Original article

Influence of DMBQ, Sorghum Root Extracts and Temperature on Haustorium Initiation of *Striga hermonthica* (Del.) Benth.

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Abstract

Two in vitro experiments were conducted in laboratory to study the influence of 2,6-dimethoxy-ρ-benzoquinone at 2.5 - 10.0 µM and sorghum root extract at 3.1 – 100 g/L on haustorium initiation of *Striga hermonthica* (Del.) Benth. at different level of temperature (20 – 35°C). Treatments were laid in a factorial completely randomized design with four replicates. Striga germilings were examined for haustorium initiation 4 days of germination. Data was collected and subjected to analysis of variance procedure. Separation of means was done using Duncan's Multiple Range Test (($P \leq 0.05$). The results revealed that, 2, 6-dimethoxy-ρ-benzoquinone (DMBQ) and sorghum root extract (SRE) at all concentrations tested has potentiality to induce haustoria in four–days-old *S. hermonthica* germlings. It seems that, the optimum concentration of DMBQ and SRE to induce haustorium initiation was 20 µM and 25 g/L, respectively. Moreover, the optimum temperature for *S. hermonthica* germlings to produce haustoria was 30°C and at temperature higher than 30°C the germiling may begin to lose this ability.

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Introduction

Striga hermonthica (Del.) Benth. (witchweed), a root parasitic plant belonging to the family *Orobanchaceae* (Olmstead *et al.,* 2001), is considered the most serious pest threatening cereal production in the semi-arid tropics including Sudan (Parker and Riches, 1993). About 21 million hectares of the cereal production area in Africa is estimated to be infested by *Striga*, causing an annual grain loss of about 8 million tons (Gressel *et al.,* 2004). In Sudan, sorghum (*Sorghum bicolor* (L.) Moench, *Poaceae*) is the most important cereal crop in terms of production and consumption (Ibrahim *et al.,* 1995). More than 500,000 ha under rainfed cultivation are heavily infested with *Striga*, which commonly results in significant yield losses of 70 – 100% (Babiker, 2002). *Striga* is completely dependent on the host for its survival, and its life cycle is closely linked with that of the host plant (Haussmann *et al.,* 2000). The complex biology of the parasite limits the development of successful control methods that can be accepted and adopted by subsistence farmers (Elzein and Kroschel, 2003).

This parasite produces a large number of small seeds, which are dormant and require a conditioning period in a warm moist environment before they have the potential to germinate (Yukihiro et al., 2006). Once the seeds of *S. hermonthica* have germinated, radicle has to come in contact with host root in order to parasitize it. In contact with host root the elongation of the radicle stops and a specialized organ of attachment, a haustorium, is initiated in response to a second derived signal (Butler, 1995). This process has been shown to depend on a haustorium-

imitating substance. Substance responsible for initiating haustorial development in *S. asiatica* has been identified as 2,6-diemthoxy-p-benzoquinone (2,6-DMBQ) (Smith *et al*., 1990). Lynn and Chang (1990) and Riopel *et al*., (1990) revealed that 2,6-DMBQ can't normally be detected in the exudates from sorghum roots although it is present in extract of the sorghum root. Many phenolic and flavanoid substances can also initiate haustoria in both S. asiatica and S. hermonthica (Chang and Lynn, 1986), presumably acting as the substrate for the production of 2,6-DMBQ via this enzyme system. In *S. asiatica*, the radicles are most responsive and more able to form haustoria within 4 days of germination. However, at 30°C it may begin to lose this ability even 2 days after germination (Parker and Reches, 1993). The process of haustorial development and penetration of the host is similar in *S. hermonthica* (Olivier *et al.,* 1991) and *S. asiatica* (Ramaiah *et al.,* 1991). Sticky hairs on the young haustorium help the parasite germiling to adhere to any surface. After attachment by these hairs, intrusive cells develop at the root tip and penetrate the cortex of the host. Penetration is aided by enzymatic secretion leading to separation of the host cortex cells. The haustorium sometimes fails to complete its penetration of the cortex (Ramaiah *et al.,* 1991) and may also fail to cross the endodermis, which sometimes provides a barrier (Saunders, 1933). In *S. asiatica*, the time from first penetration of the epidermis to established connection with the host stele is 60 hours (Ramaiah *et al.,* 1991). Therefore, the present investigation was conducted to study effects of 2,6-dimethoxy-ρ-benzyoquinone (DMBQ), sorghum root extract (SRE) and temperature on haustorium initiation of *S. hermonthica*.

Materials and Methods

Seed conditioning

In these experiments, disc technique described by Dafaallah (2006) was used. About 80-100, Glass Fiber Filter Paper (GFFP) (Whatman GF/C) discs (0.5 mm diameter) was placed on one layer GFFP in glass Petri-dish (GPD) 9 cm i. d. *Striga* seeds (25-100) were sprinkled on each disc. The seeds, moistened with 4.5 ml sterilized- distilled water, sealed with Para film, covered with black polyethylene bag and incubated at 30○C in the dark for 12 days (pre-conditioning).

Germination of pre-conditioned seeds with GR 24

GR 24 (1 mg) was dissolved in 1 ml acetone. Sterilized-distilled water was added to give a volume of 10 ml resulting in stock solution of 0.1 g/L (100 ppm). GR 24 concentration was prepared by sequential dilution of the stock solution with sterilized-distilled water to give 0.1 ppm solution. Discs containing conditioned *Striga* seeds were placed on top of similar glass fiber filter paper discs in a Petri dish. 30µl of each GR 24 concentration was applied to each pair of discs. The Petri dishes were sealed with Para film, placed in black polyethylene bags and incubated at 30°C in the dark for 2 days for germination.

Haustorium Initiation of *S. hermonthica* with 2, 6-dimethoxy-ρ-benzyoquinone (DMBQ) and sorghum root extract (SRE) at different temperature level

2, 6-diemthoxy-1,4-benzoquinone (DMBQ) (1.77 mg) was dissolved in 1 ml sterilized-distilled water. Sterilized-distilled water was added to give a volume of 10 ml resulting in stock solution of 0.177 g/L (1000 µM). The test solutions (DMBQ) at 2.5 to 80µM were prepared by dilution of the stock solution with sterilized-distilled water. Sorghum root extract (SRE) prepared by maceration of 1 g freshly harvested roots in 10 ml water resulting in stock solution of 100 g/L (100 ppm). Various dilutions of (SRE) at 3.1-100.0g/l were prepared. The test solutions of (DMBQ) at 2.5 to 80µM, and (SRE) at 3.1-100.0g/l were added in a total of 40 µL to each disc, placed in a plate, containing 2 days *Striga* germilings. A control with sterilized-distilled water was included for comparison. The plates, sealed with Para-film, covered with black polyethylene were incubated at 20 - 30○C in the dark for 2 days. *Striga* germilings were examined for haustorium induction 2 days after treatments with DMBQ or SRE. Treatments were laid in a factorial completely randomized design (CRD) with four replications. *Striga* seeds were examined for haustorium initiation 2 days later. Data was collected and subjected to analysis of variance (ANOVA). Where the test was significant, separation of means was done using Duncan's Multiple Range Test ($P \leq 0.05$).

Results and Discussion

Effects of 2, 6-dimethoxy-ρ-benzoquinone (DMBQ) and temperature on haustorium initiation of *S. hermonthica*

Irrespective of temperature, 2, 6-dimethoxy-ρ-benzoquinone at all

concentrations tested, 2.5 - 10 μM has potentiality to induce haustoria (10.3 – 64.9%) in four–days-old *Striga* germlings (Table 1). Haustorium induction was significantly increased, 10.3 - 66.8%, with further increase in DMPQ concentration, 2.5 - 20 μM. However, haustorium induction was significantly decreased, 33.6 and 27.4%, with further increase in DMPQ concentration, 40 and 80 μM, respectively.

Table 1. Influence of 2,6-dimethoxy-ρ-benzoquinone (DMBQ) on haustorium induction of *S. hermonthica*

Treatment	Haustorium (%)						
	DMBQ concentration (μM)						
	0	2.5	5	10	20	40	80
	0.0 g	10.3 f	15.8 f	64.9 b	66.8 a	33.6 c	27.4 d
S.E±	0.25						
CV%	8.1 %						

Means followed by the different letters are significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Regardless of 2, 6-dimethoxy-ρ-benzoquinone concentration, temperature at all degrees tested has considerable effects on four–days-old *Striga* germlings to produce haustoria (Table 2). Haustorium induction was 19.9% at 20°C. Haustorium imitation was significantly increased, 31.8 and 43.7%, with further increment in temperature, 25 and 30°C, respectively.

Table 2. Influence of temperature on haustorium induction of *S. hermonthica* induced by 2,6-dimethoxy-ρ-benzoquinone

Treatment	Haustorium (%)			
	Temperature			
	20°C	25 °C	30°C	35°C
	19.9 d	31.8 b	43.7 a	29.7 c
S.E±	0.16			
CV%	8.1 %			

Means followed by the different letters are significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

On the other hand, haustorium induction was significantly decreased to 29.7% at 35°C. At 20°C, 2,6-dimethoxy-ρ-benzoquinone at 2.5 μM induced 0.7% of four–days-old *Striga* germlings to produce haustoria, but, haustorium imitation was not significant in comparison to a control (0 μM DMBQ) (Fig. 1).

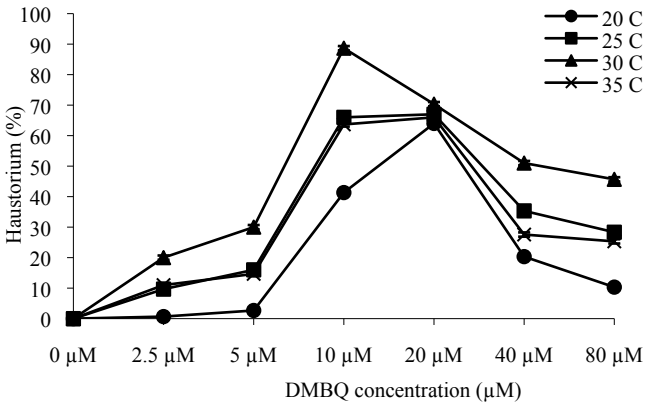

Fig. 1. Influence of 2,6-dimethoxy-ρ-benzoquinone (DMBQ) and temperature on haustorium induction of *S. hermonthica*. Vertical bar represents ± standard error.

Further increase in DMPQ concentration, 5 - 20 μM, resulted in significant increase in haustorium induction, 2.7 – 64%. However, DMPQ at 40 and 80 μM, significantly reduced haustorium imitation, 20.3 and 10.3%, respectively. At 25°C, haustorium induction was significantly increased, 9.7 - 67%, with further increase in DMPQ concentration, 2.5 - 20 μM. However, haustorium induction was significantly decreased, 33.5 and 28.3%, with further increment in DMPQ concentration, 40 and 80 μM, respectively. At 30°C, haustorium induction was significantly increased, 20 – 88.7%, with further increase in DMPQ concentration, 2.5 - 10 μM. On the other hand, haustorium induction was significantly decreased, 70.3 and 45.5%, with further increase in DMPQ concentration, 20 and 80 μM. Induction of haustorium, at 35°C, was significantly increased, 11 – 66%, with further increase in DMPQ concentration, 2.5 - 20 μM. However, haustorium induction was significantly decreased, 27.5 and 25.3%, with further increment in DMPQ concentration, 40 and 80 μM, respectively.

These results are in agreement with those reported by Parker and Riches (1993) who showed that, in *S asiatica*, the radicles are most responsive and best able to form haustoria within 4 days of germination and at 30°C may begin to lose this ability even 2 days after germination. Previous finding by Lynn and Chang (1990) showed that 2,6-DMBQ is responsible for initiating

haustorial development in *S asiatica*. Keyes and *et al.*(2000) reported that when two-days-*S asiatica* seedling was incubated at 30°C in 1 ml of H_2O containing 10 μM DMBQ, the photograph showed a typical swollen root tip with haustorial hair formation at the site of arrows, after 24 h under these condition. In the terminal haustoria formed in *S. asiatica* organogenesis is manifested primarily in the redirection of cellular swelling events. The cell distal to the meristem switch from longitudinal to radial growth and the circumscribed pre-epidermal cells from haustorial hairs. The extent of swelling required for the new organ can be significant, with an increase in diameter from twofold to fourfold, and the rate dramatic, development being complete within 24 h of induction. The swollen cells create minimally a larger surface area likely to be critical for host attachment, and it is within this swollen tip that the haustorial primordial form, giving rise to the infection peg and ultimately the mature host interface (Riopel and Timko, 1995). Haustoria-like structures (Riopel and Baird 1987) and the early stages of nodule development (Crespi and Galvez 2000) are induced by cytokinins, whereas IAA appears to be important for initiating, morphogenesis, and continued viability of lateral roots (Deklerk and *et al.* 1999). Compounds that inhibit auxin transport (Hirsch and *et al.* 1989) also induce nodule-like structures. Keyes *et al.,* 2000 observed that auxins are very potent inhibitors of haustorium induction when two- days-old *S asiatica* seedlings were incubated at 30°C in 1 ml of H_2O containing either kinetin or 10 μM DMBQ with α-IAA.

Effects of sorghum root extract (SRE) and temperature on haustorium initiation of *S. hermonthica*

Irrespective of temperature, sorghum root extract at a concentrations tested, 3.1 – 100 g/L, has potentiality to induc haustoria (7.7 – 47.9%) in four–days-old *Striga* germlings (Tab 3).

Table 3. Influence of sorghum root extract (SRE) on haustoriu induction of *S. hermonthica*

Treatment	Haustorium (%)						
	SRE concentration (g/L)						
	0 .0	3.1	6.3	12.5	25	50	100
	0.0 g	7.7 f	16.1 e	21.9 d	47.9 a	39.8 c	41.5 b
S.E±	0.25						
CV%	8.1%						

Means followed by the different letters are significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

Haustorium induction was significantly increased, 7.7 – 47.9%, with further increase in SRE concentration, 3.1 - 25 g/L. On the other hand, haustorium induction was considerably decreased (39.8 and 41.5%), with further increase in SRE concentration, 50 and 100 g/L, respectively. Regardless of sorghum root extract concentration, temperature at all degrees tested has significant influence on four–days-old *Striga* germlings to produce haustoria (Table 4). Haustorium induction was 14.7% at 20°C. Haustorium imitation was significantly increased, 25.6 and 35.6%, with further increment in temperature, 25 and 30°C, respectively.

Table 4. Influence of temperature on haustorium induction of *S. hermonthica* induced by sorghum root extract (SRE)

Treatment	Haustorium (%)			
	Temperature			
	20°C	25 °C	30°C	35°C
	14.7 d	25.6 b	35.6 a	23.9 c
S.E±	0.16			
CV%	8.1 %			

Means followed by the different letters are significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

However, haustorium induction was significantly decreased to 23.9% at 35°C. At 20°C, sorghum root extract at 3.1 g/L induced 5% of four–days-old *Striga* germlings to produce haustoria and haustorium imitation was significant in comparison to a control (0 g/L SRE) (Fig. 2).

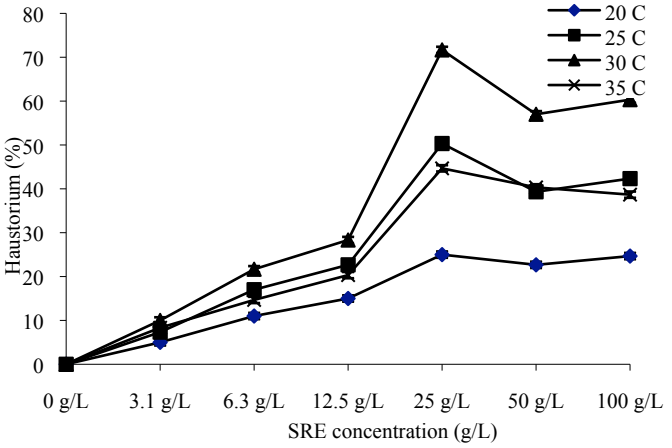

Fig. 2. Influence of sorghum root extract (SRE) and temperature on haustorium induction of *S. hermonthica*

Further increase in SRE concentration, 6.3 - 25 g/L, resulted in significant increase in haustorium induction, 11 – 25%. On the other hand, SRE at 50 and 100 g/L, significantly reduced haustorium imitation, 22.7 and 24.7%, respectively. Moreover, there was no significant different haustorium imitation between SRE at 50 and 100 g/L. At 25°C, haustorium induction was significantly increased, 7.3 – 50.3%, with further increase in SRE concentration, 3.1 - 25 g/L. However, haustorium induction was significantly decreased, 39.3 and 42.3%, with further increment in SRE concentration, 50 and 100 g/L, respectively. At 30°C, haustorium induction was significantly increased, 10 – 71%, with further increase in SRE concentration, 3.1 - 25 g/L. On the other hand, haustorium induction was significantly decreased, 57 and 60.3%, with further increase in ERE concentration, 50 and 100 g/L. Induction of haustorium, at 35°C, was significantly increased, 8.3 – 44%, with further increase in SRE concentration, 3.1 - 25 g/L. However, haustorium induction was significantly decreased, 40.3 and 38.7%, with further increment in SRE concentration, 50 and 100 g/L, respectively.

These results are in line with those reported by Parker and Riches (1993) who showed that, in *S. asiatica*, the radicles are most responsive and best able to form haustoria within 4 days of germination and at 30°C may begin to lose this ability even 2 days after germination. Lynn and Chang (1990) and Riopel *et al.* (1990) revealed that the interesting observation that 2,6-DMBQ cannot be normally detected in the exudates from sorghum roots although it is present in extracts of sorghum root. Moreover, haustorium initiation involves cell division and differentiation (Riopel *et al.,* 1990).

Conclusions

* 2, 6-dimethoxy-ρ-benzoquinone (DMBQ) and sorghum root extract (SRE) at all concentrations tested has potentiality to induce haustoria in four–days-old *S. hermonthica* germlings.

* The optimum concentration of DMBQ and SRE to induce haustorium initiation was 20 μM and 25 g/L, respectively.

* The optimum temperature for *S. hermonthica* germlings to produce haustoria was 30°C and at temperature higher than 30^0C the radicles may begin to lose this ability.

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