Plant Biotechnology 23, 419–424 (2006)

Gene Note

Cloning and molecular analysis of radish (*Raphanus sativus* L.) cDNAs encoding heterodimeric γ-glutamyltransferases

Yoshihiro Nakano[1], Rafael Prieto[1,a], Masakazu Hara[2], Jiro Sekiya[1,a,*]

[1] Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan;
[2] Faculty of Agriculture, Shizuoka University, 836 Ohya, Shizuoka 422-8529, Japan
* E-mail: sekiyaj@kyotogakuen.ac.jp

Received December 28, 2005; accepted April 27, 2006 (Edited by D. Shibata)

Abstract γ-Glutamyltransferase (GGT) catalyzes the hydrolysis and transpeptidation of the γ-glutamyl moiety of γ-glutamyl peptides. Based on the N-terminal amino acid sequences of purified radish heterodimeric GGTs, we cloned and characterized three radish full-length cDNAs (*RsGGT1*, *RsGGT2* and *RsGGT3*) encoding putative heterodimeric GGT isoforms. RsGGT proteins contained conserved amino acid residues that are required for the catalytic activity and the post-translational processing of GGT proteins in *E. coli* and mammals. Expression analysis indicated that *RsGGT* showed different organ expression patterns. The overexpression of *RsGGT1* and *RsGGT2* cDNAs, but not that of *RsGGT3* cDNA, resulted in an increase of NaCl-extractable bound GGT activity in transgenic tobacco plants. These results suggest that *RsGGT1* and *RsGGT2* cDNAs encode heterodimeric bound GGT isoforms.

Key words: Expression, γ-glutamyltransferase, glutathione, radish, *Raphanus sativus* L.

γ-Glutamyltransferases (GGTs) are widely distributed in different organisms. GGTs catalyze the transfer of the γ-glutamyl moiety of γ-glutamyl peptides to amino acids, peptides, or water. In plants, GGTs are thought to be involved in glutathione (GSH) catabolism (Schneider and Rennenberg 1992; Bergmann and Rennenberg 1993; Martin and Slovin 2000; Storozhenko et al. 2002; Shaw et al. 2005; Nakano et al. 2006), the synthesis of different γ-glutamyl peptides (Kawasaki et al. 1982), and secondary metabolism (Lancaster and Shaw 1994)

Plant GGTs are classified into soluble and bound GGTs, which are solubilized by low and high ionic strength buffer, respectively (Nakano et al. 2004). Several plants, including radish, Arabidopsis and rice, possess both soluble and bound forms, whereas tobacco and onion have only the bound form. Soluble GGTs were studied in tobacco cells (Steinkamp and Rennenberg 1984), garlic (Ceci et al. 1992) and radish (Nakano et al. 2006). On the other hand, bound GGTs were characterized in legumes (Kasai et al. 1982), onion (Lancaster and Shaw 1994; Shaw et al. 2005), tomato (Martin and Slovin 2000) and radish (Nakano et al. 2006). In radish, soluble and bound GGT activities are localized to the symplast and the apoplast, respectively (Nakano et al. 2004). With regard to their molecular structures, radish GGTs are classified into two classes: monomeric GGT and heterodimeric GGT, both of which present different substrate specificities (Nakano et al. 2006).

The precise characterization of *GGT* is required to understand the function of GGT isoforms. Plant cDNAs encoding heterodimeric GGTs were isolated and characterized: *AtGGT* from Arabidopsis, (Kushnir et al. 1995; Storozhenko et al. 2002), and *AcGGT* from onion (Shaw et al. 2005). However, as far as we know, a comparative study of different GGT isoforms from the same plant has not yet been performed. We previously purified two soluble heterodimeric GGT proteins (GGTI and GGTII) from radish cotyledons (Nakano et al. 2006). Based on the N-terminal amino acid sequences of the small subunits, here we describe the cloning and the characterization of radish cDNAs encoding three heterodimeric GGT isoforms.

Radish (*Raphanus sativus* L. cv. Comet) seeds were germinated on wetted paper towels under continuous light condition. Seven days after imbibition, the

Abbreviations: GGT, γ-glutamyltransferase; GSH, glutathione; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; RT, reverse transcription.

[a] Present address: Department of Bioscience and Biotechnology, Faculty of Bioenvironmental Science, Kyoto Gakuen University, Sogabe, Kameoka, Kyoto 621-8555, Japan

The nucleotide sequences reported in this paper have been submitted to DDBJ/GenBank/EMBL nucleotide sequence database under accession numbers AB098475 (*RsGGT1*), AB102676 (*RsGGT2*) and AB180896 (*RsGGT3*).
This article can be found at http://www.jspcmb.jp/

seedlings were transferred to half-strength modified Hoagland solution (Wilson et al. 1978) and grown in a glasshouse. The nutrient solution was changed once a week. After 30 days, the plants were incubated at 4°C for 10 days to promote bolting, and then, they were returned to the glasshouse.

Gene database search revealed that, besides At4g39640, which is equivalent to *AtGGT* (Storozhenko et al. 2002) in the Arabidopsis genome, there are at least two more genes encoding putative full-length heterodimeric GGTs, At4g39650 and At4g29210. The Arabidopsis genome contains another potential heterodimeric GGT gene that is named At1g69820. However, analysis of the At1g69820 deduced amino acid sequence (accession no. NP177140) revealed a putative protein that apparently has a complete small subunit but lacks approximately 70% of the GGT large subunit. The N-terminal amino acid sequences of radish GGT small subunits (Nakano et al. 2006) were highly identical to those of the small subunits deduced from Arabidopsis *AtGGT* (accession no. Z49240), At4g39650 (accession no. AK220806), and At4g29210 (accession no. NM119065) cDNAs. Thus, using an *AtGGT* RT-PCR cDNA fragment as the labeled probe, we isolated two cDNAs from a λ ZAP-II cDNA library constructed from radish cotyledon mRNA (Hara et al. 2000). Total RNA was extracted from Arabidopsis seedlings using the RNeasy plant mini kit (Qiagen) following the manufacturer's instructions. First-strand cDNA was synthesized from the total RNA using oligo dT primer and ReverTra Ace (Toyobo), as described by the manufacturer. Polymerase chain reaction (PCR) was carried out under conventional conditions using 5′-ATCAGTGTTCAGGCCTCTCG-3′ and 5′-CCTTCTTCACTCGAACACCG-3′primers, which led to the amplification of an 801 bp *AtGGT* cDNA fragment. This cDNA fragment was subcloned into the pGEM-T easy vector (Promega) and its nucleotide sequence was verified by sequencing. DNA sequencing was performed using the DYEnamic ET Terminator Cycle Sequencing kit (Amersham Biosciences) and the 373A DNA sequencing system (Applied Biosystems) according to the manufacturer's instructions. After that, the 801 bp *AtGGT* cDNA fragment was labeled using the Alkphos direct kit (Amersham Biosciences) and used as a probe to screen the radish cotyledon λ ZAP-II cDNA library, as described by Sambrook et al. (1989). Positive phage clones that were recovered from the second round of screening were converted into their corresponding pBluescript SK^- vector (Stratagene) forms by *in vivo* excision following the manufacturer's instructions. Two of the analyzed cDNA clones, *RsGGT1* (accession no. AB098475) and *RsGGT2* (accession no. AB102676), contained full-length open reading frames (ORFs) that are highly homologous to those of *AtGGT* and At4g39650 cDNAs. The 3′-UTR of *RsGGT1* and *RsGGT2* was isolated by the 3′-RACE method. First-strand radish cDNA was synthesized using 5′-GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTT-TTT-3′ as the dT adapter primer. The 3′-UTR of *RsGGT1* was isolated from the first-strand radish cDNA pool by PCR amplification using *RsGGT1* 5′-1 (5′-GATGAACGCTGCTCTGA-3′) and 3′-adapter (5′-GGCCACGCGTCGACTAGTAC-3′) primers, and this was followed by semi-nested PCR amplification using *RsGGT1* 5′-2 (5′-GCGGGTCTCTTCACCGCT-TGG-3′) and 3′-adapter primers. As for *RsGGT2*, primary PCR amplification was performed using *RsGGT2* 5′-1 (5′-TGATGCGTCCGTGGCAGCTG-3′) and 3′-adapter primers, and secondary PCR amplification was performed using *RsGGT2* 5′-2 (5′-GGTTAAGATAGCTGGCGGGA-3′) and 3′-adapter primers.

In order to isolate a possible At4g29210 orthologue in radish, we followed an RT-PCR strategy based on the use of degenerate primers against the highly conserved N-terminal amino acid sequence (TSHFSIVDSD) of the small subunits (Nakano et al. 2006) and At4g29210 cDNA specific primers. Primary PCR was performed on 5-day-old radish cotyledon cDNA using the degenerate primer 1 (5′-ACRAGYCATTTYTCSATMG-5′) and the At4g29210 primer 1 (5′-CTCCACCTGATAGC-TCCT-3′). Nested PCR was performed using the degenerate primer 2 (5′-CATTTYTCSATMGTRGAY-WSBGAT-3′) and the At4g29210 primer 2 (5′-CTCTGCTAGAAACATCTTG-3′). This RT-PCR amplification led to the isolation of a 468 bp cDNA fragment that was named *RsGGT3*. A 2181 bp full-length *RsGGT3* cDNA (accession no. AB180896) was cloned by 3′-RACE and 5′-RACE methods. 3′-RACE analysis was performed using *RsGGT3* 5′-1 (5′-CGACGACCGTGAACTACCCT-3′), *RsGGT3* 5′-2 (5′-TTCAAGTTTTCCTTAACTGC-3′) and 3′-adapter primers. On the other hand, *RsGGT3* 5′-RACE analysis was performed using the 5′-Full RACE Core Set (Takara) according to the manufacturer's protocol. PCR amplifications were performed using RsGGT3 5′-1 and *RsGGT3* 3′-1 (5′-CCAAGCACCGCCACCAACTC-3′) primers for primary PCR, and RsGGT3 5′-2 and *RsGGT3* 3′-2 (5′-CCCTGTAGAAGGAGAC-AAGACTC-3′) primers for secondary PCR.

Analysis of the *RsGGT1*, *RsGGT2* and *RsGGT3* cDNA nucleotide sequences revealed ORFs encoding proteins of 572, 575 and 635 amino acids and having deduced molecular weights of 61644, 61468, and 68991, respectively. Sequence homology analysis indicated that the deduced amino acid sequence of the RsGGT1 protein was 78% identical to that of the RsGGT2 protein and 46% identical to that of the RsGGT3 protein (Figure 1). The RsGGT2 protein shared 46% amino acid sequence

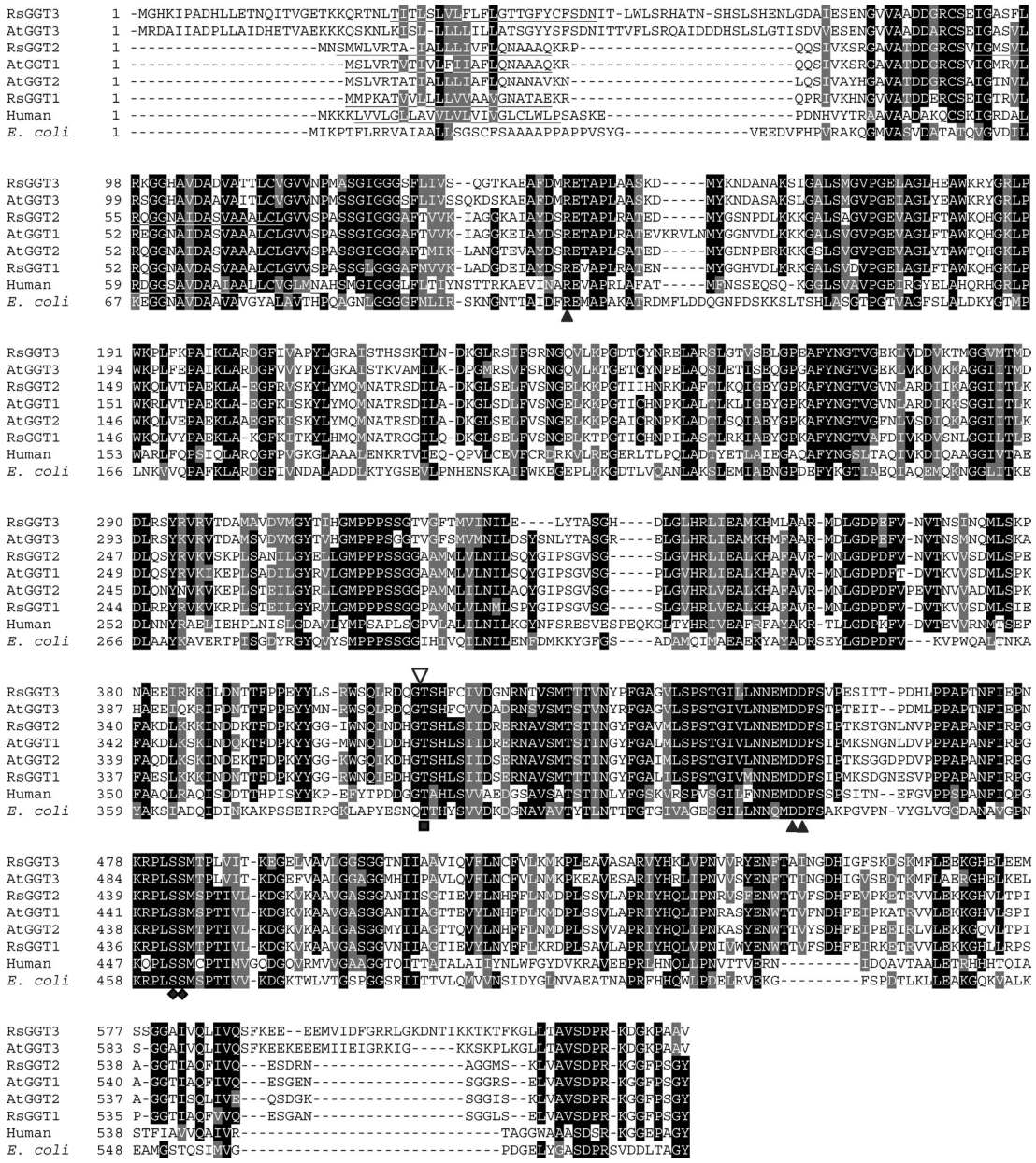

Figure 1. Multiple alignment of radish putative GGTs with GGTs of other plants and organisms. ClustalX program (Thompson et al. 1997) and Boxshade server (http://www/ch.embnet.org/software/BOX_form.html) were used for the alignment. Underlined are the hydrophobic regions predicted by SOSUI system (http://sosui.proteome.bio.tuat.ac.jp/sosui_submit.html). Amino acid residues required for γ-glutamyl donor binding are marked with closed triangles. Closed diamonds indicate amino acid residues necessary for catalytic activity. A threonine residue essential for post-translational processing and catalysis is indicated by a closed square. An open arrow indicates the site of post-translational processing.

identity with the RsGGT3 protein. On the other hand, RsGGT1 shares 77%, 75% and 48% identity with At4g39640, At4g39650, and At4g29210 deduced protein sequences, respectively. In a similar way, RsGGT2 shares 84%, 81% and 49% identity with At4g39640, At4g39650 and At4g29210, respectively. In contrast, RsGGT3 shares 46%, 45% and 74% identity with At4g39640, At4g39650 and At4g29210, respectively. Radish and Arabidopsis putative GGT proteins were ca. 30% identical to human (accession no. P19440) and *E. coli* (accession no. M28722) GGTs (Figure 1).

Structural analysis of the deduced radish GGT amino acid sequences (Figure 1) revealed that they contain amino acid residues necessary for mammalian heterodimeric GGT function (Taniguchi and Ikeda 1998). Among them are one arginine and two aspartic acid residues (R107, D422 and D423 in human GGT), which are believed to be of critical importance for binding to the γ-glutamyl donor substrate, and two serine residues, which are necessary for GGT catalytic activity (S451 and S452) (Taniguchi and Ikeda 1998) (Figure 1). Radish GGTs presented also a putative protease cleavage

site that includes the conserved threonine residue (T381) (Hashimoto et al. 1995) (Figure 1). After protein processing, this threonine becomes the N-terminal residue of *E. coli* GGT (Suzuki and Kumagai 2002) and it is also necessary for enzyme catalysis (Inoue et al. 2000). The same case applies to the heterodimeric type of radish GGTs (Nakano et al. 2006).

Plant heterodimeric GGTs contain a putative hydrophobic membrane-anchoring region at the N-terminus that may represent a membrane localization domain (Figure 1). In accord with this, it was reported that AtGGT overexpressed in tobacco plants was a membrane-bound protein that might be associated with the plasma membrane, as in the case of mammalian GGTs (Storozhenko et al. 2002). In contrast, radish heterodimeric and monomeric GGTs were purified and characterized as symplastic soluble enzymes and cell wall bound enzymes, respectively (Nakano et al. 2004; Nakano et al. 2006). These results strongly suggest that the N-terminal hydrophobic domain of at least some of the radish heterodimeric GGT isoforms may be cleaved after protein sorting. Besides, the fact that the onion heterodimeric bound AcGGT enzyme was purified in the truncated form lacking the putative N-terminal membrane-anchoring domain (Shaw et al. 2005) suggests that AcGGT is not a membrane-bound protein, but rather, a cell wall associated protein.

It is noteworthy that none of the three deduced RsGGT amino acid sequences match completely with the purified radish GGT small subunit peptide sequence TSHFSIVDSD (Figure 1), which may be explained by the difference in protein sequence identity between GGTs from Japanese radish, which was used for enzyme purification, and *GGT*s from radish (cv. Comet), which was used for cDNA cloning, or by the possibility that at least one more heterodimeric GGT isoform remains to be identified in radish.

RsGGT expression in different organs of radish plants at different growth stages was analyzed by the semi-quantitative RT-PCR method. For RT-PCR analysis, we used the primer set Act2 (5′-GTTGGTGATGAAGCACAA-3′ and 5′-CAAGACTTCTGGGCATCT-3′) to amplify a 425 bp actin2 cDNA fragment as the internal standard for gene expression. On the other hand, a 425 bp *RsGGT1* cDNA fragment, a 425 bp *RsGGT2* cDNA fragment, and a 315 bp *RsGGT3* cDNA fragment were amplified using primer sets Rsg1 (5′-GTGAAGTTGG-CCGATGGAG-3′ and 5′-GTCTTTGACGATGTCAA-ACG-3′), Rsg2 (5′-GGTTAAGATAGCTGGCGGGA-3′ and 5′-ATCTCTAGCTAGGTTTACTC-3′) and Rsg3 (5′-CGACGACCGTGAACTACCCT-3′ and 5′-TAGCACTCGCTACAGCTTCT-3′), respectively.

Figure 2 illustrates the different *RsGGT* expression profiles. High *RsGGT1* expression was noted in cotyledons of germinating seedlings and immature seeds. In contrast, *RsGGT1* expression was below the detection limit in 5-day-old cotyledons, 5-day-old roots, 5-day-old stems, immature siliques, and the axis. *RsGGT2* expression was noted in all the tested organs; however, the expression levels differed among the organs. *RsGGT2* expression was notably high in germinating cotyledons and the axis, whereas it was low in stem, edible part of the root and immature seeds. On the other hand, *RsGGT3* expression was noted after seed germination, and the expression was especially high in stem, 30-day-old roots, and immature seeds (Figure 2). These results indicate that radish GGT isoforms may play different roles at the germination, vegetative growth and reproductive stages.

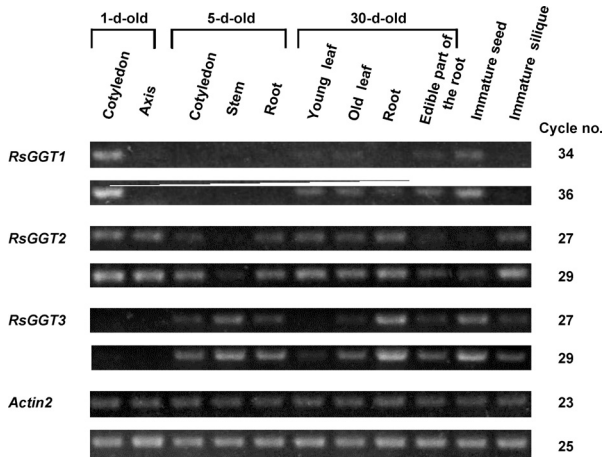

Figure 2. Expression analysis of radish *GGTs* in different organs by semi-quantitative RT-PCR. The first and the sixth leaves of 30-day-old plants are referred to as old leaf and young leaf, respectively. Siliques of about 3 cm in length were opened and immature seeds collected. These seeds were referred to as immature seed. After that, the remaining silique tissues were collected and referred to as immature silique. The amount of the template and the number of PCR cycles were determined by preliminary experiments to ensure that amplification occurs in the linear range and to allow quantification of cDNA fragments. RT-PCR products were verified by direct sequencing. PCR fragments were separated on 1.5% agarose gel and stained with ethidium bromide. We performed three independent experiments to ensure reproducibility of the results.

Tobacco plants (*Nicotiana tabacum* cv. SR-I) overexpressing *RsGGT1*, *RsGGT2* and *RsGGT3* cDNAs under the control of the CaMV 35S promoter were generated via *Agrobacterium*-mediated leaf disc transformation. *RsGGT* cDNAs were subcloned in sense orientation into the modified pBI121 vector, in which the GUS gene was removed and an *Xho* I restriction site was added (Koizumi et al. 1999). We used pBluescript-*RsGGT1* plasmid DNA as the template for PCR amplification of a 1836 bp *RsGGT1* cDNA fragment using the *RsGGT1* 5′-Xho I primer 5′-CTCGAGATGATGCCAAAAGCGACGG-3′ and the pBluescript T7 primer. The resulting *RsGGT1* PCR product was subcloned into the modified pBI121 vector

(pBI-*RsGGT1*). The *RsGGT2* cDNA, which included 5-UTR and 3-UTR sequences, was excised from the pBluescript-*RsGGT2* plasmid by *Eco* RI-*Kpn* I digestion, and subcloned into the modified pBI121 vector using the DNA blunting kit (Takara) according to the manufacturer's protocol (pBI-*RsGGT2*). The *RsGGT3* cDNA was amplified by PCR performed on pGEM Teasy-*RsGGT3* plasmid DNA using the 5′-*Bam* HI primer 5′-GGATCCAAGGGGAAAGAGAGAGA-3′ and the 3′-*Xho* I primer 5′-CTCGAGCATTAT-GACAGGAGTAGG-3′. The resulting *RsGGT3* PCR product was subcloned into the modified pBI121 vector (pBI-*RsGGT3*). The RsGGT cDNA sequences from pBI-*RsGGT1*, pBI-*RsGGT2*, and pBI-*RsGGT3* clones were verified by sequencing.

The modified pBI121 vector control and the pBI-*RsGGT* plasmids were used to transform *Agrobacterium tumefaciens* strain LBA4404, as described by Sugiyama et al. (2004). *Agrobacterium*-mediated transformation of tobacco plants was performed according to Sugiyama et al. (2004). Seven *RsGGT1*, 2 *RsGGT2*, and 12 *RsGGT3* T2 tobacco plants were selected based on their corresponding RsGGT transgene expression, which was confirmed by RT-PCR analysis (Figure 3).

Vector control and RsGGT transgenic tobacco plants were evaluated by fractionation of soluble and NaCl extractable bound GGT isoforms and GGT activity was determined as described by Nakano et al. (2004). As shown in Table 1, leaves of *RsGGT1* (1-8 and 1-11) and *RsGGT2* (2-1 and 2-2) transgenic lines exhibited, respectively, 2.5 to 3.6-fold and 8 to 16-fold higher bound GGT activity than the vector control plants. In contrast, we did not find any significant differences among the soluble GGT activities of vector control, *RsGGT1* and *RsGGT2* plants. These results indicate that *RsGGT1* and *RsGGT2* cDNAs encode GGTs expressed in tobacco plants as bound isoforms. On the other hand, vector control and *RsGGT3* (3-1, 3-5 and 3-7) plants presented similar levels of both soluble and bound GGT activities (Table 1). In fact, none of the 12 analyzed transgenic lines overexpressing *RsGGT3* exhibited a significant increase in GGT activity compared with the vector control plants. This apparent lack of RsGGT3 activity might be explained by an abnormal folding and/or post-translational processing of the protein in tobacco. However, at present, we cannot disregard the possibility that *RsGGT3* will encode a protein without GGT activity. The fact that heterodimeric GGTs were purified from the radish soluble fraction (Nakano et al. 2006) indicates that *RsGGT3* and/or yet to be identified radish cDNAs encode heterodimeric soluble GGT isoforms. The biological significance of the different heterodimeric RsGGT isoforms should be determined in further studies.

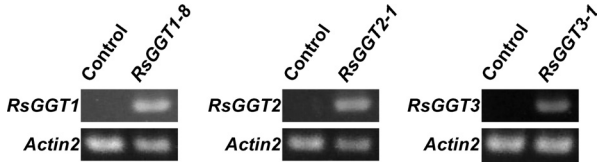

Figure 3. Heterologous expression of *RsGGTs* in tobacco plants. Vector control and three types of *RsGGT* transgenic tobacco plants were evaluated for *RsGGT* mRNA accumulation in leaves. RT-PCR analysis was performed as given in Figure 2. PCR cycles were as follows: 30 cycles for *actin2*, and 38 cycles for *RsGGT1*, *RsGGT2* and *RsGGT3*.

References

Bergmann L, Rennenberg H (1993) Glutathione metabolism in plants. In: de Kok LJ, Stulen I, Rennenberg H, Brunold C, Rauser WE (eds) *Sulfur Nutrition and Sulfur Assimilation in Higher Plants*. SPB Academic Publishing, The Hague, pp 109–123

Ceci LN, Curzio OA, Pomilio AB (1992) γ-Glutamyl transpeptidase/γ-glutamyl peptidase in sprouted *Allium sativum*. *Phytochem* 31: 441–444

Hara M, Fujii Y, Sasada Y, Kuboi T (2000) cDNA cloning of radish (*Raphanus sativus*) myrosinase and tissue-specific expression in root. *Plant Cell Physiol* 41: 1102–1109

Hashimoto W, Suzuki H, Yamamoto K, Kumagai H (1995) Effect of site-directed mutations on processing and activity of γ-glutamyltranspeptidase of *Escherichia coli* K-12. *J Biochem* 118: 75–80

Inoue M, Hiratake J, Suzuki H, Kumagai H, Sakata K (2000) Identification of catalytic nucleophile of *Escherichia coli* γ-glutamyltranspeptidase by γ-monofluorophosphono derivative of glutamic acid: N-terminal thr-391 in small subunit is the nucleophile. *Biochem* 39: 7764–7771

Kasai T, Ohmiya A, Sakamura S (1982) γ-Glutamyltranspeptidases in the metabolism of γ-glutamyl peptides in plants. *Phytochem* 21: 1233–1239

Kawasaki Y, Ogawa A, Sasaoka S (1982) Occurrence and some properties of a novel γ-glutamyltransferase responsible for the synthesis of γ-L-D-alanine in pea seedlings. *Biochim Biophys Acta* 716: 194–200

Koizumi N, Ujino T, Sano H, Chrispeels MJ (1999) Overexpression of a gene that encodes the first enzyme in the biosynthesis of asparagines-linked glycans makes plants

Table 1. Soluble and bound GGT activities in leaves of tobacco plants transformed with RsGGTs.

Fraction	GGT activity (nkat mg^{-1} protein)							
	Control	*RsGGT1-8*	*RsGGT1-11*	*RsGGT2-1*	*RsGGT2-2*	*RsGGT3-1*	*RsGGT3-5*	*RsGGT3-7*
Soluble GGT	0.011±0.001	0.013±0.002	0.017±0.002	0.019±0.004	0.026±0.005	0.010±0.002	0.009±0.001	0.013±0.004
Bound GGT	0.312±0.022	1.141±0.066	0.829±0.093	5.132±1.580	2.560±0.127	0.206±0.030	0.305±0.102	0.343±0.045

Data are means±SD (n=3).

resistant to tunicamycin and obviates the tunicamycin-induced unfolded protein response. *Plant Physiol* 121: 353–361

Kushnir S, Babiychuk E, Kampfenkel K, Belles-Boix E, Van Montagu M, Inze D. (1995) Characterization of *Arabidopsis thaliana* cDNAs that render yeasts tolerant toward the thiol-oxidizing drug diamide. *Proc Natl Acad Sci USA* 92: 10580–10584

Lancaster JE, Shaw ML (1994) Characterization of purified γ-glutamyl transpeptidase in onions: evidence for in vivo role as a peptidase. *Phytochem* 36: 1351–1358

Martin ML, Slovin JP (2000) Purified γ-glutamyl transpeptidases from tomato exhibit high affinity for glutathione and glutathione *S*-conjugates. *Plant Physiol* 122: 1417–1426

Nakano Y, Okawa S, Yamauchi T, Koizumi Y, Sekiya J (2004) Occurrence of two forms of γ-glutamyltransferase in radish plants. *Plant Biotech* 21: 243–246

Nakano Y, Okawa S, Yamauchi T, Koizumi Y, Sekiya J (2006) Purification and properties of soluble and bound γ-glutamyltransferases from radish cotyledons. *Biosci Biotechnol Biochem* 70: 369–376

Sambrook J. Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed. Cold Spring Harbor Laboratory, New York

Schneider A, Rennenberg H (1992) Degradation of glutathione in heterotrophic tobacco cells. *Phyton* 32: 111–115

Shaw ML, Pither-Joyce MD, McCallum JA (2005) Purification and cloning of a γ-glutamyl transpeptidase from onion (*Allium cepa*). *Phytochem* 66: 515–522

Steinkamp R, Rennenberg H (1984) γ-Glutamyl transpeptidase in tobacco suspension cultures: catalytic properties and subcellular localization. *Physiol Plant* 61: 251–256

Storozhenko S, Belles-Boix E, Babiychuk E, Herouart D, Davey MW, Sloosten L, Van Montagu M, Inze D, Kushnir S (2002) γ-Glutamyltranspeptidase in transgenic tobacco plants. Cellular localization, processing, and biochemical properties. *Plant Physiol* 128: 1109–1119

Sugiyama A, Nishimura J, Mochizuki Y, Inagaki K, Sekiya J. (2004) Homoglutathione synthesis in transgenic tobacco plants expressing soybean homoglutathione synthetase. *Plant Biotechnol* 21: 79–83

Suzuki H, Kumagai H (2002) Autocatalytic processing of γ-glutamyltranspeptidase. *J Biol Chem* 277: 43536–43543

Taniguchi N, Ikeda Y (1998) γ-Glutamyl transpeptidase: catalytic mechanism and gene expression. In: Purich DL (ed) *Advances in Enzymology and Related Areas of Molecular Biology Vol 72* John Wiley & Sons, New York, pp 239–278

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 24: 4876–4882

Wilson LG, Bressan RA, Filner P (1978) Light-dependent emission of hydrogen sulfide from plants. *Plant Physiol* 61: 184–189