

# Shoot-produced, light-dependent factors are partially involved in the expression of the arbuscular mycorrhizal (AM) status of AM host and non-host plants

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## Summary – Zusammenfassung

Arbuscular mycorrhizal (AM) colonization and hyphal attachment to the roots of a host plant, bean, and a non-host plant, lupin, were compared when grown either with light or in the dark with the AM fungus *Glomus mosseae*.

When grown with light, bean roots were heavily colonized whereas lupin roots showed no signs of colonization, no formation of appressoria and only scarce hyphal attachment to the roots. In contrast to roots of plants grown with light, to living roots of beans and lupins grown in the dark many hyphae were attached and appressoria were formed. The role of shoot produced, light-dependent factors in the expression of the AM mycotrophic status of AM host and non-host plants is discussed.

**Key words:** arbuscular mycorrhiza / carbohydrates / glomales / photosynthesis

## Sprossbürtige, lichtabhängige Fakoren sind teilweise für den Wirts-/Nichtwirtsstatus von Pflanzen gegenüber arbuskulären Mykorrhizapilzen verantwortlich

Bohne als Wirtspflanze und Lupine als Nichtwirtspflanze für den arbuskulären Mykorrhizapilz *Glomus mosseae* wurden im Dunkeln und mit Licht kultiviert. Die Wirkung der Lichtbehandlungen auf die Wurzelkolonisierung und das Hyphenwachstum auf der Wurzeloberfläche wurden verglichen. Unter Lichtbedingungen waren die Bohnenwurzeln stark kolonisiert, während keine Kolonisierung der Lupinenwurzeln beobachtet werden konnte, wenige Hyphen auf der Wurzeloberfläche wuchsen, und keine Appressorien gebildet wurden. Wenn Bohnen und Lupinen jedoch im Dunkeln angezogen wurden, wuchs eine Vielzahl von Hyphen auf der Wurzeloberfläche, die sowohl auf Bohnen-, als auch auf Lupinenwurzeln Appressorien bildeten. Aufgrund dieser Ergebnisse wird die Rolle von sprossbürtigen, lichtabhängigen Fakoren für die Ausbildung der Mycorrhiza in Wirts- und Nichtwirtspflanzen diskutiert.

## 1 Introduction

Under natural conditions about 80 % of all plant species form a symbiotic association with arbuscular mycorrhizal fungi (AMF). AMF are a small group of obligate biotrophic fungi in the order *Glomales* (Zygomycotina), which in association with plant roots, improve plant nutrition. The development of the AM symbiosis can be divided roughly into two different stages: i) the precolonization stage, the fungus is not yet in contact with the root, and ii) the colonization stage, starting with the appressoria formation, the penetration of the root and finalizing with the growth of intraradical hyphae and the formation of AM structures such as arbuscules. AMF receive carbohydrates exclusively from the host plant (Smith and Read, 1997), indicating the importance of the host's photosynthesis for the AM fungal growth. Thus it seems not surprising that light intensities affect root colonization by AMF (Smith and Read, 1997). Various studies report that low light conditions reduce root colonization by AMF, whereas higher light intensities increase the percentage of colonization (e.g. Hayman, 1974; Daft and El Giahmi, 1978; Tester et al., 1986; Son

and Smith, 1988; Smith and Gianinazzi-Pearson, 1990; Miller and Kling, 2000).

As with low light intensities the level of available carbohydrates in roots is reduced, the effect of light on root colonization by AMF was attributed to an altered carbohydrate level. No data are available on AM root colonization of plants grown under the most extreme low light conditions (no light), when no photoassimilates are produced and thus no carbohydrates should be available for the feeding of AMF.

In the generally AM mycotrophic family of the Fabaceae, only lupins are AM non-host plants (Harley and Harley, 1987). The mechanism to explain the inability of lupins to form the AM symbiosis are still unclear. Grafting experiments with lupin shoots on pea roots pointed toward a shoot-produced compound, which is transported to the root, to be responsible for the inability of lupins to form the AM symbiosis (Gianinazzi-Pearson and Gianinazzi, 1992).

From these observations it can be concluded that not only in AM host plants but also in the AM non-host plant lupin, shoot produced factors play at least a partial role in the expression of the AM mycotrophic status.

In the present work we grew two species of the Fabaceae, the AM host plant *Phaseolus vulgaris* (bean) and the non-

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host plant *Lupinus albus* (lupin), with and without light in presence of the AMF *Glomus mosseae*. The colonization characteristics of the AMF in the host and non-host plant were studied when grown under these conditions.

## 2 Materials and methods

### 2.1 Biological material and growing conditions

Bean (*Phaseolus vulgaris* cv. Golden Sands) was used as a AM host plant. Two lupin (*Lupinus albus*) cultivars (kindly provided by Südwestdeutsche Saatzucht, Rastatt / F.R.G.) were tested: cultivar "Ida" with a low alkaloid content ("sweet" lupin), and cultivar "Blanca" with a high alkaloid content ("bitter" lupin) in the seeds. Seeds were surface sterilized by soaking in 0.75 % sodium hypochlorite for 5 min, rinsed with tap water and germinated in vermiculite in the dark (25 °C).

Plants were grown in a growth chamber (day/night cycle: 16 h, 22 °C/8 h, 20 °C; rel. humidity 50 %; light intensity 310  $\mu\text{E m}^{-2} \text{sec}^{-1}$ ) in a steam-sterilized (40 min, 121 °C) mixture of equal parts (v) of sand, vermiculite, montmorillonite, silica, and soil.

Beans were also used as a source of inoculum of *Glomus mosseae* (Nicol. & Gerd.) (Gerd. & Trappe) (BEG 12) in the compartment system described below.

### 2.2 Experimental set-up

The compartment system developed by Wyss et al. (1991) was used. Beans for further inoculation were grown in the presence of an inoculum of *G. mosseae* in the central "inoculum-plant" compartment. This compartment was equipped with nylon screens (60  $\mu\text{m}$  mesh size) as side walls, allowing the AM mycelium to pass but not the roots. When the symbiosis was fully established in the bean roots (3 weeks after planting) shoots were cut.

The test plants bean and lupin were germinated in the dark and at an age of 4 days they were transplanted (under green light to prevent chlorophyll formation) into a series of "test-plant" compartments similarly equipped with side walls of nylon screen (for details see Wyss et al., 1991). The "test-plant" compartments were joined with the central "inoculum-plant" compartments so that the two nylon screens were in intimate contact and hyphae could pass from the central "inoculum-plant" compartment to the "test-plant" compartment. After transplantation to the "test-plant" compartments seedlings were i) either exposed to light or ii) kept in the dark.

Plants were harvested 22 days after inoculation.

To assess the viability of the plants grown in the dark several beans and lupins were exposed to light 20 days after inoculation and the ability to form chlorophyll was visualized.

### 2.3 Measurement of root colonization and hyphal attachment to the roots

Roots were cleared and stained according to the method of Phillips and Hayman (1970). Estimation of colonization and of hyphal attachment to the roots was performed according to the modified gridline intersect method with a dissecting microscope (Newman, 1966). Data are given as percentage of root length colonized and percentage of roots with attached hyphae. With a light microscope roots were also screened for fungal structures (hyphal attachment, appressoria formation, internal structures).

The experiment was repeated three times using 3 replicates per treatment. Similar results were obtained in all experiments. Means and standard deviations of a typical experiment are shown.

## 3 Results

All plants grown in the dark were yellow-white indicating the absence of chlorophyll. No leaves apart from the cotyledons were formed. Bean and lupin plants exposed to light after 20 days in the dark, turned green and formed secondary leaves indicating the viability of the plants.

Beans grown with light showed an intense root colonization (Tab. 1; Fig. 1), however, no colonization was found in beans grown in the dark. A higher number of roots of beans grown in the dark showed AM hyphal attachment (Fig. 2) compared to roots of beans grown with light (Tab. 1). The fungus formed appressoria on roots of bean plants grown in the dark (Fig. 3) and abnormal swollen fungal colonization structures which seemed to be confined to the outer cell layers of the root (Fig. 4), but no further intraradical hyphae, arbuscules or vesicles could be observed.

No root colonization could be observed neither in lupins grown in the dark nor in lupins grown with light (Tab. 2). With light single hyphae were attached to the main root of "sweet" lupins, but no appressoria could be detected. No hyphae were attached to roots of "bitter" lupins. However, multiple hyphae were attached to roots of "sweet" and "bitter" lupins when grown in the dark (Fig. 5; Tab. 2). Hyphae growing along the grooves between cells (Fig. 6) and appressoria-like structures (Fig. 7) were found in "sweet" and "bitter" lupins grown in the dark.

## 4 Discussion

In plants colonized by AMF, the site of sugar transfer from the plant to the fungus is still a matter of debate (Harrison, 1999a, b). Reviewing the literature Smith and Read (1997) suggested carbon uptake rather by the intercellular hyphae, whereas Blee and Anderson (1998) and Vierheilig et al. (2001) proposed the arbuscules as being "...the key site of interchanges of carbon between root cells and .....AMF".

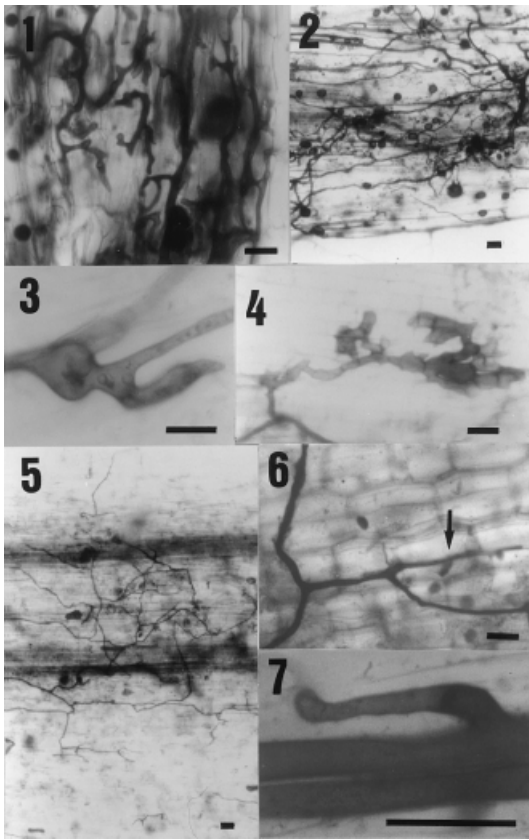
Under low light conditions a reduced root colonization (intraradical hyphae and arbuscules) has been reported (Hayman, 1974; Daft and El Giahmi, 1978; Tester et al., 1986; Son and Smith, 1988; Smith and Gianinazzi, 1990; Miller and Kling, 2000), indicating the requirement of adequate carbohydrate levels for further fungal spreading in the root once the first carbon uptake organs have been formed (intercellular hyphae or arbuscules). Interestingly in our experiment in host plants cultivated under the most extreme low light conditions (no light) neither intraradical

**Table 1:** Percentage of root colonization and percentage of roots with hyphal attachment of bean grown under normal light conditions or in the dark.

**Tabelle 1:** Anteil der Wurzelkolonisation und der Wurzeln mit Hyphen bei Bohnen, kultiviert unter normalen Lichtverhältnissen und im Dunkeln.

	with light	without light
% of root colonization	76 $\pm$ 19	0
% of roots with attached hyphae	26 $\pm$ 5	76 $\pm$ 9

Each value is the mean and the standard error of the mean of three plants.



**Figures 1–7:** Light micrographs of bean and lupin roots inoculated with *G. mosseae* (Bars = 25 µm). Plants were grown with light or in the dark.  
**1:** Colonized root of bean grown with light with intraradical hyphae.  
**2:** Root of bean grown in the dark showing hyphal attachment, but no signs of root colonization.  
**3:** Appressorium on a root of bean grown in the dark.  
**4:** Abnormal fungal structures in the roots of bean grown in the dark.  
**5:** Hyphal attachment to roots of lupin (sweet) grown in the dark.  
**6:** Hyphae growing along the groove (arrow) between cells on roots of lupin (sweet) grown in the dark.  
**7:** Appressorium on roots of lupin (sweet) grown in the dark.  
**Abbildung 1–7:** Wurzel von Bohnen und Lupinen, inokuliert mit *G. mosseae* (Balken = 25 µm). Pflanzen wurden mit Licht oder im Dunkeln kultiviert.

**1:** Kolonisierte Wurzeln von mit Licht kultivierten Bohnen, mit Hyphen.  
**2:** Wurzeln von im Dunkeln kultivierten Bohnen, mit Hyphen, aber ohne Kolonisierung.  
**3:** Appressorien auf Wurzeln von im Dunkeln kultivierten Bohnen.  
**4:** Annormale Pilzstrukturen an Wurzeln von im Dunkeln kultivierten Bohnen.  
**5:** Hyphen auf Wurzeln von im Dunkeln kultivierten Lupinen.  
**6:** Hyphen zwischen den Zellen (Pfeil), auf Wurzeln von im Dunkeln kultivierten Lupinen.  
**7:** Appressorien auf Wurzeln von im Dunkeln kultivierten Lupinen

hyphae nor arbuscules could be observed. As carbohydrates are supposed to be taken up only by specific fungal uptake organs (intercellular hyphae or arbuscules), the importance of carbohydrate as a nutritional factor during root penetration seems questionable. However, carbohydrates might be involved in the development of the AM symbiosis in two different ways: i) as nutrients for the fungus after the formation of the fungal uptake organ and ii) before the formation of the fungal uptake organ as non-nutritional signal molecules. Thus they might be responsible for the

**Table 2:** Percentage of root colonization and percentage of roots with hyphal attachment of lupin grown under normal light conditions or in the dark.

**Tabelle 2:** Anteil der Wurzelkolonisation und der Wurzeln mit Hyphen bei Lupinen, kultiviert unter normalen Lichtverhältnissen und im Dunkeln.

	Sweet Lupin		Bitter Lupin	
	with light	without light	with light	without light
% of root colonization	0	0	0	0
% of roots with attached hyphae	0.5 ± 0.4	62 ± 11	0	47 ± 7

Each value represents the mean and the standard error of the mean of three plants

initiation of root penetration and the formation of the fungal carbohydrate up-take organs in the root. An indication for carbohydrates as non-nutritional signal molecules in the initiation of root penetration could be the abnormal swollen fungal structures in roots of beans grown in the dark.

Possibly, even in a non-photosynthesizing plant, a first signal for root penetration is present, however, further signals for the development of the fungus are missing. Interestingly similar swollen AMF structures have been described recently in a mycorrhiza-defective tomato mutant (Gao et al., 2001).

Sugars as carbon sources do not prolong the growth of AM hyphae not connected to living roots (Hepper, 1984), however, they can be taken up by germ tubes of germinated spores (Bago et al., 1999) and when applied at low concentrations to AMF inoculated plants they can enhance root colonization and arbuscule formation (Gryndler et al., 1998). In our experiment on roots of plants grown without light, thus supposedly with an exudation of carbohydrates towards nil, the fungus grew abundantly on the root surface. The hyphal attachment to the root was even more frequent than in plants grown with light. In a recent study by Giovannetti et al. (1993) a similar observation was reported. When plants were decapitated and thus photosynthesis completely halted, many hyphae grew attached to roots. These data seem to exclude carbohydrates to be the decisive factor for root penetration, however, a hyphal growth stimulating factor which is produced independently from photosynthesis seems to be involved. Recently Bradbury et al. (1993) and Vierheilig and Piché (1996) found a higher number of appressoria on roots of mycorrhizal resistant mutants of usually mycorrhizal plants. They proposed the production of a factor stimulating continuously fungal hyphae to develop new appressoria. The observed hyphal attachment is probably due to a similar factor, responsible for the continuous hyphal growth on the root surface until the signal for root penetration is perceived. Thus, when the penetration signal (possibly carbohydrates) is missing the fungus continues to grow on the root surface.

Buee et al. (2000) and Douds and Nagahashi (2000) recently suggested that factors in root exudates of AM host plants, which stimulate hyphal branching trigger the developmental stage of the fungus needed for a successful root colonization. The branching could also allow the fungus

to find a site for the formation of appressoria when close to the root surface.

In our experiment with the fungus growing abundantly on the root surface and forming appressoria on the AM host and the non-host plant, both grown in the dark, this could indicate that: i) Branching factors are also present in root exudates of plants grown in the dark. However, as in non-host plants a branching factor never has been detected (Buee et al., 2000; Nagahashi and Douds, 2000) the implication of a branching factor in the hyphal growth on the root surface and the formation of appressoria in plants grown in the dark is rather unlikely; ii) no branching factors exuded by the roots are necessary for the hyphal attachment to the root and/or appressoria formation. Recently a similar hypothesis as the latter, has been brought forward by Nagahashi and Douds (1997), inoculating in vitro isolated cell walls from Ri T-DNA transformed roots of carrots with an AMF. The formation of appressoria on the isolated cell walls indicated a contact recognition to be responsible for appressoria formation and seemed to exclude signals secreted from intact cells.

However, looking at the non-host plant lupin with appressoria formed on its roots when grown in the dark, our results seem in contrast with those reported by Nagahashi and Douds (1997). They found that on isolated cell walls from Ri T-DNA transformed roots of the AM non-host plant sugarbeet (a Chenopodiaceae) no AMF appressoria formation occurred. However, this contact recognition might be plant family dependent. Whereas to our knowledge no appressoria have been reported yet on roots of any member of the Chenopodiaceae, all members of the Leguminosae, with the exception of lupins, are AM host plants.

Looking at the colonization characteristics in the non-host plant lupin grown with or without light revealed an interesting picture. No AM colonization could be detected either in “sweet” or in “bitter” lupins with light. This observation corroborates the findings of Avio et al. (1990). Moreover, we found no hyphal attachment to roots of “bitter” lupin and only single hyphae were attached to roots of “sweet” lupin. This nearly total absence of fungal attachment to roots of lupins grown with light has been attributed to an inhibitory factor diffusing into the rhizosphere (Giovannetti et al., 1993). In “sweet” lupins fungitoxic alkaloids are found in lower concentrations as in “bitter” lupins (Wink, 1984), thus the attachment of some hyphae to the roots of “sweet” lupin is probably correlated with the lower level of alkaloids in these roots. Compounds diffusing into the rhizosphere have been suggested to be responsible for the non-susceptibility of other AM non-host plants (recently reviewed by Vierheilig et al., 1998) and new results with the non-host sugarbeet (Nagahashi and Douds, 2000) seem to confirm root exudation of inhibitory compounds as a general mechanism for non-susceptibility of AM non-host plants.

Lupins grown in the dark showed no signs of root colonization, however, many hyphae were attached to the roots and appressoria were formed. Having in mind the shoot-produced nature of compounds involved in the

nonsusceptibility of lupins to AMF (Gianinazzi-Pearson and Gianinazzi, 1992), the hyphal attachment to roots of lupins grown in the dark can be attributed to two different reasons: i) the shoot-produced factor is light dependent, ii) the shoot produced-factor is not transported to the root when lupins are kept in the dark.

The mechanisms responsible for the mycorrhizal/non-mycorrhizal status of plants are still unclear. From the data presented it can be concluded that not only in AM host plants but also in the AM non-host plant lupin, shoot produced, light-dependent factors play at least a partial role in the expression of the AM mycotrophic status.

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