeds (Table 1). As salt concentrations increased to 100 and 150 µM, *Striga* seeds germination percentage was significantly reduced to 51 and 79%, respectively, irrespective to synthetic stimulant concentrations. The lowest seed germination (18%) was observed in 150 µM salt level. 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 *Striga* 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.

Haustorium initiation in response to sorghum roots macerate is inhibited by the higher level of salt. Moreover, the inhibitory effects showed dependence on the level of salt used and the source of the haustorium factor. Inhibition of haustorium initiation in *Striga* by salt may be attributed to phytotoxic substances, inhibitors or extracelluar enzymes that degrade, curtail release of the haustorium factor from the host root, degrade and/or reduce production of H_2O_2 in *Striga* radicle tip (Hassan *et al.*, 2008; Mabrouk *et al.,* 2006; Keys *et al.,* 2000).

With respect to pot experiment, results revealed that salinity reduced and delayed *Striga* emergence on sorghum. Soil saturated with Nacl between 50 to 75 mM reduced *Striga* infestations by 65-100%, respectively. Also pot treated with 50 mM delayed *Striga* emergence for more than two weeks. However, at 75 mM the parasite was completely absence. Furthermore, this result agree 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 this studies, salinity may preventing germination and/ or by establishing infections on tomato roots. In addition, salinity also might affect root exudation, of chemicals required for *Striga* seed germination. Hence, seed germination may not happen.

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