Effect of Medium Composition on Production of *Striga hermonthica* (Del.) Benth Germination Stimulant(s) by *Menispermum dauricum* (DC.) Root Cultures

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Germination of *Striga hermonthica* (Del.) Benth, a noxious parasitic weed on cereals, requires an exogenous stimulant produced by the roots of host and some nonhost plant species. Root cultures of *Menispermum dauricum* (DC.), a nonhost broad-leaved herbaceous plant, produced a group of substances that induce the parasite germination. This paper reports the establishment of a high-stimulant-producing *M. dauricum* root culture by manipulation of culture composition. A modified B5 medium (MB5) containing 35.7 mM nitrogen at a NO_3^-/NH_4^+ ratio of 1:42, 0.1 mM Fe^{2+}, 1.0 mM Ca^{2+}, 0.55 mM inorganic phosphorus, 0.28 mM inositol, 4.1 μM nicotinic acid, 3.7 μM pyridoxine hydrochloride, 14.8 μM thiamin hydrochloride, 1 μM 1-naphthaleneacetic acid (NAA), and 4% sucrose sustained root growth for a longer period and increased root biomass by >30% and stimulant production by 5-fold, in comparison to the standard B5 medium supplemented with 3% sucrose and 1 μM NAA.

Keywords: *Root culture; germination stimulant; Striga hermonthica*

INTRODUCTION

Striga hermonthica (Del.) Benth, an economically important root parasitic weed, constitutes a threat to the production of several poaceous crops in arid and semiarid tropical Sub-Saharan Africa. Losses in grain yield of sorghum [*Sorghum bicolor* (L.) Moench] and millet [*Penisetum glaucum* (L.) R. Br.] due to *Striga* damage are often high (>70%) in heavily infested fields (Babiker et al., 1987). Prodigious seed production together with prolonged longevity and special germination requirements makes *Striga* a difficult weed to control. To germinate, a *Striga* seed has to be subjected to warm and moist conditions prior to exposure to an exogenous stimulant, including sorgoleone, sorgolactone, alectrol, and strigol, exuded by the roots of host and some nonhost plant species. Induction of germination by artificial stimulants has been the prime objective of several research efforts. Several germination stimulants were identified from host and some nonhost plants (Cook et al., 1966; Hauck et al., 1992; Müller et al., 1992; Butler, 1993), and many of their analogues were synthesized (Johnson et al., 1976; Mangnus et al., 1992). However, further work revealed their extreme instability in soils. On the basis of circumstantial evidence and experimentation, several workers concluded that germination stimulants from susceptible hosts and their synthetic mimics are likely to be unstable (Babiker et al., 1987; Fate et al., 1990; Joel et al., 1995). The need to probe nonhost plants for potent, more stable stimulants or leading compounds from which more effective and stable derivatives can be synthesized has been emphasized (Joel et al., 1995).

Recently Ma et al. (1996), on the basis of preliminary screening of several tissue cultures, reported the potential of *Menispermum dauricum* (DC.) root cultures as a possible source of novel *Striga* germination stimulants. *M. dauricum* roots cultured in a B5 medium supplemented with 1 μM 1-naphthaleneacetic acid (NAA) and 3% sucrose, henceforth referred to as B5N6, produced compounds that induce high germination of *S. hermonthica* seeds. However, establishment of high-stimulant-producing cultures is imperative for further characterization of the active substance(s). The extremely low production of *Striga* germination stimulants by host plants has been a major obstacle to isolation and further characterization of the stimulants (Butler, 1993; Siame et al., 1993). Flores et al. (1987) reported that production of secondary metabolites by root cultures was enhanced by manipulation of culture parameters. In this paper we report the influence of culture media composition on root growth and production of *Striga* germination stimulant(s) by cultured *M. dauricum* roots.

MATERIALS AND METHODS

Source of Plant Materials. Seeds of *S. hermonthica* were collected in 1992/1993, from under sorghum, at the Gezira Research Station, Sudan. *M. dauricum* roots were obtained from established cultures (Sugimoto et al., 1994).

Surface Sterilization and Preconditioning of *Striga* Seeds. Seeds were surface sterilized by immersion in ethanol and sonication for 3 min with occasional swirling followed by thorough washing with distilled water. The seeds were preconditioned for 12−15 days on 8 mm disks of glass fiber filter paper as described by Parker et al. (1977).

Stimulant Production, Germination Assay, and Statistical Analysis. Laboratory experiments were undertaken to study and verify the influence of composition of culture media on root growth and production of *Striga* germination stimulants by *M. dauricum* root cultures. In all experiments,

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unless mentioned otherwise, excised *M. dauricum* roots were cultured in the dark at 27 °C on a rotary shaker (70 rpm) in a B5 medium supplemented with 1 μM NAA and 3% sucrose. The roots, placed in 100 mL flasks, were allowed to grow for 6 weeks before harvest. Treatments were replicated three times. At harvest, culture filtrates were collected. The roots were thoroughly washed with distilled water, freeze-dried, and weighed. Culture filtrates were assayed for activity. Aliquots (20 μL each) of the respective filtrate were applied directly to preconditioned *Striga* seeds placed on 8 mm disks of glass fiber filter paper. The treated seeds were incubated in the dark at 30 °C and examined for germination 24 h later. Distilled water and culture media, in which no roots were grown, were included as controls when appropriate. Germination data were transformed to arcsin, examined by analysis of variance, and then back-transformed. Details of individual experiments are given below.

Effect of Composition of Culture Medium. Nine laboratory experiments were undertaken to study the influence of culture medium, medium strength, and composition pertaining to nitrogen level, NO_3^-/NH_4^+ ratio, concentrations of Fe^{2+}, Ca^{2+}, Mg^{2+}, inorganic phosphorus, sucrose, vitamins, and hormones on root growth, and stimulant production. All media, unless mentioned otherwise, were supplemented with 1 μM NAA and 3% surcrose. Four basal selected media MS, EM, GA, and B5, prepared as described by Murashige and Skoog (1962), Gamborg et al. (1968), and Yamamoto et al. (1989), were employed to study the effects of culture medium. For medium strength, five media were used. Medium strength was varied from 0.25- to 1.25-fold of the standard B5 medium. The effects of nitrogen and NO_3^-/NH_4^+ ratio were studied in two separate experiments. In the first experiment five media were prepared. Nitrogen concentration in the medium was varied from 8.9 to 44.6 mM, and the NO_3^-/NH_4^+ ratio (1:42) was as specified for the B5 medium (Gamborg et al., 1968). In the second experiment, the NO_3^-/NH_4^+ ratio was varied from 1:0 to 1:84. Total nitrogen concentration (26.8 mM) was as specified for the B5 medium (Gamborg et al., 1968). For studying the effects of Fe^{2+}, Ca^{2+}, Mg^{2+}, and inorganic phosphorus, all media were based on B5 medium. Fe^{2+} (0−0.2 mM), Ca^{2+}, Mg^{2+} (each at 0−2.0 mM), and inorganic phosphorus (0−3.3 mM) were added in forms of $FeSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, $MgSO_4 \cdot 2H_2O$, and $NaH_2PO_4 \cdot 2H_2O$, respectively. For the effects of sucrose on root growth and stimulant production seven media were used. The concentration of sucrose was varied from 1 to 7% (w/v). For the effects of vitamins five media, based on B5 medium, were prepared. Vitamins and their ratio were as specified for the B5 medium. The total concentration of vitamin was, however, varied from 0- to 2-fold. For the effects of hormones, the standard B5 medium supplemented or not supplemented with NAA, indoleacetic acid (IAA), and indolebutyric acid (IBA), each at 1, 5, and 10 μM, was used. For verifications of experimental results, excised *M. dauricum* roots were cultured in a modified B5 medium (MB5) for 1−8 weeks in 200 mL flasks. The meidum composition (Table 1) was based on concentrations of the components that elicited increased activity of filtrates in the germination assay. *M. dauricum* roots cultured in B5N6 were included for comparison.

RESULTS

Effect on Root Growth. On the basis of dry weight, obtained 6 weeks after subculturing, *M. dauricum* roots cultured in MS medium displayed limited growth (0.065 g). Those cultured in GA and EM media exhibited moderate growth (0.09−0.11 g). Roots grown in B5N6 medium displayed the highest growth (0.36 g). Root dry weight increased consistently with increasing medium strength from 0.25- to 1.25-fold of the standard B5 medium (Figure 1). However, a further increase in medium strength curtailed root growth, albeit not significantly. Root dry weight displayed a negligible change with increasing nitrogen concentration from 8.9 to 26.8 mM (Figure 2). At a nitrogen concentration of 35.7 mM a significant increase in root biomass was obtained. However, further increase in root dry weight was accomplished when the nitrogen level was raised to 44.6 mM. Root growth was maximum at an NO_3^-/NH_4^+ ratio of 1:14 (Figure 3). Increasing the proportion of NH_4^+ in the medium or using NO_3^- as the sole source

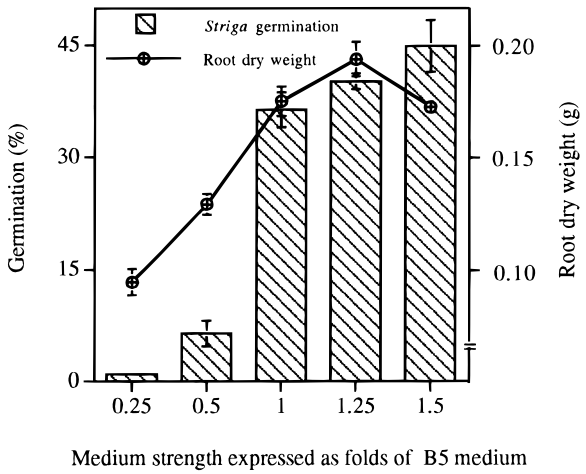

Figure 1. Influence of media strength on cultured *M. dauricum* root growth and production of *Striga* germination stimulant(s).

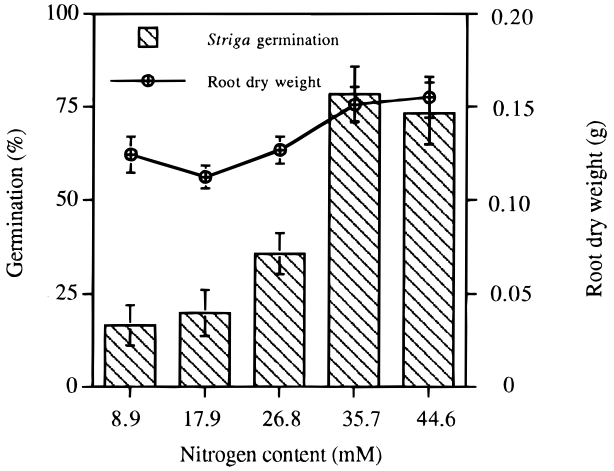

Figure 2. Influence of nitrogen concentration on *M. dauricum* cultured root growth and production of *Striga* germination stimulant(s).

Table 1. Composition of MB5 and B5 Medium (Milligrams per Liter)

	MB5	B5
KNO_3	3337	2500
$(NH_4)_2SO_4$	169	134
$MgSO_4 \cdot 7H_2O$	250	250
$CaCl_2 \cdot 2H_2O$	150	150
$NaH_2PO_4 \cdot 2H_2O$	85.5	171
$FeSO_4 \cdot 7H_2O$	27.8	27.8
Na_2EDTA	37.3	37.3
$MnSO_4 \cdot 4H_2O$	10	10
$ZnSO_4 \cdot 7H_2O$	2	2
$CuSO_4 \cdot 5H_2O$	0.039	0.039
$CoCl_2 \cdot 6H_2O$	0.025	0.025
KI	0.75	0.75
H_3BO_3	3.0	3.0
$Na_2MoO_4 \cdot 2H_2O$	0.25	0.25
inositol	50	100
nicotinic acid	0.5	1.0
pyridoxine hydrochloride	0.5	1
thiamin hydrochloride	5	10

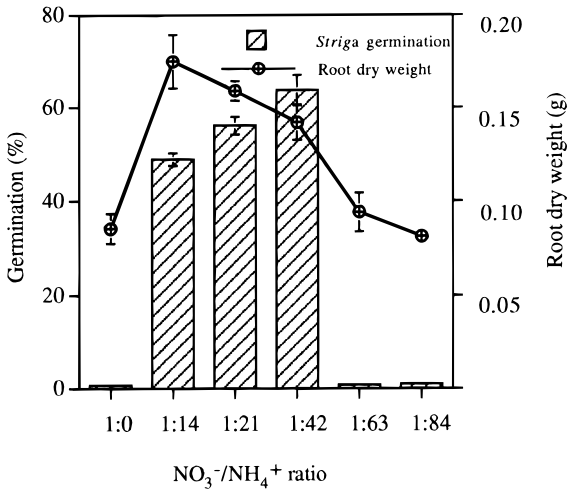

Figure 3. Influence of NO_3^-/NH_4^+ nitrogen ratio on cultured *M. dauricum* root growth and production of *Striga* germination stimulant(s).

of nitrogen reduced root dry weight significantly. Media free of Fe^{2+} or Ca^{2+} (Table 2) or inorganic phosphorus (Figure 4) sustained little root growth. Addition of Fe^{2+} (0.05 mM), Ca^{2+} (0.5 mM), and inorganic phosphorus (0.55 mM) increased root biomass significantly. A further increase in concentration of the nutrients had a negligible effect on root growth. A medium lacking Mg^{2+} supported considerable root growth (Table 2). Addition of Mg^{2+}, up to 2 mM, resulted in no significant increase in root biomass.

Roots cultured in a medium containing 1 or 2% sucrose exhibited, relatively, limited growth (Figure 5). A considerable increase in root growth occurred when the sucrose concentration was raised to 3 and 4%. However, no further significant increase in root biomass was attained when the sucrose content was increased to 4% or more.

Roots cultured in a medium free of vitamins displayed little root growth (Table 3). Changing vitamin contents to between 0.5- and 1.5-fold of a standard B5 medium increased root biomass by >48%. However, a further increase in vitamin concentration curtailed root growth considerably.

Roots cultured in a medium free of auxins demonstrated relatively little growth (Figure 6). Root biomass increased consistently with increasing IAA and IBA concentrations from 1 to 10 μM. NAA at 1 μM promoted considerable root growth in comparison to both IAA and IBA. However, a significant decrease in root biomass was achieved when the NAA concentration was raised to 5 and 10 μM.

Roots grown in MB5 medium consistently displayed better growth than those grown in B5N6 medium (Figure 7). Roots cultured for 3 and 8 weeks in MB5 medium yielded 31 and 47% more dry weight than those cultured for similar periods in B5N6 medium. Furthermore, root growth was maintained for only 7 weeks in B5N6 medium, whereas it was sustained for at least 8 weeks in MB5 medium.

Effect on Stimulant Production. Distilled water and culture media, in which no roots were grown, did not induce *Striga* to germinate (data not shown). Undiluted filtrates from cultures raised in EM and MS media induced little germination (14−17%) (Table 4). A complete loss of activity occurred on a 50-fold dilution. Undiluted and 10-fold-diluted filtrates from cultures grown in a GA medium induced high germination (83−95%). However, moderate activity (58% germination) was attained on a 50-fold dilution. Filtrates from cultures made in a B5N6 medium maintained high germination (79−93%) up to 50-fold dilution.

A 50-fold-diluted filtrate from cultures maintained in one-fourth- or half-strength B5 medium supplemented with 1 μM NAA and 3% surcose elicited negligible (1 and 6%) germination (Figure 1). Similarly diluted filtrates from cultures grown in media with strength adjusted to 1- and 1.5-fold of a B5 medium induced 35−37% germination.

Filtrates from cultures grown in media containing nitrogen at 8.9 and 17.9 mM induced 15−20% germination (Figure 2). A substantial increase in germination was achieved when the nitrogen concentration was raised to between 17.9 and 35.7 mM. A further increase in nitrogen did not increase germination.

Filtrates from cultures grown in media in which the NO_3^-/NH_4^+ ratio was adjusted to 1:0, 1:63, and 1:84 induced no germination on 50-fold dilution (Figure 3). Similarly diluted filtrates from cultures maintained in media in which the NO_3^-/NH_4^+ ratio was adjusted to 1:14, 1:21, and 1:42 elicited 49, 55, and 63% germination, respectively.

Filtrates from cultures maintained in a medium free of Fe^{2+} induced negligible germination (Table 2). Filtrates from cultures grown in a medium supplemented with 0.05 mM Fe^{2+} induced high germination. Undiluted and 5-, 10-, and 50-fold-diluted filtrate induced 78, 75, 61, and 29% germination, respectively. Increasing the Fe^{2+} content to 0.1 mM resulted in a slight increase in filtrate activity. However, a further increase in Fe^{2+} content to 0.15 and 0.2 mM substantially decreased germination.

Filtrate from cultures grown in a medium free of Ca^{2+} displayed no activity on *Striga* seeds. Filtrate from cultures supplemented with 0.5 mM Ca^{2+} elicited low to moderate germination. Undiluted and 5-, 10-, and 50-fold-diluted filtrates induced 52, 47, 32, and 20% germination, respectively. Increasing Ca^{2+} contents to 1.0 mM, considerably increased filtrate activity. Undiluted and 5-, 10-, and 50-fold-diluted filtrate induced 82, 70, 61, and 31% germination, respectively. A further increase in Ca^{2+} contents had negligible effects on germination (Table 2).

Filtrates from cultures maintained in a medium free of Mg^{2+} elicited considerable germination. Undiluted and a 5-fold-diluted filtrate induced 78 and 72% germination, respectively. Filtrate activity increased with Mg^{2+} concentration, reaching a maximum at 1.0 mM, and then declined on further increase in Mg^{2+} content (Table 2).

Filtrates from cultures grown in a medium free of inorganic phosphorus induced considerable germination (75%) (Figure 4). Increasing phosphorus concentration to 0.55 mM increased filtrate activity. Increasing phosphorus concentration to >1.1 mM reduced germination. Filtrates from cultures grown in a medium containing sucrose at 1 and 2% induced low (1−10%) germination (Figure 5). A sharp rise in filtrate activity occurred when the sucrose content was raised to 3 and 5%. An increase in sucrose to 6% or more depressed germination from the maximum at 5%.

Undiluted and 5- and 10-fold-diluted filtrates from cultures grown free of vitamins induced 37, 17, and 1% germination, respectively (Table 3). Similarly diluted

Table 2. Effects of Fe^{2+}, Ca^{2+}, and Mg^{2+} on *M. dauricum* Cultured Root Growth and Production of *Striga* Germination Stimulant(s)

			germination (%) at dilution of			
treatment	concn (mM)	root dry wt (g)	undiluted	5-fold	10-fold	50-fold
$FeSO_4 \cdot 7H_2O$	0	0.038	(5.3)[a] 1	(5.7) 1	(5.1) 1	(5.5) 1
	0.05	0.114	(62.0) 78	(59.8) 75	(51.3) 61	(32.2) 29
	0.1	0.110	(74.6) 93	(64.8) 82	(57.1) 71	(28.2) 22
	0.15	0.113	(65.8) 83	(53.0) 64	(49.0) 57	(21.4) 13
	0.2	0.118	(62.3) 78	(56.3) 69	(30.5) 26	(18.6) 10
		SE = ±0.01		SE = ±(2.27)		
$CaCl_2 \cdot 2H_2O$	0	0.072	(5.4) 1	(5.0) 1	(6.5) 1	(5.6) 1
	0.5	0.123	(45.9) 52	(43.4) 47	(34.7) 32	(26.8) 20
	1.0	0.128	(64.6) 82	(56.5) 70	(51.3) 61	(33.9) 31
	1.5	0.125	(64.6) 81	(52.1) 62	(40.5) 42	(22.1)14
	2.0	0.120	(46.2) 52	(35.1) 33	(24.6) 17	(11.4) 4
		SE = ±0.01		SE = ±(4.25)		
$MgSO_4 \cdot 7H_2O$	0	0.102	(62.1) 78	(58.0) 72	(28.5) 23	(18.1) 10
	0.5	0.107	(74.8) 93	(64.1) 81	(38.5) 39	(24.2) 17
	1.0	0.114	(75.2) 94	(70.3) 89	(52.8) 63	(28.6) 23
	1.5	0.110	(65.5) 83	(55.1) 67	(39.2) 40	(28.9) 23
	2.0	0.118	(66.2) 84	(56.0) 69	(42.8) 46	(21.1) 13
		SE = ±0.01		SE = ±(2.38)		

[a] Parentheses indicate arcsin transformed.

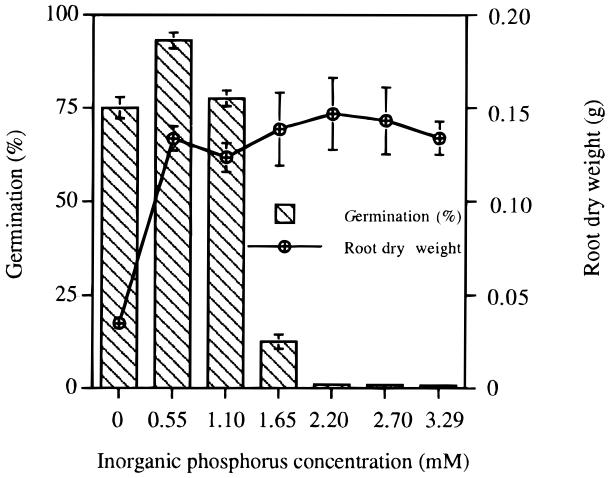

Figure 4. Influence of inorganic phosphorus on cultured *M. dauricum* root growth and production of *Striga* germination stimulant(s).

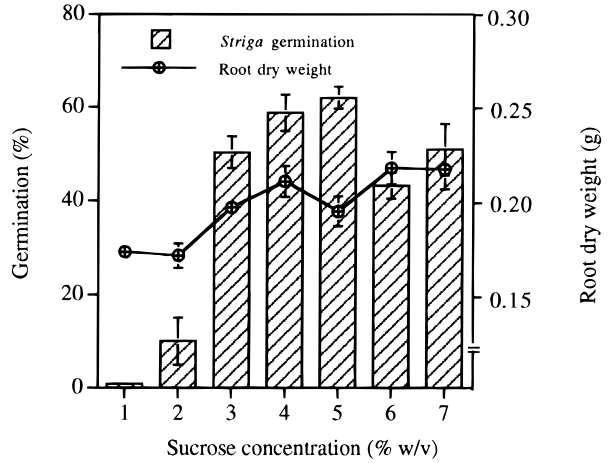

Figure 5. Influence of sucrose on cultured *M. dauricum* root growth and production of *Striga* germination stimulant(s).

filtrates from cultures grown in a medium with vitamin content adjusted to 0.5-fold of the standard B5 medium elicited 94, 90, and 80% germination, respectively. Filtrate from cultures supplemented with vitamin content equivalent to 1-fold of the standard B5 medium gave and maintained high germination (94−71%) up to 10-fold dilution. However, on 50-fold dilution poor germination was attained. Increasing vitamin contents to 1.5-fold of the standard B5 medium reduced germination. Undiluted and 5-fold-diluted filtrates elicited high (93%) and moderate (65%) germination, respectively. Higher dilutions (10- and 50-fold) resulted in poor germination. A further increase in vitamin to 2-fold of the standard B5 medium resulted in complete loss of activity.

A 50-fold-diluted filtrate from cultures grown in a B5 medium supplemented with IAA, IBA, and NAA, each at 1 μM, induced 15, 25, and 37% germination, respectively (Figure 6). Similarly diluted filtrates from cultures supplemented with IAA, IBA, and NAA, each at 5 and 10 μM, induced little (1−7%) germination.

Roots cultured in MB5 medium produced more *Striga* germination stimulant than those grown in a B5N6 medium (Figure 7). A 100-fold-diluted filtrate from roots grown for 3−7 weeks in B5N6 medium induced 1−23% germination. Increasing the culture period to 8 weeks reduced germination. Similarly diluted filtrates from cultures grown in MB5 medium for 3−5 weeks elicited considerable (36−40%) germination. Increasing the culture period to 6 weeks resulted in a sharp increase in filtrate activity (78% germination). The resulting high filtrate activity was maintained throughout the duration of the experiment (8 weeks).

DISCUSSION

It is evident from the results that *M. dauricum* root growth and production of *Striga* germination stimulants were influenced by nutrient levels in the culture medium. In general, within the range of the concentrations examined in this study, nutrients at low levels enhanced both root growth and stimulant production. However, at high levels they either were inhibitory or had negligible effects. These findings are consistent with several studies on the influence of nutrients on the production of secondary metabolites by root cultures and intact plants (Putnam, 1985; Flores et al., 1987, Sugimoto et al., 1988, 1994).

Table 3. Effect of Vitamin Concentration on Root Growth and Production of *Striga* Germination Stimulant(s) *by M. dauricum* Root Culture

treatment (FOSC)[a]	root dry wt (g)	germination (%) at dilution of			
		undiluted	5-fold	10-fold	50-fold
0	0.078	(37.1)[b] 37	(24.5) 17	(5.1) 1	(5.6) 1
0.5-fold	0.135	(76.4) 94	(70.3) 90	(63.4) 80	(53.9) 65
1.0-fold	0.116	(76.2) 94	(75.1) 93	(57.5) 71	(26.6) 20
1.5-fold	0.133	(74.3) 93	(53.6) 65	(22.0) 14	(5.45) 1
2.0-fold	0.071	(5.6) 1	(5.3) 1	(5.2) 1	(5.2) 1
	SE = ±0.01		SE = ±(2.11)		

[a] FOSC, folds of B5 medium standard concentration. [b] Parentheses indicate arcsin transformed.

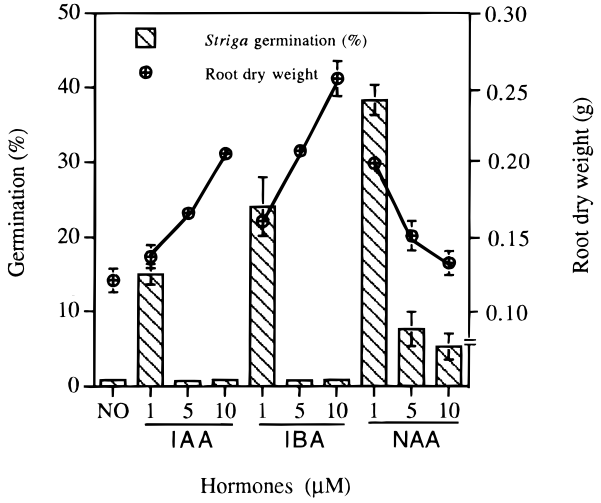

Figure 6. Influence of hormones and their concentrations on cultured *M. dauricum* root growth and production of *Striga* germination stimulant(s).

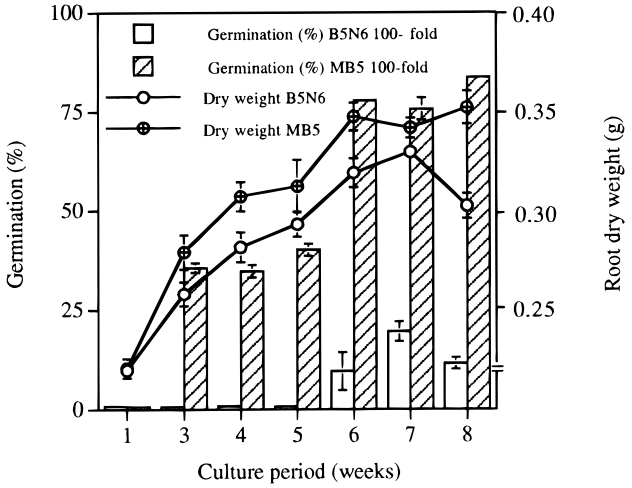

Figure 7. *M. dauricum* root growth and production of *Striga* germination stimulant(s) as influenced by culture medium.

The individual nutrients varied in their importance with respect to root growth and stimulant production. In the absence of Fe^{2+} or Ca^{2+}, cultured *M. dauricum* roots displayed negligible growth and produced little of germination stimulant (Table 2). Lack of inorganic phosphorus decreased root growth but had little effect on stimulant production (Figure 4). Mg^{2+} was less critical. A medium free of Mg^{2+} supported considerable root growth and stimulant production.

Nitrogen enhanced both root growth and stimulant production (Figure 2). Moreover, both stimulant production and root growth were influenced by the NO_3^-/NH_4^+ ratio (Figure 3). Increased production of the *Striga* germination stimulant from *M. dauricum* with

Table 4. Influence of Basal Medium on Production of *Striga* Germination Stimulant(s) by *M. dauricum* Root Cultures[a]

	germination (%)			
	GA	B5	EM	MS
undiluted	(77.5)[b] 95	(74.3) 93	(22.1) 14	(24.0) 17
10-fold dilution	(65.3) 83	(70.5) 89	(8.8) 2	(9.1) 3
50-fold dilution	(49.6) 58	(62.7) 79	(5.3) 1	(5.5) 1
		SE = ±(3.45)		

[a] All media were supplemented with 1 μM NAA and 3% sucrose. [b] Parentheses indicate arcsin transformed.

increasing nitrogen content of the medium and its dependence on nitrogen source are contrary to reports of host-derived germination stimulants. Nitrogen, irrespective of source, is reported to decrease stimulant production by host plants (Parker and Riches, 1993).

In general, culture conditions that were not conducive to root growth did not promote stimulant production. However, contrary to a previous paper (Ma et al., 1996), root growth and stimulant production were not always closely associated. Stimulant production seems to be more sensitive to fluctuations in composition of the culture medium than root growth. EM and GA media supported about equal root growth; however, stimulant production was much greater in the GA medium (Table 4). The hormones NAA, IAA, and IBA at 1 μM promoted both root growth and stimulant production (Figure 6). However, at high concentration (5 and 10 μM) NAA suppressed both root growth and stimulant production. IAA and IBA, on the other hand, were conducive to root growth but suppressive to stimulant production (Figure 6). Accordingly, it seems plausible that the relationship between root growth and stimulant production is not that of a cause and effect and that more subtle interactions are involved. Previously, Ma et al. (1996) suggested that accumulation of the *Striga* germination stimulant in *M. dauricum* root cultures was a function of production and utilization.

The present work demonstrates the possibility of establishing a high-stimulant-producing *M. dauricum* root culture by manipulation of medium composition. A modified B5 medium (MB5), in which total nitrogen was adjusted to 35.7 mM at an NO_3^-/NH_4^+ ratio of 1:42, inorganic phosphorus to 0.55 mM, inositol to 0.28 mM, nicotinic acid to 4.1 μM, pyridoxine hydrochloride to 3.7 μM, and thiamin hydrochloride to 14.8 μM and supplemented with 1 μM NAA and 4% sucrose is an optimum medium for growing *M. dauricum* root cultures with a high capacity for production of *Striga* germination stimulants. This medium maintained root growth for a longer period, increased root biomass by >30%, and increased stimulant production by 5-fold when compared to a B5N6 medium (Figure 7).

Much attention has focused on the isolation and identification of *Striga* germination stimulants from

exudates of intact plants. Several compounds, namely, strigol, sorgolactone, alectrol, and sorgoleones, were isolated (Cook et al., 1966; Hauck et al., 1992; Siame et al., 1993). However, the difficulty of attaining completely aseptic conditions and the unstability and extremely low production of these compounds and, hence, the need for a large number of plants, have been major constraints for furthering this work. Tissue culture systems, in addition to being less laborious, allow control of the environment and eliminate problems incited by contaminating organisms. The present study indicates the feasibility of increasing the production of the *Striga* germination stimulant by manipulation of the composition of the culture medium. Increasing production of the active substance may enhance isolation and further characterization of the stimulant(s).

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LITERATURE CITED

Babiker, A. G. T.; Hamdoun, A. M.; Rudwan, A.; Mansi, M. G.; Faki, H. H. Influence of soil moisture on activity and persistence of the strigol analogue GR24. *Weed Res.* **1987**, *27*, 173−178.

Butler, L. G. Chemical communication between the parasitic weed *Striga* and its crop host. A new dimension in allelochemistry. In *Allelopathy, Organisms, Processes and Applications*; Inderjit, K. M., Dakshini, M., Einhelling, F. A., Eds.; American Chemical Society: Washington, DC, 1993; pp 158−168.

Cook, C. E.; Whichard, L. P.; Turner, B.; Wall, M. E. Germination of witchweed (*Striga lutea* Lour.): isolation and properties of a potent stimulant. *Science* **1966**, *154*, 1189−1190.

Fate, G.; Chang, M.; Lynn, D. G. Control of germination in *Striga asiatica*: Chemistry of spatial definition. *Plant Physiol.* **1990**, *93*, 201−207.

Flores, H. E.; Hoy, M. W.; Pickard, J. J. Secondary metabolites from root cultures. *Trends Biotechnol.* **1987**, *5*, 64−69.

Gamborg, O. L.; Miller, R.; Ojima, K. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res.* **1968**, *50*, 148−151.

Hauck, C.; Müller, S.; Schildknecht, H. A germination stimulant for parasitic flowering plants from *Sorguhm bicolor*, a genuine host plant. *J. Plant Physiol.* **1992**, *139*, 474−478.

Joel, D. M.; Steffens, J. C.; Mathews, D. E. Germination of weedy root parasites. In *Seed Development and Germination*; Kigel, J., Galili, G., Eds.; Dekker: New York, 1995; pp 567−597.

Johnson, A. W.; Rosebery, D.; Parker, C. A novel approach to *Striga and Orobanche* control using synthetic germination stimulants. *Weed Res.* **1976**, *16*, 223−227.

Ma, Y. Q.; Babiker, A. G. T.; Ali, I. A.; Sugimoto, Y.; Inanaga, S. *Striga hermonthica* (Del.) Benth germination stimulant-(s) from *Menispermum dauricum* (DC.) root culture. *J. Agric. Food Chem.* **1996**, *44*, 3355−3359.

Mangnus, E. M.; Zwanenburg, B. Synthesis, structural characterization, and biological evaluation of all four enantiomers of strigol analogue GR7. *J. Agric. Food Chem.* **1992**, *40*, 697−700.

Müller, S.; Hauck, C.; Schildknecht, H. Germination stimulants produced by *Vigna unguiculata* Walp cv Saunders Upright. *J. Plant Growth Regul.* **1992**, *11*, 77−84.

Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant* **1962**, *15*, 473−497.

Parker, C.; Riches, C. R. *Parasitic Weeds of the World Biology and Control*; CAB International: Wallingford, U.K., 1993; p 332.

Parker, C.; Hitchcock, A. M.; Ramaiah, K. V. The germination of *Striga* species by crop root exudates; techniques for selecting resistant crop cultivars. In *Proceedings Asian-Pacific Weed Science Society 6th Conference*; Asian-Pacific Weed Science Society: Jakarta, 1977; pp 67−74.

Putnam, A. R. Weed Allelopathy. In *Weed Physiology, Reproduction and Ecophysiology*; Duke, S. O., Ed.; CRC Press: Boca Raton, FL, 1985; Vol. 1, pp 131−156.

Siame, B. A.; Weerasuriya, Y.; Wood, K.; Ejeta, G.; Butler, L. G. Isolation of strigol, a germination stimulant for *Striga asiatica* from host plants. *J. Agric. Food Chem.* **1993**, *41*, 1486-1491.

Sugimoto, Y.; Sugimura, Y.; Yamada, Y. Effects of culture conditions on bisbenzylisoquinoline alkaloid production in cultured roots of *Stephania cepharantha*. *Agric. Biol. Chem.* **1988**, *52*, 1495−1498.

Sugimoto, Y.; Yoshida, A.; Uchida, S.; Yamada, Y. Dauricine production in cultured roots of *Menispermum dauricum*. *Phytochemistry* **1994**, *36*, 679−683.

Yamamoto, Y.; Kinoshita, Y.; Watanabe, S.; Yamada, S. Anthocyanin production in suspension culture of high producing cells of *Euphorbia millii*. *Agric. Biol. Chem.* **1989**, *53*, 417−423.

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