

## Sequence Similarity of Glutathione *S*-Transferase between a Land Plant and an Arbuscular Mycorrhizal Fungus

Hiromichi SAWAKI

*Bio-oriented Technology Research Advancement Institution (BRAIN), Laboratory of Soil Microbiology, Department of Ecology, National Grassland Research Institute, 768 Senbon-matsu, Nishinasuno, Tochigi 329-2793, Japan*

*Present address: Glycogene Function Team, Research Center for Glycoscience, The National Institute of Advanced Industrial Science and Technology, AIST Tsukuba Central 2, Tsukuba, Ibaraki 305-8568, Japan*  
*Corresponding author* E-mail address: hiro-sawaki@aist.go.jp

Received 29 March 2002, accepted 13 June 2002

### Abstract

Arbuscular mycorrhiza is the most ubiquitous and oldest symbiosis between land plants and fungi in terrestrial areas. However, details of the symbiosis are largely unknown. DNA sequence information on arbuscular mycorrhizal fungi is currently being accumulated. An expressed sequence tag (EST) clone from *Glomus intraradices* encodes glutathione *S*-transferase (GST), *GiGSTF1*, whose sequence is significantly similar to that of plant phi-class GSTs, which is a subgroup of a large gene family in plant GST. This study analyzes the phylogenetic relationship between plant and fungal GSTs, and the origin of plant phi-class GST is discussed from the standpoint of plant evolution.

**Keywords:** coevolution, gene-transfer, molecular-evolution, symbiosis.

### Abbreviations

AM, arbuscular mycorrhiza; GST, glutathione *S*-transferase; EST, expressed sequence tag.

### Introduction

Arbuscular mycorrhiza (AM) is the most ubiquitous symbiosis between land plant and fungi. AM fungi consist of more than 150 species (Walker and Trappe, 1993; Walker and Vestberg, 1998), and they are monophyletic, belonging to the class Glomeromycota (Schuessler *et al.*, 2001). In contrast to other microbe-plant symbioses, the AM symbiosis is characterized by very low, and often absent host-symbiont specificity. This may have resulted from long coevolution with the host plant. It has been estimated that AM fungi originated 400–460 million years ago, based on molecular-clock analysis and the fossil record (Simon *et al.*, 1993; Remy *et al.*, 1994; Redecker *et al.*, 2000). It is reasonable to assume that the earliest land plants lacked functional roots, and therefore that they depended on AM-like symbiotic fungi for nutrition and water uptake from the environment.

It is well known that molecular studies support the endosymbiosis hypothesis on the origin of

eukaryotic cells. The prolonged coexistence of nuclei and organelles in a cell allows gene-transfer events to occur repeatedly. Evidence for such events was found at the level of sequence similarity among genes in nuclei and ancestral sister organisms of organelles (Horiike *et al.*, 2001; Rujan and Martin, 2001).

Glutathione *S*-transferase (GST) forms a large gene family consisting of eight classes in various organisms, including bacteria, mammals, and plants. Land plants contain four classes of GSTs: phi, zeta, tau, and theta. The phi and tau classes are plant specific (Edwards *et al.*, 2000). *Arabidopsis thaliana*, the smallest genome among flowering plants that have been sequenced completely, inherits 47 genes from GST and its homologs. An AM fungal GST was found in expression sequence tag (EST) data of *Glomus intraradices*, an AM fungus (Sawaki and Saito, 2001). Here, I report sequence similarity between the GST genes derived from a land plant and an AM fungus, and from this I infer gene transfer from ancestral AM fungi to early land plants.

### Materials and Methods

The putative peptide sequence of GST in an

arbuscular mycorrhizal fungus, *Glomus intraradices* was deduced from nucleotide sequence of an EST clone, which was deposited under accession number AU082837 in a public DNA database, DDBJ (Sawaki and Saito, 2001). The AU082837 peptide was compared over 159 amino acid residues with GST and its homologs in other organisms: a land plant *Arabidopsis thaliana*; fungi consisting of *Saccharomyces cerevisiae*, *Issatchenkia orientalis*, and *Schizosaccharomyces pombe*; a green alga, *Coccomyxa* sp.; and a cyanobacterium *Synechocystis* sp. PCC6803. Forty-seven peptide sequences in *A. thaliana*, annotated as GST or its homolog, were retrieved (via the Internet: <http://www.tigr.org/>) from the *Arabidopsis thaliana* database at The Institute for Genomic Research. Sequences from other organisms were similarly downloaded from the NCBI and EMBL databases. Computational phylogenetic analyses of the peptide sequences were performed using ClustalX (Thompson *et al.*, 1997) and BioEdit (Hall, 1999) software, as described previously (Sawaki *et al.*, 1998; Sawaki and Saito, 2001).

## Results

*Glomus intraradices* EST clone AU082837 inherited the open-reading frame of 159 amino acids, which encodes a partial-peptide-lacking initiation codon (Sawaki and Saito, 2001). The peptide sequence of AU082837 manifested strong similarity with GST peptides derived from various land plants including *Arabidopsis thaliana*, *Zea mays*, and *Oryza sativa* by BLAST search (data not shown). The AU082837 coding peptide was analyzed phylogenetically with 47 GST sequences annotated in complete genome sequence of *A. thaliana*. **Fig. 1** shows four clusters on the phylogenetic tree, which agrees well with morphology from the full-length sequence data set of *A. thaliana*, and in the figure they are denoted using nomenclature proposed by Edwards *et al.* (2000). The resulting phylogenetic tree indicates that the AU082837 peptide was clustered with phi-class GSTs with a high bootstrap frequency. Therefore, the AU082837 peptide sequence is designated as *GiGSTF1* in the present report in accordance with the above nomenclature. *GiGSTF1* and the most similar peptide in *A. thaliana*, At3g03190.60247.t00041, were compared with GSTs from other organisms: algae, cyanobacteria, and fungi. **Fig. 2** pairs *A. thaliana* phi-class GST with *GiGSTF1* with a high bootstrap frequency. This pair was further clustered with three fungal GSTs. In contrast, algal GST was clustered with those of cyanobacteria. No cyanobacterial

genes indicated similarity with *A. thaliana* phi-class GST greater than that of *GiGSTF1*.

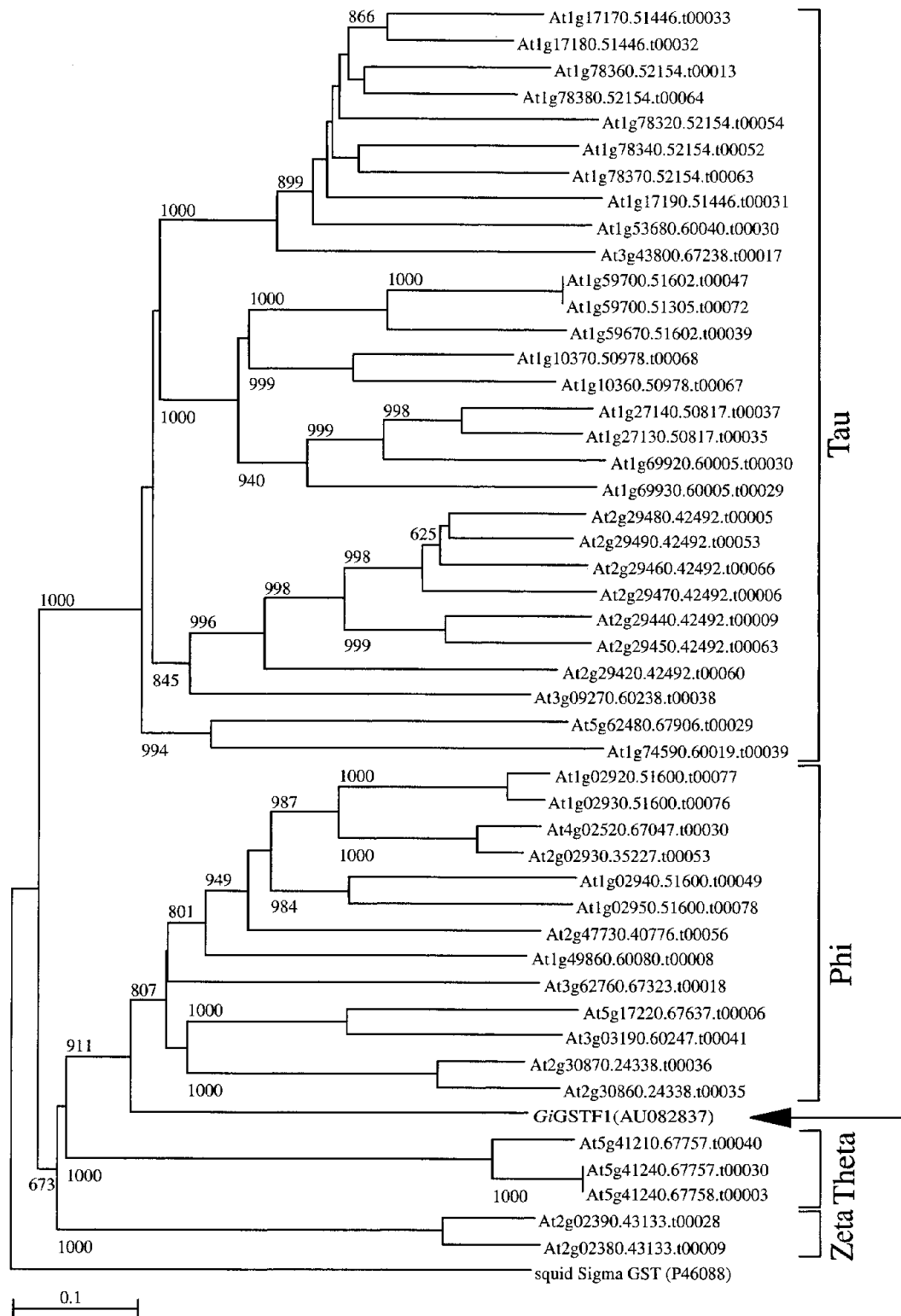
## Discussion

*GiGSTF1* indicated significant similarity with plant phi-class GST, as shown in **Fig. 1** and **2**. Phi-class GST is a plant-specific group (Edwards *et al.*, 2000). The phylogenetic tree in **Fig. 2** shows that the plant phi-class GST belongs to the fungal GST cluster. In contrast, Edwards *et al.* (2000) described that cyanobacterial GST Q55139 indicated similarity with plant phi-class GST. In the present study, the algal GST was more similar to the cyanobacterial GSTs than to the plant phi-class GST. These results suggest that plant-specific phi-class GST originated from fungi rather than from green algae or cyanobacterium.

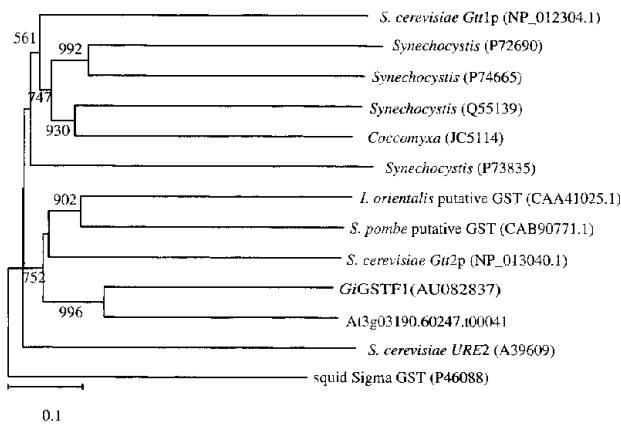
Molecular studies of ribosomal genes support the hypothesis that land plants developed from a monophyletic ancestor, a fresh-water green alga (Kranz *et al.*, 1995; Graham *et al.*, 2000). However, the sequence similarity of phi-class GST between plant and AM fungus appeared to conflict with this hypothesis, so that alternative hypotheses were proposed on the origin of land plants. Pirozynski and Malloch (1975) proposed that land plants are descendents of further symbiosis between algae and fungi. From the standpoint of morphology, the tip-growth and cell-polarization processes of land plants are absent in primitive green algae. It was hypothesized that these genetic capacities were donated from symbiotic fungi (Jorgensen, 1993).

Several studies have presented molecular evidence of gene transfer from organelles to the nucleus (Nugent and Palmer, 1991, 1993; Kadowaki *et al.*, 1996; Martin *et al.*, 1998; Kubo *et al.*, 1999; Kubo and Kadowaki, 2001). Kubo and Kadowaki (2001) pointed out that transfer of the *sdh4* gene from the mitochondria to the nucleus happened independently in three plant species – *Arabidopsis*, pea, and rice – after these species diverged. This capability of gene incorporation into the nucleus has probably been maintained in land plants up to the present day. The gene-transfer event should be detectable as a sequence similarity of the genes.

Present AM fungi form the most ubiquitous mutual endosymbiosis, exchanging soil-derived nutrition and photosynthates with various land-plant roots. Molecular considerations infer that early fungi and plants colonized land first (Heckman *et al.*, 2001). The earliest land plants, which lacked roots as a nutrition pump, would have depended on AM-like symbiotic fungi for incorporating nutrition, as supported by fossil records. Land



**Fig. 1** Clustering analysis of glutathione S-transferases in a land plant and an arbuscular mycorrhizal fungus. Peptide sequences in *Arabidopsis thaliana* corresponding to *Gi*GSTF1 were analyzed using ClustalX software. Phi, zeta, tau, and theta on the right side of the figure represent four classes in plant glutathione S-transferase, designated according to Edwards *et al.* (2000). *Gi*GSTF1 represents the peptide sequence translated from the EST clone AU082837 in *Glomus intraradices*, an arbuscular mycorrhizal fungus. The other 47-peptide sequences of glutathione S-transferase in *A. thaliana* are represented as Atxgxxxxx according to The Institute of Genome Research. Bootstrap frequencies higher than 600 per 1000 random replicates are represented on each branch. Squid Sigma GST, accession number P46088 in the SWISS-PROT database, is the out-group sequence.



**Fig. 2** Clustering analysis of glutathione *S*-transferases in fungi and plant phi-class glutathione *S*-transferase. Peptide sequences of an alga *Coccomyxa* sp. (JC5114), fungi *Saccharomyces cerevisiae* *Gut1p* (NP\_012304), *Gut2p* (NP\_013040), *URE2* (A39609), *Schizosaccharomyces pombe* (CAB90771), *Issatchenkia orientalis* (CAA41025), and cyanobacteria *Synechocystis* sp.; and PCC6803 hypothetical proteins (P73835, P74665, P74690, and Q55139) corresponding to *GiGSTF1* were phylogenetically analyzed using ClustalX. Numbers in the parentheses represent accession numbers in the public databases (Genbank or SWISS-PROT). Other information as described in **Fig. 1**.

plants and AM-like fungi have coexisted symbiotically for a long period, and so it is possible that gene-transfer events have occurred repeatedly. The sequence similarity of GSTs may be the molecular evidence for the AM-like fungi in fossil records being the orthologous ancestor of present-day AM fungi.

The role of GST proteins in plant is still largely unknown, although their expression is regulated in various stress conditions and by environmental stimuli, which implies that they have protective functions in plants (Edwards *et al.*, 2000). In transgenic plants, overexpression of the GST gene with the glutathione peroxidase gene conferred stress tolerance to tobacco seedling plants (Roxas *et al.*, 1997, 2000). Early land plants would have required a high stress tolerance to endure the existing environmental stresses, and the associated protective functions could have been obtained by gene incorporation from the symbiotic AM fungi. Phi-class GST might be one of the protective genes incorporated into plant during adaptation process.

I speculate that early land plants could only survive under the adverse conditions of the time with the help of nutrition uptake by symbiotic ancestral AM fungi. During this long-term symbio-

sis, early land plants incorporated genes from the symbiotic fungi to evolve their root organs for nutrition uptake from environment and the development of protection systems against various stresses. I therefore propose that AM fungi contributed to plant survival and evolution.

## Acknowledgements

The author is grateful to Dr. G. K. Agrawal for his critical reading of the manuscript. This study was supported in part by PROBRAIN (Program for Promotion of Basic Research Activities for Innovative Bioscience).

## References

- Edwards, R., Dixon, D. P., Walbot, V., 2000. Plant glutathione *S*-transferases: enzymes with multiple functions in sickness and in health. *Trends Plant Sci.*, **5**: 193–198.
- Graham, L. E., Cook, M. E., Busse, J. S., 2000. The origin of plants: body plan changes contributing to a major evolutionary radiation. *Proc. Natl. Acad. Sci. U. S. A.*, **97**: 4535–4540.
- Hall, T. A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.*, **41**: 95–98.
- Heckman, D. S., Geiser, D. M., Eidell, B. R., Stauffer, R. L., Kardos, N. L., Hedges, S. B., 2001. Molecular evidence for the early colonization of land by fungi and plants. *Science*, **293**: 1129–1133.
- Horiike, T., Hamada, K., Kanaya, S., Shinozawa, T., 2001. Origin of eukaryotic cell nuclei by symbiosis of Archaea in Bacteria is revealed by homology-hit analysis. *Nat. Cell Biol.*, **3**: 210–214.
- Jorgensen, R., 1993. The origin of land plants: a union of alga and fungus advanced by flavonoids? *Biosystems*, **31**: 193–207.
- Kadowaki, K., Kubo, N., Ozawa, K., Hirai, A., 1996. Targeting presequence acquisition after mitochondrial gene transfer to the nucleus occurs by duplication of existing targeting signals. *EMBO J.*, **15**: 6652–6661.
- Kranz, H. D., Miki, D., Siegler, M. L., Capesius, I., Sensen, C. W., Huss, V. A., 1995. The origin of land plants: phylogenetic relationships among charophytes, bryophytes, and vascular plants inferred from complete small-subunit ribosomal RNA gene sequences. *J. Mol. Evol.*, **41**: 74–84.
- Kubo, N., Harada, K., Hirai, A., Kadowaki, K., 1999. A single nuclear transcript encoding mitochondrial RPS14 and SDHB of rice is processed by alternative splicing: common use of the same mitochondrial targeting signal for different proteins. *Proc. Natl. Acad. Sci. U. S. A.*, **96**: 9207–9211.
- Kubo, N., Kadowaki, K., 2001. The gene encoding mitochondrial succinate dehydrogenase subunit 4 has been successfully transferred to the nuclear genome in pea, while leaving an original sequence as a Pseudogene in

- the mitochondrial genome. *Plant Biotechnol.*, **18**: 283–287.
- Martin, W., Stoebe, B., Goremykin, V., Hapsmann, S., Hasegawa, M., Kowallik, K. V., 1998. Gene transfer to the nucleus and the evolution of chloroplasts. *Nature*, **393**: 162–165.
- Nugent, J. M., Palmer, J. D., 1991. RNA-mediated transfer of the gene *coxII* from the mitochondrion to the nucleus during flowering plant evolution. *Cell*, **66**: 473–481.
- Nugent, J. M., Palmer, J. D., 1993. Characterization of the *Brassica campestris* mitochondrial gene for subunit six of NADH dehydrogenase: *nad6* is present in the mitochondrion of a wide range of flowering plants. *Curr. Genet.*, **23**: 148–153.
- Pirozynski, K. A., Malloch, D. W., 1975. The origin of land plants: a matter of mycotrophism. *Biosystems*, **6**: 153–164.
- Redecker, D., Kodner, R., Graham, L. E., 2000. Glomalean fungi from the Ordovician. *Science*, **289**: 1920–1921.
- Remy, W., Taylor, T. N., Hass, H., Kerp, H., 1994. Four hundred-million-years-old vesicular arbuscular mycorrhizae. *Proc. Natl. Acad. Sci. U. S. A.*, **91**: 11841–11843.
- Roxas, V. P., Lodhi, S. A., Garrett, D. K., Mahan, J. R., Allen, R. D., 2000. Stress tolerance in transgenic tobacco seedlings that overexpress glutathione S-transferase/glutathione peroxidase. *Plant Cell Physiol.*, **41**: 1229–1234.
- Roxas, V. P., Smith, R. K. Jr., Allen, E. R., Allen, R. D., 1997. Overexpression of glutathione S-transferase/glutathione peroxidase enhances the growth of transgenic tobacco seedlings during stress. *Nat. Biotechnol.*, **15**: 988–991.
- Rujan, T., Martin, W., 2001. How many genes in Arabidopsis come from cyanobacteria? An estimate from 386 protein phylogenies. *Trends Genet.*, **17**: 113–120.
- Sawaki, H., Saito, M., 2001. Expressed genes in the extraradical hyphae of an arbuscular mycorrhizal fungus, *Glomus intraradices*, in the symbiotic phase. *FEMS Microbiol. Lett.*, **195**: 109–113.
- Sawaki, H., Sugawara, K., Saito, M., 1998. Phylogenetic position of an arbuscular mycorrhizal fungus, *Acaulospora gerdemannii*, and its synanamorph *Glomus leptotichum*, based upon 18S rRNA gene sequence. *Mycoscience*, **39**: 477–480.
- Schuessler, A., Schwarzott, D., Walker, C., 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Micol. Res.*, **105**: 1413–1421.
- Simon, L., Bousquet, J., Levesque, R. C., Lalonde, M., 1993. Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. *Nature*, **363**: 67–69.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., Higgins, D. G., 1997. The CLUSTAL\_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.*, **25**: 4876–4882.
- Walker, C., Trappe, J. M., 1993. Names and epithets in the *Glomales* and *Endogonales*. *Micol. Res.*, **97**: 339–344.
- Walker, C., Vestberg, M., 1998. Synonymy amongst the arbuscular mycorrhizal fungi: *Glomus claroideum*, *G. multisubstansum* and *G. fistulosum*. *Ann. Bot.*, **82**: 601–604.