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Research Paper

Colonization by Arbuscular Mycorrhizal Fungi of Sorghum Leads to Reduced Germination and Subsequent Attachment and Emergence of *Striga hermonthica*

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arbuscular mycorrhiza, root exudate, sorghum, striga, strigolactones, germination

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ABSTRACT

Two sorghum cultivars: the *Striga*-tolerant S-35 and the *Striga*-sensitive CK60-B were grown with or without arbuscular mycorrhizal (AM) fungi, and with or without phosphorus addition. At 24 and 45 days after sowing (DAS) of sorghum, root exudates were collected and tested for effects on germination of preconditioned *Striga hermonthica* seeds. Root exudates from AM sorghum plants induced lower germination of *S. hermonthica* seeds than exudates from non-mycorrhizal sorghum. The magnitude of this effect depended on the cultivar and harvest time. A significantly (88–97%) lower germination of *S. hermonthica* seeds upon exposure to root exudates from AM S-35 plants was observed at both harvest times whereas for AM inoculated CK60-B plants a significantly (41%) lower germination was observed only at 45 DAS. The number of *S. hermonthica* seedlings attached to and emerged on both sorghum cultivars were also lower in mycorrhizal than in non-mycorrhizal plants. Again, this reduction was more pronounced with S-35 than with CK60-B plants. There was no effect of phosphorus addition on *Striga* seed germination, attachment or emergence. We hypothesize that the negative effect of mycorrhizal colonization on *Striga* germination and on subsequent attachment and emergence is mediated through the production of signaling molecules (strigolactones) for AM fungi and parasitic plants.

INTRODUCTION

Sorghum is one of the major food grain crops in the world, especially in developing countries. In savannah regions of Africa where sorghum is one of the major staple foods, it is particularly overwhelmed by infestation by the root hemiparasite *Striga hermonthica* (Del.) Benth. (Orobanchaceae). Efforts to combat *Striga* had very little effect to date. Reasons for the limited success in *Striga* control include prolific seed production, close coupling of its life cycle to that of its host, and the fact that effective control methods are not within the reach of farmers practicing subsistence agriculture.[1]

The symbiosis between sorghum roots and arbuscular mycorrhizal (AM) fungi influences the *S. hermonthica*–sorghum interaction. Colonization by AM fungi reduced attachment and emergence of *S. hermonthica* on sorghum.[2-5] The underlying mechanisms for the lower performance of *Striga* on cereal crops upon AM fungal colonization are unknown. Preliminary investigations suggested that colonization by AM fungi also reduced seed germination of *S. hermonthica*.[4] In order to germinate, *Striga* seeds require signal molecules that are exuded by the roots of their hosts, called germination stimulants. These signal molecules belong to the class of the strigolactones.[6] Strigolactones have recently also been identified as signal molecules in the earliest stage of the interaction between plant roots and AM fungi.[7-9] This double role for strigolactones suggests that the non-mycorrhizal Orobanchaceae have hijacked the molecular communication between host plants and AM fungi.

Vierheilig[10] reviewed the regulatory mechanisms during the interaction between host plants and AM fungi. He noted that AM plants show autoregulation, i.e., plants that are colonized by an AM fungus suppress subsequent colonization by AM fungi through altered root exudation.[11-12] Vierheilig[10] proposed the hypothesis of "one mechanism–two symptoms", i.e., the possibility that changed root exudates not only autoregulate further mycorrhizal colonization but also repress soil pathogens that are attracted to the root by the same compounds in the exudate. This hypothesis has not been investigated for *Striga*.

The objective of this study was therefore to investigate whether root exudates from mycorrhizal and non-mycorrhizal sorghum plants differ in their ability to induce germination of *S. hermonthica* seeds. To study this we used two cultivars of sorghum and reared

them in the presence or absence of AM fungi and with or without phosphorus addition. We investigated the effect of these treatments on the induction of *S. hermonthica* seed germination, attachment and emergence.

MATERIALS AND METHODS

Plant material and growth conditions. The experiments were conducted at Wageningen University during July-August 2003 in a greenhouse with simulated tropical conditions. Daylength was regulated at 12 h using black screens. The average temperature in the greenhouse varied between 25-36°C during the day and 23-35°C at night. Relative humidity varied between 53–68% during the day and 68–71% at night. Sorghum was grown in a mixture of 1:3 of arable soil and coarse sand. This soil/sand mixture was gamma-radiated at 10 kGy. The arable soil had a pH-KCl of 6.7, organic matter content of 1.3% and total N content of 0.05%. Plants were grown in 2-litre black plastic pots filled with 2 kg of the sterilized soil/sand mixture, with drainage holes in the bottom. The design was a full factorial with three factors: AM fungal inoculum (absent and 50 g per pot), P (0 and 30 kg P ha^{-1} as triple superphosphate) and *S. hermonthica* [0 and 5 mg (≈1000 germinable seeds) per pot]. The eight treatments were applied in five replicates to two sorghum cultivars, the *Striga*-tolerant S-35 and the *Striga*-sensitive CK60-B in a randomized complete block design. Plants were harvested at 24 and 45 days after sowing (DAS).

The AM fungal soil inoculum was a mixture of propagules of *Glomus clarum* Nicolson and Schenck and *Gigaspora margarita* Becker and Hall.[2] The *S. hermonthica* seeds were harvested from plants growing on sorghum in Mali in 1998. The seeds for each pot were mixed with quartz sand (50 g) in aluminum trays and kept moist for 10 days in the greenhouse to precondition. A similar amount of quartz sand without *S. hermonthica* seeds was treated similarly. The sand/*S. hermonthica* seeds mix, inoculum of AM fungi, P fertilizer or their combinations were mixed with the top 6 cm soil in the pots in the relevant treatments. The control treatments were established at the same time: moist quartz sand was mixed with soil in the upper 6 cm of the pots that did not receive *Striga* seeds, whereas 50 g of gamma-radiated soil inoculum of AM fungi was mixed through the soil in pots that did not receive inoculum of AM fungi. A microbial wash[13] was used in all treatments by suspending 50 g mycorrhizal inoculum in 500 ml water and passing the solution through a filter. Three pregerminated sorghum seeds were planted in each pot and thinned to one per pot just after emergence. Plants were watered with tap water throughout the experiment. No fertilizer was applied apart from triple superphosphate at the start of the experiment. *Striga* emergence and the number of shoots were recorded daily. At each harvest, the soil was carefully washed off the roots and the numbers of attached *Striga* recorded.

Sorghum plants from the treatments without *Striga* were placed in glass beakers with sufficient (70 ml for the first and 250 ml for the second harvest) demineralized water to keep the whole root system immersed. The roots were kept in darkness by wrapping aluminum foil around the beakers. After 36 h the solution in the beakers was collected and used in a *Striga* germination bioassay.

Germination bioassay. *Striga* seeds were surface sterilized in 2% sodium hypochlorite containing 0.02% (v/v) Tween 20 for 5 minutes, subsequently rinsed with sterile demineralized water, and dried for 30 minutes in a laminar air flow cabinet. For preconditioning, 80–100 surface sterilized *Striga* seeds were placed on moistened (with 50 µl of sterile demineralized water) glass microfibre filter paper (GMFP) discs (1 cm diam.), placed in sterile Petri dishes lined with one layer of Whatman filter paper (90 mm diam.). To keep the seeds moist during preconditioning, the filter paper was wetted with 3 ml of sterile demineralized water. The Petri dishes were sealed with parafilm, wrapped in aluminum foil and subsequently kept in an incubator at 30°C for 21 days to ascertain optimal sensitivity to germination stimulants.[14] After preconditioning, the GMFP discs containing *Striga* seeds were dried for 20 minutes in a laminar air flow cabinet and 5 GMFP discs placed in the middle of each Petri dish lined with a ring (1.0 cm wide) of filter paper. The filter paper ring was moistened with 0.9 ml sterile demineralized water. To each cluster of seeds on a GMFP disc, 50 µl root exudate was applied. As positive control, 50 µl of GR24 (0.1 mg l^{-1}), a synthetic germination stimulant (provided by the Department of Organic Chemistry, Radboud University, Nijmegen, The Netherlands), was used to ascertain germinability of seeds. Sterile demineralized water served as negative control. All Petri dishes were sealed with parafilm, wrapped in aluminum foil and incubated at 30°C for five days. Germinated and non-germinated seeds were counted under a dissecting microscope (× 50 magnification). Seeds were considered germinated when germ tubes were clearly visible.

Fractional root colonization and biomass determination. After root exudates were collected, approximately 2 g (fresh weight) of roots were cut and chopped into roughly 1-cm fragments, stained in 0.01% Trypan blue in lacto-glycerol and evaluated for mycorrhizal colonization with the gridline-intersect method. Subsequently, root and shoot fresh weight were determined and the plant material dried at 80°C for 24 h in an oven to determine dry matter content.

Data analysis. Because we could not exclude the possibility that germination of *Striga* seeds stimulates neighboring seeds in vitro, we used the non-parametric Mann-Whitney *U* test for fractional germination. In one treatment with the cultivar S-35, no *Striga* emerged. Because these data did not meet the requirements for ANOVA, the Kruskal-Wallis test was used to test for significance of effects of mycorrhiza and phosphorus on number of attached or emerged *Striga*. Data on sorghum biomass were subjected to ANOVA using the statistical package SAS® System for Windows (8th edition).

RESULTS

Sorghum root colonization by AM fungi. In the non-mycorrhizal treatments, sorghum roots remained free of AM fungal colonization. Colonization levels at 24 DAS varied between 3 and 5% and at 45 DAS between 27 and 44%. Colonization levels were similar between the treatments irrespective of cultivar differences or P addition.

Germination of preconditioned *S. hermonthica* seeds on exposure to root exudates. No *Striga* seeds germinated on exposure to sterile demineralized water. Root exudates collected from non-mycorrhizal sorghum plants induced germination of *Striga* seeds. Germination was higher at the second than at the first harvest. At first harvest (24 DAS) *Striga* germination varied between 6 and 13%, whereas 45 DAS *Striga* germination varied between 41 and 52%, only slightly lower than germination induced by GR 24 (62%). There was no significant effect of P fertilization on *Striga* germination. Root exudates from AM sorghum plants induced lower *Striga* germination (Table 1).

The magnitude of the mycorrhizal effect was cultivar-dependent. The reduction in germination by mycorrhizal root exudates of S-35 was significant at both harvests whereas the effect of mycorrhizal root exudates of CK60-B was only significant at the second harvest. With root exudates from mycorrhizal S-35 plants, germination of *Striga* seeds was 3–12% (second and first harvest respectively) of that

Table 1 **Percentage germination of *S. hermonthica* seeds after exposure to GR24 (0.1 mg l^{-1}) or to sorghum root exudates**

	S-35		CK60-B	
Treatment	**Harvest 1**	**Harvest 2**	**Harvest 1**	**Harvest 2**
GR24	52.6	61.7	52.6	61.7
—AM—P	13.3	48.5	6.2	41.1
—AM+P	12.3	49.8	5.7	52.3
+AM—P	1.6	2.5	1.3	29.1
+AM+P	1.4	0.9	4.0	26.4
Mann-Whitney U-test				
Mycorrhiza	**	**	n.s.	**
Phosphorus	n.s.	n.s.	n.s.	n.s.

Root exudates were obtained from two sorghum cultivars (S-35, CK60-B) grown for 24 (harvest 1) or 45 (harvest 2) days with AM fungal inoculation (-AM = no inoculation; +AM = addition of 50 g per pot) and/or P (-P = no addition of P fertilizer; +P = addition of the equivalence of 30 kg ha-1 of triple superphosphate). Values are means of five replicates. Mann-Whitney U-test: n.s. not significant ($P > 0.05$); **$P < 0.01$.

of non-mycorrhizal plants. In contrast, germination of *Striga* seeds after exposure to root exudates from mycorrhizal CK60-B plants was 45–59% (first and second harvest respectively) of that of non-mycorrhizal plants (Table 1).

Attached and emerged *Striga*. In none of the treatments *Striga* seedlings were observed on sorghum roots at 24 DAS. At 45 DAS significantly higher numbers of *Striga* had attached and emerged in the non-mycorrhizal treatments. In the AM treatments, both the numbers of attached, but still below ground, and emerged *Striga* were significantly lower. This reduction in numbers was much larger for S-35 than for CK60-B. Almost no (94% reduction) *Striga* were attached to the root systems of treatments of S-35 that received AM fungal inoculum compared to 53% reduction in CK60-B. Number of *Striga* emerged showed the same trend. There was no P effect on the number of attached and emerged *Striga* (Table 2).

Sorghum performance. At the first harvest there were no significant effects of *Striga*, AM fungal inoculation or P addition on sorghum biomass. By 45 DAS mycorrhizal plants of both cultivars had a significantly lower biomass than non-mycorrhizal plants. *Striga* reduced above-ground sorghum biomass in CK60-B but had no effect on root biomass. In S-35 *Striga* caused a small decrease in stem biomass but a large increase in root biomass. P application increased biomass in CK60-B, especially of roots, and had no effect on S-35 (data not shown).

DISCUSSION

Mycorrhizal mediation of *Striga* seed germination. Germination of *Striga* seeds, which is induced by root exudates, was significantly lower with exudates of AM sorghum plants. As far as we are aware, this suppression due to AM colonization has not been demonstrated before. We have now also confirmed suppression of *Striga* seed germination by AM maize (Bouwmeester HJ et al., unpublished).

The lower germination with root exudates of AM-sorghum can be explained in three ways. (i) The formation of metabolites that are responsible for the induction of *Striga* germination is Downregulated upon mycorrhizal colonization; (ii) (Plant) metabolites that are up-regulated upon mycorrhizal colonization inhibit *Striga* germination; (iii) Mycorrhizal colonization induces mycorrhizosphere effects that negatively impact on *Striga* germination.

Table 2 **Effect of AM fungal inoculation and P addition on the number of *Striga* attached or emerged on two sorghum cultivars (S-35, CK60-B) at 45 DAS**

	S-35		CK60-B	
	Attached	**Emerged**	**Attached**	**Emerged**
—AM—P	8.2a	2.8a	18.6a	11.2a
—AM+P	7.6a	3a	16.6a	6.6a
+AM—P	0.4b	0b	8.8b	5.4b
+AM+P	0.6b	0.2b	7.8b	6.2b

For explanation of parameters see (Table 1). Values are means of five replicates. In each column means followed by the same letter are not significantly different (Kruskal-Wallis test, $P < 0.05$).

Ad (i). downregulated compounds. Strigolactones are the prime candidate for an explanation for reduced *Striga* seed germination upon mycorrhizal colonization. Strigolactones act as chemical signals for host plant recognition by AM fungi and as germination stimulants for *Striga* and *Orobanche* species.[7-9] Root exudates (most likely strigolactones) induce a chemotropic reaction in *Glomus mosseae*, up to 1 mm from the root,[15] comparable to the few mm over which the strigolactones act as germination stimulants. We envisage a mechanism whereby a host plant exudes strigolactones to attract AM fungi, but upon colonization reduces strigolactone exudation, resulting in autoregulation of mycorrhization and in lower *Striga* germination.

Ad (ii) upregulated compounds. Levels of cyclohexenones, which arise through carotenoid degradation, were positively correlated with root colonization by AM-fungi.[16] Germination stimulants of the Orobanchaceae are also derived from carotenoids.[17] Fester et al.[18] suggested that cyclohexenone levels accumulate with the concomitant degradation of arbuscules. However, Vierheilig et al[19] reported that cyclohexenones are not accumulating systemically. Because mycorrhizal autoregulation is systemic[10] and because we hypothesize that mycorrhizal autoregulation and reduced *Striga* seed germination is caused by the same underlying mechanism, we consider upregulation of cyclohexenone levels as explanation for reduced *Striga* germination unlikely. We are currently investigating whether mycorrhizal suppression of *Striga* seed germination is also systemic.

Ad (iii). Mycorrhizosphere effects. Experiments with AM and non-mycorrhizal plants as a standard should involve a microbial wash.[13] Through this microbial wash differences in bacterial composition between treatments and control should be eliminated. However, their use in experiments involving root exudates is not without difficulties. Certain bacteria live in close association with or are attached to AM fungi.[20-21] Members of the bacterial genus *Paenibacillus*, described from the mycorrhizosphere of sorghum, stimulate mycorrhization and simultaneously show antagonistic activities against fungal pathogens and *Phytophthora*.[22-23] Strains of *Paenibacillus validus* exude compounds that enhance hyphal branching after spore germination of *Glomus intraradices*.[24-25]

While our experiments do not, therefore, strictly show that the AM fungus is the sole causal agent for reduced germination of *S. hermonthica* seeds, we argue, in the light of the double role of strigolactones, that the mycorrhizal explanation is most parsimonious. Possible ways to test our proposed mechanism (mycorrhizal fungi autoregulate subsequent mycorrhization and simultaneously downregulate *Striga* germination through reduced production of strigolactones) is through the use of leaf materials, because strigolactones are produced in roots and shoots of sorghum[9] and most likely maize.[26] In such systems effects on the rhizosphere community could be eliminated.

Alternatively, AM fungi can be grown under monoxenic conditions. In such a system other (micro-)organisms should be largely ruled out, although the contamination and increased secondary metabolite production on transformed roots could still be problematical.[27]

Effects of mycorrhizal colonization on *Striga* attachment and emergence. Mycorrhizal sorghum plants also showed a significantly lower attachment and emergence of *Striga*. These results are consistent with earlier reports.[2-5] While it is likely that this effect is explained through mycorrhizal effects on *Striga* germination, we cannot exclude further roles for AM fungi in affecting the performance of *Striga*. Mycorrhizal colonization can increase levels of cell wall-bound phenolics[28] and induce expression of defense-related genes.[29] Both mechanisms provide direct protection against antagonists. Improved nutrient status as a consequence of mycorrhizal colonization can also result in enhanced protection against pathogens.[30]

Effects of sorghum cultivar and phosphorus. There were no differences in AM fungal colonization between both sorghum cultivars, even though mycorrhizal colonization of S-35 reduced *Striga* seed germination more than that of CK60-B. Gworgwor and Weber,[3] however, noted that *Glomus mosseae*, which colonized sorghum plants to a higher degree than other AM fungi, also had a stronger suppressive effect on *Striga* emergence.

The lower *Striga* germination with exudates of AM colonized sorghum was not due to an improved P status of sorghum. There were no effects of P on number of germinating, attaching and emerging *Striga*, both in the mycorrhizal and non-mycorrhizal condition. No growth response of sorghum to P was observed either, indicating that plants were most likely not P-limited. Vierheilig[10] reviewed the effect of P status of the plant on fungal responses to plant signals. Root exudates of P-fertilized plants result in a reduced stimulation of spore germination and hyphal growth. Recently, Yoneyama and coworkers[31] showed that P-deficient red clover seedlings strongly promoted the exudation of the strigolactone orobanchol. Mechanisms how P availability affects root exudate composition and how this relates to both mycorrhizal colonization and *Striga* seed germination are currently under investigation in our laboratories.

Implications for *Striga* management. Changes in root exudate composition after colonization by AM fungi are an important factor in the tripartite interaction between sorghum, *Striga* and AM fungi. It could potentially also contribute to *Striga*-control, if mycorrhiza-affected changes in exudate (strigolactone) production in the field occur rapidly enough. Hart and Reader[32] studied colonization strategies of 21 AM fungal isolates. Four species of *Glomus* and one species of *Acaulospora* colonized *Allium porrum* within one week. Root colonization of sorghum was observed seven days after addition of inoculum (Lendzemo VW, unpublished). We are currently investigating the rate at which strigolactone production responds to mycorrhizal colonization.

Finally, an important caveat may also be necessary. Because strigolactones are required by AM-fungi for rapid colonization of their hosts, selection of crop cultivars for lower induction of *Striga* germination may be traded off against selection for reduced mycorrhizal colonization. Considering the prevailing agro-ecological circumstances this could be detrimental as the important staple foods in sub-Saharan Africa are both dependent on and responsive to AM fungi. Rather, we suggest that agronomic research efforts should be directed towards improving rapid colonization by AM fungi, immediately after germination of the crop seed, and a rapid response of the host to this colonization by reducing strigolactone exudation. This may involve a larger reliance on the mycorrhizal network than on spore inoculum.

References

1. Oswald A. *Striga* control—Technologies and their dissemination. Crop Prot 2005; 24:333-42.
2. Lendzemo VW, Kuyper TW. Effects of arbuscular mycorrhizal fungi on damage by *Striga hermonthica* on two contrasting cultivars of sorghum, *Sorghum bicolor*. Agric Ecosyst Environ 2001; 87:29-35.
3. Gworgwor NA, Weber HC. Arbuscular mycorrhizal fungi-parasite-host interaction for control of *Striga hermonthica* (Del.) Benth. in sorghum [*Sorghum bicolor* (L.) Moench]. Mycorrhiza 2003; 13:277-81.
4. Lendzemo VW. The tripartite interaction between sorghum, *Striga hermonthica*, and arbuscular mycorrhizal fungi. Ph.D. Thesis. Wageningen University, 2004:112.
5. Lendzemo VW, Kuyper TW, Kropff MJ, Van Ast A. Field inoculation with arbuscular mycorrhizal fungi reduces *Striga hermonthica* performance on cereal crops and has the potential to contribute to integrated *Striga* management. Field Crops Res 2005; 91:51-61.
6. Bouwmeester HJ, Matusova R, Sun Zhongkui, Beale MH. Secondary metabolite signaling in host-parasitic plant interactions. Curr Opin Plant Biol 2003; 6:358-64.
7. Akiyama K, Hayashi H. Strigolactones: Chemical signals for fungal symbionts and parasitic weeds in plant roots. Ann Bot 2006; 97:925-31.
8. Akiyama K, Matsuzaki K, Hayashi H. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 2005; 435:824-7.
9. Besserer A, Puech-Pagès V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais JC, Roux C, Bécard G, Séjalon-Delmas N. Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. PloS Biol 2006; 4:1239-47.
10. Vierheilig H. Regulatory mechanisms during the plant—Arbuscular mycorrhizal fungus interaction. Can J Bot 2004; 82:1166-76.
11. Pinior A, Wyss U, Piché Y, Vierheilig H. Plants colonized by AM fungi regulate further root colonization by AM fungi through altered root exudation. Can J Bot 1999; 77:891-7.
12. Vierheilig H, Lerat S, Piché Y. Systemic inhibition of arbuscular mycorrhiza development by root exudates of cucumber plants colonized by *Glomus mosseae*. Mycorrhiza 2003; 13:167-70.
13. Koide RT, Li MG. Appropriate controls for vesicular arbuscular mycorrhizal research. New Phytol 1989; 111:35-44.
14. Matusova R, Van Mourik T, Bouwmeester HJ. Changes in the sensitivity of parasitic weed seeds to germination stimulants. Seed Sci Res 2004; 14:335-44.
15. Sbrana C, Giovannetti M. Chemotropism in the arbuscular mycorrhizal fungus *Glomus mosseae*. Mycorrhiza 2005; 15:539-45.
16. Maier W, Peipp H, Schmidt J, Wray V, Strack D. Levels of terpenoid glycoside (blumenin) and cell-wall bound phenolics in some cereal mycorrhizas. Plant Physiol 1995; 109:465-70.
17. Matusova R, Rani K, Verstappen FW, Franssen MC, Beale MH, Bouwmeester HJ. The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp. are derived from the carotenoid pathway. Plant Physiol 2005; 139:920-34.
18. Fester T, Hause B, Schmidt D, Halfmann K, Schmidt J, Wray V, Hause G, Strack D. Occurrence and localization of apocarotenoids in arbuscular mycorrhizal plant roots. Plant Cell Physiol 2002; 43:256-65.
19. Vierheilig H, Maier W, Wyss U, Samson J, Strack D, Piché Y. Cyclohexenone derivative- and phosphate levels in split-root systems and their role in the systemic suppression of mycorrhization in precolonized barley plants. J Plant Physiol 2000; 157:593-9.
20. Mansfeld-Giese K, Larsen J, Bodker L. Bacterial populations associated with mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices*. FEMS Microb Ecol 2002; 41:133-40.
21. Toljander JF, Artursson V, Paul LR, Jansson JK, Finlay RD. Attachment of different soil bacteria to arbuscular mycorrhizal fungal extraradical hyphae is determined by hyphal vitality and fungal species. FEMS Microbiol Lett 2006; 254:34-40.
22. Budi SW, Van Tuinen D, Martinotti G, Gianinazzi S. Isolation from the *Sorghum bicolor* mycorrhizosphere of a bacterium compatible with arbuscular mycorrhiza development and antagonistic towards soilborne fungal pathogens. Appl Environ Microbiol 1999; 65:5148-50.
23. Selim S, Negrel J, Govaerts C, Gianinazzi S, Van Tuinen D. Isolation and partial characterization of antagonistic peptides produced by *Paenibacillus* sp. strain B2 isolated from the sorghum mycorrhizosphere. Appl Environ Microbiol 2005; 71:6501-7.
24. Hildebrandt U, Janetta K, Bothe H. Towards growth of arbuscular mycorrhizal fungi independent of a plant host. Appl Environ Microbiol 2002; 68:1919-24.
25. Hildebrandt U, Ouziad F, Marner FJ, Bothe H. The bacterium *Paenibacillus validus* stimulates growth of the arbuscular mycorrhizal fungus *Glomus intraradices* up to the formation of fertile spores. FEMS Microbiol Lett 2006; 254:258-67.
26. Emechebe AM, Ahonsi MO. Ability of excised root and stem pieces of maize, cowpea and soybean to cause germination of *Striga hermonthica* seeds. Crop Prot 2003; 22:347-53.
27. Bago B, Cano C. Breaking myths on arbuscular mycorrhizas in vitro biology. In: Declerck S, Strullu DG, Fortin JA, eds. In vitro culture of mycorrhizas. Berlin: Springer Verlag, 2005:111-38.
28. Grandmaison J, Olah GM, Vancalsteren MR, Furlan V. Characterization and localization of plant phenolics likely involved in the pathogen resistance expressed by endomycorrhizal roots. Mycorrhiza 1993; 3:155-64.
29. Taylor J, Harrier LA. Expression studies of plant genes differentially expressed in leaf and root tissues of tomato colonised by the arbuscular mycorrhizal fungus *Glomus mosseae*. Plant Mol Biol 2003; 51:619-29.

30. Borowicz VA. Do arbuscular mycorrhizal fungi alter plant-pathogen relations? Ecology 2001; 82:3057-68.
31. Yoneyama K, Yoneyama K, Takeuchi Y, Sekimoto H. Phosphorus deficiency in red clover promotes exudation of orobanchol, the signal for mycorrhizal symbionts and germination stimulant for root parasites. Planta 2007, (in press).
32. Hart MM, Reader RJ. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytol 2002; 153:335-44.