**Characterization and abiotic stress-responsive expression
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Characterization and abiotic stress-responsive expression analysis of *SGT1* genes in***Brassica oleracea***

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Abstract

SGT1 genes are involved in enhancing plant responses to various biotic and abiotic stresses. *Brassica oleracea* is known to contain two types of *SGT1* genes, namely *Suppressor of G2 allele of SKP1* and *Suppressor of GCR2*. In this study, through systematic analysis, four putative *SGT1* genes were identified and characterized in *B. oleracea*. In phylogenetic analysis, the genes were formed *BolSGT1a*, *BolSGT1b* (both *Suppressor of G2 allele of SKP1* types) and *BolSGT1* (*Suppressor of GCR2*) groups. Functional domain analysis and organ-specific expression patterns suggested possible roles for *BolSGT1* genes during stress conditions. *BolSGT1* genes showed significant changes in expression in response to heat, cold, drought, salt, or ABA treatment. Interaction network analysis supported the expression analysis, and showed that the *BolSGT1a* and *BolSGT1b* genes are strongly associated with co-regulators during stress conditions. However, the *BolSGT1* gene not showed any strong association. Hence, *BolSGT1* might be a stress resistance-related gene that functions without a co-regulator. Our results show that *BolSGT1* genes are potential target genes to improve *B. oleracea* resistance to abiotic stresses such as heat, cold, and salt.

Keywords

Abiotic stress resistance, *BolSGT1*, *Brassica oleracea* var. *capitata*, Gene expression

Introduction

Brassica oleracea is a major vegetable crop around the world, but its establishment, growth and yield are highly affected by biotic and abiotic stresses such as bacteria, viruses, fungi, and drought, cold, and salt (Ahmed et al. 2012; Thamilarasan et al. 2014). These stresses increase the level of reactive oxygen species (ROS), which causes injuries to the plants through oxidative damage at the cellular level. Plants have developed mechanisms for tolerance against biotic and abiotic stresses involving cellular, physiological, and developmental changes through expression of resistance-related genes that can be activated within hours or days (Yabuta et al. 2009). Interestingly, biotic resistance-related genes such as *thaumatin-like* genes in wheat, *DREB*, *AtRALFL8*, *AtMGL* and some *cytochrome P450* genes in *Arabidopsis* exhibit crosstalk with abiotic stress responses and can be expressed during both forms of stresses (Agarwal et al. 2006; Atkinson et al. 2013; Deihimi et al. 2013; Fujita et al. 2006; Narusaka et al. 2004).

SGT1 (*suppressor of the G2 allele of skp1*) was first identified in yeast as suppressor of *skp1-4*, a temperature-sensitive mutation of *SKP1* that arrests cell division in G2/M phase (Kitagawa et al. 1999). *SGT1* is expressed in association with several resistance-related genes like *HSP-90*, *Prf*, *RAR1*, *Nod1*, and forms protein complexes that function in various biological processes in plants (Azevedo et al. 2002; Catlett & Kaplan 2006; Kud et al. 2013). *B. oleracea* contains *suppressor of the G2 allele of skp1*, as well as another related gene, *suppressor of GCR2*.

Some SGT1 proteins contain three functional domains, including tetratricopeptide repeats (TPRs), CHORD SGT1 (CS) and SGT1-specific sequence (SGS) (Kumar & Kirti 2015). TPR domains are generally involved in inter-protein associations, whereas CS and SGS domains are responsible for stress resistance (Botër et al. 2007; Nyarko et al. 2007).

Arabidopsis contains two SGT1 proteins, AtSGT1a and AtSGT1b, which bind to RAR1, a component required for the resistance through R proteins (Azevedo et al. 2006). Another type of SGT1 protein is characterized by the so-called SGT1 domain, similar to that found in human suppressor SGT1 protein (Sato et al. 1999).

These earlier reports revealed that *SGT1* plays an important role as a resistance-related gene and is expressed during various stress conditions in plants. Currently, no detailed studies reported the expression of *SGT1* genes in abiotic stress responses in *B. oleracea*. In this study, *SGT1* genes are identified in the *B. oleracea* genome and characterized them by *in silico* analysis. Also examined their expression in response to abiotic stresses, finding evidence suggesting that these resistance genes respond during all stresses.

Materials and methods

Sequence analysis

Coding DNA and protein sequences for SGT1 of *B. oleracea* and *B. rapa* were retrieved from the *Brassica* database (<http://www.brassicadb.org/brad/>) using key word searches. Genomic structures of *BolSGT1* genes were determined by the Gene structure display server (<http://gsds.cbi.pku.edu.cn/>). Pairwise and multiple alignments were used for analyses of similarity and homology. Functional domains were analyzed with SMART (http://smart.embl-heidelberg.de/smart/set_mode.cgi?NORMAL=1). Predictions of pI, N-Glyc position and molecular mass were made using the entire ORF and the pI/Mw tool at Expasy (<http://web.expasy.org/protparam/>). The subcellular localizations of *BolSGT1* genes were predicted using the PSORT software programs (Nakai & Horton 1999). Predicted protein interactions were identified using STRING software (Search Tool for the Retrieval of

Interacting Genes/Proteins, <http://string-db.org/>) (Szkarczyk et al. 2011).

Phylogenetic tree

Phylogenetic analysis was conducted using MEGA6.06. Predicted protein sequences of *BolSGT1* and pathogenesis-related protein (thaumatin-like protein) genes were used for phylogenetic tree construction using the neighbor-joining method with the complete deletion option. Tree reliability was assessed using 1000 bootstrap replicates, and the numbers indicated for each clade represent bootstrap support values given as percentages (Thamilarasan et al. 2014).

Plant materials and abiotic stress conditions

Cabbage line 106 (*B. oleracea* var. *capitata*) plants were grown in Department of Horticulture, Sunchon National University, South Korea. Fresh roots, stems, leaves and flower buds of *B. oleracea* were harvested, frozen immediately in liquid nitrogen, and stored at -80 °C for RNA isolation followed by organ-specific expression. Plant treatments (cold, drought, heat, salt and ABA) were started at morning (8 am) and samples were collected at appropriate time courses and frozen immediately in liquid nitrogen. For the stress-responsive expression analysis, seeds of cabbage line 106 (*B. oleracea* var. *capitata*) were sown and grown aseptically on half-strength MS agar medium in a culture room under a 16-h light photoperiod at 25 °C. After three weeks of growth, the seedlings were treated at 42 °C for 0, 0.5, 1 and 3 h time courses for heat stress. For salt and ABA treatments, the seedlings were transferred to fresh liquid MSH (half-strength MS medium without sucrose) medium containing 250 mM NaCl or 100 mM abscisic acid (ABA). To induce cold stress, the seedlings were maintained at 4 °C. Drought treatment was applied by keeping the seedlings

on the filter paper at 28 °C. The samples were subjected to stresses for 0 (control), 1, 3, 6, 12 and 24 h. Afterward, the second and third leaves were collected and frozen immediately in liquid nitrogen and stored at -80 °C until RNA isolation. The total RNA was extracted from the 100 mg of frozen samples of the roots, stems, leaves and flower buds of healthy plants and leaves of plants treated with abiotic stress using an RNeasy mini kit (Qiagen, USA), after which RNA was treated with RNase-free DNase (Promega, USA) to remove genomic DNA contamination. Quantified and checked the purity of isolated RNA by ND-1000 Spectrophotometer and NanoDrop v3.7 software (NanoDrop Technologies, USA). Synthesis of cDNA from RNA extracts was performed with Superscript III® First-strand Synthesis Supermix kit (Invitrogen, USA) following the manufacturer's instructions.

Expression analysis

Gene-specific primers were designed from coding DNA sequences of *SGT1* genes by using Primer3 software for RT-PCR and Real-time PCR (qPCR). Primers were designed with 18-21 bp length, 45-55% of GC content and 58-60 °C annealing temperature as default parameters. Actin, a commonly used housekeeping gene for expression study, so this gene in *B. oleracea* were used as a reference gene (Table 1) in RT-PCR and qRT-PCR experiments (Dekkers et al. 2012; Hee-Jeong et al. 2014). RT-PCR was performed for organ-specific expression using 50 ng cDNA (1 µl), 20 pmol primer pairs (2 µl), Emerald master mix (8 µl), and sterile H₂O to a total volume of 20 µl. Condition was set at 30 cycles of 60 °C for 30 s and 72 °C for 45 s, followed by a final extension at 72 °C for 5 min. The PCR products were resolved on a 2% agarose gel. For qRT-PCR 1µl cDNA with 10 µl iTaq SYBR Green Supermix with ROX (California, USA) was used with three step amplification (annealing temperature 60 °C) and melting peak with two technical replicates. Amplification, detection, and data analysis were carried out using a Light Cycler® 96 Instrument (Roche Diagnostics,

United States). Efficiency was determined based on different cDNA concentration and primer specificity using melting peak (Supplementary file 1).

Results and Discussion

Identification and characterization of *SGT1* genes

Extensive searches were conducted in the *Brassica* database to identify *SGT1* genes, and retrieved sequences based on annotation. Among the 17 *B. oleracea* genes, eight were annotated as *SGT1a*, seven as *SGT1b* (*suppressor of G2 allele of skp1*) and two as *SGT1* (*suppressor of GCR2*) based on *Arabidopsis* nomenclature in *Brassica* database. Coding sequences of eight *SGT1a* genes range from 159 to 1,032 bp, and the seven *SGT1b* genes range from 168 to 963 bp. The two *SGT1* genes are 1,947 and 342 bp. Sequence similarities were identified between *SGT1a* genes, which also showed the highest similarity between the genes, and between *SGT1b* genes. Likewise, the two *SGT1* genes showed high levels of similarity to each other. Even though the remaining genes are complete sequences, they lack functional domains and are short in length. Despite the presence of promoter region in these genes, they showed no expression during organ-specific analysis. Hence, we omitted these genes for further analysis. In pairwise alignments, short sequences with high similarity between the sequences were found, suggesting that *SGT1* genes may have been duplicated in the *B. oleracea* genome (Supplementary File 2). These duplicated genes in *B. oleracea* evolved during evolutionary events and 4,365 duplicated genes are identified in 1,825 gene clusters. Duplication and triplication of the genes are a result of evolutionary events in *Brassicaceae* lineage, during this event genes possibly be mutated into pseudogenes or non-functional genes (Liu et al. 2014; Lynch et al. 2001; Town 2006; Yao et al. 2015). In *Arabidopsis*, Noel et al. (2007) reported that *SGT1* encodes three functional domains (TPR,

CS and SGS) that are essential for activation of suppressor of G2 allele of SKP1 protein. Domain analysis revealed that among eight annotated *SGT1a* genes, only one (Gene id: Bol006218) encoded all three functional domains, and named that gene as *BolSGT1a*. Similarly, among the seven genes annotated as *SGT1b*, only two (Gene ids: Bol006784 and Bol025710) encoded proteins containing all three functional domains, and named these genes as *BolSGT1b_1* and *BolSGT1b_2*. The remaining genes encoded only one domain (TPR/SGS) or no domains. Only one of the two annotated *SGT1* genes encoded the SGT1 functional domain and that gene was named as *BolSGT1*. Additionally, RT-PCR was performed for genes that did not encode functional domains and expression was not observed (data not shown). This domain and expression analysis suggested that the functional domains are essential for expression of *BolSGT1* genes. The domain-containing sequences were used for further studies, and are described in Table 3.

Genomic DNA sequences for all four genes were obtained from the *B. oleracea* genome sequence. Exon positions were determined for the *BolSGT1* genes (Table 2). Amino acid sequence characteristics, molecular weight and pI for the four predicted BolSGT1 proteins are presented in Table 3. Both the BolSGT1a and BolSGT1b proteins were predicted to be located in cytoplasm, mitochondrial matrix space and the endoplasmic reticulum, whereas BolSGT1 was predicted to be in the chloroplast and cytoplasm. Previously, subcellular localization in *Arabidopsis* was studied by Noel et al. (2007), who found that SGT1 and HSC70 proteins co-localize in the cytosol and in the nucleus to regulate biotic and abiotic stress responses. The predicted characteristics of the BolSGT1 proteins are consistent with similar functions in stress responses in *B. oleracea*. Based on these results, further analysis of these *BolSGT1* genes are carried out.

Phylogenetic analysis

Fu et al. (2009) reported that *Glycine max SGT1* is 60 to 65% identical to *A. thaliana SGT1*, and greater than 70% identical to tobacco *NbSGT* and barley *HvSGT*. Chung et al., (2006) generated a phylogenetic tree of *SGT1* genes from different plant species, which showed that *AtSGT1* and *BolSGT1* are closely related. However, no studies have reported the phylogenetic relationships of *BolSGT1a*, *BolSGT1b* and *BolSGT1* among different plant species. Accordingly, constructed a phylogenetic tree using 17 *SGT1* protein sequences from five species including *A. thaliana*, *B. oleracea*, *B. rapa*, *B. napus*, and *Camelina sativa* from the *Brassicaceae* family (Fig. 1). Additionally, pathogenesis-related protein (thaumatin-like protein) is used as a outgroup gene in the phylogenetic tree (Fig. 1) which is also expressed during abiotic stress (Deihimi et al. 2013). In the phylogenetic tree, the proteins clearly formed separate *SGT1a*, *SGT1b* and *SGT1* groups, indicating that all three proteins arose before the divergence of these species. The tree shows that *SGT1* is diverged from *SGT1a* and *SGT1b*, whereas *SGT1a* and *SGT1b* are more closely related. Consistent with the close relationship of the two species, *BolSGT1a* is most closely related to *BrSGT1a* and *BolSGT1b* is clustered with *BrSGT1b*. Likewise, *BolSGT1* is grouped with *SGT1* of *B. rapa*.

Organ-specific expression

Organ-specific expression analysis was conducted using mRNA isolated from roots, stems and leaves (second and third leaf) of three weeks old non-treated seedlings, and flower buds were collected from 14 weeks old seedlings of *B. oleracea* by RT-PCR at different experimental profiles. Expression is observed in all four *BolSGT1* genes in all tested organs. *BolSGT1a* was highly expressed in root, stem and leaves but showed lower expression in flower bud. *BolSGT1b_1* was highly expressed in stem and moderately expressed in leaves

and flower bud, whereas it had lower expression in root. *BolSGT1b_2* showed high expression in flower bud, moderate expression in stem and leaves and lower expression in root. *BolSGT1* was strongly expressed in stem, moderately expressed in leaves and flower bud but expressed in root only to very low levels (Fig. 2). Organ-specific expression analysis showed that the *BolSGT1* genes are differentially expressed in all organs at various experimental conditions (data not shown). This propose that *BoSGT1* genes are functional during essential plant development stages. Together, our results indicate that *BolSGT1* genes are expressed to different degrees in different organs. *SGT1* genes are expressed in different organs of *Capsicum annuum* and *Haynaldia villosa* which are involved in different development stages and substantially expressed during different stresses (Chung et al. 2006; Xing et al. 2013). This similarity suggests that the *BolSGT1* genes may also play roles during abiotic stress conditions. Hence, the *BolSGT1* genes were studied using quantitative PCR in the abiotic treated *B. oleracea*.

Analysis of *BolSGT1* gene expression during abiotic stresses

To analyze the expression pattern of *BolSGT1* genes, RT-qPCR was carried out on samples from plants treated with abiotic stresses such as heat, cold, drought, and salt, and with ABA. Our results revealed that *BolSGT1a* expression was highly up-regulated by heat stress until 1 h and subsequently decreased. During salt and cold stress, *BolSGT1a* expression increased until 6 h and 1 h, respectively, and then decreased. Drought and ABA treatments did not induce notable expression during the time courses. During ABA stress, *BolSGT1a* decreased and showed similar expression after treatment at 1 h and 3 h, followed by a decrease and similar expression at 6 h, 12 h and 24 h.

BolSGT1b_1 and *BolSGT1b_2* showed similar expression patterns in response to heat,

cold, and drought stresses. During heat and drought stress, no significant differential expression pattern was observed. During cold stress, expression gradually increased until 3 h, followed by a decrease. In salt stress, *BolSGT1b_2* was up-regulated until 6 h, and then suddenly decreased at 12 h. ABA treatment led to decreased *BolSGT1b_1* expression. However, *BolSGT1b_2* responded positively at 1 h and then decreased until 12 h. *BolSGT1* expression in response to salt stress and ABA treatment was up-regulated and downregulated, respectively. During salt stress *BolSGT1* expression gradually increased until 3 h, slightly decreased at 6 h, and then increased. During ABA treatment, *BolSGT1* expression suddenly increased at 1 h, followed by a gradual decrease (Fig. 3).

These observations support the idea that *BolSGT1* genes could play roles during stress conditions. In *Arabidopsis*, *SGT1a* is more highly up-regulated in heat stress than other *SGT1* genes (Noël et al. 2007). Although *BolSGT1a* and *BolSGT1b* genes share similar domain structures, they display contrasting expression patterns during heat stress (Fig. 3), which is similar to the situation in *Arabidopsis* (Yabuta et al. 2009). In cold stress, *BolSGT1a* and *BolSGT1b* genes were downregulated, unlike *BolSGT1*. Along with previous reports, our results suggest that HSP90/SGT1 complex might be co-regulated during cold stress condition, which is expressed as downregulation, and may be slightly induced by heat stress (Fu et al. 2009). In *Arabidopsis*, *HSC70-1*, encoding an interactor of SGT1, shows significant responses during salt stress (Cazalé et al. 2009). Our results also revealed that *BolSGT1a*, *BolSGT1b_2* and *BolSGT1* have a differential expression pattern during salt stress conditions, which might be co-regulated by *HSC70-1* (Cazalé et al. 2009). In *A. diogeni*, *SGT1* was up-regulated during ABA treatment (Kumar & Kirti 2015), consistent with the report of Cazale et al., (2009) that *HSC70/SGT1* act directly or indirectly during stress conditions. Our results revealed the *BolSGT1* is repressed and other all *BolSGT1* genes have no differential

expression pattern during ABA treatment, responses to which may therefore not be co-regulated by *HSC70/SGT1*. Previous results revealed that *BolSGT1* genes may be co-regulated and induced by all stresses excluding drought; in drought stress *BolSGT1* genes were not expressed well, suggesting that they may not function in drought stress responses (Fu et al. 2009).

To further explore the possible functions of these genes, generated an interaction network by *in silico* methods, which revealed many genes as co-regulated with *BolSGT1* genes. Interestingly, many proteins that may act as co-regulators (HSC70-1, ATHSP90.1, SRFR1, PBS2, RPM1 and AFB5) of *BolSGT1*s (*BolSGT1a* and *BolSGT1b*) showed strong interaction with all proteins except *BolSGT1* (Fig. 4). In *SGT1*, the CS domain is involved in interaction with Hsp90 and RAR1 (Takahashi et al. 2003; Wang et al. 2015). However, *BolSGT1* has not shown any interaction with RAR1, though previously reported in other plants. Our results suggest that *BolSGT1* does not strongly interact with other proteins during stress conditions, but might be expressed individually. Notably, although *AtSGT1* is functionally annotated as an unknown gene, based on our results, can suggest that *BolSGT1* is a stress-related gene involved in drought, salt, and ABA responses in *B. oleracea*.

Conclusion

In summary, four *SGT1* genes identified are in *B. oleracea* and characterized their expression patterns in response to different abiotic stresses. This is the first systematic investigation of *B. oleracea SGT1* genes. Conducted the quantitative PCR analysis of *BolSGT1* genes, and found that they have potential for use as target genes to improve plant resistance to abiotic stresses such as heat, cold, and salt in *B. oleracea*. Interaction network analysis supported the expression analysis, and showed that these genes are associated with co-

regulators during stress conditions, with the exception of *BolSGT1*. *BolSGT1* was functionally annotated as an unknown gene; our results show that it can be considered a stress-related gene that is expressed individually in response to drought, salt, and ABA.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

- Agarwal, P. K., Agarwal, P., Reddy, M. K., & Sopory, S. K. 2006, December. Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. *Plant Cell Reports*. doi:10.1007/s00299-006-0204-8
- Ahmed, N. U., Park, J.-I., Seo, M.-S., Kumar, T. S., Lee, I.-H., Park, B.-S., & Nou, I.-S. 2012. Identification and expression analysis of chitinase genes related to biotic stress resistance in *Brassica*. *Molecular Biology Reports*, **39**(4): 3649–3657. doi:10.1007/s11033-011-1139-x
- Atkinson, N. J., Lilley, C. J., & Urwin, P. E. 2013. Identification of genes involved in the response of *Arabidopsis* to simultaneous biotic and abiotic stresses. *Plant Physiology*, **162**(4): 2028–41. doi:10.1104/pp.113.222372
- Azevedo, C., Betsuyaku, S., Peart, J., Takahashi, A., Noël, L., Sadanandom, A., Shirasu, K. 2006. Role of SGT1 in resistance protein accumulation in plant immunity. *The EMBO Journal*, **25**(9): 2007–16. doi:10.1038/sj.emboj.7601084
- Azevedo, C., Sadanandom, A., Kitagawa, K., Freialdenhoven, A., Shirasu, K., & Schulze-Lefert, P. 2002. The RAR1 interactor SGT1, an essential component of R gene-triggered disease resistance. *Science (New York, N.Y.)*, **295**(5562): 2073–2076. doi:10.1126/science.1067554
- Botër, M., Amigues, B., Peart, J., Breuer, C., Kadota, Y., Casais, C., Guerois, R. 2007. Structural and functional analysis of SGT1 reveals that its interaction with HSP90 is required for the accumulation of Rx, an R protein involved in plant immunity. *The Plant Cell*, **19**(11): 3791–804. doi:10.1105/tpc.107.050427
- Catlett, M. G., & Kaplan, K. B. 2006. Sgt1p is a unique co-chaperone that acts as a client adaptor to link Hsp90 to Skp1p. *The Journal of Biological Chemistry*, **281**(44): 33739–48. doi:10.1074/jbc.M603847200
- Cazalé, A.-C., Clément, M., Chiarenza, S., Roncato, M.-A., Pochon, N., Creff, A., Noël, L. D. 2009. Altered expression of cytosolic/nuclear HSC70-1 molecular chaperone affects development and abiotic stress tolerance in *Arabidopsis thaliana*. *Journal of Experimental Botany*, **60**(9): 2653–64. doi:10.1093/jxb/erp109
- Chung, E., Ryu, C.-M., Oh, S.-K., Kim, R. N., Park, J. M., Cho, H. S., Choi, D. 2006. Suppression of pepper SGT1 and SKP1 causes severe retardation of plant growth and compromises basal resistance. *Physiologia Plantarum*, **126**(4): 605–617. doi:10.1111/j.1399-3054.2006.00631.x
- Deihimi, T., Niazi, A., & Ebrahimie, E. 2013. Identification and expression analysis of TLPs as candidate genes promoting the responses to both biotic and abiotic stresses in wheat. *Journal of Plant Biology & Molecular Omics*, **6**(2): 107–115. Retrieved from
- Dekkers, B. J. W., Willems, L., Bassel, G. W., van Bolderen-Veldkamp, R. P., Ligterink, W., Hilhorst, H. W. M., & Bentsink, L. 2012. Identification of Reference Genes for RT-qPCR Expression Analysis in *Arabidopsis* and Tomato Seeds. *Plant and Cell Physiology*, **53**(1): 28–37. doi:10.1093/pcp/pcr113
- Fu, D., Ghabrial, S., & Kachroo, A. 2009. GmRAR1 and GmSGT1 are required for basal, R gene-mediated and systemic acquired resistance in soybean. *Molecular Plant-Microbe Interactions* : MPMI, **22**(1): 86–95. doi:10.1094/MPMI-22-1-0086
- Fujita, M., Fujita, Y., Noutoshi, Y., Takahashi, F., Narusaka, Y., Yamaguchi-Shinozaki, K., & Shinozaki, K. 2006. Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. *Current Opinion in Plant Biology*,

- 9(4): 436–42. doi:10.1016/j.pbi.2006.05.014
- Hee-Jeong, J., Nasar Uddin, A., Jong-In, P., Senthil Kumar, T., Hye-Ran, K., Yong-Gu, C., & Ill-Sup, N. 2014. Analysis of S-locus and expression of S-alleles of self-compatible rapid-cycling *Brassica oleracea* “TO1000DH3”. *Molecular Biology Reports*, **41**(10): 6441–8. doi:10.1007/s11033-014-3526-6
- Kitagawa, K., Skowyra, D., Elledge, S. J., Harper, J. W., & Hieter, P. 1999. SGT1 Encodes an Essential Component of the Yeast Kinetochore Assembly Pathway and a Novel Subunit of the SCF Ubiquitin Ligase Complex. *Molecular Cell*, **4**(1): 21–33. doi:10.1016/S1097-2765(00)80184-7
- Kud, J., Zhao, Z., Du, X., Liu, Y., Zhao, Y., & Xiao, F. 2013. SGT1 interacts with the Prf resistance protein and is required for Prf accumulation and Prf-mediated defense signaling. *Biochemical and Biophysical Research Communications*, **431**(3): 501–505. doi:10.1016/j.bbrc.2013.01.028
- Kumar, D., & Kirti, P. B. 2015. Pathogen-induced SGT1 of *Arachis diogeni* induces cell death and enhanced disease resistance in tobacco and peanut. *Plant Biotechnology Journal*, **13**(1): 73–84. doi:10.1111/pbi.12237
- Liu, S., Liu, Y., Yang, X., Tong, C., Edwards, D., Parkin, I. a P., Paterson, A. H. 2014. The *Brassica oleracea* genome reveals the asymmetrical evolution of polyploid genomes. *Nature Communications*, **5**(May): 3930. doi:10.1038/ncomms4930
- Lynch, M., O’Hely, M., Walsh, B., & Force, A. 2001. The probability of preservation of a newly arisen gene duplicate. *Genetics*, **159**(4): 1789–804.
- Nakai, K., & Horton, P. 1999. PSORT: a program for detecting sorting signals in proteins and predicting their subcellular localization. *Trends in Biochemical Sciences*, **24**(1): 34–35. doi:10.1016/S0968-0004(98)01336-X
- Narusaka, Y., Narusaka, M., Seki, M., Umezawa, T., Ishida, J., Nakajima, M., Shinozaki, K. 2004. Crosstalk in the responses to abiotic and biotic stresses in *Arabidopsis*: analysis of gene expression in cytochrome P450 gene superfamily by cDNA microarray. *Plant Molecular Biology*, **55**(3): 327–42. doi:10.1007/s1103-004-0685-1
- Noël, L. D., Cagna, G., Stuttmann, J., Wirthmüller, L., Betsuyaku, S., Witte, C.-P., Parker, J. E. 2007. Interaction between SGT1 and cytosolic/nuclear HSC70 chaperones regulates *Arabidopsis* immune responses. *The Plant Cell*, **19**(12): 4061–76. doi:10.1105/tpc.107.051896
- Nyarko, A., Mosbahi, K., Rowe, A. J., Leech, A., Boter, M., Shirasu, K., & Kleanthous, C. 2007. TPR-Mediated self-association of plant SGT1. *Biochemistry*, **46**(40): 11331–41. doi:10.1021/bi700735t
- Sato, T., Jigami, Y., Suzuki, T., & Uemura, H. 1999. A human gene, hSGT1, can substitute for GCR2, which encodes a general regulatory factor of glycolytic gene expression in *Saccharomyces cerevisiae*. *Molecular and General Genetics*, **260**(6): 535–540. doi:10.1007/s004380050926
- Szklarczyk, D., Franceschini, A., Kuhn, M., Simonovic, M., Roth, A., Minguez, P., Von Mering, C. 2011. The STRING database in 2011: Functional interaction networks of proteins, globally integrated and scored. *Nucleic Acids Research*, **39**(Database issue): D561–8. doi:10.1093/nar/gkq973
- Takahashi, A., Casais, C., Ichimura, K., & Shirasu, K. 2003. HSP90 interacts with RAR1 and SGT1 and is essential for RPS2-mediated disease resistance in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America*, **100**(20): 11777–11782. doi:10.1073/pnas.2033934100

- Thamilarasan, S., Park, J.-I., Jung, H.-J., & Nou, I.-S. 2014. Genome-wide analysis of the distribution of AP2/ERF transcription factors reveals duplication and CBFs genes elucidate their potential function in *Brassica oleracea*. *BMC Genomics*, **15**(1): 422. doi:10.1186/1471-2164-15-422
- Town, C. D. 2006. Comparative Genomics of *Brassica oleracea* and *Arabidopsis thaliana* Reveal Gene Loss, Fragmentation, and Dispersal after Polyploidy. *THE PLANT CELL ONLINE*, **18**(6): 1348–1359. doi:10.1105/tpc.106.041665
- Wang, G.-F., Fan, R., Wang, X., Wang, D., & Zhang, X. 2015. TaRAR1 and TaSGT1 associate with TaHsp90 to function in bread wheat (*Triticum aestivum* L.) seedling growth and stripe rust resistance. *Plant Molecular Biology*, **87**(6): 577–589. doi:10.1007/s11103-015-0298-x
- Xing, L., Qian, C., Cao, A., Li, Y., Jiang, Z., Li, M., Chen, P. 2013. The Hv-SGT1 Gene from *Haynaldia villosa* Contributes to Resistances Towards Both Biotrophic and Hemi-Biotrophic Pathogens in Common Wheat (*Triticum aestivum* L.). *PLoS ONE*, **8**(9): e72571. doi:10.1371/journal.pone.0072571
- Yabuta, Y., Nishizawa-Yokoi, A., Ono, K., & Shigeoka, S. 2009. *Arabidopsis* Sgt1a as an important factor for the acquirement of thermotolerance. *Plant Science*, **177**(6): 676–681. doi:10.1016/j.plantsci.2009.08.005
- Yao, Q.-Y., Xia, E.-H., Liu, F.-H., & Gao, L.-Z. 2015. Genome-wide identification and comparative expression analysis reveal a rapid expansion and functional divergence of duplicated genes in the WRKY gene family of cabbage, *Brassica oleracea* var. *capitata*. *Gene*, **557**(1): 35–42. doi:10.1016/j.gene.2014.12.005

Table 1. Primers for expression analysis

Primers for RT-PCR			
Gene	Forward	Reverse	Product length (bp)
<i>BoActin</i>	GCTATGTATGTCGCTATTC	TTGGAAGTCTCCATCTC	329
<i>BolSGT1a</i>	GCCGGTAAAAGTTGATCAGAC	TCAATCTTGTCCTCCAGTCCTTC	420
<i>BolSGT1b_1</i>	CCACTCCACCACTAGCAAC	AAACCGCTGATGCGACATTGG	372
<i>BolSGT1b_2</i>	AAGATGGATGGCGAAGGAAC	AACAGGAGCAGCATCAGC	454
<i>BolSGT1</i>	CACCTCCAAATCATCGACTTC	TCTAACCTAACGCGATGC	519
Primers for RT-qPCR			
<i>BoActin</i>	TTCTCTCTTCCACACGCCAT	CTTGTCTGCGGGTAATTCG	265
<i>BolSGT1a</i>	AAGTTGATCAGACCCCTGTG	TTGGAGATGATACGCTTCCTC	194
<i>BolSGT1b_1</i>	CCACTCCACCACTAGCAAC	ATAAGCTTCCTCTCCAGCAAC	200
<i>BolSGT1b_2</i>	AAGATGGATGGCGAAGGAAC	ACTCGATGGCTTTGTTGGC	200
<i>BolSGT1</i>	CACCTCCAAATCATCGACTTC	ATGCGGAAGAGGAGGAAGAC	200

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Table 2. Sequence characteristics of *BolSGT1* genes used in this study

Name	Accession number	Nucleotide length (bp)	ORF (bp)	Chromosome number	Exon positions
<i>BolSGT1a</i>	Bol042107	1,879	1,032	C06	1-160
					516-585
					683-807
					888-969
					1056-1152
					1227-1292
					1369-1507
					1587-1879
<i>BolSGT1b_1</i>	Bol006784	2,022	963	C09	1-160
					406-475
					553-677
					776-893
					990-1086
					1177-1242
					1326-1464
					1566-1645
<i>BolSGT1b_2</i>	Bol025710	2,189	687	C03	1915-2022
					1-108
					968-1064
					1148-1274
					1388-1512
					1697-1766
					2030-2189
<i>BolSGT1</i>	Bol009238	2,178	1,947	C03	1-186
					264-543
					628-966
					1037-2178

ORF - Open reading frame, bp – base pair

Table 3. Sequence characteristics of BolSGT1 proteins used in this study

Name	Accession number	Protein								
		Length (aa)	Domain				N-Glyc (Asn) position	Mol. wt (kDa)	pI	Instability index
			TPR	CS	SGS	SGT1				
<i>BolSGT1a</i>	Bol042107	343	2-103	145-221	260-341	-	51, 312	38.535	5.49	42.39
<i>BolSGT1b_1</i>	Bol006784	320	2-103	157-233	273-318	-	51, 289	35.302	5.23	41.94
<i>BolSGT1b_2</i>	Bol025710	228	2-103	160-207	192-226	-	51, 102, 197	24.859	4.71	45.16
<i>BolSGT1</i>	Bol009238	648	-	-	-	22-609	-	73.167	4.69	54.64

aa - amino acid, pI – isoelectric point, kDa- kilodalton

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Figure captions

Fig. 1. Phylogenetic tree of SGT1 proteins of the Brassicaceae constructed by the neighbor-joining method. The tree was grouped into SGT1a, SGT1b and SGT1 clades. At-*A. thaliana*, *Bol-B. oleracea*, *Bra-B. rapa*, *Bn-Brassica napus*, *Cs-Camelina sativa*. Accession numbers are listed in Supplementary file 3.

Fig. 2. Organ-specific expression of *BolSGT1* genes. R- Root, S-Stem, L-Leaf, Fb-Flower bud, *Bo-Brassica oleracea*

Fig. 3. Real-time PCR expression analysis of *BolSGT1* genes after stress treatment in *B. oleracea*. The error bars represent the standard error of the means of two technical replicates.

Fig. 4. Interaction network (confidence view) of *BolSGT1* genes identified in *B. oleracea* and related genes in *Arabidopsis*. Stronger associations are represented by thicker lines.

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338x190mm (300 x 300 DPI)

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338x190mm (300 x 300 DPI)

Abbreviation

<i>BolSGT1a</i>	<i>Brassica oleracea</i> Suppressor of G2 allele of SKP1a
<i>BolSGT1b</i>	<i>Brassica oleracea</i> Suppressor of G2 allele of SKP1b
<i>BolSGT1</i>	<i>Brassica oleracea</i> Suppressor of GCR2
SKP1	S-phase kinase-associated protein 1
<i>AtSGT1a</i>	<i>Arabidopsis thaliana</i> Suppressor of G2 allele of SKP1a
<i>AtSGT1b</i>	<i>Arabidopsis thaliana</i> Suppressor of G2 allele of SKP1b
<i>AtSGT1</i>	<i>Arabidopsis thaliana</i> Suppressor of GCR2
GCR2	GlyColysis Regulation
DREB	Dehydration-responsive element-binding
<i>AtRALF</i>	<i>Arabidopsis thaliana</i> plant rapid alkalization factor
<i>AtMGL</i>	<i>Arabidopsis thaliana</i> Methionine gamma-lyase
Hsp90	Heat shock protein 90
Prf	Protein release factor
RAR1	Retinoic acid receptor 1
Nod1	Nucleotide-binding oligomerization domain-containing protein 1
RNA	Ribonucleic acid
SMART	Smart Modular Architecture Research Tool
N-Glyc	N-glycosylation
RT-PCR	Reverse transcriptase Polymerase Chain Reaction
RT-qPCR	Real time quantitative PCR
pmol	Picomolar
ml	Milli litre
μl	Micro litre
HSC70-1	Heat shock cognate protein 70-1
PBS2	Pphb susceptible 2
SRFR1	Suppressor of RPS4-RLD 1
AFB5	Auxin F-Box protein 5
RPM1	Resistance to <i>Pseudomonas syringae</i> PV <i>maculicola</i> 1

338x190mm (300 x 300 DPI)

Supplementary File 2. Pairwise alignment of 17 BolSG

Group	Gene id.	Bol009532	Bol007888	Bol028593
<i>BolSGT1a</i>	Bol006218 (243 bp)	77 %	65 %	34 %
	Bol009532 (168 bp)		92 %	67 %
	Bol007888(279 bp)			54 %
	Bol028593 (228 bp)			
	Bol010362 (267 bp)			
	Bol043319 (180 bp)			
	Bol012349 (159 bp)			
<i>BolSGT1b</i>	Bol042107 (1032 bp)			
	Bol006784 (963 bp)			
	Bol017231 (276 bp)			
	Bol026963 (168 bp)			
	Bol000515 (207 bp)			
	Bol000937 (453 bp)			
	Bol043318 (471 bp)			
<i>BolSGT1</i>	Bol025710 (687 bp)			
	Bol009238 (1947 bp)			
	Bol008059 (342 bp)			

T1 genes.

Bol010362 Bol043319 Bol012349 Bol042107 Bol006784 Bol017231 Bol026963 Bol000515

62 %	37 %	75 %	78 %	57 %	47 %	75 %	51 %
94 %	24 %	96 %	86 %	67 %	62 %	92 %	41 %
93 %	33 %	94 %	80 %	43 %	42 %	92 %	52 %
53 %	27 %	66 %	79 %	69 %	45 %	68 %	16 %
	32 %	93 %	80 %	44 %	44 %	94 %	49 %
		25 %	78 %	72 %	15 %	27 %	23 %
			86 %	70 %	62 %	94 %	40 %
				72 %	54 %	84 %	76 %
					54 %	67 %	52 %
						61 %	18 %
							41 %

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Bol000937	Bol043318	Bol025710	Bol009238	Bol008059
32 %	19 %	48 %	31 %	23 %
38 %	21 %	60 %	44 %	27 %
16 %	34 %	48 %	30 %	30 %
20 %	16 %	44 %	32 %	31 %
17 %	39 %	35 %	40 %	22 %
82 %	40 %	77 %	32 %	37 %
26 %	23 %	66 %	46 %	34 %
80 %	42 %	69 %	13 %	18 %
65 %	41 %	88 %	9 %	31 %
22 %	19 %	44 %	37 %	16 %
37 %	22 %	60 %	47 %	27 %
28 %	28 %	35 %	46 %	34 %
	38 %	69 %	21 %	27 %
		34 %	24 %	22 %
			18 %	21 %
				82 %

Supplementary File 3. Accession number of sequences used for Phylogenetic tree

Name	Accession number
Bolsgt1a	Bol042107
BolSGT1b_1	Bol006784
BolSGT1b_2	Bol025710
BolSGT1	Bol009238
BolTha	Bol026250
BraSGT1a_1	Bra019295
BraSGT1a_2	Bra013710
BraSGT1b_1	Bra035239
BraSGT1b_2	Bra000741
BraSGT1	Bra024390
BraTha	Bra015980

Sequence collected from Brassica database

Name	Accession number
AtTha	AT1G73620
ATSGT1a	AT4G23570
ATSGT1b	AT4G11260
ATSGT1	AT5G65490

Sequence collected from TAIR database

Name	Accession number
BnSGT1	CDY14571.1
CsSGT1b	XP_010422073.1
CsSGT1	XP_010463019.1
BnSGT1a	CDY10946.1
BnSGT1b	CDY13243.1
BnTha	NP_177503.1

Sequence collected from Genbank

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