

## Tissue Culture Technique as New Approach to Combat *Striga Hermonthica*

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### ABSTRACT

This study was conducted to evaluate the effect of media type, sugars, Ammonium nitrate, Potassium phosphate, auxins and cytokinin in different concentrations on *in vitro Striga* seed germination. Medium B5 was showed to induce *Striga* germination after short period of time. *Striga* germination reached 100% after 10 day of culture. This may be due to chemical components of this media that induced *Striga* germination. Results indicated that *Striga* seeds germination was affected by sugars. Sucrose and glucose at 60g/l were the most inhibitory to *Striga* seeds germination. It reduced *Striga* seeds germination by 20 and 60% after 50 and 45 days, respectively. The depressive effects of the *Striga* germination increased with increasing sugar concentrations. Furthermore, all Ammonium nitrate and Potassium phosphate concentrations tested delayed *Striga* germination. Different concentrations of auxins and cytokinines were tested to evaluate their effects on seeds germination of *Striga*. *Striga* seeds treated with different auxins displayed various results. Both 2, 4-D and NAA auxins, irrespective of concentrations level displayed no *Striga* seeds germination. However, IAA and IBA displayed 100% germination as compared to the control. Cytokinines (kin and BAP) induced *Striga* seeds germination. Moreover, it was concluded that morphogenesis of cultured *S. hermonthica* is influenced by exogenous growth regulators.

**Key words:** *Striga hermonthica*, suppression, growth regulators, NaCl.

### Introduction

*Striga* species, so-called witchweeds, are obligate root hemiparasites belonging to the *Orobanchaceae*, and represent the biggest weed threat to agriculture of sub-Saharan Africa. In particular, *Striga hermonthica* and *Striga asiatica*, which infect sorghum, maize, millet, and upland rice, cause considerable yield losses [2,8,20]. Early growth stages, such as seed germination stimulated by host root exudates and tubercle development, are key phases for *Striga* development. Inhibition of these early phases could be a general strategic option for

parasitic plants management. The seeds of all orboanchaceae germinate in soil under natural conditions only in response to specific chemical exudates from the host plant [23].

Many management strategies have been tried against *S. hermonthica* and *Orobanche* species, but few of them have proved reliable and these are only economical in high-value agriculture.

These include host plant resistance, use of trap-crops, and the improvement and maintenance of soil fertility through cereal-legume rotation/intercropping or application of organic or inorganic nitrogen [6,18,21].

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Trap-crops are non hosts which stimulate the germination of *Striga* seeds, but are not parasitized by the weed (Khalel, 1992). They cause suicidal germination of the weed, which reduces the seed bank in the soil. Some varieties of cowpea, groundnut and soybean have potential to cause suicidal germination of *S. hermonthica* and improve soil fertility [6,21]. Kim *et al.*, [17] have shown that high rates of nitrogen reduced the level *Striga* infestation and damage in maize and greatly increased yields. Some transgenic [14] and tissue culture derived [1] herbicide-resistant crops enable the early control of parasitic weeds before or during attachment to the host. This study describes the influence of different media, sugars, salts, auxins and cytokinines on germination of *Striga* seeds *in vitro*. *In vitro* culture of *Striga* plants is affected by different factors including, growth media components, and sugars and salts auxins and cytokinins. Therefore the objectives of these studies are to develop *in vitro* screening method for salinity, sugars, auxins and cytokinins in suppressing *Striga* seeds germination.

### Materials and methods

This study was carried out in the Laboratory of Plant Cell and Tissue Culture, Commission for Biotechnology and Genetic Engineering, National Center for Research Khartoum, Sudan. *Striga hermonthica* seeds, used in this study, were collected from parasitic plants growing under sorghum in 2008 at the Abu Nama Station Farm in Sinnar State. Seeds were cleaned by placement in a measuring cylinder (1000 ml) containing tap water. Floating materials containing debris and immature light seeds were discarded. The seeds were washed several times with tap water and tween 20 to free them from sand. Then seeds were surface-sterilized for 5 min in sodium hypochlorite (20%) and rinsed five times with sterile distilled water.

#### Experimental Studies:

A series of laboratory experiments were undertaken to investigate the effect of different media, sugars, salts, auxins and cytokinins on *in vitro* seed germination of *Striga*. Five to six replicates were used in these studies.

#### Effects of Different Basal Media on *in Vitro Striga Seeds Germination*:

Four different basal media namely, full-salt strength MS [19] medium, half-salt strength MS medium, Full -salt strength B5 [10] medium and half -salt strength B5 medium were tested to evaluate their effects on *in vitro Striga* seeds germination. All procedures were carried out under strictly aseptic

conditions. Then the culture was incubated at 25°C±2 with a 16 h photoperiod. Data on seeds germination were recorded twice after 10, 13 and 15 days.

#### Effects of Sucrose and Glucose on *in Vitro Striga Seeds Germination*:

From the previous experiment we selected the best media which supported germination of *Striga* for other treatments. Two types of sugars (sucrose and glucose) were used in this experiment. Sterilized *Striga* seeds were transferred directly to culture bottle containing B5 basal media supplemented with different sugars concentrations 10.0, 20.0, 30.0, and 60g/l. The culture was incubated at 25°C±2 with a 16 h photoperiod for 60 days. All procedures were carried out under strictly aseptic conditions. Data on effect of sugar type and concentration on seeds germination were recorded on 10, 30, 45 and 50 days period.

#### Effects of Ammonium Nitrate and Potassium Phosphate on *in Vitro Striga Seed Germination*:

In order to assess the effect of HN<sub>4</sub> NO<sub>3</sub> and KH<sub>2</sub> PO<sub>4</sub> on *in vitro* germination, sterilize seeds were cultured in culture bottles containing B5 basal media supplemented with different concentrations of HN<sub>4</sub> NO<sub>3</sub> (0.82, 1.65, 3.30, and 6.60 g/l) and KH<sub>2</sub> PO<sub>4</sub> with (0.85, 1.70, 3.40, and 6.80 g/l). Then culture bottle were incubated at 25°C±2 with a 16 h photoperiod for 60 days. All procedures were carried out under strictly aseptic conditions. Data on effects of Ammonium nitrate and Potassium phosphate on *in vitro Striga* seed germination were recorded after 10 and 15 days.

#### Effect of Auxins on *in Vitro Striga Seed Germination*:

To assess the effect of auxins on *in vitro* seed germination, *Striga* seed were cultured in culture bottles containing B5 basal media supplemented with four kinds of auxins namely indole -3- acetic acid (IAA), naphthalene acetic acid (NAA), indole-3-butyric acid (IBA) and 2,4-dichloro-phenoxyacetic acid (2,4-D) at different concentration (5.0, 10.0, 15.0, and 20.0 mg l<sup>-1</sup>). Data on seeds germination were recorded after 10 - 20 days.

#### Effect of Cytokinines on *in Vitro Striga Seed Germination*:

To assess the effect of cytokinines on *in vitro* seed germination, *Striga* seed were cultured in culture bottles containing B5 basal media supplemented with two kinds of cytokinins, Benzylamino purine (BAP) and Kinetin (Kin) at different concentrations (5,10, 15, and 20 mg l<sup>-1</sup>).

Data on seeds germination were recorded after 10-15 days.

## Results and discussion

### *Effects of Different Basal Media on in Vitro Striga Seeds Germination:*

Four basal media were evaluated for their effect on *Striga* seeds germination. Result showed that all media tested displayed highest germination (100 %) after 15 days, except B5 medium which induced *Striga* germination after a short period (10 days) (Table 1 and Fig.1). This may be due to the chemical components of this media. These results are agreement with Hassan *et al.*, 2008 finding who reported that the culture medium may influence germination and subsequent development of the parasite. Calli with many root-like protrusions and attachment organs from media B5-3 and B5-5 were the most successful for *in vitro* infection with *O. ramosa* and *O. aegyptiaca*, while those from medium B5-6 were the most successful for *in vitro* infection with *O. minor* [24].

### *Effects of Sucrose and Glucose on in Vitro Striga Seeds Germination:*

Result indicated that *Striga* seeds germination was affected by sugars (Table 2 and Fig 2). In among all sugars concentrations, media B5 supplemented with sucrose and glucose at 10 and 20 g/l were the least inhibitory to *Striga* germination (100%). *Striga* seeds cultured on media supplemented with sucrose and glucose at 40g/l reduced and delayed *Striga* germination. It reduced germination to 60 and 90% after 30 days, respectively as compared to the control. Sucrose and glucose at the highest concentration (60g/l) were the most inhibitory. It reduced *Striga* seeds germination by 20 and 60% after 50 and 45 days, respectively. However, sucrose at 60g/l suppressed germination (60%) significantly as compared with control. These results indicate that the depressive effect on *Striga* germination increased with increasing sugar concentrations (Table 2 and Fig. 2). Brown *et al.*, [4] reported that certain other sugars, e.g. D-fructose, D-fructose 1:6-diphosphate and L-sorbose, promoted slight germination of *Striga* seeds.

### *Effect of Ammonium Nitrate and Potassium Phosphate on in Vitro Striga Seeds Germination:*

*In vitro* germination of *Striga* was observed after 15 days on two different salts element at different concentrations (Fig.3). The results indicated that all Ammonium nitrate and Potassium phosphate concentrations tested delay *Striga* germination.

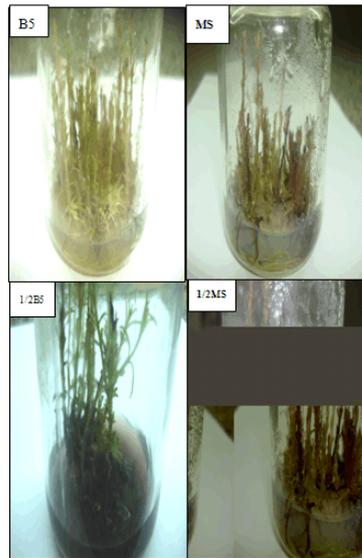
It delayed germination 5 days as compared to the control (Table 3). Ungar [22] reported that High substrate salinity is a major limiting factor for plants growth. Salinity affected germination through osmotic pressure and toxicities of specific salts and nutritional imbalances. Some elements, such as sodium, chlorine, and boron, have specific toxic effects on plants. Furthermore, these results agree with those of Hassan *et al.* (2010) who reported that sorghum plant treated with NaCl at 50-75 mM reduced and delayed *Striga* emergence.

### *Effect of Auxin and Cytokinines on in Vitro Striga Seeds Germination:*

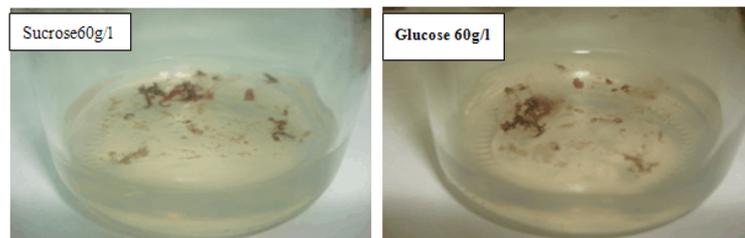
Germination of *Striga* seeds in this investigation has been studied on B5 medium supplemented with various plant growth regulators. Different concentrations of auxin and cytokinines were tested to evaluate their effects on seeds germination of *Striga*. Germination *in vitro* was observed after 15 days on control of *Striga* seeds. *Striga* seeds treated with different auxins displayed various results (Table 4 and Fig 4). Results showed that, both 2, 4-D and NAA auxins irrespective of concentrations displayed no germination of *Striga* seeds (100% germination inhibition). However, IAA and IBA displayed 100% germination after 20 days (Table 4, Fig 4). It delayed seeds germination for 5 days as compared to the control. Delayed infestation by the parasite was reported to cause less damage than early infestations [7]. These results in agreement with Cai *et al.*, [5] findings, who reported that shoots grew in a medium supplemented with IAA and those of kinetin, but did not in a medium containing NAA plus IBA. However, our results disagree with those of the same authors who reported that on replacement of glucose and IAA with sucrose and 2,4-D, respectively, *Striga* seeds germinated, and the heart-shaped embryos differentiated into calli. In our studies 2, 4-D displayed no *Striga* seeds germination; this may be due to difference in concentrations used and/or effect on *Striga* germination. Moreover, morphogenesis of cultured *S. hermonthica* is influenced by exogenous growth regulators.

Furthermore, *Striga* perturb hormonal balance of plants and is more predominant in soils of poor fertility, particularly those deficient in phosphorus and nitrogen. *Striga* germination and morphogenesis are influenced by hormones. Ethylene and strigolactones, cytokinines induce germination of the parasite. IAA suppresses haustorium initiation, while cytokinines induce haustorium initiation and shoot development [11].

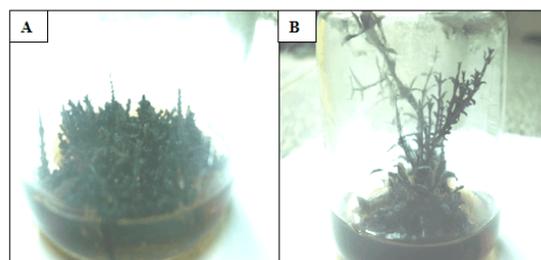
With respect to cytokinines (BAP and kin) result showed that there is no effect on *Striga* seeds germination as compared to the control (Table 5 and Fig 5).



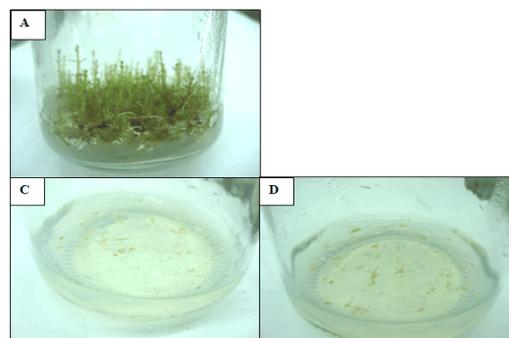
**Fig. 1:** Effects of different basal media on in vitro Striga seed germination after four weeks of culture.



**Fig. 2:** Effect sucrose and glucose concentration at 60g/l concentrations on in vitro Striga seed germination supplemented in B5 medium.

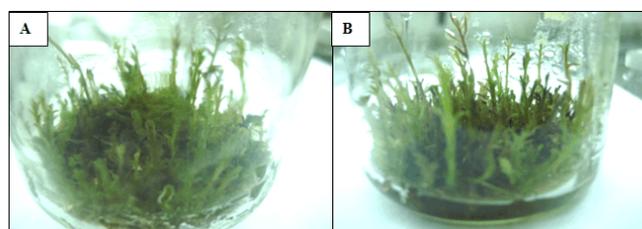


**Fig. 3:** Striga seed germination in B5 media supplemented with (A) Ammonium nitrate (6.6g/l) and (B) Potassium phosphate (6.8g/l).



**Fig. 4:** Effect of auxins on in vitro Striga seeds germination cultured on B5 medium after five weeks.  
 A: In vitro seeds germination on B5 medium supplemented with 5 mg<sup>-1</sup> IBA..

- B: In vitro seeds germination on B5 medium supplemented with 10 mg<sup>l</sup><sup>-1</sup> kin.
- C: In vitro seeds germination on B5 medium supplemented with 10 mg<sup>l</sup><sup>-1</sup> NAA.
- D: In vitro seeds germination on B5 medium supplemented with 5 mg<sup>l</sup><sup>-1</sup> 2,4-D.



**Fig. 5:** Effect of cytokinines on in vitro seeds germination of *Striga* cultured on B5 medium after five weeks of culture

- A: In vitro seeds germination on B5 medium supplemented with 15 mg<sup>l</sup><sup>-1</sup> kin.
- B: In vitro seeds germination on B5 medium supplemented with 10 mg<sup>l</sup><sup>-1</sup> BAP.

**Table 1:** Effect of different basal media on *in vitro Striga* seeds germination after four weeks of culture.

Basal media strength	Day to 100% germination
MS	15
½ MS	15
B5	10
½B5	13

Key:  
 MS: full-salt strength medium  
 ½ MS: half-salt strength medium  
 B5: Full -salt strength B5 medium  
 ½B5: half -salt strength B5 medium

**Table 2:** Effect of different sucrose and glucose concentrations on *in vitro Striga* seeds germination.

Sugar(g/l)	con.	Germination %	Day of germination
Sucrose	10	100	30
	20(control)	100	10
	40	60	30
Glucose	60	20	50
	-	-	-
	10	100	30
	20	100	10
	40	90	30
	60	60	45

**Table 3:** Effect different Ammonium nitrate and Potassium phosphate concentrations on *in vitro Striga* seeds germination.

Salt (g/l)	con.	Day to 100% germination
B5(control)	10	
HN <sub>4</sub> NO <sub>3</sub>	0.82	15
	1.65	15
	3.3	15
	6.6	15
		15
KH <sub>2</sub> PO <sub>4</sub>	0.85	15
	1.7	15
	3.4	15
	6.8	15
		15

**Table 4:** Effect of auxins on *in vitro Striga* seeds germination.

Auxins (g/l)	con.	germination %	Day of germination
Control		100	15
2,4-D	5	0.00	-
	10	0.00	-
	15	0.00	-
	20	0.00	-
NAA	0.00	-	-

**Table 4:** Continue.

	5	0.00	
	10	0.00	-
	15	0.00	-
	20	0.00	-
IAA	-	-	
	5	100	20
	10	100	20
	15	100	20
	20	100	20
IBA	-	-	
	5	100	20
	10	100	20
	15	100	20
	20	100	20

**Table 5:** Effect of cytokinin on *in vitro* *Striga* seeds germination.

Cytokinines (g/l)	Day to 100% germination	
BAP	-	
	5	15
	10	15
	15	15
	20	15
Kin	-	
	5	15
	10	15
	15	15
	20	15
Control		15

Hence determination of the most optimal concentrations of plant growth regulators as medium constituents is one of the most important aspects among other *in vitro* factors.

**Conclusions:**

*Striga* seeds treated with different auxins displayed various results. Both 2, 4-D and NAA auxins, displayed no *Striga* seeds germination. However, IAA and IBA displayed 100% germination. Cytokinines (kin and BAP) induced *Striga* seeds germination. Moreover, it was concluded that morphogenesis of cultured *S. hermonthica* is influenced by exogenous growth regulators. Determination of the most optimal concentrations of plant growth regulators as medium constituents is one of the most important aspects among other *in vitro* factors.

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