

Short Communication

The arbuscular mycorrhizal host status of plants can not be linked with the *Striga* seed-germination-activity of plant root exudates

Der Wirtspflanzenstatus der vesikulären-arbuskulären Mykorrhiza korreliert nicht mit der *Striga*-Keimungsaktivität von Pflanzenwurzelexsudaten

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Abstract

Root exudates from sorghum, a *Striga* and arbuscular mycorrhizal fungal (AMF) host plant, and a number of *Striga* non-host plants which are AM host or AM non-host plants were collected and their effect on seed germination of *Striga hermonthica* was tested. *Striga* seeds germinate exclusively in presence of strigolactones, thus, *Striga* seeds germination is an indicator for the presence of strigolactones. Strigolactones are also thought to be essential signals (branching of AMF) for a successful root colonization by AMF. Root exudates of AM host plants stimulated *Striga* seed germination or showed no effect, whereas root exudates of AM non-host plants never showed an effect on *Striga* seed germination. This means that *Striga* seed germination stimulation by compounds in root exudates can not necessarily be linked with the host and nonhost status of plants to AMF. This absence of an effect on *Striga* seed germination of root exudates of AM host plants can be linked i) either with different levels of strigolactones in the root exudates of different AM host plants or ii) with the fact that not the same compounds are involved in *Striga* seed germination and in signaling during the establishment of the AM symbiosis.

Key words: arbuscular mycorrhiza, germination assays, *Striga*, strigolactones, root exudates

Zusammenfassung

Wurzelexsudate von Sorghum-Hirse, einem Wirt der parasitischen Samenpflanze *Striga* und von arbuskulären Mykorrhizapilzen (AMP), sowie einer Reihe von Nichtwirten von *Striga*, die entweder Wirte oder Nichtwirte von AMP sind, wurden gesammelt und ihre Wirkung auf die Samenkeimung von *Striga hermonthica* untersucht. *Striga*-Samen keimen nur in Gegenwart von Strigolactonen, weshalb ihre Keimung als Indikator für Strigolactone dient. Strigolactone gelten daneben als essenzielle Signale für eine erfolgreiche Wurzelbesiedlung durch AMP. Wurzelexsudate von AMP-Wirten stimulierten zum Teil die Keimung von *Striga*-Samen, während Wurzelexsudate von AMP-Nichtwirten niemals die Samenkeimung anregten. Dies bedeutet, dass die Stimulierung der Samenkeimung bei *Striga* durch Komponenten von Wurzelexsudaten

keinen Rückschluss auf den Wirtsstatus von Pflanzen gegenüber AMP erlaubt. Die fehlende Wirkung der Wurzelexsudate von AMP-Wirten auf die Samenkeimung von *Striga* kann entweder auf i) der unterschiedlichen Konzentration von Strigolactonen in den Wurzelexsudaten von AMP-Wirtspflanzen beruhen oder ii) darauf, dass nicht dieselben Exsudatkomponenten für die Samenkeimung und als essenzielle Signale für die AMP-Besiedlung von Wurzeln verantwortlich sind.

Stichwörter: arbuskuläre Mykorrhiza, Keimungstests, *Striga*, Strigolactone, Wurzelexsudate

1 Introduction

Arbuscular mycorrhizal fungi (AMF) are a group of obligate mutualistic fungi that associate with roots to form the arbuscular mycorrhizal symbiosis. Strigolactones are apocarotenoids exuded by roots in extremely low concentrations which stimulate seed germination of the parasitic weeds *Striga* and *Orobancha* (BOUWMEESTER et al. 2007; STEINKELLNER et al. 2007) and act as signaling molecules for the establishment of the AM symbiosis due to their activity on AM hyphal branching (AKIYAMA et al. 2005). Recently, strigolactones have been identified as a new class of plant hormones regulating shoot branching, suggesting that all plant species produce these compounds (GÓMEZ-ROLDAN et al. 2008; UMEHARA et al. 2008). However, an activity on AM hyphal branching of root exudates due to strigolactones has been reported only for AM host plants of but not for AM nonhost plants (BUÉE et al. 2000; NAGAHASHI and DOUDS 2000).

Germination of *Striga* or *Orobancha* seeds after root exudate application clearly indicates the presence of strigolactones in the exudates. Data on the effect of root exudates on the germination of *Striga* and *Orobancha* seeds are available from a number of plants from different studies, however, the experimental set-up in these studies is always varying e.g. different seed patches are tested and/or the collections of root exudates differed. Thus, comparing the data from different studies on the effect of root exudates from different plants on *Striga* and *Orobancha* seed germination and concluding from these data on the level of seed germination-stimulating compounds in root exudates is critical. To our knowledge there is

only one study with *Orobancha* (WESTWOOD 2000), but none with *Striga*, where the effect of compounds released by roots of different plants on seed germination was directly compared, thus giving indications on differing levels of seed germination-stimulating compounds depending on the tested plant.

In the present study, by testing the effect of root exudates from sorghum (a *Striga* and AMF host plant) and a number of *Striga hermonthica* non-host plants which are AM host or AM non-host plants we wanted to obtain data on the presence of *Striga* seed-germination-stimulating compounds in root exudates of *Striga* non-host plants and their possible involvement in the determination of the AM status of AM host and non-host plants.

2 Materials and methods

2.1 Plant material and collection of root exudates

The root exudates of a range of plants (see Table 1) were tested. Apart from the tree species, seeds of all plant species were surface sterilized in 1% aqueous NaOCl for 5 min and pregerminated in perlite for 5 days. Thereafter four plantlets of each plant species were transferred to pots filled with autoclaved (for 20 min at 121 °C) substrate: a mix of arable soil, fine sand (< 2 mm) and expanded clay in the ratio 1:1:1 v/v/v in pots.

Seeds of *Populus tremula* and *Pinus sylvestris* were surface sterilized in H₂O₂, rinsed several times with sterile water and germinated thereafter on agar. *Quercus robur* seeds were germinated in moistened perlite. Plantlets were transferred to pots containing autoclaved peat.

Salix caprea plants were obtained from tissue cultures. Plantlets were transferred to pots with clay granules (SERAMIS; Mars, Inc., Hackettstown, NJ, USA) as a substrate.

Fagus sylvatica seeds were surface sterilized in H₂O₂, rinsed several times with sterile water and germinated thereafter in wet sand at 4 °C. Plantlets were transferred to pots to an autoclaved mixture of seedling peat substrate, sand and perlite (1:1:1).

Four replicate pots per plant species were set up. All pots were placed in the greenhouse at the Universität für Bodenkultur in spring 2007. The seedlings were watered regularly on a daily basis. Three weeks after emergence (except trees: *P. tremula* and *Q. robur*: 2 months; *P. sylvestris* and *F. sylvatica*: 3 months; *S. caprea*: 4 months), the roots of all the seedlings were washed free of adjoining substrate and placed in beakers of varying sizes depending on the volume of each root system. Distilled water was added to the beakers to barely cover each root system. The beakers were wrapped with aluminium foil to simulate dark conditions as previously in the substrates. The seedlings were placed under these conditions for 24 hours. Subsequently, each seedling was removed; the root system cut off and blotted with tissue paper then weighed with an electronic balance. The solution in each beaker was diluted with distilled water to the ratio 1 g root fresh weight to 10 ml of water. This solution was used as fresh root exudates on pre-conditioned *S. hermonthica* seeds.

2.2 Preconditioning *Striga* seeds

Ten milligrams of *S. hermonthica* seeds harvested from sorghum as host at Maroua (latitude 10° 30' and 11° N and longitude 14° and 14° 30' E) Cameroon in October 2005) were surface sterilized in a laminar flow in 2% NaOCl (for

Table 1: Effect of root exudates of AM host and nonhost plants on the germination of *Striga* seeds

Plant species	Family	Scientific name	Germination (%)	Mycorrhizal status
Control	GR24 (10-7M)		53.7 a	
CK60B sorghum	Gramineae	<i>Sorghum bicolor</i>	32.1 b	AM
Barley	Gramineae	<i>Hordeum vulgare</i>	1 e	AM
Cucumber	Cucurbitaceae	<i>Cucumis sativus</i>	25.5 b	AM
Zucchini	Cucurbitaceae	<i>Cucurbita sp</i>	0 f	AM
Basil	Lamiaceae	<i>Ocimum basilicum</i>	0 f	AM
Leek	Alliaceae	<i>Allium porrum</i>	0 f	AM
Carrot	Apiaceae	<i>Daucus carota</i>	0 f	AM
Tomato	Solanaceae	<i>Solanum lycopersicum</i>	0 f	AM
Common Bean	Fabaceae	<i>Phaseolus vulgaris</i>	15.5 c	AM
Soybean	Fabaceae	<i>Glycine max</i>	2.3 d	AM
Pea	Fabaceae	<i>Pisum sativum</i>	2.2 d	AM
Lupine	Fabaceae	<i>Lupinus spp</i>	0 f	non-AM
Radish	Cruciferae	<i>Raphanus sativus</i>	0 f	non-AM
Green cabbage	Cruciferae	<i>Brassica oleracea</i>	0 f	non-AM
Kohlrabi	Cruciferae	<i>B. oleracea</i>	0 f	non-AM
Spinach	Amaranthaceae	<i>Spinacia oleracea</i>	0 f	non-AM
Tartary buckwheat	Polygonaceae	<i>Fagopyrum tataricum</i>	0 f	non-AM
Buckwheat	Polygonaceae	<i>F. esculentum</i>	0 f	non-AM
Poplar	Salicaceae	<i>Populus tremula</i>	0 f	AM/EM
Willow	Salicaceae	<i>Salix caprea</i>	0 f	AM/EM
Pine	Pinaceae	<i>Pinus sylvestris</i>	0 f	EM
European beech	Corylaceae	<i>Fagus sylvatica</i>	0 f	EM
Oak	Fagaceae	<i>Quercus robur</i>	0 f	EM

AM = arbuscular mycorrhizal host; non-AM = AM nonhost; EM = ectomycorrhizal host. Values followed by different letters are significantly different according to the Student-Newman-Keuls test at 5%.

details see LENZEMO et al. 2007). After surface sterilization the dry surface sterilized *Striga* seeds were carefully tapped onto 1-cm diameter glass fibre filter paper (GFFP) (about 50 to 80 seeds per disc to ease counting). The Petri dishes were sealed with Parafilm, then wrapped in aluminium foil and incubated for conditioning at 28°C in the dark for 21 days.

2.3 Germination test

For germination assays, the preconditioned seeds on the 1-cm GFFP discs were removed from the Petri dishes and dried on tissue paper for 30 minutes at room temperature. The GFFP discs containing seeds were transferred to new lids of 9 cm Petri dishes (5 discs per Petri dish), lined with a filter paper (Whatman® filter paper No. 2) ring wetted with 1 ml of distilled water. 50 µl of the root exudates solution of each seedling or a GR24 solution (10⁻⁷ M) were added to each GFFP discs containing *Striga* seeds. GR24 is a synthetic strigolactone analogue kindly provided by Prof. B. Zwanenburg (Department of Organic Chemistry; Radboud University, Nijmegen, The Netherlands).

A positive control (50 µl of GR24) and a negative control (50 µl of distilled water per disc) were included. The dishes were sealed with Parafilm and incubated in the dark at 28°C for 48 hours. The Petri dishes were observed under a binocular (stereo) microscope for germination of seeds. Seeds are considered germinated when the radicle comes out of the seed coat.

3 Results

A germination of more than 50% could be observed with the strigolactone analogue GR24 (10⁻⁷ M) showing that the used *Striga* seeds were viable. Also root exudates of the *Striga* host sorghum and the *Striga*-nonhosts cucumber and common bean showed a clear stimulation of seed germination. Root exudates of soybean, pea and barley only slightly stimulated seed germination, whereas in presence of root exudates of all other tested plants no seed germination could be observed.

4 Discussion

Due to their role as plant hormones regulating shoot branching, strigolactones are thought to be present in all plant species (GÓMEZ-ROLDAN et al. 2008; UHEYAMA et al. 2007). When we tested the effect of root exudates of a diverse range of plants on the seed germination of *Striga* we found that root exudates either stimulated *Striga* seed germination indicating the presence of strigolactones or showed no effect.

At first sight the data we obtained with root exudates from AM nonhost plants and their effect on *Striga* seed germination seemed to indicate that in root exudates of AM nonhost plants strigolactones are absent. Comparing the effect of root exudates of AM nonhost and host plants on *Striga* seed germination we found that exudates of none of the tested AM nonhost plants such as radish, green cabbage, kohlrabi (all Brassicaceae), lupine and several plants forming ectomycorrhizal associations exhibited a *Striga*-seed germination stimulatory effect. These observations seem to confirm that in root exudates of AM nonhost plants strigolactones are absent and thus, also can not induce AM hyphal branching (BUÉE et al. 2000; NAGAHASHI and DOUDS 2000).

However, these data are in contrast with data obtained in studies with *Orobanchae*. Lupine, an AM nonhost plant in the normally mycotrophic leguminosae, recently has been reported to exude strigolactones which stimulate *Orobanchae* seed germination, although strigolactone levels are much lower compared to legumes which are AM host plants (YONEYAMA et al. 2008). WESTWOOD (2000) found *Orobanchae* seed germination in presence of *Arabidopsis thaliana*, a member of the

Brassicaceae and an AM nonhost plant, although it was reduced by 40% compared to carrot and tobacco and most recently several strigolactones have been identified in root exudates of *A. thaliana* (GOLDWASSER et al. 2008). Moreover, in several studies it has been shown that root exudates of rape and cabbage, both Brassicaceae, stimulate the germination of *Orobanchae* seeds (JACOBSON and LEVY 1986; BENHARRAT et al. 2003), indicating the presence of strigolactones in the root exudates of these AM nonhost plants.

As strigolactones also seem to be present in root exudates of AM nonhost plants which did not exhibit an effect on *Striga* seed germination in our experiment and do not induce AM hyphal branching (BUÉE et al. 2000; NAGAHASHI and DOUDS 2000), *Orobanchae* seed germination might be more sensitive to the presence of specific strigolactones than *Striga* seed germination and AM hyphal branching. Our results indicate that the level of strigolactones is reduced in all AM nonhost plants we tested.

Root exudates of all AM host plants tested so far have been shown to exhibit an AM hyphal branching activity (BUÉE et al. 2000; NAGAHASHI and DOUDS 2000), thus indicating the presence of strigolactones. Sorghum is an AM host plant and also a host for *Striga*, thus it was not surprising that in our experiment the highest level of *Striga* seed germination was reached in presence of sorghum root exudates. To our surprise in presence of root exudates from the other tested AM host plants extremely varying effects could be observed. Whereas in presence of cucumber and common bean root exudates intermediate levels of *Striga* seed germination were observed, low levels of germination occurred with barley, soybean and pea and no germination was observed with basil, zucchini, leek, carrot and tomato root exudates. Strigolactones levels even varied extremely in root exudates from member of the same plant family, as high germination levels were observed with sorghum and low with barley (both Gramineae) and intermediate levels were observed with cucumber, whereas no germination occurred with zucchini (both Cucurbitaceae). To our knowledge, these differences in *Striga* seed germination stimulating compounds are not reflected in a different susceptibility of these plants to AMF.

Taken together, our data with root exudates of AM host plants inducing different degrees of *Striga* seed germination could either indicate that the status of plants as AM hosts is not defined by a similar level of strigolactones in their root exudates and/or that different strigolactones are involved in *Striga* seed germination and in signaling during the establishment of the AM symbiosis (branching of AMF).

To summarize, our data indicate that *Striga* seed germination stimulation by compounds in root exudates can not necessarily be linked with the host and nonhost status of plants to AMF, as not only root exudates of AM nonhost plants but also root exudates of AM host plants showed no effect on *Striga* seed germination. This absence of an effect on *Striga* seed germination of root exudates of some AM host plants can be linked i) either with different levels of strigolactones in the root exudates of AM host plants or ii) with the fact that not the same compounds are involved in *Striga* seed germination and in signaling during the establishment of the AM symbiosis (branching of AMF).

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