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Review

"Chromoplast" development in arbuscular mycorrhizal roots

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Abstract

The accumulation of apocarotenoids in arbuscular mycorrhizal (AM) roots suggests a dramatic reorganization of the plastids responsible for the biosynthesis of these compounds. This review describes the cytological and biochemical characterization of this phenomenon. The results presented suggest that plastids are key organelles for the establishment of the symbiotic interface of the AM symbiosis. In addition, a complex interplay of various plant cell components during the different functional phases of this interface is suggested. Arbuscule degradation appears to be of particular interest, as it correlates with the formation of the most extensive plastid structures and with apocarotenoid accumulation.

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Keywords: Arbuscular mycorrhiza; Root plastids; Apocarotenoids; Mycorradicin; Cyclohexenone derivatives; Stromules

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1. Introduction

The work reviewed in this article started after the discovery of two metabolites accumulating specifically in arbuscular mycorrhizal (AM) roots, i.e., mycorradicin (Klingner et al., 1995a) and blumenin. The latter is member of a group of related compounds with marked variation in derivatization and glycosylation (Maier et al., 1995). This group is often summarily referred to as cyclohexenone derivatives according to the common basic chemical structure. Mycorradicin, on the other hand, occurs *in planta* not in its free form but as a complex esterification product. It was soon postulated that both groups of compounds, mycorradicin and cyclohexenone derivatives are produced by the cleavage of a common precursor carotenoid molecule (Klingner et al., 1995a; Walter et al., 2000). While the presumed

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carotenoid degradation products were first observed in members of the Poaceae (Maier et al., 1997), later studies demonstrated their occurrence in other plant species as well (Maier et al., 1999, 2000; Fester et al., 2002a, 2005; Schliemann et al., 2006). Experiments analyzing carotenoid biosynthesis detected a mycorrhiza-specific activation even in plant species not accumulating the compounds in question, suggesting a universal occurrence of this phenomenon (Fester et al., 2005). In general, the activation of carotenoid biosynthesis obviously has a strong impact on the organelles involved and is a typical feature of plastid differentiation resulting in chromoplasts (Camara et al., 1995). The existence of such chromoplasts has been proposed for AM roots of *Ornithogalum umbellatum* already in 1977 by Scannerini and Bonfante-Fasolo. It later became clear, however, that intact carotenoids are not accumulating to a measurable extent in AM roots (Fester et al., 2002b) and that the mentioned carotenoid degradation products are not stored in plastids (Klingner et al., 1995b; Fester et al., 2002a). Nevertheless, the large amount of accumulating compounds – at least in plant species like *Zea mays* or *O. umbellatum* – suggested a profound reorganization of the plastids responsible for this accumulation. The research detailed in this review aimed at obtaining a comprehensive picture of this part of the response of a plant cell to colonization by an AM fungus. The results reveal that plastid organization is not uniformous throughout the colonization process. They suggest plastids to be key organelles during formation and decomposition of the symbiotic structures and they provide perspectives for further experiments regarding the functional role of apocarotenoid accumulation.

2. Cytological changes of plastids upon colonization of root cortical cells by AM fungi

Arbuscules are elaborate, highly branched fungal structures within individual root cortical cells in AM symbioses (for review see e.g. Bonfante-Fasolo, 1984; Gianinazzi-Pearson, 1996; Hause and Fester, 2005). In these cells, a major part of the cell lumen is occupied by the newly formed symbiotic interface, while the cytosol is increasing considerably in volume. This process is accompanied by the proliferation of plant cell organelles according to electron microscopic analyses (Bonfante-Fasolo, 1984; Gianinazzi-Pearson, 1996). Plastids have first been studied in *O. umbellatum*, which exhibits a particularly strong yellow colouration of AM roots (Scannerini and Bonfante-Fasolo, 1977). The compound responsible for this colouration is the already mentioned mycorradicin (Fester et al., 2002a), present *in planta* in the form of complex esters (Schliemann et al., 2006) and not as intact carotenoids as proposed initially (Scannerini and Bonfante-Fasolo, 1977). Further electron microscopic studies resulted in the observation of irregularly formed, ‘‘elongate’’ plastids in AM roots of a number of plants (Dexheimer et al., 1990). Whereas electron microscopy provides precise representations of subcellular cross-sections, the reconstruction of threedimensional structures from electron microscopical recordings is laborious and problematic. The introduction of confocal laser scanning microscopy (CLSM) and its application to green fluorescent protein (GFP)-labelled cellular components has revolutionized this area of research, as may be exemplified by the data reviewed below.

A first analysis in *Nicotiana tabacum* transformed with a plastid targeted GFP revealed the massive proliferation of plastids in colonized cells. This proliferation referred to an increase in numbers of the organelles, and to the formation of large tubular elongations. In some cases, these tubules appeared to connect all plastids of a given cell forming a network-like structure covering the arbuscules (Fig. 1; Fester et al., 2001). These elongations were partially comparable to tubular plastid extensions designated as stromules (stroma filled tubules; Koehler et al., 1997; Tobin, 1997). Nevertheless, they often exhibited larger diameters and occured in considerably higher numbers when compared

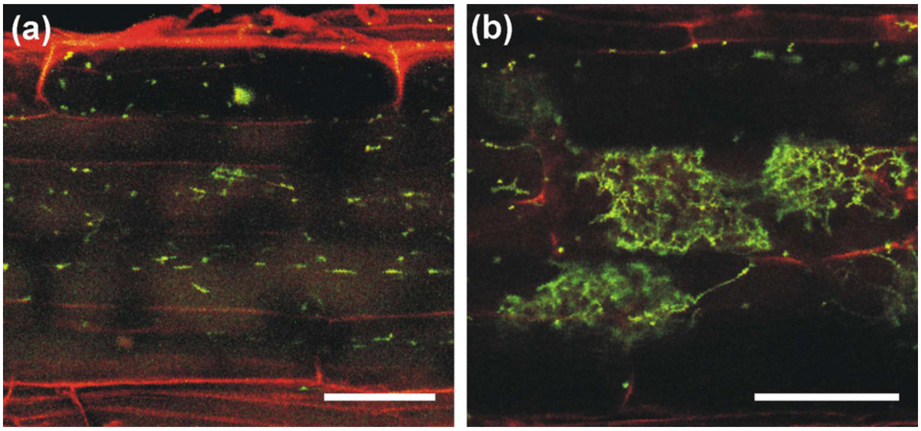

Fig. 1. Plastid proliferation and network formation in AM roots of *Nicotiana tabacum*. Representative micrograph of a non-colonized root cortical cell: (a) containing only a few plastids along the cytoplasmic seam and a colonized cell; (b) recorded by CLSM. Autofluorescence of the cell wall is given in red colour, fluorescence of a plastid-targeted GFP in green colour. Bar represents 50 μm.

to stromules described for root plastids so far. Regarding the intensity of tubule formation they rather resembled plastids from cell suspension cultures of *N. tabacum*, which also show the formation of complex plastid networks (Koehler and Hanson, 2000). Similar plastid networks have been described for fruits of *Lycopersicon esculentum* (Pyke and Howells, 2002) and for epidermal cells of *N. tabacum* (Arimura et al., 2001). In addition, both in cells from suspension cell cultures and in root cortical cells colonized by AM fungi, the formation of octopus- or millipede-like plastid structures surrounding the plant cell nucleus was observed (Fester et al., 2001). This feature possibly refers to the fact that the plant cell nucleus is depending on nucleotides produced by plastids. Accordingly, in non-colonized root cortical cells, often plastids are located close to plant cell nuclei.

The analysis of GFP-labelled plastids in AM roots from *Medicago truncatula* after an *Agrobacterium rhizogenes*-mediated transformation with suitable constructs revealed similar structures as in *N. tabacum* (Lohse et al., 2005). Octopus-like plastid structures surrounding plant cell nuclei, however, were not observed in this plant and the extent of network formation appeared to be reduced. The colocalization of plastids with fungal structures stained by a plant lectin specific for chitin (wheat germ agglutinin) revealed a close correlation of plastid shape and the structure of the symbiotic interface. While multiple lens-shaped plastids were observed in the vicinity of arbuscules consisting of fine, highly branched fungal hyphae, arbuscules composed of fewer coarse branches were rather surrounded by tubular, partially connected plastids (Lohse, 2006; Lohse et al., 2006). Because these arbuscules often showed features of decomposition like a general blurring of shape and a reduced staining for chitin, they were assumed to be older, decomposing structures. Whereas the tubular plastids surrounding these arbuscules did not form complete networks as in *N. tabacum*, a considerable number of signals for the plastid division protein FtsZ could be localized along these plastids (Lohse, 2006; Lohse et al., 2006). This observation might indicate that the tubular plastids surrounding decomposing arbuscules undergo continous fission and fusion events thus constituting a dynamic network. Similar mechanisms for the dynamic integration of a plant cell compartment have been described for plant cell mitochondria in *Arabidopsis thaliana* by Arimura et al. (2004).

3. Essential features of apocarotenoid accumulation in AM roots

The compounds accumulating in AM roots are various glycosylated C_{13} isoprenoids containing a cyclohexenone ring and a C_{14} polyenic, dicarboxylic acid of yellow colouration named mycorradicin esterified in a complex way to unknown components (Fig. 2). There are several lines of evidence suggesting that these compounds are degradation products of a common precursor carotenoid and that the

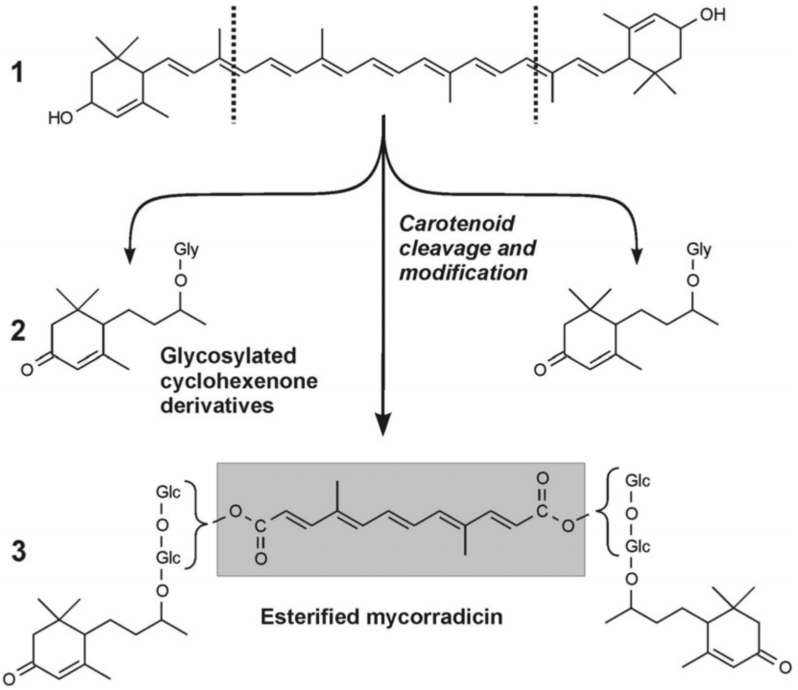

Fig. 2. Hypothetical scheme of apocarotenoid formation. Carotenoid (1) cleavage initially leads to the production of a C_{14}-dialdehyde and of two C_{13}-ketones. The latter compounds are reduced, modified and glycosylated resulting in various cyclohexenone derivatives (2). The C_{14}-dialdehyde, in contrast is oxidised and the resulting dicarboxylic acid (mycorradicin, marked by the grey box) is esterified to various compounds leading to a complex mixture of non-identified esters (3). In *Zea mays*, these esters also contain a cyclohexenone derivative (blumenol C cellobioside) as depicted here. The metabolites described are accumulating in large amounts in a number of plants (Table 1), while the levels of potential precursor carotenoids are very low.

IPP precursor for the biosynthesis of this carotenoid is produced by the plastid-located methylerythritolphosphate (MEP) pathway. The participation of the MEP-pathway was demonstrated by labelling experiments using ^{13}C-glucose and NMR-analysis of the accumulating cyclohexenone derivatives (Maier et al., 1998). In accordance with these results, Northern blot experiments showed the AM-specific increase of transcript levels of the first two enzymes of this pathway, 1-deoxy-xylulose-5-phosphate synthase, DXS (Walter et al., 2000) and 1-deoxy-xylulose-5-phosphate reductoisomerase, DXR (Hans et al., 2004), in AM roots. A similar increase in transcript levels in AM roots has been observed for two enzymes of carotenoid biosynthesis, phytoene desaturase (PDS, Fester et al., 2002b) and ζ-carotene desaturase (ZDS, J. Hans, M.H. Walter, unpublished; Lohse et al., 2005) and for one carotenoid cleaving dioxygenase (CCD, Lohse et al., 2005). Further evidence for the proposed pathway derives from the observation that the inhibition of this pathway for example in mutant maize plants defective in carotenoid biosynthesis (Fester et al., 2002a), by application of the PDS inhibitor norflurazon (Fester et al., 2002b), or in RNAi-plants referring to the MEP pathway enzyme DXR (Floss et al., unpublished) leads to reduced levels of the accumulating compounds.

Cyclohexenone derivatives have been described for AM roots from a number of members of the Poaceae (Maier et al., 1995, 1997), from *N. tabacum* and *L. esculentum* (Maier et al., 1999; Maier et al., 2000) and recently from *Lotus japonicus* (Fester et al., 2005) and *M. truncatula* (Schliemann et al., unpublished). Mycorradicin, in contrast, was initially only found in AM roots of *Zea mays* and of some other members of the Poaceae (Klingner et al., 1995b). The initial failure of extracting mycorradicin in many other cases has been shown to be due to the integration of this compound in a complex mixture of various esterification products (Fester et al., 2002a). The characterization of these products in AM roots from *Z. mays* revealed that they contain a significant amount of a glycosylated cyclohexenone derivative (blumenol C cellobioside) besides mycorradicin (Fester et al., 2002a). Thus, after the oxidative carotenoid cleavage reaction and after various modifications of the cleavage products, a part of the C_{13} fragment is integrated into complex esterification products involving the other (C_{14}) cleavage fragment in *Z. mays*. The resulting complexes are not soluble in water and are deposited in the form of small droplets accumulating initially in the cytosol and finally in the vacuole. The kinetics of their accumulation as well as an electron microscope analysis provided evidence that they are mainly produced during decomposition of arbuscules (Fester et al., 2002a). Besides this bound form of the C_{13} fragment, another fraction of cyclohexenone derivatives occurs as soluble glycosides. An overview about the variability of these compounds is given by Strack et al. (2003) and Strack and Fester (2006). Because mycorradicin in general can only be extracted from AM plant roots after alkaline hydrolysis, its integration into various esterification products seems to be a common phenomenon (Fester et al., 2002a). These complexes, however, do not necessarily contain the other carotenoid cleavage fragment as has been shown for *O. umbellatum* (Schliemann et al., 2006).

Upon hydrolysis of the mentioned complexes by mild alkaline treatment, mycorradicin can be liberated from AM roots of a large number of plant species. The amounts of mycorradicin extracted after this treatment from AM roots are variable and correlate with the intensity of the yellow colouration of roots (Fester et al., 2002a). For this reason and due to similar absorption spectra of mycorradicin and of the yellow droplets in the cytosol and vacuoles of AM *Z. mays* roots, mycorradicin seems to be the main compound responsible for this yellow colouration (Klingner et al., 1995b; Fester et al., 2002a). Due to its characteristic absorption spectrum in the visible range, mycorradicin can be detected with higher sensitivity and specificity in AM roots when compared to glycosylated cyclohexenone derivatives. It often occurs, however, in smaller amounts than suggested by stoichiometric relation to the accumulating cyclohexenone derivatives (Table 1).

Summarizing the data regarding the accumulation of mycorradicin and cyclohexenone derivatives, there are a number of monocotyledonous plants not accumulating cyclohexenone derivatives and a number of dicotyledonous plants not accumulating mycorradicin. Nevertheless all monocotyledonous plants lacking cyclohexenone derivatives, which were checked for mycorradicin, showed to accumulate this compound (Fester et al., 2002a; Table 1). Similarly, all dicotyledonous plants without mycorradicin, which were checked for phytoene accumulation after application of the PDS inhibitor norflurazon, contained increased levels in mycorrhizal compared to non-mycorrhizal roots (Fester et al., 2005; Table 1) indicating an activation of carotenoid biosynthesis in AM roots of these plants. Apparently both cleavage products of the initial carotenoid oxidation can be subjected to further transformations, as is already suggested by the non-stoichometric relation of the compounds observed (Table 1). The high flux through the carotenoid biosynthetic pathway indicated by the PDS inhibition experiments is only reflected by traces of ζ-carotene detected specifically in AM roots from *Z. mays* and *M. truncatula* (Fester et al., 2002b). As no other possible precursor carotenoids could be observed so far, the biosynthesis of the accumulating apocarotenoids appears to proceed rapidly, without the accumulation of detectable intermediates.

4. Plastid metabolism in AM roots

To obtain a comprehensive picture of further changes in plastid metabolism upon colonization of root cortical cells by AM fungi, data referring to transcript and metabolite levels were combined (Lohse et al., 2005). This combination allowed the circumvention of inherent problems of

Table 1
Mycorrhiza-specific accumulation of apocarotenoids and activation of carotenoid biosynthesis in selected plant species demonstrating the general occurrence of the phenomenon and the broad range of concentrations

Taxon	1	2	3	Taxon	1	2	3
Monocotyledonous plants				Dicotyledonous plants			
Allium cepa		5.5		*Centaurea cyanus*		n.d.	2.6
Allium porrum		1		*Cucumis sativus*		96	
Asparagus densiflorus		<1		*Cucurbita pepo*		14	
Avena sativa	125			*Digitalis purpurea*		<1	
Hordum vulgare	346			*Lotus japonicus*	330[c]	1.9	11.5
Nardus stricta	n.d.	4		*Lycopersicon esculentum*	198[a]	0.9	
Ornithogalum umbellatum		130		*Medicago sativa*		17	
Poa nemoralis	209			*Medicago truncatula*	137[c]	17	17[b]
Panicum miliaceum	27	15		*Nicotiana tabacum*	896[a]	16	31[b]
Secale cereale	142			*Petroselinum crispum*		n.d.	5.3
Sorghum bicolor	n.d.	4		*Phaseolus vulgaris*		0.8	
Triticum aestivum	414			*Ruta graveolens*		n.d.	30
Zea mays	n.d.[d]	310	23[b]	*Tagetes erecta*		n.d.	27.4

Concentrations of metabolites are given in nmol (g fresh weight)$^{-1}$. Unless indicated otherwise, values of cyclohexenone derivatives (**1**, given as the total of the various derivatives present) are taken from Maier et al. (1997), values for mycorradicin (**2**) are taken from Fester et al. (2002a) and values for phytoene after norflurazon application (**3**) are taken from Fester et al. (2005). In a few cases, low levels of apocarotenoids were also observed in roots of nonmycorrhizal plants. The structural diversity of cyclohexenone derivatives is reviewed by Strack et al. (2003) or Strack and Fester (2006).

[a] Values from Maier et al. (2000).

[b] Values from Fester et al. (2002b).

[c] Values from Fester et al. (2005).

[d] Cyclohexenone derivatives were observed in this plant in later experiments (Walter et al. (2000)) n.d. not detectable; blank spaces, not determined.

the two approaches and finally led to a number of plastid biosynthetic pathways apparently activated in AM roots. Metabolite profiling revealed increased steady state levels of various fatty acids as well as of a number of amino acids, while the steady state levels of malate and fumarate were reduced. In the case of fatty acids, it is possible to distinguish a set of predominantly plant and a set of predominantly fungus-derived compounds (Olsson, 1999). Steady state levels of members from both sets are increased upon mycorrhization, indicating an activated lipid biosynthesis by the fungus as well as activation of plant fatty acid biosynthesis. Increased transcript levels of key enzymes of plant fatty acid biosynthesis support this interpretation. In the case of amino acids, the differentiation between a plant or a fungal origin of a given compound is not possible. The observation of increased transcript levels referring to key plant enzymes of amino acid biosynthesis, however, suggests that the changes observed indicate an activation of plant amino acid biosynthesis. Decreased levels of malate and fumarate, finally, only can refer to the plant pools of these metabolites. They probably indicate an increased consumption of these precursors of many plastid biosynthetic products.

The changes of metabolites and transcripts in AM roots reported above only represent a small portion of the actual changes, because the analyses referred to whole root systems. Root cortical cells colonized by AM fungi only constitutes a small portion of the cells in the complete root systems. In addition, on a cellular level, different phases of colonization can be observed, corresponding – as detailed below – to different metabolic activities. The increase in plastid-located fatty acid and amino acid biosynthesis, e.g., has to be accompanied by an increase in the activity of the mitochondrial respiratory chain and of either cytosolic glycolysis or of plastid pentose phosphate pathway. Apart from a marked proliferation of mitochondria upon AM colonization, no experimental evidence for such an increased activity could be found (Lohse et al., 2005).

5. Correlation of plastid processes to specific symbiotic stages

Immunocytological simultaneous localization of plastids, plastid located proteins and fungal structures has revealed that plastid structure and metabolism are not uniformous during AM colonization, but change in correlation to the development of fungal structures (Lohse, 2006; Lohse et al., 2006). The experiments could differentiate in particular an early and a late phase of arbuscule development. The early phase is characterized by an intense division of plastids (studied by immunolocalization of the plastid division protein FtsZ) resulting in the biogenesis of a large number of small, lens-shaped plastids. The late phase, in contrast, is characterized by elongate plastids, surrounding the decomposing arbuscular structures (Fig. 3). As already mentioned, these tubular plastids were decorated by a considerable number of FtsZ division rings allowing speculations about the existence of a dynamic plastid network formed by repeated fusion and fission events. Immunolocalization of the MEP pathway enzyme DXR revealed in addition that the presence of this enzyme is dependent on the developmental state of fungal

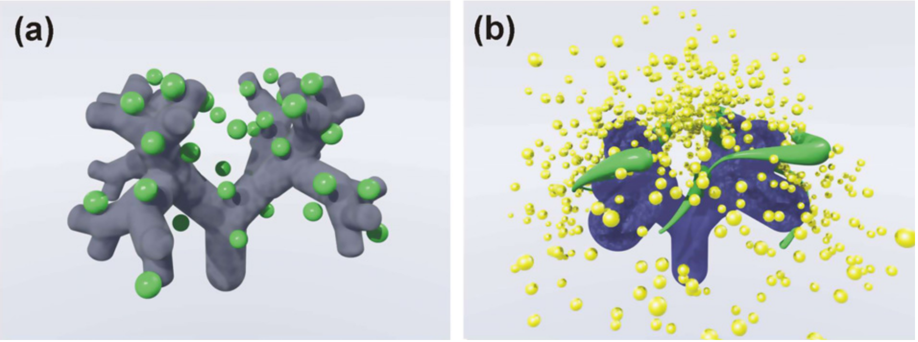

Fig. 3. Schematic representation of a developing: (a) and a decomposing; (b) arbuscule (blue colour). The lens-shaped plastids (green colour) surrounding the growing arbuscules are most likely involved in the biosynthesis of fatty acids and amino acids, necessary, e.g., for the formation of the periarbuscular membrane separating the plant cell cytosol from the fungal structures. The most likely biochemical task of the tubular plastids (green colour) surrounding the decomposing arbuscule (b, blue colour) regards recycling of compounds liberated from this structure. Besides, these plastids are involved in production of apocarotenoids. In AM roots of many plant species a part of these apocarotenoids is accumulating in the form of small yellow droplets (yellow colour) in the plant cell cytosol and vacuole. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

structures. In a first localization in AM roots from *Z. mays* the enzyme was observed in elongate plastids mainly around fully mature or initially decomposing arbuscules (Hans et al., 2004), in *M. truncatula* its presence clearly correlated with arbuscule decomposition (Lohse et al., 2006). A parallel analysis of plastid shape and presence of the enzyme was possible in *M. truncatula*, where plastids were labelled by the GFP. Here the enzyme was almost exclusively present in elongate plastids and not in smaller lens-shaped plastids occurring in the initial phase of arbuscule development (Lohse et al., 2006). This correlation of arbuscule degradation and presence of DXR nicely complements earlier evidence (based on electron microscopical analysis and on apocarotenoid accumulation kinetics) correlating apocarotenoid accumulation and arbuscule degradation (Fester et al., 2002a).

6. Summary and conclusions

By producing basic metabolic building blocks like fatty acids and amino acids as well as a number of secondary metabolites, plastids are the main biosynthetic organelles of plant cells (reviewed e.g. by Tetlow et al., 2005). Accordingly they are responsible for a number of biochemical tasks during arbuscule formation and degradation. The increase in fatty acid and amino acid biosyntheses upon root colonization is reflected by respective changes of metabolite and transcript levels on the whole root level (Lohse et al., 2005). It appears reasonable to assume that a large part of this activation occurs in the early phase of arbuscule formation, when the newly formed periarbuscular membrane, the increased volume of the plant cell cytosol, and the intense proliferation of various organelles require a particularly large amount of fatty acids and amino acids. In addition, the involvement of plastids in the assimilation of ammonia is necessary for the uptake of nitrogen, which is supplied by the fungus in the reduced, inorganic form (Govindarajulu et al., 2005). The marked proliferation of plastids and their ubiquitous distribution between individual arbuscular branches reflects these tasks. The late phase of arbuscule decomposition, in contrast, is presumably characterized by the breakdown of components of the symbiotic interface, including in particular the periarbuscular membrane, and plant and fungal wall components (Kinden and Brown, 1976). Even the transfer of substantial quantities of mineral nutrients from the collapsing arbuscule to the colonized plant cell has been proposed (Kinden and Brown, 1976). Because of their biosynthetic capacities plastids are certainly involved in these processes. While liberated fatty acids are recycled by the cooperation of glyoxysomes and mitochondria (Tolbert, 1981; Kindl, 1993) plastids are particularly involved in carbohydrate and amino acid metabolism. Carbohydrates liberated from the host or fungal cell wall possibly either are stored in the form of starch or provide carbon skeletons for the assimilation of ammonia liberated during chitin degradation. The latter process might result in the formation of asparagine, which is used as a transport metabolite for nitrogen in *M. truncatula* (Colebatch et al., 2004) and which is observed in increased amounts in AM roots of this plant (Lohse et al., 2005). Interestingly, similarities of the rhizobial and AM interaction regarding the biosynthesis of asparagine were not only observed referring to the metabolite, but also referring to the transcript levels (Lohse et al., 2005).

The tubular plastids observed around decomposing arbuscules are apparently formed by the fusion of tubular extensions of the lens-shaped plastids from the earlier symbiotic phase. Several functional reasons have been suggested for the formation of such tubular plastid extensions in general (Gray et al., 2001). The most significant possibilities in the case of decomposing arbuscules seem to be an increase in the total plastid surface and the possibility for the exchange of signals, metabolites and proteins between individual plastids, i.e., the integration of the plastid compartment. The formation of a dynamic plastid network by fusion and fission events in the late phase of arbuscule development in *M. truncatula* would support this possibility. Referring to the increase in the total surface of tubular plastids, it is interesting to note that apocarotenoid biosynthesis, which relies on a number of enzymes from the inner plastid envelope, is correlated with the presence of tubular plastids both in *M. truncatula* as well as in Z. mays. The reason for this particular plastid activity remains enigmatic. The correlation of apocarotenoid and reactive oxygen species (ROS) accumulation during arbuscule decomposition (Salzer et al., 1999; Fester and Hause, 2005) allows the possibility that carotenoid biosynthesis – as in above ground plant parts (Bouvier et al., 1998) – is activated in AM roots by these ROS. Experiments designed to show a protective effect of the activation of carotenoid biosynthesis against ROS damage, however, failed so far (unpublished results). The apocarotenoids in AM roots are part of the large group of carotenoid cleavage products found in nature. Many of these compounds are connected to organismic interactions (Giuliano et al., 2003; Bouvier et al., 2005). Given the structural similarity of cyclohexenone derivatives and the plant hormone abscisic acid or the zygomycete mating factor trisporic acid, an involvement of these compounds in signalling cannot be excluded. Such an involvement does not explain, however, the strong variation of respective concentrations in AM roots of different plant species and the extraordinarily high concentrations in the case of some monocotyledonous plants (Table 1). This variation is particularly difficult to explain, because it is not reflected by differences regarding establishment or functioning of the AM symbiosis.

7. Perspectives

One of the most fascinating topics in AM research regards the regulation of the symbiosis. In recent years, a number of components responsible for symbiotic signalling has been discovered, most of them involved in the early steps of plant-fungus recognition (Kistner and Parniske, 2002; Oldroyd et al., 2005). A few of these steps are referring to plastids, i.e. the plastid located proteins CASTOR and POLLUX necessary for the establishment of the symbiosis (Imaizumi-Anraku et al., 2005) and a carotenoid derived (Matusova et al., 2005) compound responsible for branching of fungal hyphae (Akiyama et al., 2005). Although this compound is produced by a similar pathway as the apocarotenoids described in this review, it should be kept in mind that it is accumulating and effective in far lower amounts. The plastid located proteins CASTOR and POLLUX, which have been tentatively identified as ion-channels, are mainly involved in the early steps of plant-fungus recognition, referring to plastids in rhizodermal cells. In the case of an involvement of these proteins in arbuscule formation, however, they might participate in signalling leading to the initial plastid proliferation in the vicinity of such arbuscules.

The general focus on early symbiotic steps is mainly due to the easier recognizability of mutants disturbed in these steps, when compared to, e.g., mutants disturbed in arbuscular functioning or arbuscule decomposition. As detailed in this review, however, these later steps are of similar complexity when compared to the early steps and they may be of similar importance for the regulation and performance of the AM symbiosis. The research detailed above has shown that arbuscular degradation in particular is a coordinated process involving a number of cellular compartments. Thus, the initiation of arbuscule degradation most certainly not only involves signals between the two symbionts, but also intracellular signals as, e.g., signals from the nucleus to the plastids and vice versa. A close analysis of the largely unknown biochemical processes involved in arbuscule degradation is a first necessary step for studying the respective regulatory mechanisms. In addition, such an analysis may provide the basis for understanding the enigmatic accumulation of apocarotenoids, which is closely correlating with this process.

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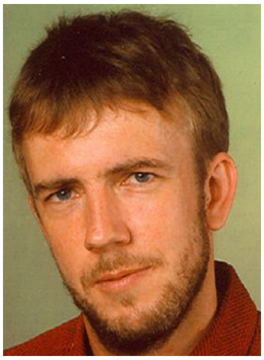

Thomas Fester studied biochemistry at the Eberhard Karls Universität Tübingen. He finished his PhD studies under the supervision of Dr. Wolfgang Schuster and Prof. Dr. Axel Brennicke in 1996 and then worked as a post-doctoral research fellow at the Leibniz-Institute of Plant Biochemistry in Halle in the department of Prof. Dr. Dieter Strack. His research is focussed on symbiosis and plant cell organelles, starting with his diploma thesis on cyanoplasts from *Cyanophora paradoxa*. While his PhD thesis covered RNA-binding proteins from plant mitochondria, current projects are referring to reactions of root cortical cell plastids to cell colonization by arbuscular mycorrhizal fungi. These projects, funded in the context of the DFG Focus Program "Molecular Basics of Mycorrhizal Symbioses", have resulted in the authorship of a Habilitation thesis, which has been submitted at the Faculty of Agriculture at the Martin Luther Universität Halle-Wittenberg. Besides his scientific projects Thomas Fester is engaged in a number of popular scientific activities which can be viewed at www.scivit.de.

Swanhild Lohse studied pharmacy at the Martin-Luther-Universität Halle-Wittenberg. Her diploma work under the supervision of Prof. Dr. Werner Roos dealt with the development of stabilising conditions for the investigation of the pH in vacuoles of cultured cells of *Eschscholtzia californica* in perfusion experiments. During her PhD studies at the Leibniz-Institute of Plant Biochemistry in Halle under the supervisiono f Dr. Thomas Fester and Prof. Dr. Dieter Strack she was investigating the development of plastids in colonised arbuscular mycorrhizal roots of *Medicago truncatula*. One focus of the work was plastid division studied by examination of the plastid division protein FtsZ. Since may 2005 Swanhild Lohse is looking for a new position.

Kristine Halfmann studied biotechnology at the Technische Universität Berlin. During her work at the Leibniz-Institute of Plant Biochemistry in Halle she investigated root exudates of mycorrhizal and non-mycorrhizal roots and analyzed the chemical composition of the accumulating apocarotenoids in *Zea mays*. After she left the institute in 2000, she has been working at the LEWA GmbH Co. in Leonberg, Germany.