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Loss-of-function in *GIGANTEA* confers resistance to PPO-inhibiting herbicide tiafenacil through transcriptional activation of antioxidant genes in Arabidopsis

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Abstract

Herbicides play a crucial role in maintaining crop productivity by reducing competition between weeds and crops. Protoporphyrinogen oxidase (PPO)-inhibiting herbicides trigger the photooxidative damage that destroys cell membranes. Tiafenacil is a recently developed pyrimidinedione-type PPO-inhibiting herbicide that has low IC_{50} values in plants and is less toxic in humans compared to other PPO inhibitors. Previous reports confirmed that mutations in Arabidopsis circadian clock-controlled gene *GIGANTEA* (*GI*) were insensitive to phytooxidants, including chloroplast biogenesis inhibitors and herbicides. Here, we examined whether GI regulates the resistance to tiafenacil. Both *gi* mutant alleles, *gi-1* and *gi-2*, were resistant to tiafenacil with survival rates of 97% and 83%, respectively, under 1 μM tiafenacil treatments, while 56% of wild-type and GI-overexpressing plants (GI-OX) survived. Both *gi* mutants were insensitive to tiafenacil-induced inhibition of photosystem efficiency and alleviated photooxidative damage. The *gi* mutants showed significant increases in transcriptional expressions and enzyme activities of antioxidants compared to wild-type and GI-OX. Moreover, loss-of-function in *GI* enhanced resistance to tiafenacil-containing commercial herbicide Terrad'or Plus®. Collectively, based on our results together with previous reports, mutations in *GI* confer resistance to herbicides with different MoAs and would be a crucial molecular target for non-target-site resistance strategies to develop herbicide-resistant crops.

Keywords: GIGANTE, Herbicide resistance, Protoporphyrinogen oxidase, Reactive oxygen species, Tiafenacil

Introduction

Weed controls are the major activities in agronomics to maintain crop yields and quality as weeds and crops compete for water, nutrients, sunlight, and space availability [1]. Weed controls have been developed from native manual control to modern mechanical, biological, and chemical controls, reducing agricultural labor for crop producers and increasing the effectiveness of controls [2]. Chemical weed control, represented as an herbicide application, is widely used to inhibit the germination or growth of the weed species on cultivated lands [3].

The Weed Science Society of America (WSSA) and the Herbicide Resistance Action Committee (HRAC) recently classified herbicides into 25 groups based on their herbicide site of action (SoA) (http://wssa.net), which refers to a specific molecular target that the herbicide binds to and its binding disrupts the biological process (referred to a mode of action, MoA) in weed plants resulting in the death [4]. Herbicide MoAs vary

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like lipid synthesis inhibitors, amino acid synthesis inhibitors, growth regulators, photosynthesis inhibitors, nitrogen metabolism inhibitors, pigment inhibitors, cell membrane disruptors, and seedling growth inhibitors.

The protoporphyrinogen oxidase (PPO) inhibitors termed Group 14 belong to cell membrane disruptors, which are the most widely used herbicides to control weeds resistant to glyphosate and acetolactate synthase (ALS) inhibitors [5]. PPO is a key enzyme in the heme/chlorophyll biosynthetic pathway and catalyzes the oxidation of protoporphyrinogen IX to protoporphyrin IX [6, 7]. PPO inhibitors lead to the accumulation of protoporphyrinogen IX in the cytosol, which allows the active oxidation of protoporphyrin IX by light and oxygen [8]. The photosensitive protoporphyrin IX leads to the production of reactive oxygen species (ROS) causing lipid peroxidation and plant death [9, 10]. PPO inhibitors are also sub-classified into diphenyl ethers, N-phenylphalimides, oxadiazoles, oxazolidinediones, phenylpyrazoles, pyridinediones, thiadiazoles, triazinones, and triazolinones [4].

GIGANTEA (GI) is a highly conserved vascular plant-specific gene displaying pleiotropic phenotypes including flowering time control, circadian period, light signaling, carbohydrate metabolism, abiotic stress responses, and other physiological processes [11–15]. One *gi* mutant allele, *gi-3*, showed resistance to oxidative agents, paraquat and hydrogen peroxide [16]. Recently, we reported that other *gi* mutant alleles, *gi-1* and *gi-2*, were tolerant to herbicide butafenacil, a pyrimidinedione chemical class PPO inhibitor [17]. These results suggest that mutation in *GI* would display cross-reactivity to various herbicides having different MoAs.

Recently, tiafenacil (Terrad'or Plus®) is developed as a new pyrimidinedione-type herbicide by FarmHannong Co., Ltd., Korea, and registered in the Rural Development Administration of Korea [18]. Tiafenacil is a low toxicity herbicide including low skin sensitization, genotoxicity, and favorable environmental safety, suggesting that tiafenacil is less toxic to growers and a highly effective herbicide to control the annual and perennial weeds [18].

Here, we investigated whether loss-of-function in Arabidopsis *GI* enhances resistance to herbicide tiafenacil. To conclude *GI* involves in herbicide tiafenacil resistance, we carried out phenotypic, molecular, and biochemical analyses using two *gi* mutant alleles, *gi-1* and *gi-2*, and *GI*-overexpressing (GI-OX) plants, and the results showed that *GI* negatively regulates resistance to herbicide tiafenacil with reduced oxidative damage via enhanced antioxidant systems. Furthermore, we also identified that *gi* mutants are resistant to tiafenacil-containing product, Terrad'or Plus®. Thus, genetic modification of *GI* would be a powerful non-target-site resistance strategy for developing herbicide-resistant crops.

Results

Mutations in *GI* confers resistance to herbicide tiafenacil

Paraquat is a fast-acting pyridinium-type herbicide having MoA as a photosynthesis inhibitor that causes oxidative stress in plants [19]. Interestingly, it has been reported that *gi* mutants (Ler ecotype background, *gi-3* to *gi-6*) were resistant to paraquat and two *gi* mutants (Col-0 ecotype background, *gi-1* and *gi-2*) were resistant to butafenacil [16, 17, 20]. It raises the possibility that loss-of-function in Arabidopsis *GI* may confer resistance to various herbicides having different SoAs and MoAs. Thus, we examined whether *gi* mutants show resistance to the recently developed herbicide tiafenacil using two *gi* mutant alleles, *gi-1* and *gi-2*, and GI-OX plants. Twenty-day-old plants grown in soils were air-sprayed onto the leaves using different concentrations of tiafenacil. Five days after tiafenacil treatments, the growth of Col-0 (wild-type) plants was gradually inhibited with increasing tiafenacil concentrations (Fig. 1A). By contrast, both *gi* mutant alleles, *gi-1* and *gi-2*, were less sensitive to tiafenacil treatments compared to Col-0, whereas the tiafenacil-induced injury symptoms of GI-OX plants were sensitive similar to Col-0 (Fig. 1A). Under 1 μM tiafenacil treatments, the survival rate of *gi-1* and *gi-2* was 96.875% and 82.813%, respectively, and that of Col-0 and GI-OX plants was 56.25% (Fig. 1B). However, the application of 5 μM tiafenacil in all plants resulted in death. The chlorophyll contents in *gi-1* and *gi-2* were significantly higher than Col-0 under all doses of tiafenacil, and those in GI-OX plants were significantly lower (Fig. 1C). These data suggest that GI negatively regulates herbicide tiafenacil resistance.

gi mutants were insensitive to tiafenacil-induced photosynthetic injury

The PPO inhibitor herbicides result in ROS accumulation in the presence of light disrupting cell membranes, and tiafenacil facilitates injury symptoms light-dependently [5, 18]. PPO inhibitors result in proto accumulation in plants exposed to light, and decrease photosynthetic activity [18]. Thus, we confirmed the Fv/Fm values in *gi* mutant alleles and GI-OX plants under light or dark conditions. Twenty-day-old plants grown in soils were air-sprayed with water (0 μM tiafenacil) or 100 μM tiafenacil and incubated 18 h in the dark with following 6 h in the light. The Fv/Fm values in all plants slightly decreased after a dark period for 18 h either in the absence or presence of tiafenacil (Fig. 2). The values in Col-0 and GI-OX plants exposed to tiafenacil were rapidly decreased

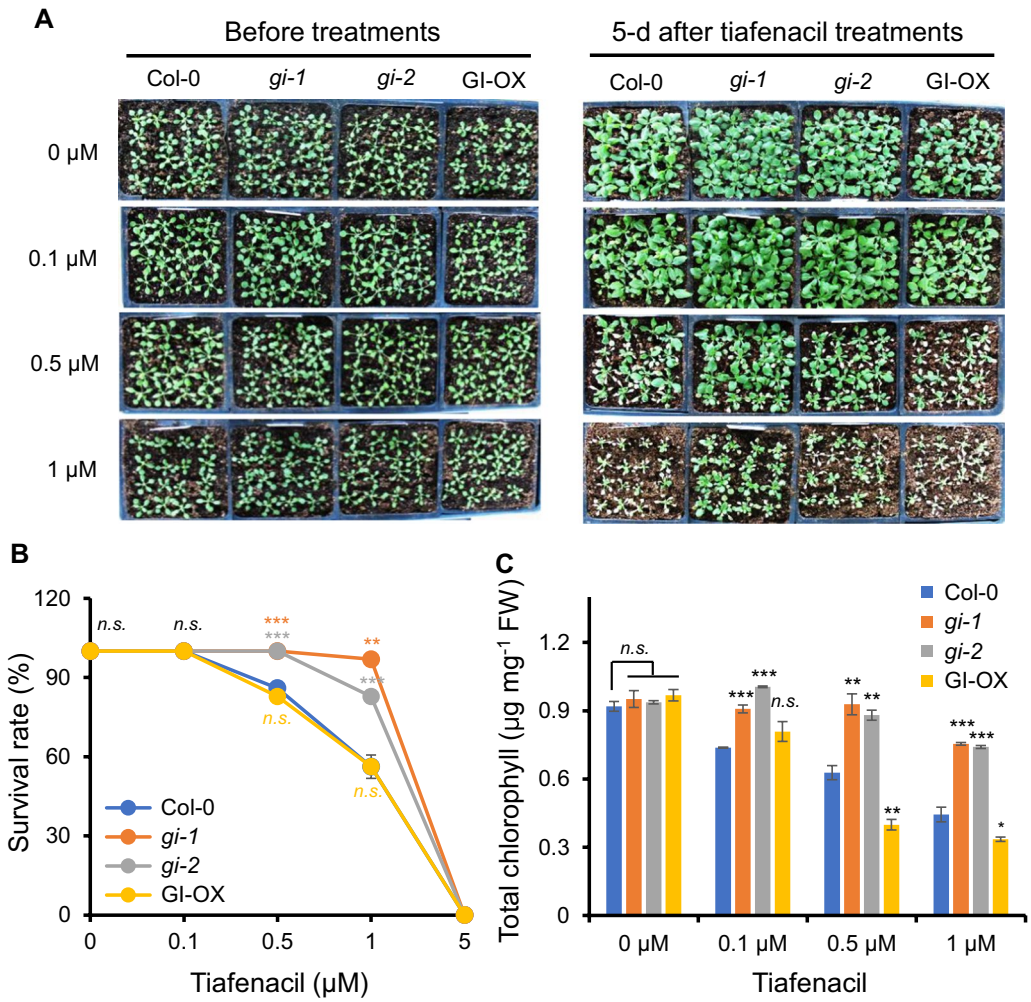

Fig. 1 Mutations in *GI* confers tiafenacil resistance in Arabidopsis. Various concentrations of tiafenacil were applied to 20-day-old wild-type (Col-0), two *gi* mutant alleles (*gi-1* and *gi-2*), and GI-OX plants by foliar spraying. **A** Phenotypes of plants under various concentrations of tiafenacil treatments. The photographs were taken before tiafenacil and 5 days after treatment. **B** Survival rate. The number of healthy plants under the treatments was counted and relatively calculated by the division of total plants examined. **C** Total chlorophyll contents. Chlorophyll contents were measured in leaves of plants shown in **A**. Values represent means ± SE of three independent biological replicates. Asterisks indicate significant differences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, *n.s.*, no significant difference) compared to Col-0 using two-tailed Student's *t*-test

during the subsequent light condition for 6 h, but those in both *gi* mutant alleles were not changed (Fig. 2B). It suggests that *gi* mutants did not show the tiafenacil-induced injury in photosynthesis efficiency.

GI negatively regulates tiafenacil-induced oxidative stress

The PPO-inhibiting herbicides including tiafenacil cause injury symptoms, such as necrotic spots and desiccation, on leaves via the herbicide-induced oxidative damage [3, 5, 18]. Thus, we analyzed leaf phenotypes of two-week-old plants three days after 0 or 0.5 μM tiafenacil treatments. Tiafenacil-treated Col-0 and GI-OX clearly showed necrotic spots on the leaves, whereas both *gi* mutant alleles exhibited minor leaf symptoms (Fig. 3A). Malondialdehyde (MDA) contents in both *gi* mutants were significantly lower than Col-0 and GI-OX plants (Fig. 3B), suggesting that mutations in *GI* reduce the tiafenacil-induced oxidative damage in Arabidopsis. Next, we analyzed endogenous ROS levels through histochemical staining and H_2O_2 contents. DAB staining showed that H_2O_2 accumulations presented as brown-color precipitates were visible in tiafenacil-treated leaves of Col-0 and GI-OX plants, whereas those in both *gi* mutant

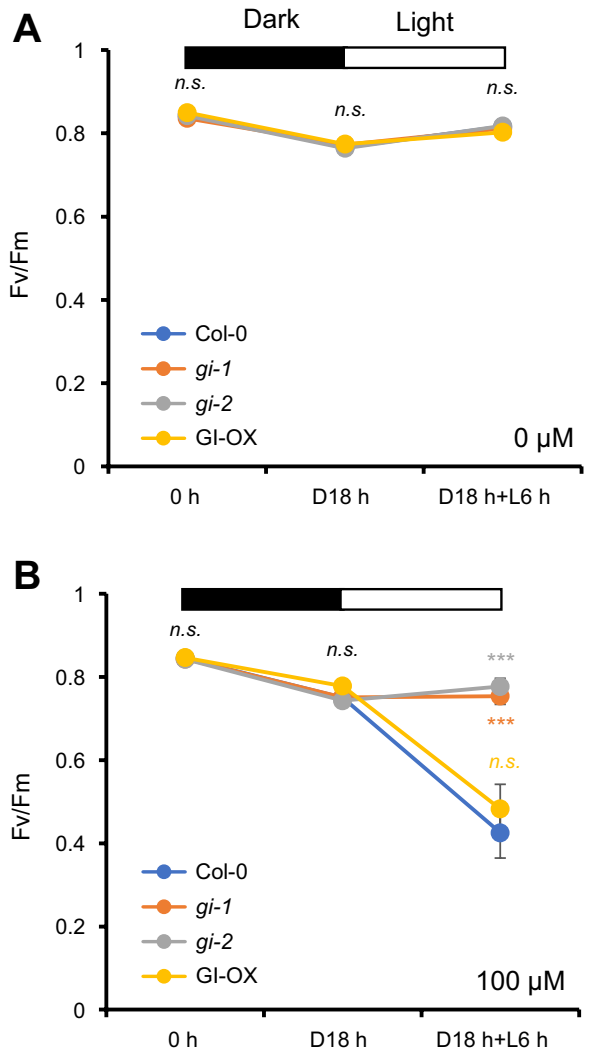

Fig. 2 GI negatively regulates tiafenacil-induced photosynthetic inhibition. Light dependency of photosystem II efficiency (Fv/Fm) as determined by chlorophyll fluorescence measurements in Col-0, two *gi* mutant alleles, and GI-OX plants under 0 μM (**A**) and 100 μM (**B**) tiafenacil treatments for 18 h in the dark (D18 h) and subsequent 6 h in the light (D18 h + L6 h). Values represent means ± SE ($n = 7$). Asterisks indicate significant differences (*** $p < 0.001$, *n.s.*, no significant difference) compared to Col-0 using two-tailed Student's *t*-test

alleles were less than Col-0 and GI-OX (Fig. 3C). We analyzed H_2O_2 quantification as more direct evidence for endogenous H_2O_2 levels in plants and data showed that tiafenacil-induced H_2O_2 accumulation in both *gi* mutants was significantly lower than that in Col-0 and GI-OX plants (Fig. 3D). These data suggest that loss-of-function in Arabidopsis *GI* alleviates the herbicide tiafenacil-induced oxidative damage with less accumulation of ROS.

gi mutants exhibit enhanced antioxidant enzyme activity

Oxidative damage by increased ROS levels in plant cells hurts biomolecules and cellular machineries, such as proteins, DNA, lipids, and various cellular compartments [21, 22]. To defend against this oxidative stress, efficient antioxidant systems are necessary to maintain ROS homeostasis via the ROS detoxifying mechanisms including superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX), and peroxiredoxin (PrxR) [23]. Thus, we examined whether enhanced tiafenacil resistance in *gi* mutant alleles is due to the elevated scavenging activity of antioxidant enzymes. The peroxidase (Prx) activity was significantly higher in both *gi* mutants compared to Col-0 and GI-OX plants, while CAT activity was not significantly different (Fig. 4A). This data is a consistent result with *gi-3* under paraquat treatments [16]. In addition, transcriptional levels of antioxidant genes including *APX*, *PrxQ*, *FeSOD3*, *MnSOD*, and *CAT1* were significantly upregulated in the tiafenacil-treated both *gi* mutants compared to Col-0 and GI-OX plants, whereas *CAT2* expression was not significantly different (Fig. 4B). These results suggest that the mutations in Arabidopsis *GI* increase the transcriptional activation of antioxidant genes, subsequently resulting in enhanced ROS scavenging capacity in *gi* mutants against tiafenacil-induced oxidative injury.

gi mutants displayed resistance to the Terrad'or Plus®

Non-selective herbicide Terrad'or Plus® containing 24% glyphosate and 0.5% tiafenacil as the active ingredient has been commercially developed by Farm-Hannong. Thus, we tested whether mutations in Arabidopsis *GI* also confer the resistance to commercial herbicide Terrad'or Plus®. Under 0.01 mg ml^{-1} Terrad'or Plus® applications to 20-day-old plants, both *gi* mutant alleles, *gi-1* and *gi-2*, showed resistance with healthy leaves, while Col-0 and GI-OX plants had severely injured symptoms with inhibition of growth and leaf bleaching (Fig. 5A). Total chlorophyll contents again indicated that *gi-1* and *gi-2* were tolerant to 0.01 mg ml^{-1} Terrad'or Plus® with significantly higher chlorophyll contents compared to Col-0 and GI-OX plants (Fig. 5B). MDA contents in both *gi* mutants under Terrad'or Plus® applications were significantly lower than Col-0 and GI-OX plants, whereas those in all water-treated plants were not significantly different (Fig. 5C). These data suggest that loss-of-function in *GI* enhances the resistance to commercial herbicide Terrad'or Plus® via reducing the herbicide-induced oxidative damage.

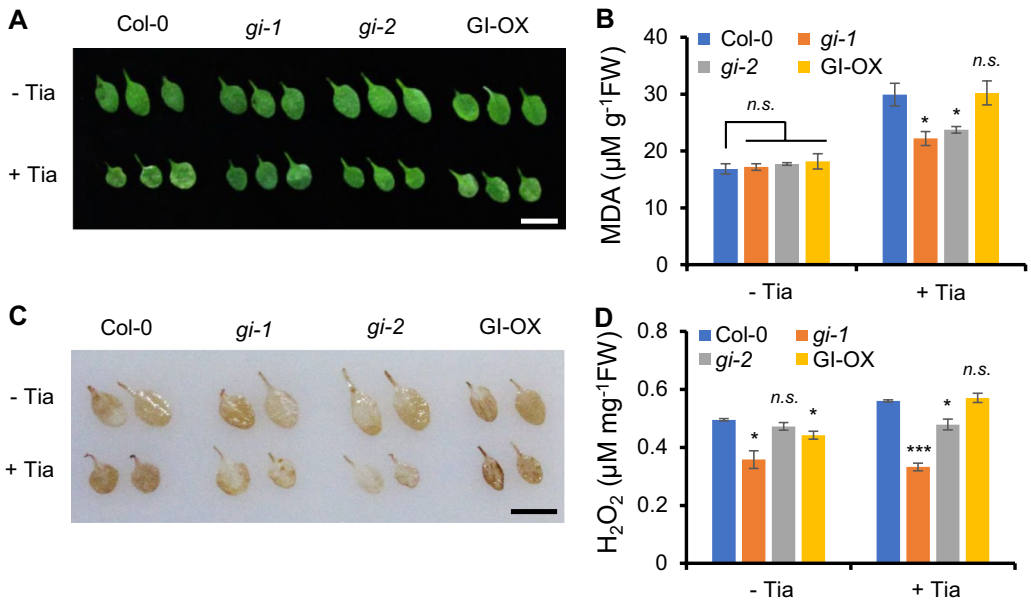

Fig. 3 Mutations in *GI* show reduced tiafenacil-induced oxidative damage. Twenty-day-old plants were exposed to water (— Tia) or 0.5 μM tiafenacil (+Tia) by foliar spray application and grown for another 3 days. **A** Necrotic symptoms. The photographs were taken 3 days after treatments. Scale bar = 1 cm. **B** MDA contents. **C** Histochemical DAB staining for H_2O_2. Scale bar = 1 cm. **D** Quantification of H_2O_2 contents. Values represent means ± SE of three independent biological replicates. Asterisks indicate significant differences (*$p < 0.05$, ***$p < 0.001$, *n.s.*, no significant difference) compared to Col-0 using two-tailed Student's *t*-test

Discussion

Weed control is essential for maintaining and increasing crop productivity [24]. Due to this reason, the global seed companies are developing herbicide-resistant crops and selling them together with herbicides. One of the best-selling non-selective herbicides is glyphosate with MoA as an amino acid synthesis inhibitor, which inhibits the shikimic acid pathway through blockade of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) [25, 26]. Genetically engineered crops having resistance to glyphosate (named Roundup-ready) were developed in soybean, maize, cotton, canola, and sugar beet [27]. Most recently, mutant variants of the *EPSPS* gene were tolerant to glyphosate and higher grain yield in rice [28]. Compared to these target-site mutations conferring herbicide resistance, non-target-site resistance would impart cross-resistance to different MoA herbicides [4, 29].

GI is involved in various physiological responses, and its mutations display valuable agronomic traits including prolonged vegetative growth and salt stress tolerance in Arabidopsis, cabbage, and poplar [13, 30–33]. We have previously found that the *gi-2* mutant is insensitive to lincomycin causing chloroplast biogenesis defects [17]. Lincomycin activates the retrograde signaling to repress the expression of photosynthesis-associated nuclear genes and functions similar to norflurazon, belonging to MoA group 12 for inhibition of phytoene desaturase [34]. In addition to these, various *gi* mutant alleles in different Arabidopsis ecotype backgrounds show resistance to herbicide paraquat and butafenacil, which have different MoA as a photosynthesis inhibitor and a cell membrane disrupter, respectively [16, 17, 20]. Thus, it raises the possibility that genetic modification in *GI* would apply to generate herbicide-resistant crops against a wide range of herbicides having different MoAs, especially herbicides inducing photooxidative damage.

PPO-inhibiting herbicides are widely applied to control the weeds from cultivated lands. Recently developed herbicide as a new pyrimidinedione-type PPO-inhibiting herbicide by FarmHannong Co. (Republic of Korea), tiafenacil (Terrad'or Plus®) is high affinity to binding to PPOs and has a relatively low half-maximal inhibitory concentration (IC_{50}) values (20–28 nM tiafenacil) against the PPOs from amaranth, soybean, Arabidopsis, and rapeseed compared to other PPO inhibitor herbicides, such as butafenacil, saflufenacil, acifluorfen, oxyfluorfen, and fomesafen [18]. Thus, we investigated GI involved in herbicide tiafenacil resistance using two loss-of-function *gi* mutant alleles and GI-OX plants. Both *gi* mutants, *gi-1* and *gi-2*, were resistant to tiafenacil treatment up to 1 μM with a significantly higher survival rate compared to wild-type plants, and the chlorophyll contents in GI-OX plants were significantly lower than wild-type (Fig. 1). Tiafenacil showed significantly lower IC_{50} to amaranth (20 nM)

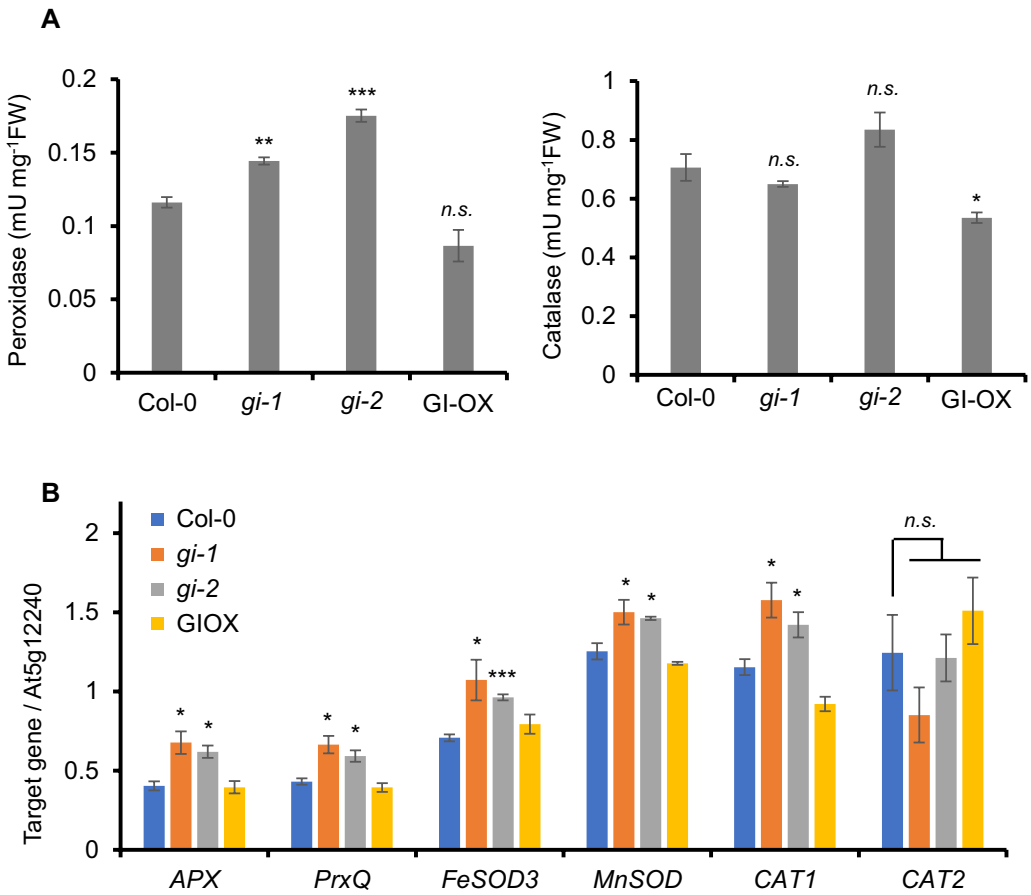

Fig. 4 Loss-of-function in *GI* enhances the activity and transcriptional expression of antioxidants under tiafenacil treatments. Twenty-day-old plants were foliar sprayed with 0.5 μM tiafenacil and grown for another 3 days. **A** Specific activities of peroxidase (*left*) and catalase (*right*). **B** Transcriptional levels of genes encoding antioxidant enzymes. The transcriptional levels of each gene were normalized to the expression of internal control, At5g12240. Values represent means ± SE of three independent biological replicates. Asterisks indicate significant differences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, *n.s.*, no significant difference) compared to Col-0 using two-tailed Student's *t*-test

and rapeseed (28 nM) than butafenacil (29 and 40 nM, respectively), suggesting tiafenacil has a greater inhibitory effect on plant growth to butafenacil [18]. Our previous study showed that both *gi* mutants were resistant to butafenacil at concentrations of up to 5 μM, while wild-type plants were dead [17]. It reflects the high sensitivity of plants to tiafenacil compared to butafenacil, which is consistent with previous report [18]. PPO-inhibiting herbicides including tiafenacil induce photo-oxidative damage to plants via photosynthetic injury in a light-dependent manner [5, 18], however both *gi* mutants were insensitive to tiafenacil-induced photosynthetic injury (Fig. 2). The injured symptoms are initially presented as necrotic spots on leaves as a consequence of oxidative stress [3, 18]. Our results showed that application of tiafenacil to *gi* mutants caused minor injured symptoms, while that to wild-type and GI-OX resulted in the formation of necrotic spots (Fig. 3A). MDA and H_2O_2 contents are indicators of herbicide-induced oxidative damage in plants, and *gi-3* mutant was significantly lower MDA and H_2O_2 contents and higher antioxidant enzyme activities compared to wild-type plants under paraquat treatments [16, 35]. Our results also showed that both *gi-1* and *gi-2* were less accumulation of MDA and H_2O_2 contents and higher antioxidant capacity compared to wild-type and GI-OX plants under tiafenacil treatments (Figs. 3B–D and 4), consistent with the previous report on paraquat and butafenacil resistance of *gi* mutants [16, 17], suggesting that mutations in *GI* caused inhibition of tiafenacil-induced oxidative damage via the enhanced antioxidant enzyme activities. Furthermore, both *gi* mutants were resistant to the tiafenacil-containing commercial herbicide Terrad'or Plus® (Fig. 5).

CRISPR/Cas9-mediated genome editing has been extensively developed as a powerful technique to generate targeted mutations in valuable crop genomes [36].

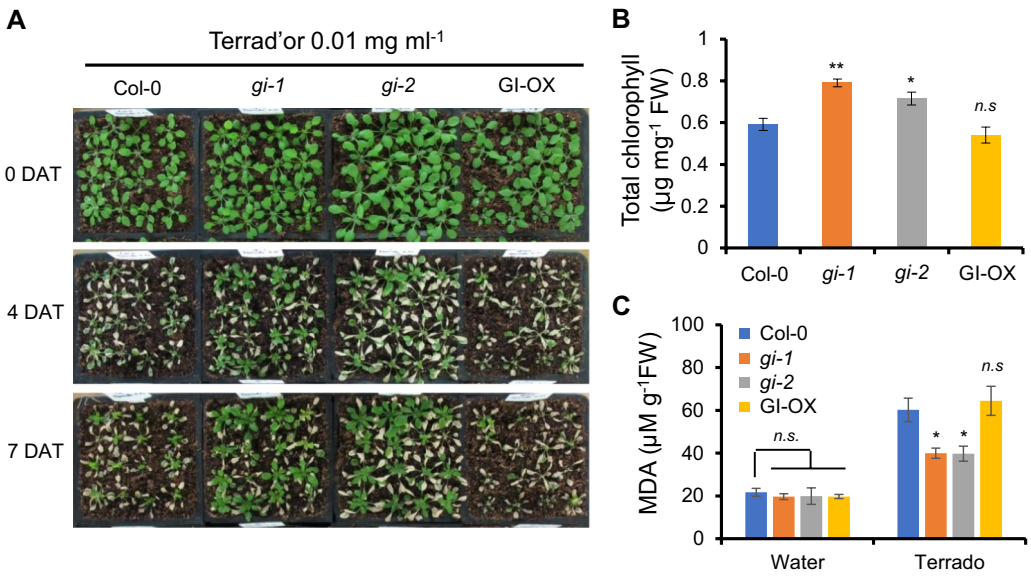

Fig. 5 *gi* mutants were resistant to Terrad'or. Twenty-day-old plants were exposed to Terrad'or Plus® (0.01 mg ml^{-1}) by foliar spray application. **A** Phenotypes of plants under herbicide Terrad'or Plus® treatments. The photographs were taken before (Top, 0 DAT), 4 days (middle, 4 DAT), and 7 days after treatment (7 DAT). **B** Total chlorophyll contents. Chlorophyll contents were measured in the leaves of 7 DAT plants. **C** MDA contents. MDA levels were measured in the leaves of 7 DAT plants. Values represent means ± SE of three independent biological replicates. Asterisks indicate significant differences (*$p < 0.05$, **$p < 0.01$, *n.s.*, no significant difference) compared to Col-0 using two-tailed Student's *t*-test

Despite technical advances, useful genetic materials are still largely absent and need to be elucidated. Through the various physiological, biochemical, and molecular functions of GI in plants reported to date, GI displays pleiotropic phenotypes such as flowering time regulation, light signaling, carbohydrate metabolism, chloroplast biogenesis, and abiotic stress responses [14, 17]. Loss-of-function in Arabidopsis *GI* causes prolonged vegetative stages, elevated starch accumulation, salt stress tolerance, and insensitivity to photooxidative agents, which are important issues in crop breedings [13, 16, 17, 30, 37]. Furthermore, mutations in *GI* conferred the cross-resistance to herbicides having different MoA [16, 17]. Thus, GI would be a potential molecular target to develop herbicide-resistant crops with other valuable agronomic traits.

Conclusions

In conclusion, tiafenacil is a recently developed pyrimidinedione-type PPO-inhibiting herbicide. Loss-of-function in Arabidopsis *GI* confers the resistance to tiafenacil and insensitive to tiafenacil-induced photooxidative damage. Both *gi* mutants, *gi-1* and *gi-2*, showed less ROS accumulation with enhanced antioxidant capacity under tiafenacil treatments compared to wild-type plants. Together with previous reports, our results suggest that mutations in *GI* mediate the resistance to a wide range of herbicides having different MoAs, and would be a pivotal molecular target for generating herbicide-resistant crops.

Materials and methods

Plant materials and growth conditions

Arabidopsis thaliana wild-type (Columbia-0 ecotype background, Col-0), *GI* (AT1G22770)-overexpressing plants (35S:GI-HA, GI-OX) and its two mutants (*gi-1* and *gi-2*) were used in this study [30, 38]. Seeds were sterilized with 30% (v/v) bleach-containing 0.02% (v/v) triton X-100 for 5 min. After stratification in the dark at 4 °C for 2 days, seeds were germinated on Murashige and Skoog (MS) medium for 1 week and transferred to soil for another 2 weeks. Plants were grown in a growth chamber (16 h light/8 h dark cycles, 100 μmol photons m^{-2} s^{-1} of white cool fluorescent light) at 22–23 °C for 20 days.

Herbicides treatments

Tiafenacil (methyl N-[2-[[2-chloro-5-[3,6-dihydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)-1(2H)-pyrimidinyl]-4-fluorophenyl]thiol-1-oxoprophyl]-ß-alaninate; CAS no. 1220411-29-9] [18] and Terrad'or Plus® [24% (v/v) glyphosate-isopropylamine and 0.5% (v/v) tiafenacil] were kindly

supported from FarmHannong Co., Ltd., Korea. Twenty-day-old Arabidopsis plants grown in soil were treated with foliar spraying of tiafenacil or Terrad'or Plus® (0.01 mg ml^{-1}) onto the leaves.

Determination of total chlorophyll and MDA contents

For measuring chlorophyll contents, herbicide-treated shoot parts of plants were soaked in 80% (v/v) acetone at 23 °C for 48 h in the dark. Chlorophyll a and b were measured spectrophotometrically at 663 and 645 nm, respectively [39]. For measuring MDA contents, herbicide-treated shoot parts of plants were homogenized in the reaction buffer containing 0.5% (w/v) thiobarbituric acid and 20% (w/v) trichloric acid followed by centrifugation at 13,000 rpm for 15 min at 4 °C. Resulting supernatants incubated at 85 °C for 20 min and the reaction was terminated by incubating on ice for 5 min. The absorbance was measured spectrophotometrically at 532 and 600 nm. MDA contents were calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} as described previously [40].

Detection of H_2O_2 by histochemical staining and quantification of H_2O_2

Hydrogen peroxide (H_2O_2) accumulation in the tiafenacil-sprayed plant leaves was stained with 3,3′-diaminobenzidine (DAB, 1 mg mL^{-1}, pH 3.8) and then immersed in 80% (v/v) ethanol to remove the chlorophyll completely to visualize dark-brown staining. The H_2O_2 contents were determined using the Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (Invitrogen) according to the manufacturer's instructions [17].

Fv/Fm determination

For measuring photosynthetic efficiency, plants were foliar sprayed with 0 μM (water) and 100 μM tiafenacil and incubated for 18 h in the dark and subsequent 6 h in the light as described previously [18]. Photosynthetic efficiency was analyzed in each leave with a photosynthesis yield analyzer mini-pam (MINI-PAM, Walz, Effeltrich, Germany) by measuring Fv/Fm value, where $Fv = Fm \pm Fo$. Fo is the minimum and Fv is the maximum value of fluorescence. To determine Fo, plants were dark-adapted for 20 min using a leaf-clip holder. The fluorescence yield was measured when an internal artificial light source was exposed.

Antioxidant enzyme assay

Twenty-day-old plant leaves exposed to 0.5 μM tiafenacil were ground and subjected to antioxidant enzyme assays. Prx and CAT activities were measured using an Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit and Amplex Red Catalase Assay Kit (Invitrogen), respectively, according to the manufacturer's instructions.

RNA extraction and qRT-PCR

Total RNA was isolated from 20-day-old Arabidopsis plants exposed to tiafenacil with Total RNA Kit (Biofact) following the manufacturer's instructions. First-strand cDNA was synthesized from 3 μg total RNA with a RevertAid First-Strand cDNA Synthesis Kit (Thermo Fisher Scientific). Synthesized cDNA was amplified by real-time quantitative PCR (qPCR) with a TOPreal™ qPCR 2X PreMIX Kit (SYBR Green with low ROX/Enzynomics) using the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories). The housekeeping gene AT5G12240 was used as the normalization control. The primers used in this study are listed in Additional file 1: Table S1.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-022-00734-6.

Additional file 1: Table S1. Primers used in this study.

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Author contributions

JYC, GIS, GA, and WYK designed the research; JYC, GIS, GA, SYJ, MGJ, and AA performed experiments; JYC, GIS, GA, MGK, and WYK analyzed data and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Declarations

Competing interests

The authors declare that they have no competing interests.

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