Mycorrhiza (1998) 7:301–306

ORIGINAL PAPER

Stephen H. Burleigh · Maria J. Harrison

A cDNA from the arbuscular mycorrhizal fungus *Glomus versiforme* with homology to a cruciform DNA-binding protein from *Ustilago maydis*

Accepted: 28 November 1997

Abstract A cDNA clone (Gv1) was isolated from a *Medicago truncatula*/*Glomus versiforme* root cDNA library by a differential screening technique. Gv1 represents a full-length *G. versiforme* mRNA encoding a 99-amino-acid polypeptide. This shares regions of identity with HMP1, a non-histone, cruciform DNA-binding protein from *Ustilago maydis*. Northern blot analysis indicated that Gv1 transcripts were present in mycorrhizal roots of *M. truncatula* and *M. sativa* (alfalfa), but absent in uncolonized roots of both species. A reverse transcription/polymerase chain reaction (RT-PCR) technique detected transcripts in the roots of the *M. sativa* non-mycorrhizal genotype MN NN-1008 colonized with *G. versiforme,* as well as in external hyphae collected from the roots of *M. sativa* colonized with *G. versiforme*. A genomic copy of Gv1 was detected by PCR in DNA isolated from the roots of *M. truncatula* colonized with *G. versiforme* and in DNA isolated from spores of *G. versiforme*. The PCR product identified in the mycorrhizal DNA preparation was identical in sequence to the cDNA, except for the presence of two introns. A homologue of Gv1 was detected in a second arbuscular mycorrhizal fungus, *Glomus intraradices*.

Key words Arbuscular mycorrhiza · Differential screening · Gv1 · HMP1 · Roots

Introduction

While the obligate nature of arbuscular mycorrhizal (AM) fungi has impeded molecular studies of this symbiosis, innovative techniques are now circumventing the once insurmountable problem of obtaining AM fungal sequences. Plasmid (Zézé et al. 1994) and phage (Franken and Gianinazzi-Pearson 1996) genomic libraries made from pure spore DNA have provided the means to identify AM fungal sequences in the absence of contaminating plant genes. Ribosomal sequences from AM fungi have been identified by PCR using AM fungal-specific 18S small-subunit primers (Simon et al. 1992), by restriction fragment length polymorphism analysis using 5.8S rRNA internal transcribed spacer universal primers (Sanders et al. 1995) and by other similar PCR-based techniques (Lanfranco et al. 1995; Abbas et al. 1996; Lloyd-MacGilp et al. 1996; Simon 1996). These sequences revealed genetic variation both within and between AM fungal species, and led to the initial molecular phylogenetic characterization of the Glomales. These data also enabled researchers to detect AM fungal species in mycorrhizae from various host plants (Di Bonito et al. 1995) and in the soil (Claassen et al. 1996).

One of the first non-ribosomal AM fungal sequences with homology to a gene from another organism, an assimilatory nitrate reductase homologue, was identified in DNA from *Glomus* spores using a PCR-based method (Kaldorf et al. 1994). Recently a phosphate transporter cDNA from *G. versiforme* (GvPT) was identified by screening a mycorrhizal cDNA library with the *PHO84* gene from *Saccharomyces cerevisiae* (Harrison and van Buuren 1995). The function of the GvPT protein was established by complementation of a yeast phosphate-uptake mutant and its origin was established by PCR amplification of spore DNA followed by sequence analysis.

Our current understanding of fungal gene expression in mycorrhizae is limited and identification of fungal cDNAs is a necessary prerequisite for the analysis of the mycorrhizal symbiosis at the molecular level. Here we report the characterization of a cDNA clone (Gv1) from *G. versiforme*, which was isolated by a differential screen to identify genes showing altered expression during the interaction between *G. versiforme* and *M. truncatula*.

S. H. Burleigh · M. J. Harrison (✉)
The Samuel Roberts Noble Foundation, Plant Biology Division, 2510 Sam Noble Parkway, Ardmore, OK 73401, USA
Fax: +1-405-221-7380; e-mail: mjharrison@noble.org

Materials and methods

Materials and growth conditions

The plant materials included *M. truncatula* Gaertn 'Jemalong' (line A17), *M. sativa* 'Kanza' and the alfalfa non-mycorrhizal genotype MN NN-1008 (kindly donated by D. K. Barnes, University of Minnesota). The fungal strains used in the experiments were *G. versiforme* (Karst Berch.) and *G. intraradices* [UT176-1, International Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM), West Virginia University, Morgantown, W.Va., USA]. Plants were grown from seed for 2 weeks, inoculated with the roots of mycorrhizal leek (*Allium porrum* L.) and grown for an additional 5 weeks prior to harvest. Non-mycorrhizal control plants were inoculated with leek roots grown without fungus. Treatments consisted of 4–5 plants per pot. Plants were watered daily with de-ionized water and fertilized weekly with half-strength Hoaglands' solution (Arnon and Hoagland 1940), pH 6.0, containing 5 mM KNO_3 and 20 μM KH_2PO_4. Details of plant and fungal culture and colonization procedures have been described previously (Harrison and Dixon 1993).

A small portion of the roots harvested from each treatment was examined for fungal colonization as described previously (Harrison and Dixon 1993). Colonization was defined as the total root length with surface and/or interior fungal structures as observed microscopically. Colonization of *M. truncatula*, *M. sativa* and MN NN-1008 with *G. versiforme* was estimated at 85%, 86%, and 42%, respectively. No fungus was detected in the uncolonized treatments. The mycorrhizae of *M. truncatula/G. versiforme* and *M. sativa/G. versiforme* were typical of an AM association, consisting of external hyphae and appressoria on the root surface and inter- and intracellular hyphae, vesicles and arbuscules within the root. The interaction between the fungus and MN NN-1008, as previously described by Bradbury et al. (1991), was limited to external hyphal growth and appressoria formation on the root surface, without any visible signs of fungal growth within the root. Since AM fungi do not form a complete association with this mutant, the term colonized in this context refers to the external colonization.

Differential screening

Duplicate filter-lifts of a *M. truncatula/G. versiforme* root cDNA library were hybridized with ^{32}P-labeled cDNAs derived from total RNA from uncolonized MN NN-1008 roots or MN NN-1008 roots colonized with *G. versiforme*. Details of the differential screen, excision of the positive plaques and sequence analysis were described previously (Burleigh and Harrison 1997). Sequence comparisons were made using BLASTX or BLASTP programs and also by PCGENE.

Northern blot analysis

RNA isolation from roots (Burleigh and Harrison 1997) and AM hyphae (Harrison and van Buuren 1995) has been described previously. Northern blotting and hybridization (Figs. 3, 4) were carried out as described by Sambrook (1989) using 10 μg of RNA per treatment. Briefly, blots were hybridized at 42 °C in 50% formamide, 4 × SSPE, 1% SDS, 0.5 × Denhardt's solution, 100 μg/ml salmon sperm DNA. The final washing conditions were 2 × SSPE, 1% SDS at 65 °C. Following hybridization with Gv1, the blots were probed with an 18S rRNA probe (pSR1-2B3). This provides a control for loading and transfer and enables comparison of expression levels between treatments of the same blot.

PCR amplification

The PCR amplification technique used to identify Gv1 transcripts from DNA preparations has been described previously (Harrison and van Buuren 1995). The oligonucleotide primers used in the reactions (5';ACTACCAAGGTGCTGTCAAGGAAACTATC, 3'; GGTCACATTAATCACTTATTTGAAA) were based on the Gv1 cDNA sequence (Fig. 1A). The PCR reaction consisted of an initial denaturation step at 94 °C for 5 min followed by 30 cycles of melting at 94 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 30 s using a Perkin Elmer Geneamp 2400 thermal cycler. Approximately 10 ng of DNA was used in each reaction (Fig. 2). The spore DNA was digested with *Eco*R1 and *Xho*1 prior to the PCR reaction, since restriction enzyme digestion has been observed to facilitate the amplification of AM spore DNA by PCR (Burleigh and Harrison, unpublished data). The PCR product identified in the root DNA isolated from mycorrhizal *M. truncatula* was purified using a Biorad Prep-A-Gene kit prior to sequencing.

Reverse transcription-PCR

The reverse transcription-PCR technique (RT-PCR) used to identify Gv1 transcripts in the various RNA preparations was carried out following Kawasaki (1990). One microgram of total RNA was used for each reverse transcription reaction (Figs. 5, 6), except for the hyphae treatment (Fig. 6), which contained only trace levels of RNA due to a limited amount of material. One microliter of these reverse transcribed products was then used for the subsequent PCR reaction. Since the amount of fungal RNA was not uniform in the RT-PCR experiments, only qualitative differences in the hybridization patterns of these treatments were considered for discussion. The products of the PCR and RT-PCR reactions were blotted and hybridized as described above.

Results

Isolation of a fungal cDNA clone (Gv1) from a mycorrhizal cDNA library

The intent of our screening strategy was to identify genes expressed during the early stages of the AM mycorrhizal symbiosis by utilizing the *M. sativa* non-mycorrhizal genotype MN NN-1008. This mutant blocks AM fungi from penetrating its roots, resulting in fungal colonization of the root surface without the development of internal fungal structures such as intercellular hyphae, vesicles and arbuscules (Bradbury et al. 1991). Thus the fungal RNA present in this tissue would represent genes expressed primarily in external hyphae and appressoria.

Approximately 70 000 plaques of a *M. truncatula/G. versiforme* cDNA library were differentially screened using labeled cDNAs derived from total root RNA isolated from MN NN-1008 and MN NN-1008 colonized with *G. versiforme*. One cDNA clone, Gv1, hybridized to the probe derived from the colonized roots, but not to the probe derived from the uncolonized roots. Sequence analysis revealed that the Gv1 cDNA is 520 bp in length, including a 20-bp poly (A)+ tail at the 3' end (Fig. 1A). Gv1 was used as a probe to rescreen the same library and two additional clones of identical size were obtained. Gv1 hybridized exclusively to a transcript of approximately 0.5 kb (see below) and was thus assumed to represent the entire transcript. The complete cDNA sequence has been deposited in the EMBL

Fig. 1A, B The nucleotide and amino acid sequence of Gv1 and its comparison to HMP1. **A** The nucleotide sequence of Gv1. Bases are numbered at right, the proposed open reading frame is in upper case, intron junctions are in bold and the oligonucleotides used as the primer-pair are underlined. **B** Comparison of translated ORF 54–353 of Gv1 with ORF 26–414 of HMP1 (* identical amino acids, **.** amino acids with similar characteristics, and – gaps in the sequence)

A

```
caaaaaaaaa accaatcagt ttaaatctat atattcatta aaaaaaattc   50
aacATGTCTG AACCATCCAG AGTCGATGCT AACTCCAAAT ACTACCAAGG  100
TGCTGTCAAG GAAACTATCG GCAAAGCGAT AGGCAATGAA CAAATGGAAG  150
CCGAAGGAAA AGCCAAAAAA CTTGAAGGTG AAGGTGAATA CAAAGCAGCA  200
CAAACAAAAG GACAAGCAGA AGGAGTTAAA GACAGCGTCA CTGGAACTGT  250
AAAAGAAAAC ACGGGTAAAG CCATCGGTAA TCAACAGATG CAAGCTGAAG  300
GCAATATCAC CAAAAATACT GGTGATGCAA AGAAAGAAGC TCATAAACAT  350
TAAatccatt aataaggaca ttttatctta ttttttttta ttattattat  400
ttagcaataa tgcaaaatcc ccatacattt tttatgtatt taaaagcttg  450
atctttttat atttaaataa atttttttcaa ataagtgatt aatgtgaccc  500
aaaaaaaaaa aaaaaaaaaa  520
```

B

```
Gv1    MSEPSRVDANSKYYQGAVKETIGKAIGNEQMEAEGKAKKLEGEGEYKAAQ 50
HMP1   MSEPSKVNGNYNSVAGTVKETIGNALGSTEWQKAGKEQHAKGEGEIKAAQ 50
       *****.*..* .  .*.******.*.*... . .**... .**** ****

Gv1    TKGQAEGVKDSVTGTVKENTGKAIGNQQMQAEGNITKNTGDAKKEAHKH  99
HMP1   AQGYAEGTKDQVSGKIDNVVGAVTGDKSKELSGKAQQESGKAQKEINS-  98
       ..* ***.** *.*.... .* ..*.. .. .*.  ...*.*.** ..
```

database under the Genbank accession number AF034574.

The protein encoded by Gv1 is 99 amino acids in length (Fig. 1B). It is predicted to be primarily hydrophobic and contain two potential protein kinase C phosphorylation sites at amino acids 65 and 70, two potential casein kinase II phosphorylation sites at amino acids 5 and 65 and a potential tyrosine kinase phosphorylation site at amino acid 46 (data not shown). The protein shares 42% amino acid identity and 82% similarity with HMP1, a cDNA encoding a non-histone cruciform DNA-binding protein from the fungus *Ustilago maydis* (Dutta et al. 1997). The amino acid identity includes four regions of almost complete identity to the HMP1 peptide (Fig. 1B).

Origin of the Gv1 cDNA clone

As the cDNA clone was identified from a mycorrhizal cDNA library, Gv1 could represent either a plant or a fungal mRNA. Gv1 did not hybridize to a Southern blot of *M. truncatula* DNA (data not shown), so the origin of the cDNA was further investigated by PCR analysis. DNA was isolated from the roots of *M. truncatula* colonized with *G. versiforme*, an uncolonized *M. truncatula* control and from spores of *G. versiforme*. The DNAs were amplified by PCR using a primer-pair based on the Gv1 cDNA sequence (Fig. 1A). The products of the reaction were separated by gel electrophoresis and stained with ethidium bromide. A single 0.4-kb fragment was visible only in the Gv1 cDNA positive control (data not shown). However, when the gel was blotted and probed with labeled Gv1 cDNA, a 0.55-kb fragment was detected in the DNA from *M. truncatula* colonized with *G. versiforme* and in DNA from spores of *G. versiforme* (Fig. 2). A smaller, 0.4-kb product was also detected in the spore DNA, which could represent a contaminant, possibly from the control DNA. This product was not observed in the mycorrhizal DNA treatment nor in spore DNA from another preliminary experiment (data not shown). No products were detected in DNA isolated from the uncolonized control nor in a minus-DNA control.

The sequence of the 0.55-kb fragment amplified from root DNA isolated from mycorrhizal *M. truncatula* was subsequently determined to be identical to Gv1, except for the presence of two introns; the first is 79 nucleotides in length and is located at bp 178 on the cDNA and the second is 73 nucleotides and located at bp 256 (Fig. 1A). Both introns were bound by a 5' GT and a 3' AG. The increased size of this PCR product (0.55 kb) was consistent with the presence of these introns relative to the product made when the Gv1 cDNA (0.4 kb) was used as a template. Together the

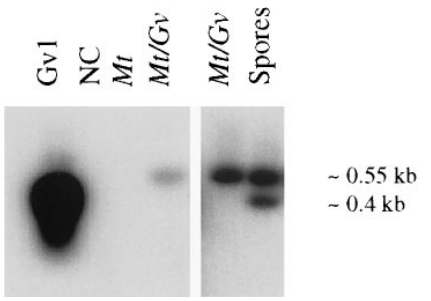

Fig. 2 Amplification of the Gv1 gene from DNA isolated from mycorrhizal roots and spores of *Glomus versiforme*. PCR products from a Gv1 cDNA control (*Gv1*), a minus-DNA control (*NC*), uncolonized *M. truncatula* roots (*Mt*), *M. truncatula* roots colonized with *G. versiforme* (*Mt/Gv*) and from *G. versiforme* spores (*spores*). DNA from the various treatments was PCR amplified, separated by gel electrophoresis, blotted and probed with ^{32}P-labeled Gv1 cDNA

PCR and sequence analyses indicate that Gv1 originated from the *G. versiforme* genome.

Gv1 transcripts are present in mycorrhizal roots of *M. truncatula*, *M. sativa* and MN NN-1008 colonized with *G. versiforme* or *G. intraradices*

Northern blot analyses were used to investigate the expression of the Gv1 gene in mycorrhizal roots of *M. truncatula*, *M. sativa* and MN NN-1008 colonized with *G. versiforme* and in mycorrhizal roots of *M. truncatula* colonized with *G. intraradices*. Total RNA was isolated from the roots, separated by gel electrophoresis, blotted and probed with labeled Gv1 cDNA. The probe hybridized to a transcript of 0.5 kb present in the roots of mycorrhizal *M. truncatula* and *M. sativa* (Fig. 3). The intensity of the band derived from RNA isolated from mycorrhizal *M. sativa* was considerably stronger than that of the band derived from mycorrhizal *M. truncatula*, despite equal loading as determined by the rDNA blot, similar levels of fungal colonization (86% and 85%, respectively) and identical growth conditions. Transcripts were not detected in uncolonized roots of these species nor in the roots of either uncolonized or colonized MN NN-1008. A transcript of 0.5 kb was also detected in the roots of *M. truncatula* colonized by an another species of mycorrhizal fungus, *G. intraradices* (Fig. 4).

While northern blot analysis did not detect Gv1 transcripts in the roots of MN NN-1008 colonized with *G. versiforme*, a 0.4-kb product was detected by RT-PCR. Total root RNA was reverse transcribed, amplified by PCR using the Gv1 primer-pair and the products separated by gel electrophoresis (Fig. 5A). This gel was subsequently blotted and probed with Gv1 (Fig. 5B). A 0.4-kb fragment was detected in the root RNA from mycorrhizal *M. sativa*, *M. truncatula* and MN NN-1008. Transcripts were not detected in root RNA from uncolonized *M. truncatula*. As shown in Fig. 5A, the products of the PCR reactions are not always visible following gel electrophoresis.

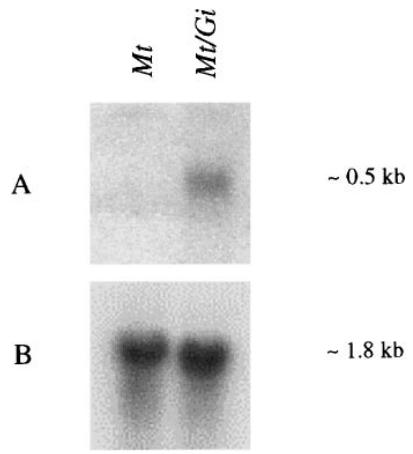

Fig. 4A, B Detection of a Gv1 homologue in *M. truncatula* colonized with *G. intraradices*. Northern blots of total RNA from the roots of *M. truncatula* (*Mt*) and *M. truncatula* colonized with *G. intraradices* (*Mt/Gi*). RNA from the two treatments was separated by gel electrophoresis, blotted and probed with **A** ^{32}P-labeled Gv1 cDNA or **B** ^{32}P-labeled pSR1-2B3 (18S rRNA)

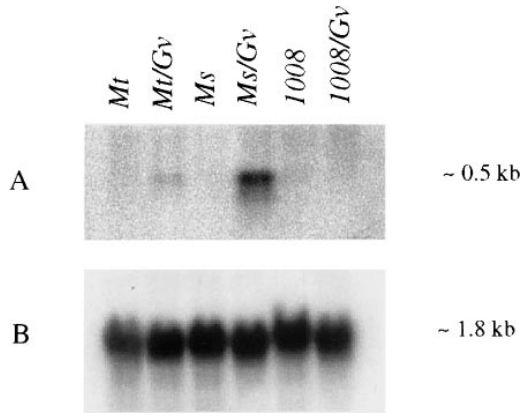

Fig. 3A, B Detection of Gv1 transcripts in *M. truncatula* and *M. sativa* roots colonized with *G. versiforme*. Northern blots of total RNA from the roots of *M. truncatula* (*Mt*), *M. truncatula* colonized with *G. versiforme* (*Mt/Gv*), *M. sativa* (*Ms*), *M. sativa* colonized with *G. versiforme* (*Ms/Gv*), MN NN-1008 (1008) and MN NN-1008 colonized with *G. versiforme* (*1008/Gv*). RNA from the various treatments was separated by gel electrophoresis, blotted and probed with **A** ^{32}P-labeled Gv1 cDNA or **B** ^{32}P-labeled pSR1-2B3 (18S rRNA)

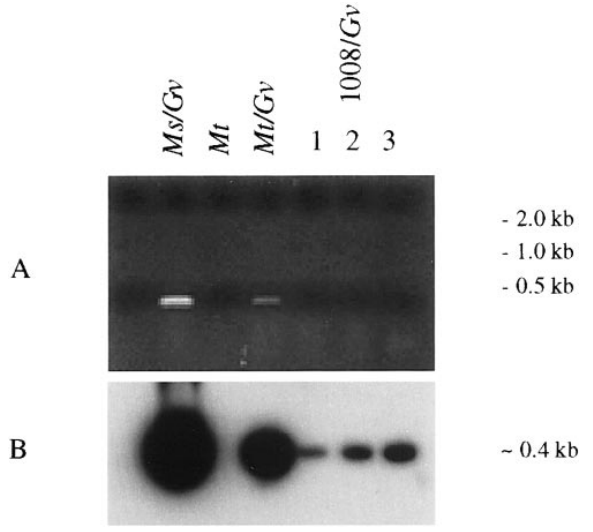

Fig. 5A, B Detection of Gv1 transcripts in the roots of the alfalfa mycorrhizal mutant MN NN-1008 colonized with *G. versiforme*. RT-PCR products from the roots of *M. sativa* colonized with *G. versiforme* (*Ms/Gv*), uncolonized *M. truncatula* (*Mt*), *M. truncatula* colonized with *G. versiforme* (*Mt/Gv*) and in three samples of MN NN-1008 colonized with *G. versiforme* (*1008/Gv*, *1–3*). RNA from the various treatments was reverse transcribed, PCR amplified, separated by gel electrophoresis, and **A** stained with ethidium bromide or **B** probed with ^{32}P-labeled Gv1 cDNA

Gv1 transcripts are present in external hyphae

To further investigate the site of expression of the Gv1 gene, samples of external hyphae were collected from *M. sativa* roots colonized with *G. versiforme*. RNA was prepared from these samples and the presence of the Gv1 transcript investigated by RT-PCR. A 0.4-kb product was detected in two out of three hyphal samples (Fig. 6), which indicated that Gv1 was expressed in the external hyphae.

Discussion

We have identified a cDNA clone, Gv1, which represents a gene from the AM fungus *G. versiforme*. The full-length cDNA is 520 bp and contains an open reading frame encoding a polypeptide of 99 amino acids. The Gv1 genomic sequence revealed that it contains two introns, both bound by a 5' GT and a 3' AG. The presence of a homologue in *G. intraradices* indicates that this gene is found in other AM species. The strong hybridization of Gv1 to this homologue under stringent conditions indicates high sequence similarity between the two genes.

Gv1 shares identity with HMP1, a cDNA encoding a non-histone cruciform DNA-binding protein from the fungus *Ustilago maydis* (Dutta et al. 1997). The HMP1 protein was shown to bind cruciform-like oligonucleotide complexes in vitro and have additional high mobility group (HMG)-like characteristics, including a small size and solubility in dilute acid (Kotani et al. 1993; Dutta et al. 1997). Binding assays demonstrated that the HMP1 protein was involved in inducing or maintaining DNA structure, but not directly involved in genetic recombination. Because the HMP1 protein had only low levels of sequence homology to other cruciform DNA-binding proteins, the authors suggested that it represented a new class of HMG-like architectural proteins (Dutta et al. 1997). The relatively high level of amino acid similarity of the Gv1 and HMP1 proteins suggests that Gv1 represents a second member of this gene family. DNA-binding assays using the Gv1 protein will be necessary to determine whether it displays similar activity to the HMP1 protein.

While we have conclusively demonstrated that Gv1 is expressed in external hyphae, from the current data we can only speculate as to additional locations of Gv1 gene expression in mycorrhiza. The relatively large differences in transcript abundance observed between the wild-type mycorrhiza of *M. sativa* and that of the non-mycorrhizal genotype MN NN-1008 is consistent with localization of Gv1 in external hyphae, since there was a relatively large difference in the degree of colonization between the two plant types (86% and 42%, respectively). However, these differences could also be explained by the presence of Gv1 transcripts in internal fungal structures associated with the wild-type mycorrhiza, such as intercellular hyphae, vesicles or arbuscules. These results could also be explained by the dependence of Gv1 expression, in external hyphae or other undetermined structures, on carbohydrates derived from the plant as a result of a functional symbiosis. The relatively large difference in transcript levels observed in the mycorrhiza of *M. sativa* and *M. truncatula* may reflect differences in the amount of external hyphae present in the two mycorrhizas, which was not quantified in these experiments. Differences in expression might also reflect AM fungal-plant compatibility in the two associations.

The PCR and RT-PCR techniques proved to be valuable tools in our study. PCR analysis followed by sequencing was vital for showing that Gv1 was fungal in origin, since a Southern blot of *G. versiforme* spore DNA using Gv1 as a probe was not successful (data not shown). While northern blot analysis failed to identify Gv1 transcripts in the roots of MN NN-1008 colonized by *G. versiforme*, the RT-PCR technique successfully detected low levels of transcript in this mutant. This technique also identified Gv1 transcripts in external hyphae, which would have been near impossible to determine using northern analysis due to the lack of sufficient fungal tissue. Conclusions based solely on our northern data, hence, would have greatly limited our current understanding of Gv1 gene expression.

Our cloning strategy was designed to identify genes expressed primarily during the early stages of the symbiosis. Clearly this would be expected to include genes expressed in the external hyphae and therefore accounts for the identification of Gv1. In this instance, the differential screening strategy proved to be relatively sensitive, since the small pool of Gv1 transcript present in the MN NN-1008 genotype was abundant enough to identify a Gv1 cDNA in the mycorrhizal library. Gv1 is the second complete AM fungal cDNA cloned thus far, and provides a useful molecular marker for the fungus in the AM symbiosis. Cloning the genomic sequence of Gv1 may have utility in the production of vectors for future attempts to transform AM fungi.

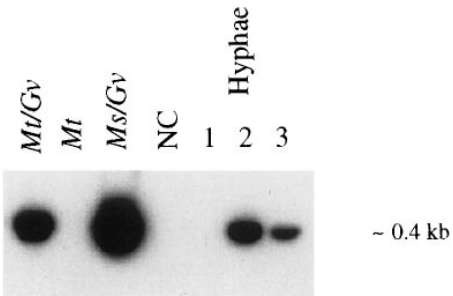

Fig. 6 Detection of Gv1 transcripts in external hyphae collected from *M. sativa* colonized with *G. versiforme*. RT-PCR products from the roots of *M. truncatula* colonized with *G. versiforme* (*Mt/Gv*), uncolonized *M. truncatula* (*Mt*), *M. sativa* colonized with *G. versiforme* (*Ms/Gv*), a minus-RNA control (*NC*) and three samples of external hyphae collected from *M. sativa* colonized with *G. versiforme* (*Hyphae, 1–3*). RNA from the various treatments was reverse transcribed, PCR amplified, separated by gel electrophoresis and probed with ^{32}P-labeled Gv1 cDNA

Acknowledgements The authors thank members of Plant Biology, Samuel Roberts Noble Foundation, for helpful discussions, X. S. Ding and B. Cassidy for critical reading of the manuscript and Allyson Wilkins for organization of the references. The work was supported by the Samuel Roberts Noble Foundation.

References

Abbas JD, Hetrick BAD, Jurgenson JE (1996) Isolate-specific detection of mycorrhizal fungi using genome specific primer pairs. Mycologia 88:939–946

Arnon DI, Hoagland DR (1940) Crop production in artificial culture solutions and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. Soil Sci 50:463–483

Bradbury SM, Peterson RL, Bowley SR (1991) Interaction between three alfalfa nodulation genotypes and two *Glomus* species. New Phytol 119:115–120

Burleigh SH, Harrison MJ (1997) A novel gene whose expression in *Medicago truncatula* roots is suppressed in response to colonization by vesicular-arbuscular mycorrhizal (VAM) fungi and to phosphate nutrition. Plant Mol Biol 34:199–208

Claassen VP, Zasoski RJ, Tyler BM (1996) A method for direct soil extraction and PCR amplification of endomycorrizal fungal DNA. Mycorrhiza 6:447–450

Di Bonito R, Elliott ML, Des Jardin EA (1995) Detection of an arbscular mycorrhizal fungus in roots of different plant species with the PCR. Appl Environ Microbiol 61:2809–2810

Dutta S, Gerhold DL, Rice M, Germann M, Kmiec EB (1997) The cloning and overexpression of a cruciform binding protein from *Ustilago maydis*. Biochim Biophys Acta 1352 (3):258–266

Franken P, Gianinazzi-Pearson V (1996) Construction of genomic phage libraries of the arbuscular mycorrhizal fungi *Glomus mosseae* and *Scutellospora castanea* and isolation of ribosomal RNA genes. Mycorrhiza 6:167–173

Harrison MJ, Dixon RA (1993) Isoflavonoid accumulation and expression of defense gene transcripts during the establishment of vesicular-arbuscular mycorrhizal associations in roots of *Medicago truncatula*. Mol Plant Microbe Interact 6:643–654

Harrison MJ, van Buuren MJ (1995) A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. Nature 378:626–629

Kaldorf M, Zimmer W, Bothe H (1994) Genetic evidence for the occurrence of assimilarity nitrate reductase in arbuscular mycorrhizal and other fungi. Mycorrhiza 5:23–28

Kawasaki ES (1990) Amplification of RNA. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols. A guide to methods and applications. Academic, San Diego

Kotani H, Kmiec EB, Holloman WK (1993) Purification and properties of a cruciform DNA-binding protein from *Ustilago maydis*. Chromosoma 102:348–354

Lanfranco L, Wyss P, Marzachi C, Bonfante P (1995) Generation of RAPD-PCR primers for the identification of isolates of *Glomus mosseae*, an arbuscular mycorrhizal fungus. Mol Ecol 4:61–68

Lloyd-MacGilp SA, Chambers SM, Dodd JC, Fitter AH, Walker C, Young JPW (1996) Diversity of the ribosomal internal transcribed spacers within and among isolates of *Glomus mosseae* and related mycorrhizal fungi. New Phytol 33:103–111

Sambrook J, Fritisch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Sanders IR, Alt M, Groppe K, Boller T, Wiemken A (1995) Identification of ribosomal DNA polymorphisms in spores of the Glomales: application to studies on the genetic diversity of arbuscular mycorrhizal fungal communities. New Phytol 130:419–427

Simon L (1996) Phylogeny of the Glomales: deciphering the past to understand the present. New Phytol 133:95–101

Simon L, Lalonde M, Bruns TD (1992) Specific amplification of 18S fungal ribosomal genes from vesicular-arbuscular endomycorrhizal fungi colonizing roots. Appl Environ Microbiol 58:291–295

Zézé A, Dulieu H, Gianinazzi-Pearson V (1994) DNA cloning and screening of a partial genomic library from an arbuscular mycorrhizal fungus. Mycorrhiza 4:251–254