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Short communication

Root exudate-stimulated RNA accumulation in the arbuscular mycorrhizal fungus *Gigaspora rosea*

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Abstract

Plant root exudates induce the transition from asymbiosis to presymbiosis in arbuscular mycorrhizal fungi. In order to get an insight into this developmental switch, two libraries of *Gigaspora rosea* and one library of *Gigaspora gigantea* were screened for fragments of genes that show enhanced RNA accumulation 1 h after addition of a semi-purified exudate fraction of carrot roots. Among 150 clones, 40 seemed to contain inserts of root exudate-induced genes. One of the genes, *GrosRbp1*, putatively encoding an RNA binding protein involved in developmental control showed RNA accumulation which correlates to the extent of stimulation of presymbiotic hyphal branching.

Keywords: Arbuscular mycorrhizal fungi; Gigaspora; Presymbiosis; RNA accumulation; Suppressive subtractive hybridisation; T-RFLP

Fungi of the phylum Glomeromycota form mutualistic interactions with roots from most plant species called arbuscular mycorrhizas (Smith and Read, 1997; Schuessler et al., 2001). This interaction is essential for the fungal partner of the symbiosis to fulfil its life cycle. Only the spores of these obligate biotrophic symbionts are able to germinate in the asymbiotic phase without the presence of a host root, but further growth of these germ hyphae and their characteristic branching during the presymbiotic phase is dependent on specific factors in plant root exudates (Giovannetti et al., 1993; Buée et al., 2000). The chemical structure of these factors was recently discovered to be a strigolactone (Akiyama et al., 2005). Differential RNA display analysis has indicated that an increase of the respiratory activity is one of the earliest responses at the developmental change from asymbiosis to presymbiosis. This was confirmed by analysis of a gene encoding a pyruvate carboxylase, by studying oxygen consumption and by microscopy showing changes in mitochondrial number and shape (Tamasloukht et al., 2003). Strigolactones were also observed to lead to this typical pattern of changes (Besserer et al., 2006).

In order to get further insight into the molecular basis of the fungal response to the presence of the plant root, it was aimed to identify more genes that are induced by the presence of the branching factor. As described for *Gigaspora rosea* (Tamasloukht et al., 2003), RNA was extracted from root exudate-stimulated hyphae of *Gigaspora gigantea*, cDNA was synthesised and subtracted by cDNA derived from non-stimulated control hyphae. In order to get a further enrichment of differentially expressed genes, subtracted and non-subtracted cDNAs were amplified with the nested primer pairs from the subtraction (Clontech, Heidelberg, Germany), one labelled with phosphoramidite fluorochrome 5-carboxy fluorescein (Eurogentec, Searing, Belgium). The PCR products were compared on an automatic sequencer following the protocol for T-RFLP analysis (Luckow et al., 2000). This showed that most differences were obtained in a size range of 1.1–1.4 kb. Subtracted cDNA was then separated by gel electrophoresis, the corresponding size range was extracted, cloned and 23 clones were sequenced (AJ968963–AJ968985). Eight cDNA fragments gave

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significant homologies to genes with a known function (BlastX search on 11 October 2006; Gish and States, 1993). These fragments were hybridised in reverse Northern blots to complex cDNA probes derived from *G. rosea* hyphae stimulated for 1 h with root exudates and from the corresponding controls of two independent experiments as described (Tamasloukht et al., 2003). In parallel, 92 *G. rosea* cDNA fragments of a subtractive library from exudate-stimulated hyphae (Tamasloukht et al., 2003) and 50 expressed sequence tags from activated spores (Stommel et al., 2001) were also screened. Among 150 cDNA fragments, 38 turned out to belong to different genes stimulated by the branching factor extracted from carrot root exudates. The fact that seven of eight inserts from the *G. gigantea* clones gave positive results underlined how useful the T-RFLP method is for the further enrichment of fragments from differentially expressed genes in cDNA populations.

Screening the sequence databases revealed that 18 fragments gave no significant similarities to any entries (*e*-value $> 10^{-5}$: AJ566917, AJ566918, AJ566919, AJ566922, AJ566924, AJ566925, AJ566926, AJ566931, AJ566932, AJ566935, AJ566939, AJ566940, AF090458, BE057063, BE057026, AF090461, BE057042, BE057045), while 20 fragments showed homology to genes with known function (Table 1). Two genes putatively encode enzymes localised in mitochondria which additionally confirmed the finding that the respiratory activity is one of the first responses of the fungus to the presence of the plant (Tamasloukht et al., 2003; Besserer et al., 2006). The other genes encoded proteins mainly involved in three basic cell processes, signal transduction, gene expression and DNA synthesis. Elements of the signalling machinery are necessary for the molecular cross talk between the plant and the fungus, which starts at the developmental switch from asymbiosis to presymbiosis while specific transcription and translation factors might be needed for the expression and modification of gene products important for further hyphal growth and branching. Such genes were also identified in *Glomus mosseae* as being induced at the next stage of the AM fungal life cycle during appressorium formation (Breuninger and Requena, 2004). The third process of the cell which seemed to be affected is DNA synthesis. It has been known for many years that AM fungi show low DNA replication activity during spore germination in the absence of a host root (Bianciotto and Bonfante, 1993; Becard and Pfeffer, 1993). Supplementing such hyphae with the branching factor increased the number of nuclei proportionally to the hyphal length (Buée et al., 2000). Based on this observation, the induction of genes

Table 1
ESTs from root exudate-induced genes

EST[a]	Length (nt)	Accession number	Similar to… from…	e-value	#1[b]	#2[b]
			Mitochondrial enzymes			
Ggig(RE)70	588	AJ968966	Pyruvate dehydrogenase E1 *Cryptococcus neoformans*	$7e^{-57}$	6.6	8.1
Gros(ASP)21	133	AF090463	Malonyl CoA-acyl carrier transacylase *Geobacter sulfurreducens*	$3e^{-09}$	3.8	3.4
			Signalling			
Gros(RE)76	306	AJ566912	MAP kinase kinase *Cryptococcus neoformans*	$4e^{-26}$	3.3	3.2
Ggig(RE)67	595	AJ968963	Serine/threonine protein kinase *Yarrowia lipolytica*	$8e^{-26}$	6.8	8.5
Gros(ASP)1	182	BE057024	Type IIB calcium ATPase *Glomus proliferum*	$4e^{-14}$	3.2	7.6
Ggig(RE)71	629	AJ968967	Leucine-rich repeat protein *Encephalitozoon cuniculi*	$3e^{-07}$	6.6	8.5
			DNA synthesis			
Gros(ASP)22	359	BE057033	Purine nucleoside phosphorylase *Schizosaccharomyces pombe*	$3e^{-08}$	3.3	3.2
Gros(ASP)52	352	BE057058	DNA helicase *Plasmodium falciparum*	$5e^{-08}$	22.8	9.9
Gros(ASP)55	517	BE057061	RNA helicase CDC28 *Schizosaccharomyces pombe*	$9e^{-65}$	2.8	5.4
			From genes to proteins			
Ggig(RE)69[c]	772	AJ968965	RNA binding protein *Aspergillus fumigatus*	$3e^{-65}$	6.1	4.2
Ggig(RE)68	545	AJ968964	40S ribosomal protein *Magnaporthe grisea*	$3e^{-77}$	4.7	4.9
Ggig(RE)G10	711	AJ968984	bHLHZip transcription factor *Tribolium castaneum*	$1e^{-06}$	2.6	3.1
Gros(ASP)9	164	AF090460	Ubiquitin *Phytophthora infestans*	$1e^{-22}$	3.1	2.8
Gros(ASP)35	429	BE057044	Protein glycotransferase *Branchiostoma belcheri*	$7e^{-21}$	5.5	10.3
Gros(ASP)39	291	BE057048	Polyubiquitin *Artemia franciscana*	$1e^{-21}$	3.2	3.3
Gros(ASP)49	268	BE057054	t-complex protein (chaperon) *Coccidioides immitis*	$4e^{-28}$	40.3	10.4
			Miscellaneous			
Gros(RE)65	267	AJ566907	H^+ ATPase *Glomus intraradices*	$2e^{-31}$	4.2	4.7
Gros(RE)D10	604	AJ566929	H^+ ATPase Glomus intraradices	$2e^{-71}$	6.2	7.2
Ggig(RE)F4	486	AJ968983	Homoserine dehydrogenase *Filobasidiella neoformans*	$9e^{-17}$	7.4	6.6
Gros(ASP)54	385	BE057060	Tetracycline efflux protein *Cryptococcus neoformans*	$5e^{-11}$	40.3	10.4

[a]ESTs obtained from SSH libraries of *G. rosea* (Gros(RE)) or *G. gigantea* (Ggig(RE)) or from a cDNA library of *G. rosea* activated spores (Gros(ASP)).
[b]Relative root exudate induction levels of expression measured by reverse Northern blot experiments using RNA from two independent experiments. A fragment of the 25S rRNA gene from *G. rosea* (AJ419662) was used for calibration.
[c]Highly homologous to Gros(RE)66 (AJ566908).