Plant Biotechnology 35, 71–79 (2018)
DOI: 10.5511/plantbiotechnology.18.0205a

Original Paper

Expression of barley *Glutathione S-Transferase13* gene reduces accumulation of reactive oxygen species by trichothecenes and paraquat in Arabidopsis plants

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Received December 30, 2017; accepted February 5, 2018 (Edited by K. Suzuki)

Abstract Glutathione S-transferases (GSTs) play an important role in the detoxification of reactive oxygen species (ROS) and toxic compounds. We found that the barley phi class *GST* (*HvGST13*) gene is upregulated by trichothecene phytotoxin produced by the fungal pathogen *Fusarium graminearum* in barley. Trichothecene phytotoxins such as DON and T-2 toxin induce accumulation of ROS and cell death in plants. It is known that the death of host cells contributes to the virulence of *F. graminearum* during the later stages of infection. To characterize the role of the *HvGST13* gene, we generated Arabidopsis plants in which *HvGST13* was overexpressed. Growth inhibition by DON and T-2 toxin was significantly alleviated in the *HvGST13*ox Arabidopsis plants compared with the wild type. Accumulation of ROS and cell death apparently decreased in *HvGST13*ox Arabidopsis plants treated with trichothecene. Paraquat herbicide is well known to induce the generation of ROS in plants. Paraquat-induced growth retardation was also suppressed in the *HvGST13*ox Arabidopsis plants compared with wild type. The inoculation of *F. graminearum* causes disease symptoms that are markedly decreased in *HvGST13*ox Arabidopsis plants compared to those in the wild type. Therefore, the *HvGST13* gene suppressed the phytotoxic activity of trichothecenes in plants, possibly by the scavenging of ROS.

Key words: *Fusarium*, glutatathione S-transferase, paraquat, reactive oxygen species, trichothecene phytotoxins.

Introduction

Phytopathogenic fungi, e.g., *Fusarium* species such as *Fusarium graminearum*, produce trichothecene mycotoxins and are etiological agents of Fusarium Head Blight (FHB) disease that causes yield and quality losses in wheat and barley (Streit et al. 2012). Trichothecenes are known as inhibitors of protein synthesis in eukaryotic cells and food contaminated with them threatens the health of humans and animals (Nishiuchi 2013). Trichothecenes have an epoxy ring at positions C-12 or C-13, which are crucial for toxicity (McCormick et al. 2011). Trichothecenes also act as phytotoxins and affect plant morphology, such as causing inhibition of root elongation and dwarfism (Masuda et al. 2007). In Arabidopsis plants, T-2 toxin, which is a type A trichothecene, induces the accumulation of reactive oxygen species (ROS) and necrotic cell death (Nishiuchi et al. 2006). Deoxynivalenol (DON), belonging to the type B trichothecenes, also induces ROS production and cell death in wheat (Desmond et al. 2008). To detoxify trichothecene phytotoxins, plants have several detoxification mechanisms, such as glucosylation (Poppenberger et al. 2003) and gluthathione (GSH)-conjugate formation (Gardiner et al. 2010). GSH conjugates are catalyzed by glutathione S-transferases (GSTs) (Edwards et al. 2000).

GST gene family in Arabidopsis contains 55 genes that

Abbreviations: DAB, 3,3′-diaminobenzidine; DHAR, dehydroascorbate reductase; DON, Deoxynivalenol; EF1G, elongation factor 1 gamma; FHB, Fusarium Head Blight; GPOX, glutathione peroxidase; GSH, gluthathione; GSTs, Glutathione S-Transferases; LPTB, lactic acid–phenol–trypan blue; MES, 2-(*N*-morpholino) ethanesulfonic acid monohydrate; MS, Murashige and Skoog; PQ, paraquat; ROS, reactive oxygen species; TCHQD, tetrachlorohydroquinone dehalogenase; WT, wild type.

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This article can be found at http://www.jspcmb.jp/
Published online March 31, 2018

can be divided into eight classes: phi (F), tau (U), theta (T), zeta (Z), lambda (L), dehydroascorbate reductase (DHAR), tetra-chlorohydroquinone dehalogenase (TCHQD), and membrane-associated proteins in eicosanoid and glutathione (Dixon and Edwards 2010). Barley genome has 84 putative members of the *GST* gene family, which are classified into eight classes: phi, tau, theta, zeta, lambda, DHAR, TCHQD, and elongation factor 1 γ (EF1G) (Rezaei et al. 2013). Tau and phi are the first-and second-largest of GST classes in plants, respectively (Dixon and Edwards 2010).

Asano et al. (2012) reported that expression of Arabidopsis phi class AtGSTF7 protein was induced following infiltrated with *F. graminearum* producing DON, while AtGSTF3 and AtGSTF9 were upregulated by inoculation of *F. sporotrichioides* producing T-2 toxin. Our previous microarray experiment using FHB-resistant barley breeding lines showed that barley's *HvGST13* gene was significantly upregulated in plants infected with *Fusarium* (unpublished data), indicating that *HvGST13* may be involved in resistance to FHB. On the basis of its amino-acid sequence alignment, barley HvGST13 protein is classified in the phi class. In addition, the application of DON in barley seedlings induced an enhanced transcript level of *HvGST13* (Figure S1). Therefore, we assumed that the *HvGST13* protein of barley can suppress phytotoxic effects of trichothecenes in host plant cells. To gain insight into the role of the *HvGST13* gene, we analyzed Arabidopsis plants in which *HvGST13* was overexpressed under trichothecene treatment.

Materials and methods

Plant materials and growth conditions

Arabidopsis thaliana ecotype Columbia-0 (Col-0) was used as wild type (WT) and genetic background for generating transgenic plants. Sterilization of the seed surface and conditions of plant growth were described previously (Miwa et al. 2017). Seeds were sown onto plates containing Murashige and Skoog (MS) medium supplemented with 2% (w/v) sucrose and 0.2% (w/v) gelrite at pH 5.8, with or without the substances being tested (10 μM DON, 0.5 μM T-2 toxin). Half-MS medium was used to evaluate the sensitivity to herbicide (0.1 μM paraquat) (Fujita et al. 2012). Both fresh weight of shoots and length of primary roots were measured at 2 and 3 weeks after sowing for control and treated seedlings, respectively. To select kanamycin-resistant plants, MS agar medium was supplemented with 0.8% (w/v) bacto agar and 50 μg ml^{-1} kanamycin. For expression study of the barley *HvGST13* gene, the FHB-resistant barley cultivar Sirius O.525 were grown in MS agar medium after sterilizing with 50% (v/v) NaClO and 0.1% (v/v) Tween-20. Seven-day-old barley seedlings were subsequently transferred to MS agar medium containing 5 μM DON and were incubated for 24 or 72 h.

Preparation of Arabidopsis transgenic plants

To generate Arabidopsis transgenic plants overexpressing *HvGST13* (AK252233.1), the coding sequence (CDS) of the *HvGST13* gene was amplified from cDNA of barley (cv. Maja) using gene-specific primers. Next, PCR products were cloned into pENTR™/D-TOPO® (Invitrogen, Carlsbad, CA, USA). The inserted sequence was confirmed and finally transferred to pK2GW7.0 binary vectors using Gateway® Technology (Invitrogen) (Karimi et al. 2002). The resulting plasmids were introduced into *Agrobacterium tumefaciens* (strain GV2260), and transgenic Arabidopsis plants were prepared using the *Agrobacterium*-mediated floral dip method (Clough and Bent 1998). Transgenic plants were screened in kanamycin-containing MS agar medium.

RNA isolation, cDNA synthesis, and RT-PCR

Total RNAs were extracted from leaf tissue of Arabidopsis and whole barley seeding using the Agilent Plant RNA Isolation Mini Kit (Agilent Technologies, Santa Carla, CA, USA). The cDNA was prepared using 1 μg total RNA and PrimeScript™ RT Master Mix (Perfect Real Time) (Takara Bio Inc., Shiga, Japan). RT-PCR was performed with Quick Taq® HS DyeMix (Toyobo Co., Ltd., Osaka, Japan), specific primers for the *HvGST13* gene (AAG CTG TAC GGG ATG ATG CT and GAA AGG GTT GAG CTT GAG G), and primers for Arabidopsis *Actin2/8* or barley *α-tubulin* genes, as described previously (Miwa et al. 2017).

Histochemical staining

To evaluate cell death, samples of plants exposed to trichothecene or paraquat and samples of plants inoculated with *Fusarium* were submerged in lactic acid–phenol–trypan blue solutions [LPTB; 2.5 mg ml^{-1} trypan blue, 25% (v/v) lactic acid, 23% (v/v) water–saturated phenol, 25% (v/v) glycerol, and H_2O] at 70°C, as described by Bowling et al. (1997). The accumulation of ROS was examined by staining samples with 3,3′-diaminobenzidine (DAB) solutions as described previously (Daudi and O'Brien 2012). Thereafter, stained leaves were observed under an Olympus SZX16 microscope (Olympus Optical Co., Ltd., Tokyo, Japan).

Leaf floating test

The method of leaf floating test developed by Fujita et al. (2012) was modified as follows. Leaves detached from 3-week-old plants were floated on a buffer containing 3 mM 2-(*N*-morpholino) ethanesulfonic acid monohydrate (MES) and 0.05% (v/v) Tween-20 with or without paraquat. Next, floated leaves were incubated at 22°C for 2 days under continuous light illumination. Subsequently, leaves were submerged in 1 ml of dimethylformamide and incubated at 4°C in the dark for overnight. The chlorophyll content of the leaves was determined, as described previously (Porra et al. 1989), and was normalized by leaf size, which was calculated using ImageJ software (http://rsb.info.nih.gov/ij/index.html).

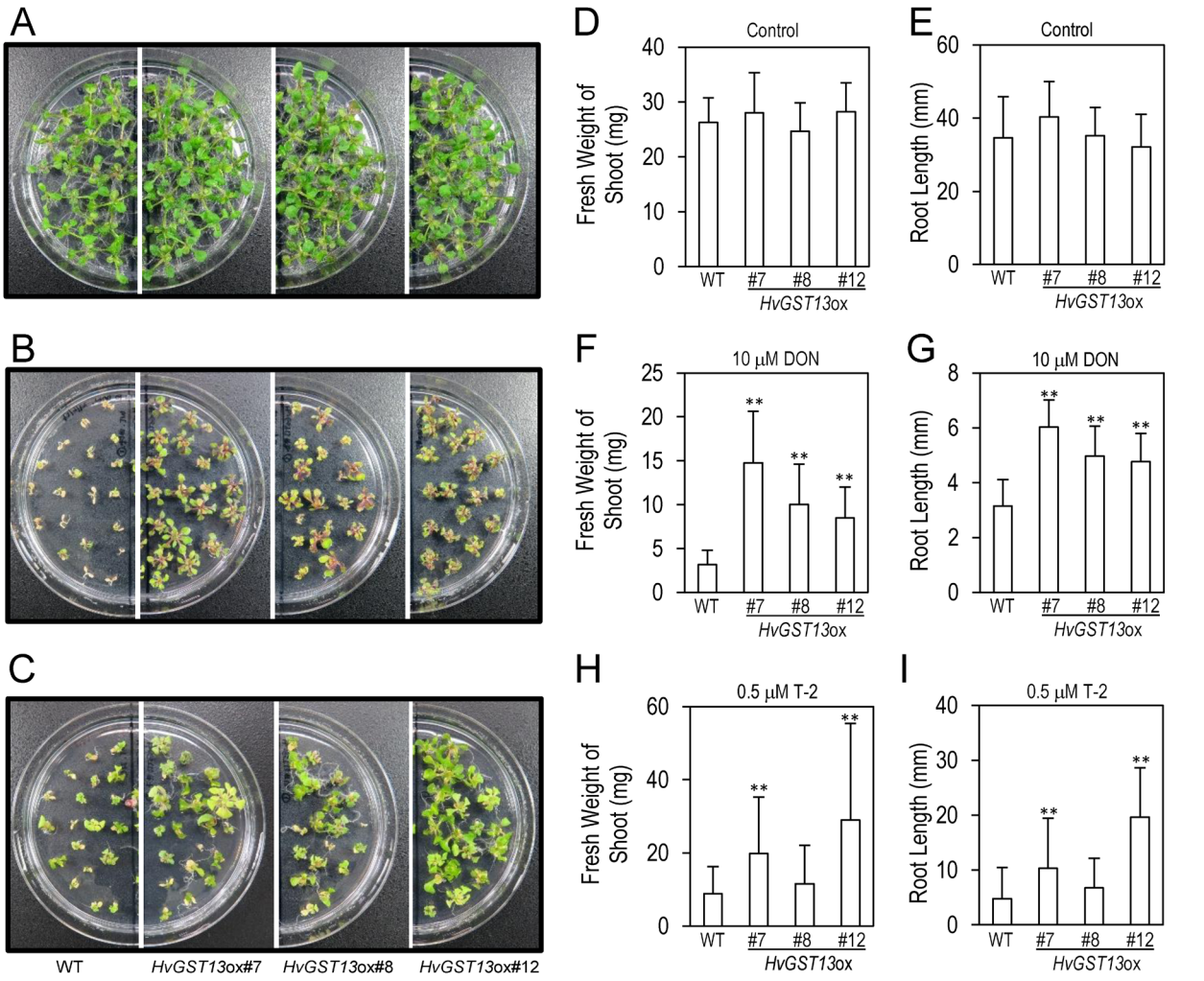

Figure 1. Arabidopsis *HvGST13*ox plants show trichothecene-resistant phenotype. Representative photographs of Arabidopsis plants grown under MS medium without trichothecenes (A), or with 10 μM DON (B) and 0.5 μM T-2-toxin (C). Fresh weight (mg) of shoots and primary root length (mm) of Arabidopsis plants grown under MS medium without (D, E), with 10 μM DON (F, G), or 0.5 μM T-2-toxin (H, I). Error bars represent the standard deviation (n=34–40). Student's t-test * p<0.05, ** <0.01.

Inoculation of Fusarium graminearum

Fusarium graminearum strain H3 was cultured in Synthetic Low Nutrient liquid medium [0.1% (w/v) KH_2PO_4, 0.1% (w/v) KNO_3, 0.1% (w/v) $MgSO_4{\cdot}7H_2O$, 0.05% (w/v) KCl, 0.02% (w/v) glucose, 0.02% (w/v) sucrose] at 22°C. A1×10^5 conidia ml^{-1} suspension was spread over the abaxial sides of the leaves of 4- to 5-week-old plants with a needleless syringe. The inoculated plants were covered with plastic sheet to maintain high humidity allowing fungal growth and placed in a growth room at 22°C for 3 days. The assessment of disease severity was based on Miwa et al. (2017), while the disease severity index was calculated according to Nalam et al. (2016).

Results and discussion

Characterization of barley HvGST13 proteins

The transgene *HvGST13* was isolated from the highly resistant barley cultivar Maja using specific primers. After isolating the RNA and synthesizing cDNA, the full length cDNA (645 bp) was obtained successfully and was subsequently transformed to Arabidopsis that was used as the WT plant (Col-0). The protein class of HvGST13 was categorized as phi GST and was highly homologous (91%) with phi TaGSTF5 (CAD20478.1) in wheat. Supplementary Figure S2 showed the alignment of amino acid sequences of barley HvGST13 and three other homologs. The N-terminal and C-terminal domains of GST proteins were conserved among these proteins. The N-terminal domain contains glutathione binding site (G-site) and first serine is important for catalytic activities (Reinemer et al. 1997). The ESR motif of G site is specifically conserved among phi class GST family proteins (Wagner et al. 2002). The C-terminal of GST proteins likely bind to substrate (H-site) (Edwards et al. 2000). According to the results of a PredictProtein (https://www.predictprotein.org/) analysis, HvGST13 has an *N*-glycosylation site, a protein kinase C phosphorylation site, a casein kinase II phosphorylation

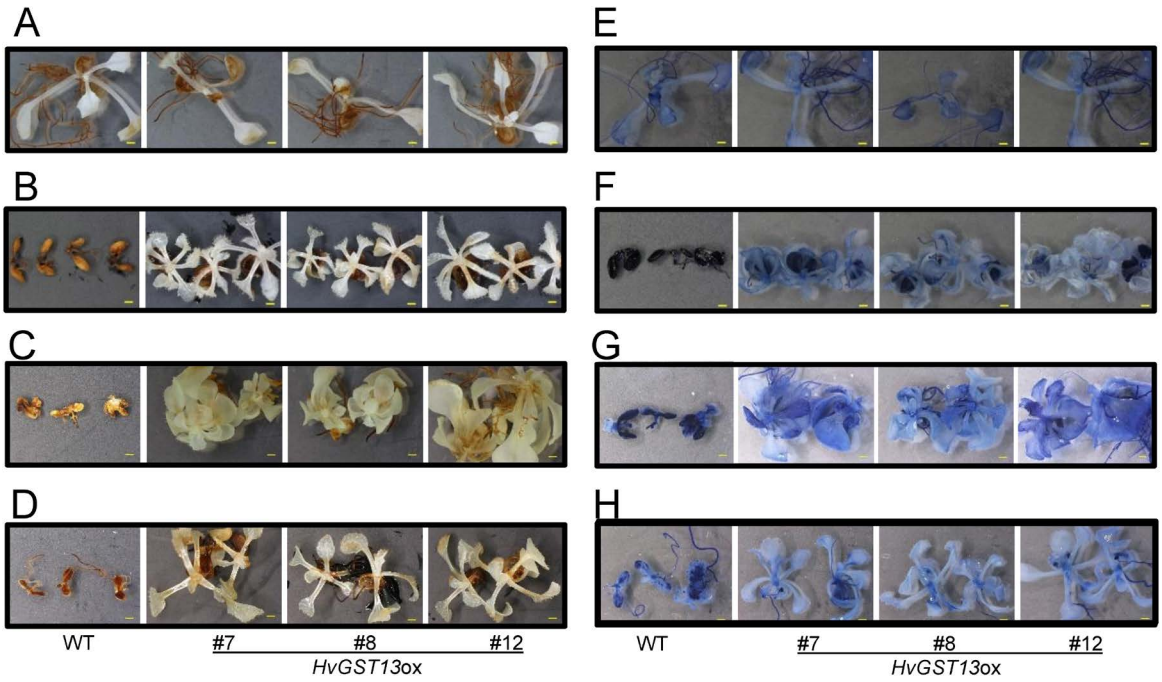

Figure 2. DAB and trypan blue staining of Arabidopsis WT and *HvGST13*ox seedlings. DAB-staining seedlings grown under MS medium without trichothecenes (A) with with 10 μM DON (B), 0.5 μM T-2 toxin (C), or 0.1 μM paraquat (D). Trypan blue staining of untreated seedlings (E) and 10 μM DON- (F), 0.5 μM T-2 toxin- (G) or 0.1 μM paraquat-treated seedlings (H). Scale bar: 1 mm.

site, an *N*-myristoilation site, and a leucine zipper pattern. In addition, HvGST13 protein neither contained Nuclear Localization Signal nor contained secretory signal peptide and was predicted to be a cytoplasmic protein. Similarly, all poplar GSTF proteins were also predicted to have a cytosolic localization (Pégeot et al. 2014). In Arabidopsis, most GSTs are also located in the cytosol; however, GSTU12 was transported to the nucleus, TCHQD was localized in the plasma membrane, and lambda GSTL2 was a peroxisomal protein (Dixon et al. 2009).

HvGST13ox Arabidopsis plants show phenotypes resistant to trichothecene phytotoxins (DON and T-2 toxin)

We examined the transcript level of the *HvGST13* gene among 13 transgenic lines using RT-PCR analysis (Supplementary Figure S3) to investigate the functional role of the *HvGST13* gene. We selected three representative lines, namely *HvGST13*ox#7, *HvGST13*ox#8, and *HvGST13*ox#12, and used homozygous plants of these lines here. WT and three lines of *HvGST13*ox Arabidopsis plants were grown in MS medium without or with 10 μM DON or 0.5 μM T-2 toxin to examine the phytotoxic effects in plants overexpressing *HvGST13*. The WT and *HvGST13*ox plants grown under MS medium without trichothecene showed a similar phenotype with normal morphology (Figure 1A). In contrast, *HvGST13*ox plants showed a phenotype that was resistant to treatment with DON (Figure 1B) or T-2 toxin (Figure 1C) in comparison with WT plants. DON-treated WT plants showed retarded growth with morphological changes, such as small yellow cotyledons, very short roots, and the absence of true leaves (Masuda et al. 2007). In contrast, the growth retardation and aberrant morphology induced by DON was significantly alleviated in the *HvGST13*ox plants.

WT plants treated with T-2 toxin showed dwarf shoots with dark-green leaves, whereas these aberrant morphologies were alleviated in *HvGST13*ox plants (Masuda et al. 2007). Both fresh weight of shoots and the root length were greater in *HvGST13*ox plants than in WT plants (Figure 1D–I). Therefore, it is likely that the *HvGST13* gene contributes to suppressing the phytotoxic effects of two different trichothecene molecular species in plants.

Accumulation of ROS and cell death by trichothecenes are suppressed in the HvGST13ox plants

Among the earliest responses following a defense response, the production of ROS such as H_2O_2 (hydrogen peroxide) is well recognized (Wojtaszek 1997). DAB staining can be used to detect the accumulation of hydrogen peroxide (Lehmann et al. 2015). DAB stains of *HvGST13*ox plants are similar to those of WT (Figure 2A). In contrast, *HvGST13*ox plants treated with DON or T-2 toxin showed a low accumulation of hydrogen peroxide compared with WT plants (Figure 2B, C), indicating that *HvGST13*ox lines were obviously capable

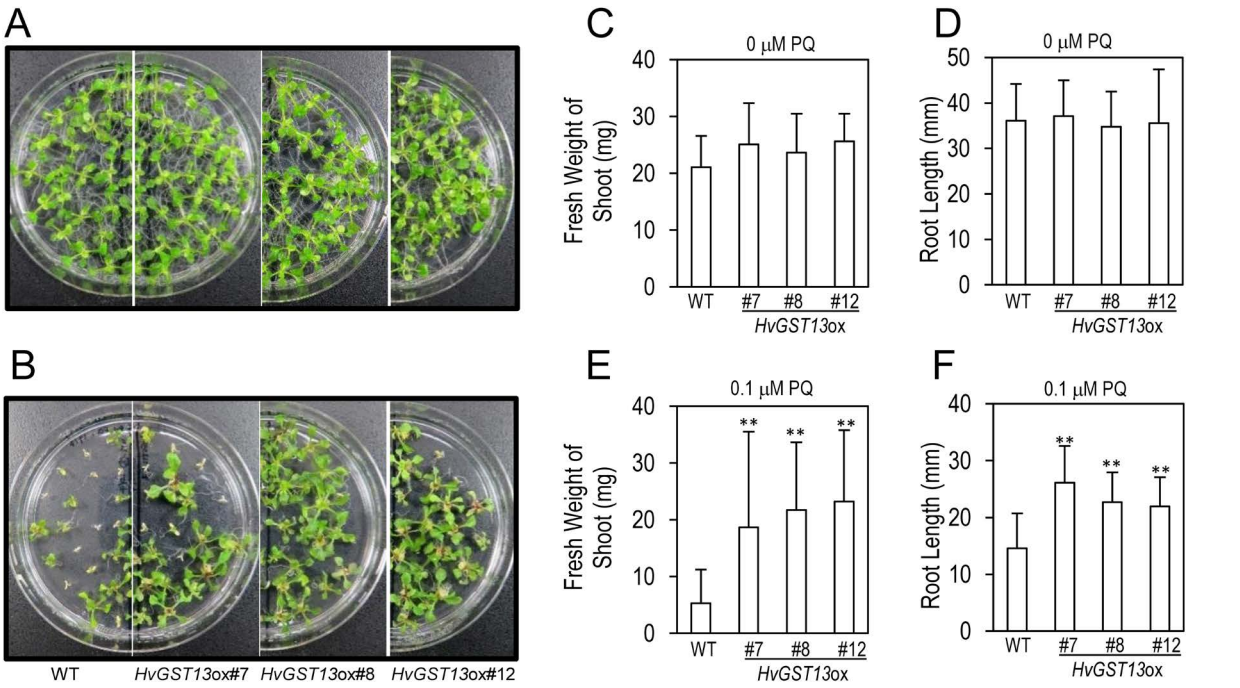

Figure 3. Arabidopsis *HvGST13*ox plants show paraquat-resistant phenotype. Untreated (A) and 0.1 μM paraquat-treated seedlings grown on half-MS medium (B). Fresh weight of shoots and root length of control (C, D) and paraquat-treated plants (E, F). Error bars represent the standard deviation (n=38–40). Student's t-test * $p<0.05$, ** <0.01.

of scavenging hydrogen peroxide by trichothecenes. Therefore, the *HvGST13* gene belongs to the phi class of the *GST* gene family (*GSTF*) which has glutathione peroxidase activity (Pégeot et al. 2014).

Trichothecenes activate the mitogen-activated pathogen kinase cascade, the accumulation of ROS and leading to cell death in Arabidopsis (Nishiuchi et al. 2006). Similarly, accumulation of hydrogen peroxide and programmed cell death in wheat were also induced by DON treatment (Desmond et al. 2008). Therefore, trichothecene-treated Arabidopsis plants were stained with trypan blue to visualize cell death. Trypan blue stains had already been observed in the roots of WT and transgenic plants without trichothecene treatment (Figure 2E). DON- and T-2 toxin-induced cell death was obvious in the shoots of WT plants (Figure 2F, G). In contrast, the true leaves of DON- and T-2 toxin-treated *HvGST13*ox plants displayed a reduced level of trypan blue stains (Figure 2F, G). These results indicate that expression of the *HvGST13* gene suppressed trichothecene-induced cell death in Arabidopsis plants.

HvGST13ox Arabidopsis plants also exhibit phenotypic resistance to paraquat

As stated above, the trichothecene-induced accumulation of ROS was possibly suppressed by the glutathione peroxidase activity of the HvGST13 protein in Arabidopsis plants. To examine whether other producers of ROS can be detoxified by *HvGST13* expression, Arabidopsis plants were grown on MS agar medium containing paraquat. Paraquat, an inhibitor of photosystem II, is a herbicide whose phytotoxicity is caused by the generation of ROS and organic hydroperoxide (Iturbe-Ormaetxe et al. 1998). When Arabidopsis seeds were sown on half-MS agar medium with 0, 0.1, and 0.5 μM paraquat; 0.5 μM paraquat completely inhibited seed germination in both WT and *HvGST13*ox Arabidopsis plants (data not shown). Transgenic and WT seedlings showed similar phenotypes on half-MS medium without paraquat (Figure 3A). Most WT plants showed severe growth defects in medium containing 0.1 μM paraquat (Figure 3B). However, Figure 3 makes clear that in most *HvGST13*ox plants, growth defects induced by 0.1 μM paraquat were significantly suppressed. In addition, the fresh weight of shoots and root length of *HvGST13*ox plants treated with 0.1 μM paraquat were significantly higher in comparison with WT plants, while there were no significant differences between WT and *HvGST13*ox grown on medium without paraquat (Figure 3C–F). In fact, the accumulation of ROS and cell death induced by paraquat were alleviated in *HvGST13*ox plants compared with WT plants (Figure 2D, H). Taken together, the accumulation of ROS by paraquat is possibly suppressed by the glutathione peroxidase activity of HvGST13 protein in Arabidopsis plants.

Expression of HvGST13 gene suppresses paraquat-induced chlorosis in Arabidopsis plants

We further evaluated the effect of paraquat on chloroplasts in WT and *HvGST13*ox plants. Arabidopsis

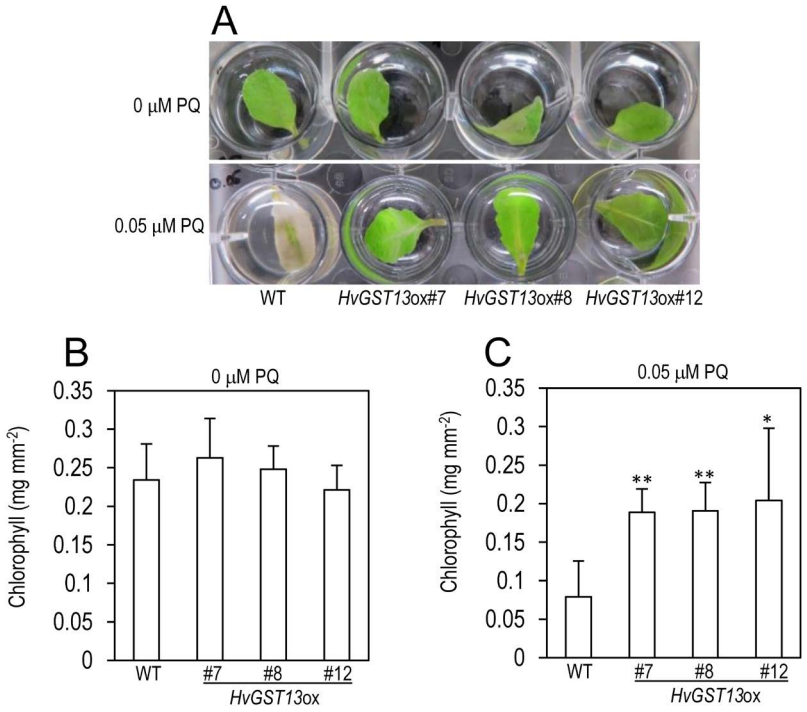

Figure 4. Leaf floating test of WT and *HvGST13*ox plants treated with or without paraquat. Detached leaves were floated on the MES buffer containing paraquat (A). Chlorophyll contents of control (B) and paraquat-treated leaves (C). Error bars represent the standard deviation ($n=6$). Student's *t*-test * $p<0.05$, ** <0.01.

leaves detached from soil-grown plants were submerged in paraquat-containing buffer. After 2 days of incubation, the chlorophyll content in leaves was measured using a spectrophotometer. In WT leaves, paraquat decreased the chlorophyll content significantly by 0.05 μM. In contrast, 0.05 μM paraquat had minor effects on the leaves of *HvGST13*ox plants (Figure 4A). Therefore, the expression of *HvGST13* suppressed the effects of chlorophyll bleaching by paraquat in Arabidopsis leaves. This result was supported by measurements of the chlorophyll concentration (Figure 4B, C).

Arabidopsis HvGST13ox plants enhance the resistance against Fusarium graminearum

Trichothecenes produced by *Fusarium* species can trigger ROS production and cell death in the host plants (Nishiuchi 2013). We recently reported that decreasing the accumulation of ROS rather enhances disease resistance against *F. graminearum* (Miwa et al. 2017). Therefore, both the accumulation of ROS and cell death in host plants possibly contribute to the virulence of *F. graminearum* in the necrotrophic stage. Therefore, we examined the effects of *HvGST13* expression on the resistance to *F. graminearum* in Arabidopsis plants. We inoculated leaves of WT and *HvGST13*ox plants with conidia of *F. graminearum* strain H3, as described previously (Miwa et al. 2017). Disease severity was classified according to Miwa et al. (2017), and the disease index was calculated using the formula given by Nalam et al. (2016). At 3 dpi, severe disease symptoms were observed in most WT leaves (Figure 5A). In addition, accumulation of ROS were apparently detected by DAB in WT plants (Figure 5B). Figure 5B also shows that the expression of *HvGST13* effectively decreases the accumulation of ROS after inoculation of *F. graminearum*. The disease severity index of all *HvGST13*ox lines decreased markedly compared to that of WT (Figure 5C). Because *F. graminearum* is a hemibiotrophic pathogen, death of the host cells by the accumulation of ROS possibly contributes to the virulence of *F. graminearum* during the late stages of infection. Similarly, overexpression of the *Gossypium arboretum* phi class *GST* (*GaGSTF9*) gene in Arabidopsis plants resulted in enhanced disease resistance against the fungal hemibiotrophic pathogen *Verticillium dahliae* (Gong et al. 2017). In that study, a conidial suspension was injected into the soil near the roots of 20 days-old Arabidopsis plants. After 14 days, the rims of leaves were dead in WT plants while they were still healthy in *GaGSTF9*ox plants. In addition, *V. dahliae*-infected *GaGSTF9*ox plants showed reduced levels of hydrogen peroxide compared with WT. The low accumulation of ROS by *GaGSTF9* expression possibly contributed to enhanced disease resistance against *V. dahliae*. In contrast, the phenotype of the Arabidopsis *gstf9* mutant was more susceptible to *V. dahliae* than the WT was (Gong et al. 2017).

GSTs are enzymes with multiple functions such as

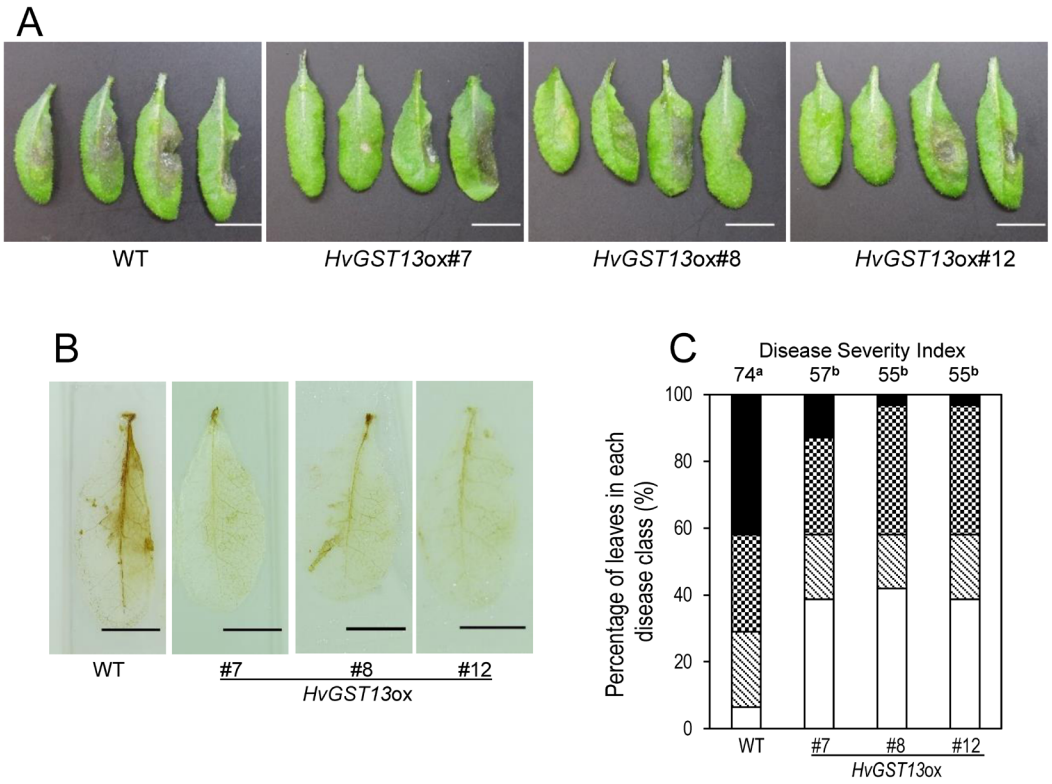

Figure 5. Enhanced disease resistance of Arabidopsis *HvGST13*ox plants against *F. graminearum*. (A) Disease symptoms of infiltrated leaves at 3 days after inoculation. (B) DAB staining of inoculated leaves. Scale bar: 1 cm. (C) Percentage of 4 classes of disease-symptoms in inoculated leaves of each line at 3 days post inoculation. Open box: normal, cross-hatched box: color change, checker board: partial aerial mycelium, closed box: expanded aerial mycelium. Disease severity index was calculated based on classifications of disease symptoms. Different letters indicate significant differences ($p<0.05$) based on Tukey's range test.

detoxification, isomerization, and peroxidation. Among all classes, two of them, namely phi (GSTF) and tau (GSTU), possess both detoxification and glutathione peroxidase (GPOX) activities (Dixon et al. 2010). In detoxification, GSTs catalyze the formation of GSH conjugates. When GST acts as a glutathione peroxidase, GST utilizes GSH to reduce an organic hydroperoxide (R–O–OH) to its less toxic monohydroxy alcohol (R–OH) and sulfenic acid (GS–OH), which subsequently can form a disulfide with another GSH to generate GS–SG without producing a GSH-conjugate end-product (Edwards et al. 2000). A number of plant GSTs revealed GST activity and or GPOX. Therefore, we suggest that *HvGST13*ox Arabidopsis plants possess at least one of them.

Mauch and Dudler (1993) reported that the expression of the *GstA1* gene, which belongs to the phi class, was induced by inoculation with *Blumeria graminis* f.sp. *tritici* and *Pucacinia recondita* f.sp. *tritici*. The excess accumulation of ROS and products of lipid peroxidation could be prevented by the glutathione peroxidase activity of the GstA1 protein (Mauch and Dudler 1993). In addition, the *AmGST2* gene in black-grass was shown to be highly expressed in a herbicide-resistant cultivar (Cummins et al. 1999). The AmGST2 protein, which belongs to the phi class, has high glutathione peroxidase (GPOX) activity toward hydroperoxide and a significantly low glutathione-conjugating activity for herbicides. In contrast, the AmGST1 protein belonging to the tau GST class had no GPOX activity but posed a high conjugating activity toward the diphenyl ether herbicide fluorodien (Cummins et al. 1999). In this study, *HvGST13*ox plants had phenotypes that were resistant to trichothecene, paraquat, disease, and decreased the accumulation of ROS. These results suggest that HvGST13 plays an important role in preventing cellular damage by scavenging ROS rather than in detoxifying toxic compounds by GSH-conjugate activity.

HvGST13 protein was similar to barley phi class HvGST6 protein (score 184, E-value 1e-63). The HvGST6 protein was also similar to AmGST2 and GstA1 and showed high glutathione peroxidase activity (Scalla and Roulet 2002). Recombinant HvGST6 protein weakly catalyzed the cleavage and conjugation of the herbicides fluorodien and fenoxaprop but exhibited a strong GPOX activity, particularly toward fatty acid hydroperoxide substrate. These results suggest that HvGST6 protein protects plant cells from oxidative damage by scavenging ROS peroxides rather than by detoxifying herbicides (Scalla and Roulet 2002). In addition, other phi class GST

proteins (Pégeot et al. 2014), such as AtGSTF9 (Nutricati et al. 2006) and poplar PttGSTF1 (Pégeot et al. 2014), were shown to have GPOX activity.

Using *AtGSTF6*/*7*/*9*/*10*Ri Arabidopsis plants that exhibited suppression of the expression of many *GSTFs* by RNA interference, Sappl et al. (2009) showed that phi class GSTs play a potential role in protecting plants from oxidative damage. As stated above, AtGSTF7 and AtGSTF9 proteins were upregulated by inoculation with *F. graminearum* and *F. sporotrichioides*, respectively (Asano et al. 2012). The *AtGSTF6*/*7*/*9*/*10*Ri plants also displayed a low expression of many phi class GST proteins compared with WT. The *AtGSTF6*/*7*/*9*/*10*Ri plants showed an increase of oxidized glutathione (GSH) but no reduction in GST activity. This demonstrates that phi class GSTs are predominantly associated with glutathione peroxidation activity, suggesting the involvement of phi GSTs in protection against oxidative stress (Sappl et al. 2009). Therefore, phi class GSTs effectively removed excess ROS by their glutathione peroxidase activity and maintained a low level of hydrogen peroxide to protect the plants from biotic stresses (Gong et al. 2017).

In this study, we showed that the expression of the *HvGST13* gene of barley in Arabidopsis plants effectively reduced the phytotoxic effects of trichothecenes and paraquat. Because the chemical structures of trichothecenes and paraquat are quite different, *HvGST13* possibly does not detoxify these substances. Alternatively, it is known that phi class GSTs display glutathione peroxidase activities in plants. Therefore, the expression of *HvGST13* possibly scavenges ROS produced by these substances, resulting in a decrease of the cellular damage in Arabidopsis plants. However, it is necessary to quantify the enzymatic activity of HvGST13 protein in the future.

Acknowledgements

We thank Kementerian Riset, Teknologi, dan Pendidikan Tinggi (Kemenristekdikti) Republik Indonesia for a student scholarship to Ninik Nihayatul Wahibah. This research was supported by grants from the Ministry of Agriculture, Forestry and Fisheries of Japan TRS1005; Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry (28007A); and the Joint Research Program implemented at the Institute of Plant Science and Resources, Okayama University in Japan. Barley seed samples were provided by the National Bio-Resource Project of the Ministry of Education, Culture, Sports, Science and Technology, Japan. This work was partly supported by KAKENHI (no. 23580060, 26450053, and 15H05780) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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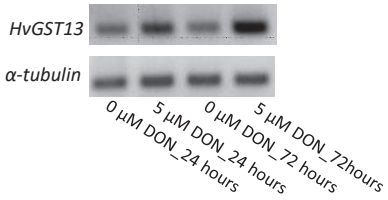

Figure S1. DON-inducible expression of *HvGST13* gene in FHB-resistant barley cultivar Sirius O.525

Figure S2. Amino acid alignment of barley HvGST13, rice OsGSTF4, wheat TaGSTF5 and Arabidopsis AtGSTF13 using Clustal Omega.

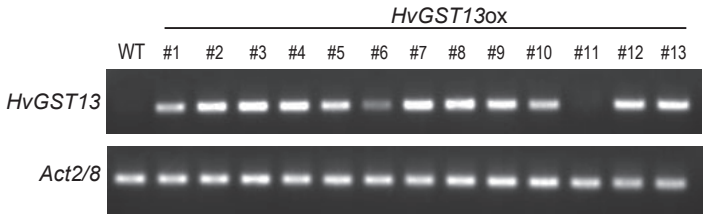

Figure S3. RT-PCR of wild type and 13 candidates of *HvGST13*ox transgenic Arabidopsis plants.