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ORIGINAL ARTICLE

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Stimulation of carotenoid metabolism in arbuscular mycorrhizal roots

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Abstract Development of arbuscular mycorrhizal roots is correlated with accumulation of various isoprenoids, i.e. acyclic C_{14} polyene 'mycorradicin' and C_{13} cyclohexenone derivatives. We present data indicating a strong stimulation of carotenoid metabolism in such roots. Carotenoid profiling revealed mycorrhiza-specific accumulation of ζ-carotene in *Zea mays* and *Medicago truncatula*. Precursor accumulation after inhibition of phytoene desaturase (Pds) activity by norflurazon indicated an increased phytoene biosynthetic capacity in mycorrhizal roots of all species analyzed. *Nicotiana tabacum* plants transformed with a *PDS* promoter-*GUS* construct showed a cell-specific induction of *PDS* promoter activity in root cells containing arbuscules. Mycorradicin biosynthesis and, partially, mycorrhization were impaired in maize mutants deficient in carotenoid biosynthesis. These data indicate that (1) mycorradicin is probably synthesized via a C_{40} precursor carotenoid, (2) carotenoid biosynthesis is induced in mycorrhizal roots, (3) induction occurs, at least partially, at the transcriptional level, and (4) that this may play a functional role during mycorrhization.

Keywords Arbuscular mycorrhiza · Apocarotenoid · *Medicago* (Fabaceae) · *Nicotiana* (Solanaceae) · Phytoene desaturase · *Zea* (Poaceae)

Abbreviations AM: arbuscular mycorrhiza · GUS: β-glucuronidase · IPP: isopentenyl diphosphate · MEP: methylerythritol phosphate · Pds: phytoene desaturase · RT-PCR: reverse transcription PCR · X-GlcU: 5-bromo-4-chloro-3-indolyl-β-D-glucuronide

Introduction

The arbuscular mycorrhizal (AM) symbiosis is a mutualistic interaction between fungal species of the order Glomales (Zygomycetes) and the roots of most terrestrial plants (for review see Smith and Read 1997). The key feature of this symbiosis is the arbuscule, a highly branched haustorium-like fungal structure within root cortical cells. Arbuscules provide the symbiotic interface between plant and fungal cells and are probably responsible for the bi-directional exchange of nutrients. The structures are functional for several days and are then quickly degraded (Smith and Read 1997). *Zea mays* root cortical cells containing collapsing arbuscules have been shown to accumulate large amounts of isoprenoids (Fester et al. 2002), most notably the acyclic C_{14} polyene 'mycorradicin', (10,10′-diapocarotene-10,10′-dioic acid; Klingner et al. 1995), and C_{13} cyclohexenone derivatives, (e.g. blumenin, 4-{3-*O*-[(2′-*O*-β-glucuronosyl)-β-glucopyranosyl]-butyl}-3,5,5-trimethyl-2-cyclohexen-1-one; Maier et al. 1995). The accumulation of these compounds has been described in AM roots from a wide variety of plants (Maier et al. 1997, 1999, 2000; Fester et al. 2002).

Previous results suggest that mycorradicin and cyclohexenone derivatives are synthesized via the plastid-located non-mevalonate methylerythritol phosphate (MEP) pathway (Maier et al. 1998; Walter et al. 2000). A strong induction of transcription of two early enzymes (1-deoxy-D-xylulose 5-phosphate synthase and 1-deoxy-D-xylulose 5-phosphate reductoisomerase) of

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this pathway has been correlated with mycorrhizal colonization of wheat roots (Walter et al. 2000).

In principle, two pathways are possible for the biosynthesis of the mycorrhiza-specific isoprenoids: a direct pathway, in which IPP from the MEP pathway is directly converted to these compounds, and an indirect one, in which IPP is first converted to a C_{40} carotenoid, which is then cleaved at the 9,10(9′,10′)-positions (Walter et al. 2000).

Here we present data obtained from mycorrhizal and non-mycorrhizal roots of three different plant species (*Z. mays*, *Nicotiana tabacum* and *Medicago truncatula*) that suggest that mycorradicin and cyclohexenone derivatives are synthesized via an unknown C_{40} precursor carotenoid. In addition, our data suggest that at least one enzyme in the carotenoid pathway, phytoene desaturase (Pds), is transcriptionally activated during mycorrhization. *Zea mays* mutants deficient in carotenoid biosynthesis allowed us to investigate the effects of the reduced carotenoid formation on the colonization of plant roots by AM fungi.

Materials and methods

Plant cultivation, AM fungus inoculation and application of norflurazon

Nicotiana tabacum L. cv. Petit Havana plants were transformed with a β-glucuronidase (*GUS*) reporter gene fused to a fragment of the *Lycopersicon esculentum PDS* gene comprising position –1,530/+564 (Corona et al. 1996). *Zea mays* L. *al1* (*albescent plant 1*) and *y9* (*pale yellow 9*) mutants were obtained from the Maize Genetics Cooperation Stock Center (http://w3.ag.uiuc.edu/maize-coop/). Seeds from *M. truncatula* Gaertn. var. Jemalong were obtained from Perkiss Seeds, Australia. In addition, *Z. mays* cv. Garant and *dwarf1* plants (Schneider et al. 1992) were used. Plants were grown in 250-ml plastic pots filled with expanded clay (Lecaton, 2–5 mm particle size, Fibo Exclay, Pinneberg, Germany) and inoculated with the AM fungus *Glomus intraradices* Schenck & Smith. Mycorrhiza formation was induced by growing the plants for at least 6 weeks in expanded clay containing 10% (v/v) of fungal inoculum. Details of plant-growing conditions have been published elsewhere (Maier et al. 1995). Norflurazon was applied to plants grown with or without mycorrhizal inoculum for 10 weeks. The plants were watered with 40 ml of a 25 mg/l norflurazon solution every second day for 10 days. Approximate percentage values for mycorrhiza formation were estimated microscopically after staining with trypan blue in lactophenol according to a procedure described by Phillips and Hayman (1970). Exact values of the percentage root length colonized by *G. intraradices* were determined microscopically with the gridline-intersection method at a magnification of ×20.

Extraction and analysis of root carotenoids

Extraction and subsequent high performance liquid chromatography (HPLC) of carotenoids was performed according to Fraser et al. (2000). Root samples (1 g) were washed and mortared in liquid nitrogen. Methanol (1.5 ml) was added and the suspension was repeatedly mixed for 5 min using a vortex. Then 1.5 ml of a buffer solution (50 mM Tris/HCl, pH 7.5, 1 M NaCl) was added and the sample mixed for 10 min. Chloroform (4 ml) was added and the sample mixed again for 10 min. Phase separation was achieved by centrifugation at 3,000 *g* for 5 min. The lower phase (3.5 ml) was removed, evaporated at room temperature (in vacuo) to dryness and dissolved in ethyl acetate prior to HPLC analysis.

The liquid chromatograph (Waters 600-MS system controller) was equipped with an S-5 μm C_{30} column (270×4 mm i.d.; YMC, Schwermbeck, Germany) coupled to a S-5 μm C_{30} precolumn. Injections of 20 μl were carried out by an automatic sampler (Waters 717 autosampler). Mobile phases consisted of 0.2% ammonium acetate in water/methanol (20/80 by volume) (B) and tert-butyl methyl ether (C) in methanol (A). Compounds were separated by a two-step linear gradient from 95% A, 5% B, 0% C, first to 75% A, 5% B, 20% C in 4 min and then to 25% A, 5% B, 70% C in 40 min. Compounds were detected photometrically (maxplot between 200 and 600 nm) by a Waters 996 photodiode array detector. Data were collected and analyzed using the Millennium software 2010 (Millipore, Eschborn, Germany). The presence of carotenoid esters was determined by saponification. This treatment was most effective when KOH was added to a final concentration of 6% w/v to the methanol suspension. The mixture was shaken at 57°C for 30 min. After incubation, the sample was cooled on ice, aqueous solution added and the extraction proceeded as described above. Esters were identified by the appearance of free carotenoid and disappearance of more non-polar compounds with identical UV/VIS spectra. Carotenoids were identified by cochromatography and comparison of spectral characteristics with authentic standards. Chromatographic, UV/VIS and mass spectrally characterized standards were prepared as described in Fraser et al. (2000). In addition, phytoene was isolated from an *Escherichia coli* engineered to produce this metabolite by the presence of the *Erwinia uredovora crt*E (geranylgeranyl diphosphate synthase) and *crt*B (phytoene synthase), kindly provided by Prof. G. Sandmann (Frankfurt, Germany). Quantities of carotenoids were estimated with β-carotene as an internal standard, calculating the absorbance coefficients of the various compounds from the values listed by Britton (1985).

Reverse transcription PCR

Total RNA was purified from *N. tabacum* roots using a modified guanidinium thiocyanate procedure (Giuliano et al. 1993). Root material (2 g) was mortared in liquid nitrogen and further homogenized in 12 ml 4 M guanidinium thiocyanate, 20 mM EDTA, 20 mM Mes (pH 7), 0.5% *N*-lauryl-sarcosinate and 130 mM β-mercaptoethanol. The suspension was mixed with an equal volume of phenol/chloroform/isoamyl alcohol (25/24/1) and centrifuged at 3,000 *g* for 20 min. The supernatant was mixed with 1/20 volume of 1 M acetic acid and 0.7 volume ethanol and incubated for 30 min on ice. RNA was precipitated by centrifugation at 3,000 *g* for 20 min and washed twice with 3 M sodium acetate (pH 5.2) and twice with 70% ethanol. The pellet was dried in vacuo and resuspended in 400 μl water.

RT-PCR was performed using Ready-To-Go RT-PCR Beads from Amersham Pharmacia starting with 2 μg *N. tabacum* root RNA. Fragments were amplified by 35 PCR cycles using the following primers and annealing temperatures. (A) *PDS*: forward primer 5′-ATG ATG ATG GAG ATT GGT ACG AG-3, reverse primer 5′-CTT TCT CAT CCA GTC CTT AAC AC-3′ (expected fragment size 331 bp, annealing temperature 55°C); (B) *ubiquitin* (used as an internal control): forward primer 5′-GAT CTT CGT GAA GAC ATT GAC TGG-3′, reverse primer 5′-AAA CCA CCA CGG AGA CGG AG-3′ (expected fragment size 223 bp, annealing temperature 58°C). Primers were designed according to a *PDS* sequence from *L. esculentum* Miller (GenBank accession number: X71023) and according to a ubiquitin sequence from *N. tabacum*. Amplification of genomic DNA can be excluded, because the genomic region corresponding to the amplified *PDS* sequence contains two introns. PCR fragments were cloned into the pGEM(R)-T Easy vector using the TA-cloning kit (Promega) and sequenced on a DNA sequencer model 4000L (Li-Cor).

Staining for GUS activity and microscopic analyses

Plants containing the *GUS* gene construct were selected after PCR amplification of a 1-kbp fragment using the following *GUS* gene

specific primers: GUS1 (TCT ACA CCA CGC CGA ACA CC), GUS2 (CAT GCA CAC TGA TAC TCT TCA CTC). DNA from F1 plant leaves was purified using the Nucleon Phytopure kit from Amersham Pharmacia Biotech.

Roots were incubated in 50 mM sodium phosphate (pH 7.0), 1 mM EDTA, 0.3% formaldehyde for 30 min at room temperature. Then the roots were washed several times in 50 mM sodium phosphate (pH 7.0), 1 mM EDTA and stained overnight at 37°C in 2 mM 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-GlcU) in the same buffer. After staining, the roots were washed with water and analyzed by bright-field microscopy using a Nikon Optiphot 2. Photographs were taken using Sensia II from Fujifilm and the slides were scanned and processed using Photoshop 4.0.

Dark blue-stained and white areas of mycorrhizal roots were chosen for embedding and sectioning. Small pieces of roots were fixed with 3% para-formaldehyde in buffered saline (PBS) for 3 h, dehydrated in a graded ethanol series and embedded in paraplast (Sigma). Sections of 10-μm thickness were collected on slides coated with poly-L-lysine, deparaffinized in xylene and hydrated. Slides were mounted in glycerol and examined with a Zeiss Axioskop2 (Zeiss, Germany). Pictures were taken with a CCD-camera (Sony, Japan) and processed with Photoshop 4.0.

Confocal laser scanning microscopy was performed using a Zeiss LSM410 equipped with a Kr/Ar laser (emission at 488 nm). Mycorrhizal roots from *Z. mays* (wild type, *dwarf1*, *al1* and *y9*) were stained with acid fuchsin according to Fester et al. (2001). Excitation at 488 nm and emission at 570 nm were used to visualize acid fuchsin staining. Series of at least 10 optical sections, each with a 1-μm interval on the *z*-axis, were collected and superimposed to create an "extended depth of focus" image using the "3D for LSM" program (Zeiss, Jena, Germany).

Results

Accumulation of carotenoids in AM roots

Small amounts of ζ-carotene (0.2 and 0.6 nmol per g fresh wt., respectively) were detected specifically in mycorrhizal roots from *Z. mays dwarf1* mutants and *M. truncatula* (Fig. 1). In addition, traces of phytofluene and lycopene (data not shown) were detected in mycorrhizal roots of *dwarf1*. Carotenoids were not detected in non-mycorrhizal roots of *dwarf1* or *M. truncatula*. In contrast, regardless of their mycorrhizal status, roots from *Z. mays* cv. Garant and *N. tabacum* plants accumulated various carotenoids. These were identified as violaxanthin and various violaxanthin and neoxanthin esters in the case of *N. tabacum*, at concentrations of up to 5.2 nmol and 2.9 nmol per g fresh wt., respectively.

Phytoene accumulation in norflurazon-treated plants

Since previously existing carotenoids in roots of *Z. mays* cv. Garant and *N. tabacum* could mask a possible de novo synthesis induced by mycorrhization, we decided to measure the flux through the pathway by taking advantage of the herbicide norflurazon. This specifically inhibits

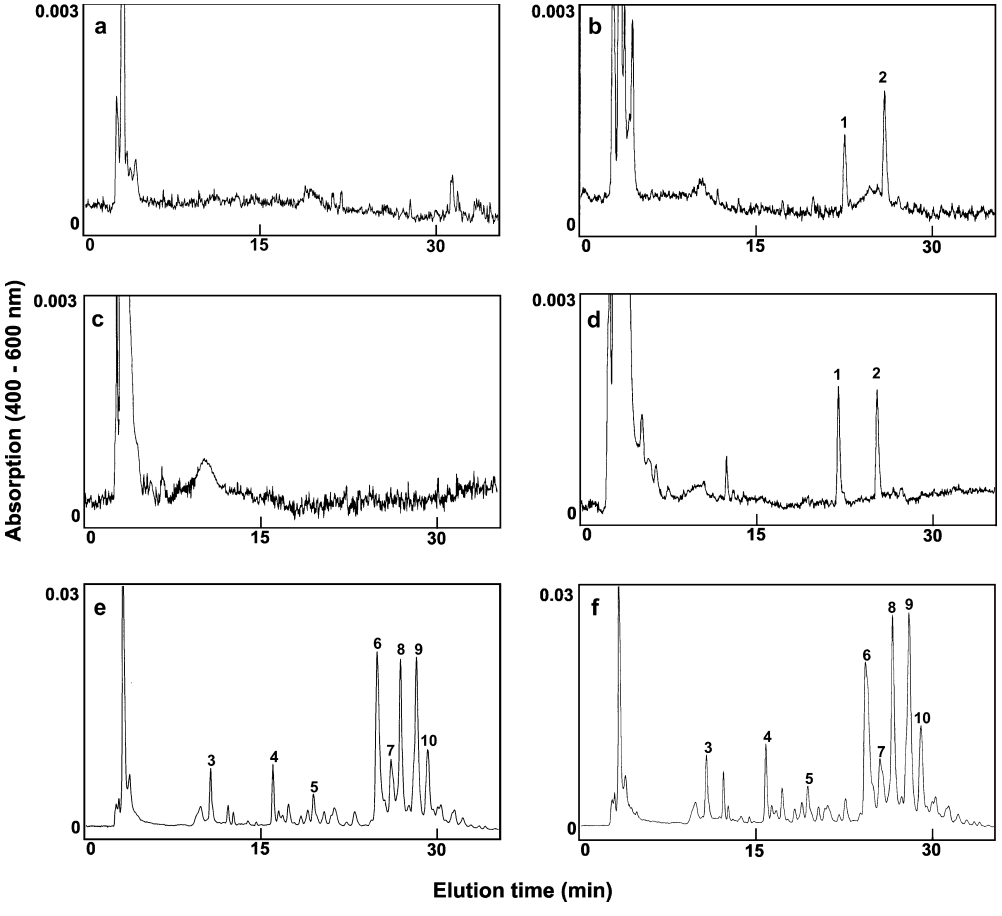

Fig. 1. High performance liquid chromatography (HPLC) (C_{30} reversed phase) elution profiles of chloroform extracts from non-mycorrhizal (**a, c, e**) and mycorrhizal (**b, d, f**) roots of *Zea mays dwarf1* (**a, b**), *Medicago truncatula* (**c, d**) and *Nicotiana tabacum* (**e, f**) recorded between 400 nm and 600 nm. Compounds were identified by comparison with known standards. All *trans* ζ-carotene (**1**) and *cis* ζ-carotene (**2**) accumulated specifically in mycorrhizal roots of *Z. mays dwarf1* (**b**, 0.6 nmol per g fresh wt.) and *M. truncatula* (**d**, 0.2 nmol per g fresh wt.). In mycorrhizal and non-mycorrhizal roots of *N. tabacum* (**e**, **f**), violaxanthin (**3**), violaxanthin esters (**4**, **6**, **7**, **8**, up to 5.2 nmol per g fresh wt.) and neoxanthin esters (**5**, **9**, **10**, up to 2.9 nmol per g fresh wt.) were detected (note that the absorbance scales in panels **e** and **f** differ from panels **a**–**d**). There were no significant differences between mycorrhizal and non-mycorrhizal roots. Similar results were obtained with roots of *Z. mays* cv. Garant (data not shown)

the second dedicated enzyme in the carotenoid pathway, phytoene desaturase (Pds; Chamovitz et al. 1991). Leaves and roots of norflurazon-treated plants accumulated a compound not present in untreated plants. The compound (λ_{max} 286 nm, shoulders at 275 and 297 nm) was identified as phytoene by spectral comparison and co-chromatography with a standard compound. The increase in phytoene in mycorrhizal versus non-mycorrhizal roots was twofold for *N. tabacum*, threefold for *Z. mays dwarf1*, fivefold for *Z. mays* cv. Garant and sixfold for *M. truncatula* (Fig. 2). These data clearly indicate that phytoene biosynthesis is more active in mycorrhizal than in non-mycorrhizal roots.

Expression of *PDS* genes in mycorrhizal roots

The low abundance of the *PDS* transcript in leaf and root tissue precludes detection by northern blot analysis (Pecker et al. 1992; Giuliano et al. 1993). Therefore, the presence of the *PDS* transcript was examined in *N. tabacum* roots using RT-PCR. Using standard conditions (2 μg RNA, 35 cycles of amplification), the *PDS* transcript was detectable in mycorrhizal but not non-mycorrhizal roots (data not shown). The fragment generated by the PCR reaction was sequenced to confirm its identity. Because the genomic region corresponding to the fragment contains two introns, amplification of genomic DNA can be excluded. Ubiquitin was used as an internal control and gave similar signals in mycorrhizal and non-mycorrhizal roots.

The activity of the *PDS* promoter was examined using transgenic *N. tabacum* plants transformed with the *GUS* reporter gene fused to the –1,530/+564 fragment from the *L. esculentum PDS* gene. This fragment has been shown to contain most of the *cis*-acting elements needed for chromoplast-associated gene expression (Corona et al. 1996). One transgenic line (1A) showed reproducible X-GlcU blue staining only in mycorrhizal roots. Non-mycorrhizal roots of this plant were typically not stained by X-GlcU (Fig. 3). Staining was not observed in mycorrhizal or non-mycorrhizal roots of wild-type plants.

Mycorrhization in *Z. mays* mutants impaired in carotenoid biosynthesis

Wild-type *Z. mays* and *dwarf1* plants, as well as carotenoid-deficient mutants *al1* and *y9* were analyzed for

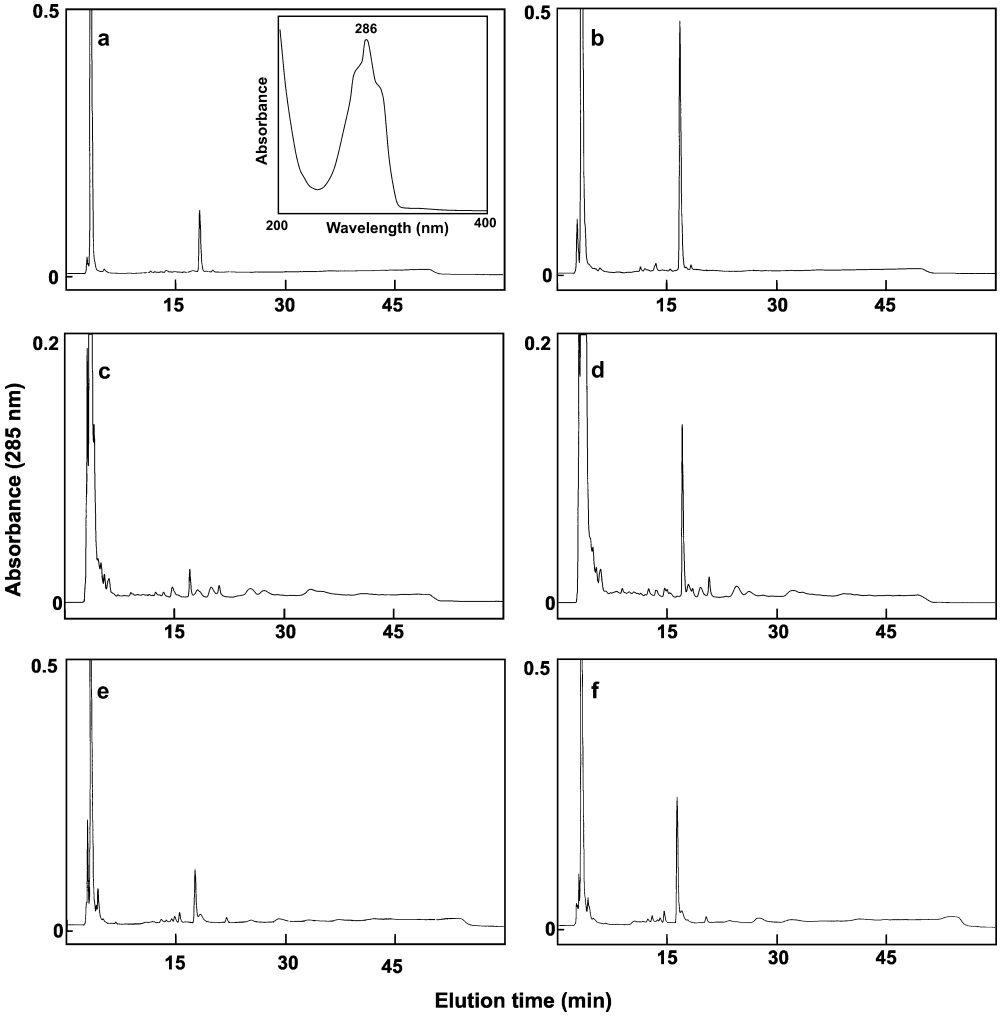

Fig. 2. HPLC (C_{30} reversed phase) elution profiles of chloroform extracts from non-mycorrhizal (**a, c, e**) and mycorrhizal (**b, d, f**) roots of *Z. mays dwarf1* plants (**a**, **b**), *M. truncatula* (**c**, **d**) and *N. tabacum* (**e**, **f**) after treatment with norflurazon, recorded at 285 nm. The concentration of phytoene (UV-visible spectrum see inset in **a**) was markedly higher in the AM roots [79 nmol per g fresh wt. in *Z. mays dwarf1* (**b**), 17 nmol per g fresh wt. in *M. truncatula* (**d**), 31 nmol per g fresh wt. in *N. tabacum* (**f**)] than in non-mycorrhizal roots [23 nmol per g fresh wt. in *Z. mays* (**a**), 3 nmol per g fresh wt. in *M. truncatula* (**c**), 15 nmol per g fresh wt. in *N. tabacum* (**e**)]. The concentration of phytoene in mycorrhizal roots of *Z. mays* cv. Garant increased to a similar extent after treatment with norflurazon (44 nmol per g fresh wt. in mycorrhizal roots versus 8 nmol per g fresh wt. in non-mycorrhizal roots; data not shown in this figure)

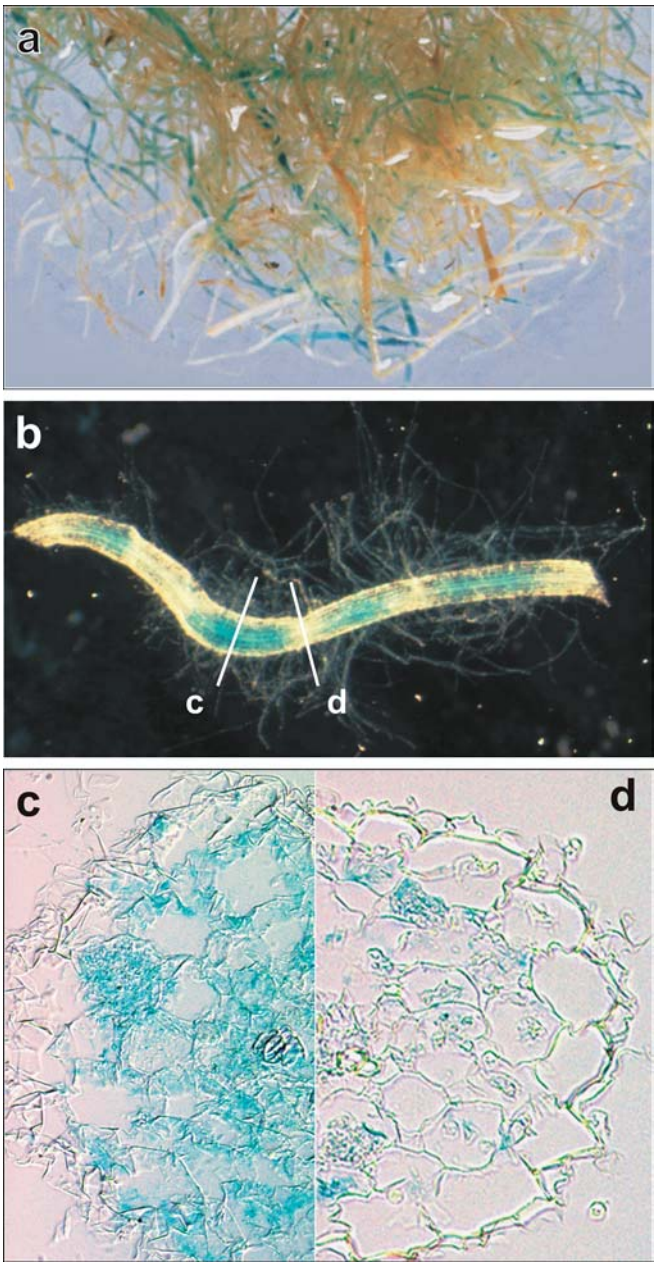

Fig. 3. GUS-stained roots (**a, b**) and root sections (**c, d**) of transgenic *N. tabacum* plants transformed with the *GUS* reporter gene fused to the *PDS* promoter from *Lycopersicon esculentum*. The roots were colonized by AM fungi. **a** Portion of the root system, **b** part of a single root, **c** cross section of a blue-stained root segment, **d** cross section through a macroscopically presented unstained root segment. Mycorrhizal and non-mycorrhizal roots from wild-type *N. tabacum* plants were not stained by the staining procedure

their susceptibility to colonization by AM fungi and production of mycorradicin. After 8 weeks inoculation, *Z. mays* wild-type and *dwarf1* plants contained considerable amounts of mycorradicin and were strongly colonized by *G. intraradices*. The carotenoid-deficient mutants, in contrast, did not contain mycorradicin and were only scarcely colonized (Fig. 4). There were no significant structural differences between AM structures in roots of wild-type/*dwarf1* mutant plants and carotenoid-deficient mutants (Fig. 4). In order to assess a possible negative influence of reduced photosynthetic rates in the carotenoid-deficient plants, we supplied *y9* mutant plants inoculated with AM fungi with 20 ml of a 50 mM glucose solution for 10 weeks three times a week. The treatment did not change the mycorrhizal status of the plants.

Discussion

Mycorradicin, a component of the so-called ''yellow pigment'', is deposited in the form of vacuolar droplets in *Z. mays* AM roots (Fester et al. 2002). We have shown previously that enzymes of the plastid-located MEP pathway are induced upon mycorrhization (Maier et al. 1998; Walter et al. 2000). Thus, IPP and DMAPP, the final products of this pathway, provide the isoprenoid pool necessary for production of mycorradicin and cyclohexenone derivatives. We assumed previously that these compounds are apocarotenoids, but this was not yet confirmed. In principle, IPP and DMAPP could be converted to these isoprenoids through a direct or through an indirect pathway, being first converted to a carotenoid, which is then converted to mycorradicin via an oxidative cleavage at the 9,10(9′,10′)-positions. ABA is also synthesized via oxidative cleavage of a precursor carotenoid, later identified as a 9-*cis*-epoxy carotenoid (Rock and Zeevaart 1991; Schwartz et al. 1997).

Unlike in the case of ABA, mutants affected specifically in mycorradicin biosynthesis are not known. Therefore, we used a carotenoid biosynthesis inhibitor (norflurazon) and mutants affected in carotenoid biosynthesis (*al1* and *y9*). In both cases, we obtained evidence that mycorradicin is synthesized through a C_{40} carotenoid. The *y9* mutant is particularly enlightening. This mutant is probably affected in carotenoid isomerase activity (Janick-Buckner et al. 2001), which is dispensable in chloroplast-containing but not in chloroplast-devoid tissues, such as endosperm and roots (Giuliano et al. 2002). Thus, this mutant is affected only to a minor extent in leaf carotenoid biosynthesis and in photosynthetic performance. Therefore, the complete inhibition of mycorradicin biosynthesis and partial inhibition of mycorrhization observed in this mutant are effects of the block in ζ-carotene desaturation/isomerization, rather than of reduced photosynthate levels, a fact further confirmed by the lack of an effect of glucose supplementation.

Pds catalyses the second dedicated step of carotenoid biosynthesis and *PDS* transcript levels are high in chromoplast-containing tissues of *L. esculentum* (Pecker et al. 1992; Giuliano et al. 1993). A large part of this regulation occurs at the transcriptional level, since the *L. esculentum PDS* promoter is able to confer chromoplast-associated expression to a *GUS* reporter gene (Corona et al. 1996). In *L. esculentum*, this promoter is active in anthers, petals, stigmas and ripening fruits, while in

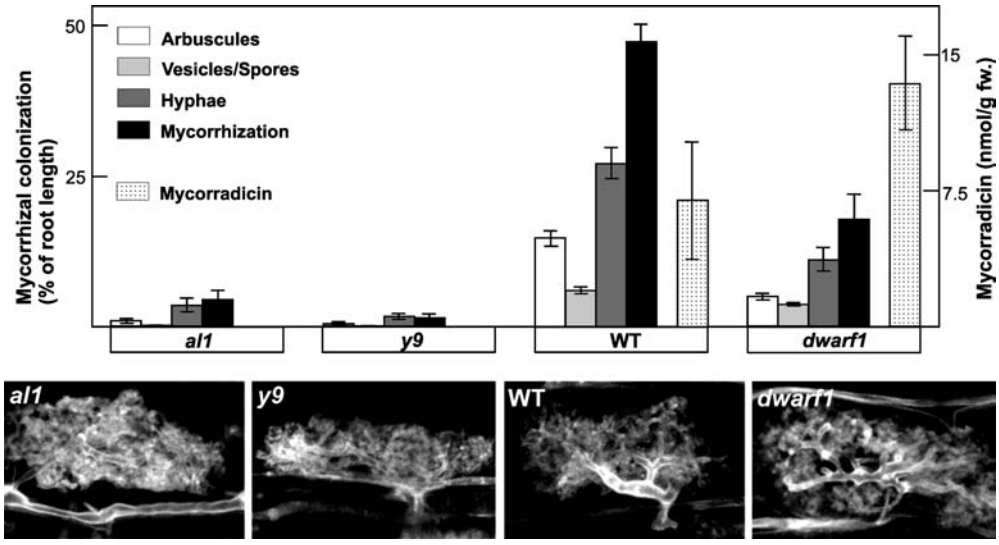

Fig. 4. AM colonization (percentage of roots containing any fungal structure) and mycorradicin contents of *Z. mays* mutants deficient in carotenoid biosynthesis [*albescent plant 1* (*al1*) and *pale yellow 9* (*y9*)] and in wild-type (*wt*) and *dwarf1 Z. mays* plants. In each case, at least three individual plants were examined. The carotenoid-deficient mutants contained no mycorradicin. Arbuscular structures from these mutants showed no obvious anomalies (see lower panel), but their numbers were markedly reduced

N. tabacum activity is restricted to anthers and stigmas. In both species, the promoter activity in roots is extremely low.

We analyzed GUS activity in mycorrhizal and non-mycorrhizal roots from transgenic *PDS::GUS N. tabacum* plants (Corona et al. 1996). Within these roots, staining correlated closely with cells containing mycorrhizal structures. In RT-PCR analyses, we detected the transcript in mycorrhizal but not in non-mycorrhizal roots. Our results indicate that AM colonization stimulates the transcription of a specific carotenoid biosynthesis gene and provide the first example of a plant carotenoid gene promoter responsive to mycorrhization. This promoter will be instrumental for describing the *trans*-acting transcription factors governing plant response to mycorrhization.

Arbuscules have limited life spans, depending on the plant species, and are constantly degraded and rebuilt (Smith and Read 1997). Arbuscules in *N. tabacum* roots are covered by extensive plastid networks (Fester et al. 2001) that constitute the major part of the plastid compartment in root cortical cells. Upon disintegration of arbuscules, these plastid networks disintegrate. The *PDS* transcriptional activity, located exclusively within root cortical cells containing arbuscules, suggests that carotenoid biosynthesis in AM roots is primarily located in the aforementioned plastid networks. This hypothesis is further supported by the finding that accumulation of apocarotenoids is correlated with the degradation of arbuscules in *Z. mays* AM roots (Fester et al. 2002).

Z. mays mutants deficient in carotenoid biosynthesis showed markedly reduced rates of fungal colonization, but were still able to form all structures typical of the symbiosis. This indicates that carotenoid/mycorradicin biosynthesis may not be essential for the formation of symbiotic structures, but may be necessary for the functioning of the AM symbiosis, at least in those plants accumulating apocarotenoids in AM roots (Fester et al. 2002).

Our data strongly suggest that the putative precursor carotenoid is located downstream of phytoene and also downstream of the ζ-carotene desaturation/isomerization, as indicated by the *y9* mutant. Its exact nature, as well as that of the cleaving enzyme, remains to be elucidated.

An interesting open question pertains to the likely signals triggering plant response to mycorrhization. One possible hypothesis is that reactive oxygen species (ROS) are produced upon mycorrhization (Salzer et al. 1999 and our unpublished data) and act as second messengers mediating carotenoid biosynthesis (Bouvier et al. 1998). The accumulated carotenoids could provide efficient protection against oxidative stress (ROS scavenging) as they are known to do in conditions of cellular oxidative stress (e.g. Britton 1995).

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