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Imazethapyr Enantioselectively Affects Chlorophyll Synthesis and Photosynthesis in *Arabidopsis thaliana*

Haifeng Qian, Xiao Han, Qiannan Zhang, Zhengqi Sun, Liwei Sun, and Zhengwei Fu*

College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou 310032, People's Republic of China

ABSTRACT: Imazethapyr (IM) is a chiral herbicide with reported enantioselective biological activities between its enantiomers. This report investigated the effect of enantioselectivity between *R*- and *S*-IM in *Arabidopsis thaliana* on chlorophyll synthesis and photosynthesis. The results suggest that *R*-IM inhibited the transcription of *chl*M to a greater extent than *S*-IM, which reduced chlorophyll synthesis. *R*-IM also showed a stronger inhibitory effect than *S*-IM on the transcription of photosynthesis-related genes, affecting linear electron transport and CO_2 fixation. IM stress enantioselectively induced transcriptional upregulation of the *ndh*H gene, a representative of the NDH complex. In contrast, the expression of *pgr*5 was downregulated, which demonstrated that IM stress enhanced adenosine 5′-triphosphate (ATP) synthesis by stimulating an NDH-dependent and not ferredoxin (FD)-independent route. This study suggested that *R*-IM has a greater toxic effect on photosynthesis than *S*-IM, affecting plant growth through chlorophyll synthesis.

KEYWORDS: *Imazethapyr, enantioselectivity, chlorophyll synthesis, photosynthesis, cyclic electron transport*

INTRODUCTION

Imazethapyr (IM) is a common broad-spectrum herbicide used to control weeds in soybean and other legume crops.[1] In China, 150−300 tons of IM are used each year to control weeds in soybean fields alone.[2] Acetolactate synthase (ALS) is the first key enzyme in the biosynthesis of branched chain amino acids (BCAA). By cloning the ALS gene from an herbicide-resistant plant and transferring it into susceptible plants, Haughn et al.[3] and Hattori et al.[4] generated IM-resistant transgenic plants, speculating that ALS is the primary site of IM action. Some researchers have demonstrated that IM inhibits the synthesis of BCAA by competitively interacting with ALS.[5,6] However, IM affects not only BCAA biosynthesis but also other metabolic pathways,[7,8] indicating a complex functional mechanism of IM in plants that has yet to be clarified. IM is a chiral compound containing one carbon chiral center. Therefore, IM has one pair of enantiomers, structural mirror images designated *R* or *S*, depending upon their absolute configuration. Similar to other chiral pesticides, IM displays enantioselectivity in biological properties,[7,9,10] because its individual enantiomers can interact enantioselectively with enzymes and biological receptors in organisms.[11,12] These previous reports also demonstrated that *R*-IM has a greater toxicity than *S*-IM to plant growth in maize, rice, and *Arabidopsis thaliana*, as demonstrated by its inhibitory effects on root elongation and ALS activity and its destructive power toward cell substructure and enantioselectivity, for example.

We previously reported that IM enantioselectively inhibited not only the biosynthesis of BCAA but also carbohydrate use in *A. thaliana*.[7] As a result of the inhibition of carbohydrate use, carbohydrates (such as glucose, maltose, and sucrose) accumulated and their amounts increased with the elongation of IM exposure.[7] As is well-known, plants capture light energy and CO_2 molecules to produce sugar molecules and carbohydrates via photosynthesis. These carbohydrates can be used as building blocks, adding to the total mass of the plant as it grows, or they can be used for cellular respiration to produce adenosine 5′-triphosphate (ATP) for processes that require energy. Therefore, it is vitally important for plant growth that carbohydrates are stored during the day to be used for growth during the night.[13,14] The disruption of carbohydrate use may be one of the primary mechanisms by which IM retards plant growth.[7] Shaner and Reider[15] also observed that the concentration of neutral sugar in maize accumulated 1.4-fold compared to the control after a 21 day exposure to IM. Chao et al.[16] found an increase in starch granules in chloroplasts of wild oat after 1 week of IM exposure, whereas Royuela et al.[17] showed that carbohydrates also accumulated in pea after 4 weeks of IM treatment. Given that carbohydrates are produced via photosynthesis and that carbohydrates are common regulators of a number of photosynthesis-related genes,[18] it is therefore possible that IM exposure will affect the process of photosynthesis. The aim of this study is to clarify whether or not and, if so, how IM enantioselectively affects chlorophyll synthesis and photosynthesis.

MATERIALS AND METHODS

Plant Growth Conditions and Chemical Reagents. *A. thaliana* (Col-0) seeds were surface-sterilized with 75% ethanol for 1 min and then were sterilized with 1% (v/v) sodium hypochlorite solution for 15 min. Sterilized seeds were vernalized at 4 °C for 2 days and then plated on MS plates [containing 0.8% (w/v) agar and 3% (w/v) sucrose] with different concentrations of IM enantiomers in a culture room equipped with cool-white fluorescent lights (approximately 300 μmol m^{-2} s^{-1}) at a constant temperature of 25 ± 0.5 °C and a 12 h light/12 h dark cycle. The treated concentration of IM enantiomers was 2.5 μg/L, which was determined by our previous reports.[7] The racemic IM

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mixture (98% purity) was provided by the Shenyang Research Institute of Chemical Industry (Shenyang, China). Enantiomers were separated from the racemic IM mixture according to Lin et al.[19] The separated enantiomers were dissolved in acetone, with a final solvent concentration of 0.05% (v/v) for each experimental solution and the control.

Analyses of Chlorophyll and Anthocyanin in Plant Tissue. Three to five seedlings in the IM enantiomer-treated and control groups were collected and immersed in *N*,*N*-dimethylformamide to extract chlorophyll according to the method by Inskeep and Bloom.[20] The chlorophyll-extracted liquids were measured for absorbance values at 647 and 664.5 nm using a spectrophotometer, and chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and total chlorophyll (total Chl) contents were calculated according to the formula reported by Inskeep and Bloom.[20] From the IM enantiomer-treated and control groups, six seedlings were ground to a powder, to which 400 μL of HCl/MeOH was added (1:99, v/v) and maintained at 4 °C overnight. Subsequently, 400 μL of water and 200 μL of chloroform were added, and the mixtures were centrifuged to separate chlorophylls. The supernatant was then used to measure anthocyanin, as indicated by a previous report.[21]

RNA Extraction, Reverse Transcription, and Real-Time Polymerase Chain Reaction (PCR) Analysis. *A. thaliana* tissues were collected and ground in liquid nitrogen to isolate the total RNA using RNAiso Reagent (TaKaRa, Dalian, China) according to the instructions of the manufacturer. First-strand cDNAs were synthesized from 500 ng of total RNA using a reverse transcriptase kit (Toyobo, Tokyo, Japan). Real-time PCR was performed with an Eppendorf MasterCycler ep RealPlex[4] (Wesseling-Berzdorf, Germany). The gene-specific primers are listed in Table 1. The real-time PCR was performed in two steps: one denaturation step at 95 °C for 1 min and 40 cycles of 95 °C for 15 s, followed by 60 °C for 1 min. Actin 2 was used as a housekeeping gene to normalize the expression profiles.

Data Analysis. The results are shown as the mean ± standard error of the mean (SEM). The data were evaluated on the basis of comparisons to the corresponding control data using the Student's *t* test, which was performed with the StatView 5.0 program. Statistically significant differences were defined when the *p* value was less than 0.05.

RESULTS AND DISCUSSION

IM Enantioselectively Affected the Chlorophyll and Anthocyanin Contents. In this study, we first analyzed the contents of chlorophyll and anthocyanin, because chlorophyll is the light capture and photosynthesis pigment and anthocyanin is the biomarker for polluted stress. As shown in Table 2, after 2 weeks of exposure, the *R*-IM treatment showed a stronger inhibitory effect on Chl *a*, Chl *b*, and total Chl compared to the control and *S*-IM treatments, with the chlorophyll contents being only approximately half of that of the *S*-IM treatment. In contrast, the *S*-IM treatment showed a weak stimulated effect on Chl *a* and total Chl, suggesting that the toxicity of *S*-IM to plant chlorophyll synthesis was not as significant as that of *R*-IM. Interestingly, we observed that the seedling leaves became purple after *R*-IM treatment but did not change significantly after *S*-IM treatment compared to the seedling leaves of the control (Figure 1). We speculated that this color change may be due to an increase in anthocyanin. Therefore, we measured the anthocyanin content and found that anthocyanin levels in *R*-IM-treated seedlings were 5.92- and 4.63-fold higher than that of the control and *S*-IM-treated groups, respectively. The change in the chlorophyll and anthocyanin contents showed similar patterns after 3 weeks of IM enantiomer exposure, with Chl *a*, Chl *b*, and total Chl decreasing to 68.5, 68.4, and 71.4% of the control, respectively, after *R*-IM treatment, while the anthocyanin level increased 4.33-fold of the control.

Table 1. Sequences of Primer Pairs Used in Real-Time PCR

gene name	primer	gene ID
*chl*H	forward: 5′-CTGGTCGTGACCCTAGAACAG-3′ reverse: 5′-GATTGCCAGCTTCTTCTCTG-3′	At5g13630
*chl*M	forward: 5′-AGCCGGGGTCGACAGTACAACAATC-3′ reverse: 5′-ACCGGCCAAGGATCTATCTTCAGTC-3′	At4g25080
*gun*4	forward: 5′-CTCCATTGCCAATCTCAC-3′ reverse: 5′-CCGAATCTACCATCACTGTG-3′	At3g59400
*rbc*L	forward: 5′-TACCTGGTGTTCTGCCTGTG-3′ reverse: 5′-GCTACTCGGTTGGCTACGG-3′	AtCg00490
*psb*A	forward: 5′-AACTAAGTTCCCACTCACGA-3′ reverse: 5′-CATCCGTTGATGAATGGCTA-3′	AtCg00020
*fnr*1	forward: 5′-CTGCAGTCTCTTTACCTTCCTCC-3′ reverse: 5′-GACAACAATCCCTTCTTCCTGTTTC-3′	At5g66190
*fnr*2	forward: 5′-GGCGACTACCATGAATGCTGC-3′ reverse: 5′-GTCTGTACCTGTTAACAATCACAC-3′	At1g20020
*ndh*H	forward: 5′-ATGGGAAATTCAATGGCAAA-3′ reverse: 5′-TCAAAGCCCCTGCTTTCTAA-3′	AtCg01110
*fd*1	forward: 5′-AATTTCATCAAAAGAGAAATTACTTGA-3′ reverse: 5′-TTGATTGATCTTATAAAAGGATGAGC-3′	At1g10960
*fd*2	forward: 5′-GAAGAAGACATTGTTTAAGCCTCA-3′ reverse: 5′-GATTGATGGTGAGCCAAACC-3′	At1g60950
*pgrl*1A	forward: 5′-CACATCTTCAACCACAGGTTC-3′ reverse: 5′-GAAGAGGAAGGTTTGCGAGA-3′	At4g22890
*pgrl*1B	forward: 5′-CAACCACACAAATCCAAAGC-3′ reverse: 5′-TTTGCGAGAAATTGCAGAAA-3′	At2g37170
*pgr*5	forward: 5′-ACCAAACCATGCTCTCCAAG-3′ reverse: 5′-CAATGGCTTTTCCTCTGAGC-3′	At2g05620
Actin 2	forward: 5′-ACCTTGCTGGACGTGACCTTACTGAT-3′ reverse: 5′-GTTGTCTCGTGGATTCCAGCAGCTT-3′	At3g18780

The synthesis of chlorophyll is an important step in chloroplast development, and the chlorophyll content directly affects plant biomass and agricultural productivity in crops.[22−24] Chlorophyll synthesis is easily affected by xenobiotics. Andrés-Colás et al.[25] demonstrated that copper caused a notable decrease in the chlorophyll content in *A. thaliana*. Qian et al.[26] demonstrated that copper and cadmium, both individually and in combination, decreased the *Chlorella vulgaris* chlorophyll content. Other environmental stresses, such as salt stress and potassium deficiency, also strongly affect the chlorophyll content.[27] This study showed that IM, a chiral herbicide, could also decrease the chlorophyll content enantioselectively, implicating chlorophyll or even chloroplast as sensitive targets for xenobiotics. In addition, *R*-IM displayed stronger xenobiotic toxicity than *S*-IM to inhibit chlorophyll synthesis, consistent with previous reports.[9,10] In contrast to photosynthesis pigments, anthocyanin levels increased after IM exposure. Previous reports showed that anthocyanin is commonly associated with stress responses,[28,29] which can be accumulated after salt- or metal-induced stress.[25,30,31] DellaPenna and Pogson[32] believed anthocyanin to be a type of antioxidant that ameliorated reactive oxygen species (ROS) accumulation and toxicity. Therefore, anthocyanin levels are also regarded as

Table 2. Effect of IM Enantiomers on Chl *a*, Chl *b*, Total Chl, and Anthocyanin Content

	Chl *a* (mg/g of FW)		Chl *b* (mg/g of FW)		total Chl (mg/g of FW)		anthocyanin (A_{535}/g of FW)	
	2nd week	3rd week	2nd week	3rd week	2nd week	3rd week	2nd week	3rd week
control	0.63 ± 0.00	0.54 ± 0.09	0.25 ± 0.02	0.19 ± 0.03	0.88 ± 0.02	0.70 ± 0.13	1.32 ± 0.03	1.13 ± 0.07
S-IM	0.72 ± 0.04[a]	0.36 ± 0.03[a]	0.28 ± 0.02	0.15 ± 0.02	1.0 ± 0.04[a]	0.50 ± 0.05[a]	1.69 ± 0.09	1.78 ± 0.17[a]
R-IM	0.40 ± 0.01[a,b]	0.37 ± 0.01[a]	0.14 ± 0.00[a,b]	0.13 ± 0.00[a]	0.54 ± 0.01[a,b]	0.50 ± 0.01[a]	7.82 ± 0.92[a,b]	4.89 ± 0.21[a,b]

[a]The values are significantly different compared to the control ($p < 0.05$). [b]The values are significantly different compared to those of the *S*-IM-treated plants ($p < 0.05$).

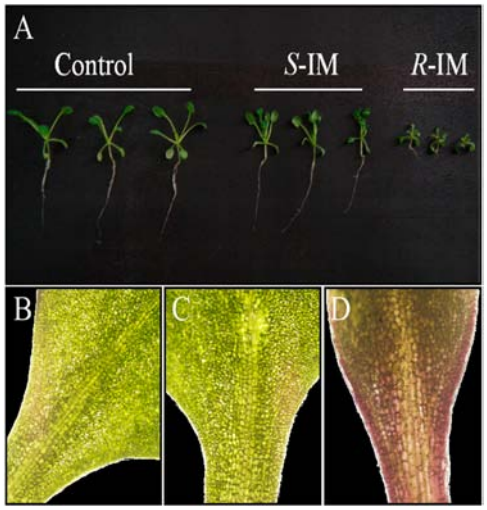

Figure 1. Color change in the seedling leaves after IM enantiomer treatment. (A) Photograph of the control and plants treated by *R*- and *S*-IM. Micrograph of the leaf in the (B) control, (C) *S*-IM-exposed, and (D) *R*-IM-exposed groups.

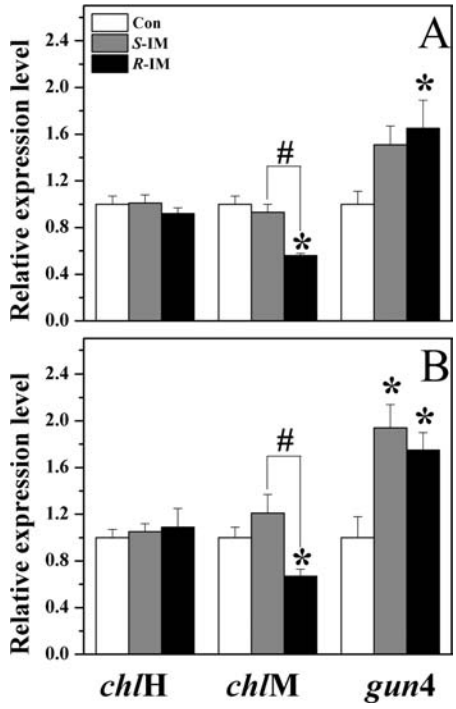

Figure 2. Effect of IM enantiomers on the transcription of chlorophyll synthesis-related genes after (A) 2 and (B) 3 weeks of exposure. (*) Statistically significant difference when compared to the control ($p < 0.05$). (#) Statistically significant difference when compared to *S*-IM-exposed plants ($p < 0.05$).

a biomarker of environmental stress. Herein, *R*-IM led to a significant increase in the anthocyanin content, indirectly demonstrating that *R*-IM also functions as stress that may induce ROS overproduction, whereas *S*-IM in this concentration did not cause significant stress for the plant.

IM Affected the Transcription of Chlorophyll Biosynthesis Genes Enantioselectively. To clarify the reason of the chlorophyll content decrease, the transcriptions of three Chl biosynthesis genes, *chl*H, *chl*M, and *gun*4, were analyzed after IM exposure. As reported by Timko,[33] the pathway of chlorophyll biosynthesis could be summarized as glutamate (Glu) → ALA → PBG → Urogen III → Coprogen III → Proto IX → Mg−Proto IX → Pchl → Chl *a* → Chl *b*. *chl*H expresses one of the subunits of Mg^{2+}−protoporphyrin IX chelatase, which can insert Mg^{2+} ions into protoporphyrin IX (Proto IX) to produce Mg^{2+}−protoporphyrin IX (Mg−Proto IX).[34] *chl*M encodes the enzyme required for the next step of chlorophyll synthesis, Mg protoporphyrin IX methyltransferase (MgPMT), which catalyzes the transfer of a methyl group to Mg−Proto IX to yield Mg^{2+}−protoporphyrin IX monomethyl ester (MgProtoMe).[35] In contrast to these two chlorophyll biosynthetic enzymes, *gun*4 is not absolutely required for the accumulation of chlorophyll but can stimulate chlorophyll biosynthesis by activating Mg−chelatase. This stimulation depends upon the binding of GUN4 to the ChlH subunit to enhance Mg−chelatase activity.[36−38] The effect of IM enantiomer treatment on the transcription of the above-mentioned three genes is shown in Figure 2. After 2 or 3 weeks of exposure, the transcription of *chl*H was not affected significantly by either *R*- or *S*-IM enantiomer treatments. The transcription of *chl*M decreased significantly after *R*-IM treatment, decreasing to only 56.5 and 60.9% of that of the control or *S*-IM-treated groups, respectively, after 2 weeks of exposure and decreasing to approximately 67.4 and 55.5%, respectively, after 3 weeks of exposure (panels A and B of Figure 2). The transcription of *gun*4 reached 1.65- and 1.75-fold of the control after 2 and 3 weeks of *R*-IM treatment, respectively, but it was only significantly stimulated by *S*-IM after 3 weeks of exposure, when it reached 1.94-fold of the control (panels A and B of Figure 2).

IM did not affect the synthesis of MgProto, because the transcription of *chl*H did not change significantly. The increase of *gun*4 did not change chlorophyll synthesis, because it can only increase the activity of Mg−chelatase indirectly. However, according to the results of *chl*H transcription in this study, we speculated that the reason that the transcription of Mg−chelatase was not stimulated with the increase of *gun*4 might be due to the fact that the activity of Mg−chelatase does not absolutely depend upon GUN4 protein.[39] We speculated that IM affects chlorophyll synthesis by decreasing the conversion of MgProto into MgProtoMe by inhibiting the transcription of MgPMT (*chl*M). *R*-IM showed a greater inhibitory effect than

S-IM on this step. Pontier et al.[40] believed *chl*M to be essential for the formation of chlorophyll and, subsequently, for the formation of Photosystems I (PSI) and II (PSII) and cytochrome b_6f complexes in *A. thaliana*. Therefore, the inhibition of chlorophyll synthesis by downregulating the transcription of *chl*M is a very efficient mechanism.

IM Affected the Transcription of Photosynthesis-Related Genes Enantioselectively. Chlorophylls are the units of light energy use. Would photosynthesis be affected by IM after the chlorophyll content was affected enantioselectively? To address this question, we analyzed the transcription of two photosynthesis-related genes (*psb*A and *rbc*L) after IM exposure. *psb*A encodes the D1 protein, located in the PSII reaction center, whose function is to transfer electrons to the plastoquinone pool. *rbc*L encodes the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo), which combines CO_2 with ribulose-1,5-bisphosphate (RuBP) to form two molecules of phosphoglyceric acid. In the present study, the transcription of *psb*A was affected by IM enantioselectively. It decreased significantly only when treated with *R*-IM, decreasing to 45% of the control and 48.5% of the *S*-IM-treated group after the 2 week treatment and decreasing to 64.7% of the control after 3 weeks of exposure (Figure 3A).

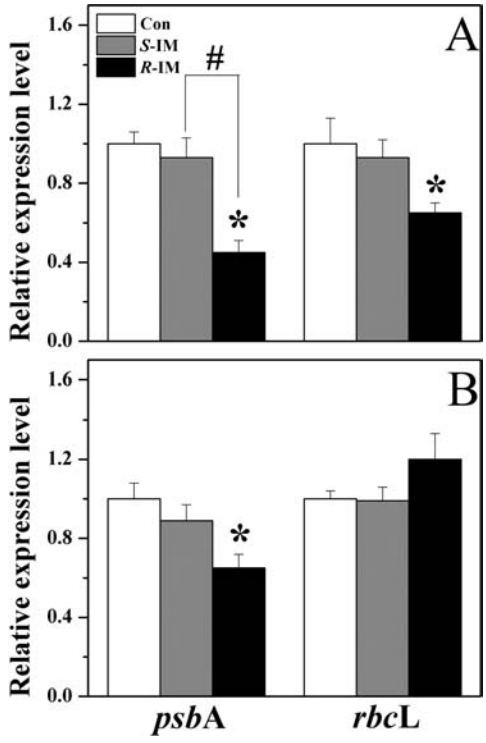

Figure 3. Effect of IM enantiomers on the transcription of photosynthesis-related genes after (A) 2 and (B) 3 weeks of exposure. (∗) Statistically significant difference when compared to the control ($p < 0.05$). (#) Statistically significant difference when compared to *S*-IM-exposed plants ($p < 0.05$).

The response of the *rbc*L gene transcription to IM treatment showed a similar pattern to that of the *psb*A gene but with some differences (Figure 3B). The transcription of the *rbc*L gene decreased to approximately 65% of the control only after treatment with *R*-IM for 2 weeks, while it was not any more significantly affected after 3 weeks of exposure.

It has been reported that atrazine, another herbicide, can also decrease the expression or activity of *psb*A, therefore affecting PSII electron transport.[41−43] The inhibition of PSII electron transport prevents the plant from absorbing light energy to convert into electrochemical energy and results in the production of triplet chlorophyll and singlet oxygen, which induce oxidative stress and final bleaching. At the same time, the inhibition of electron transport and light conversion affects the production of NADPH and reduces inorganic carbon fixation. These aspects may be the reason for the decrease of *rbc*L transcription in this study. We previously reported a similar phenomenon, in which IM affected the transcription of photosynthesis-related genes in rice as measured by microarray technology.[8] These results were also in agreement with many reports that herbicides affect the efficiency of photosynthesis or photosynthesis-related gene transcription.[44,45] We believe that the inhibitory transcription of photosynthesis-related genes is related to the decrease of the chlorophyll content.

IM Affected the Transcript of Cyclic Electron Transport (CET)-Related Genes Enantioselectively. Under normal conditions, the majority of photosynthetic energy in plants or algae is stored by the chloroplast in a process termed linear electron transport (LET). LET involves light-stimulated electron transfer from PSII to PSI to produce ATP and NADPH and then to fix CO_2 into carbohydrates for plant or algal growth. However, the inhibition of chlorophyll synthesis-related gene transcription by *R*-IM decreased the chlorophyll content, which affected light harvesting. The downregulation of key genes in the light reaction and carbon fixation showed that linear photosynthetic electron transfer was partially blocked. Therefore, one method to surpass LET may be to cycle around PSI to the CET pathway. To test this hypothesis and clarify which CET pathway was stimulated, we analyzed the transcription of several genes in CET. CET has two distinct pathways to transfer electrons circularly. One is a NDH-dependent route, in which electrons are transferred from ferredoxin−$NADP^+$ oxidoreductase (FNR) to NADPH and are then transferred to the NDH-1 and cytochrome b_6f complexes via the plastoquinone pool. The other is a ferredoxin (FD)-dependent route where electrons are transferred from P700 of PSI to the plastoquinone pool via FD, hypothetical ferredoxin−plastoquinone reductase (FQR), PGR5, and PGRL1 proteins.[31,46,47] Therefore, we selected *fd*1, *fd*2, *pgr*5, *pgrl*1A, and *pgrl*1B of the FD-dependent pathway and *fnr*1, *fnr*2, and *ndh*H of the NDH-dependent pathway for transcription analysis after exposure to IM enantiomers.

Figure 4A shows the gene transcription after 2 weeks of IM exposure. The transcription of the four genes in the NDH-dependent route did not change significantly after *R*- or *S*-IM treatments. In the FD-dependent route, the transcription of *fd*1, *pgrl*1A, and *pgr*5 decreased significantly only after *R*-IM treatment, decreasing to approximately 59.3, 47.5, and 56.1% of the control, respectively. The transcription of *pgrl*1B increased to 1.71-fold of the control after *R*-IM treatment and was unaffected by the *S*-IM treatment. Gene transcription after 3 weeks of exposure is shown in Figure 4B. The transcription of *ndh*H in the NDH-dependent route increased significantly with the *R*- and *S*-IM treatments after 3 weeks of exposure, increasing to 1.51- and 1.23-fold of the control, respectively, and the transcription of *ndh*H in the treatment of *R*-IM was significant higher than that in *S*-IM. The transcription of other genes in this route did not change significantly after the exposure to IM enantiomers. In the FD-dependent route, the

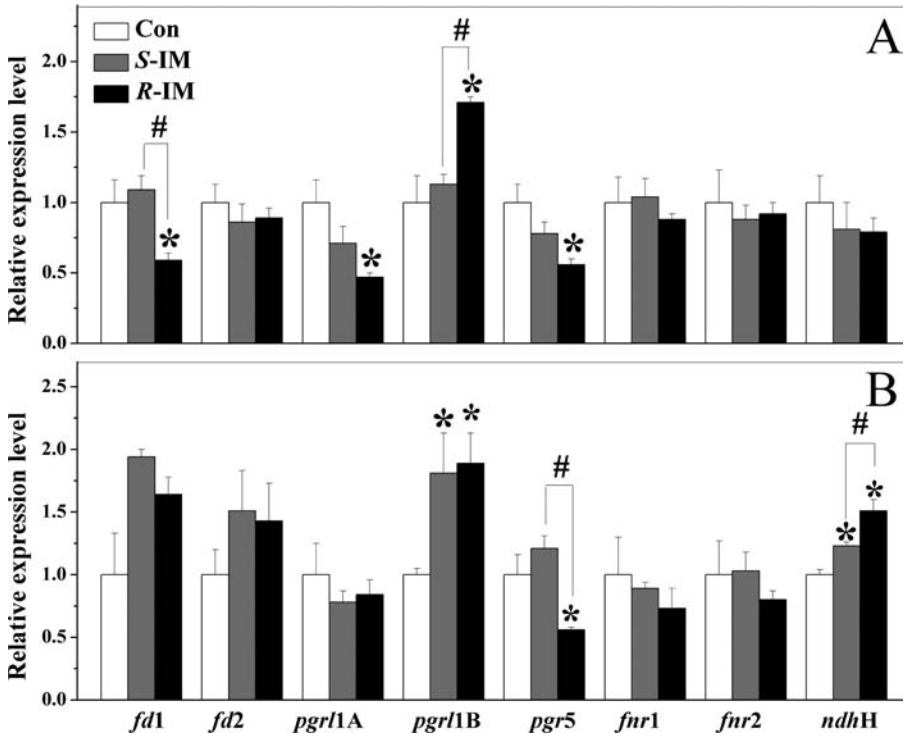

Figure 4. Effect of IM enantiomers on the transcription of CET genes after (A) 2 and (B) 3 weeks of exposure. (∗) Statistically significant difference when compared to the control ($p < 0.05$). (#) Statistically significant difference when compared to *S*-IM-exposed plants ($p < 0.05$).

transcription of *pgrl*1B was stimulated by the *R*- and *S*-IM treatments. The transcription of *pgr*5 was downregulated only by the *R*-IM treatment to approximately 56% of the control.

Many reports have demonstrated that CET is stimulated to produce more ATP to drive protein repair and transport upon several environmental stresses, such as drought, high light, and prolonged dark acclimation.[31,48−51] In the present study, we found that *R*-IM treatment increased the transcription of *pgrl*1B and decreased the transcription of *fd*1, *pgrl*1A, and *pgr*5 in FD-dependent CET. As reported by Lehtimäki et al.,[31] *pgrl* mRNA is composed of 99% *pgrl*1A mRNA and 1% *pgrl*1B mRNA, suggesting that the FD-dependent route of CET might be downregulated by *R*-IM. However, the transcription of *ndh*H, a representative of the NDH complex, was upregulated by *R*-IM after 3 weeks of exposure, implying that the NDH complex pathway rather than the FD-dependent pathway may be the dominating route of CET in *A. thaliana* to produce more ATP upon IM exposure. This was somewhat different than what was seen with water stress, which stimulated the FD-dependent pathway to activate CET.[31]

This study analyzed the effect of the enantioselective phytotoxicity of IM on chlorophyll synthesis and photosynthesis in *A. thaliana*. IM affected the chlorophyll content in an enantioselective manner. *R*-IM exhibited a greater inhibitory effect on chlorophyll synthesis than *S*-IM by inhibiting the transcription of *chl*M, one of the key enzymes in the biosynthesis of chlorophyll. Moreover, *R*-IM affected the transcription of photosynthesis-related genes to decrease linear electron transport and CO_2 fixation to a greater extent than *S*-IM. CET in plant seedlings was stimulated to resist IM stress by the NDH-dependent route rather than by the FD-dependent route. These results also demonstrated that *R*-IM had a stronger herbicidal effect than *S*-IM. Therefore, using *R*-IM alone would reduce the total IM usage and relieve the environment risk of thousands of tons of unnecessary chemicals that may have adverse impacts on nontarget species, including humans.

AUTHOR INFORMATION

Corresponding Author

*Telephone/Fax: +86-571-8832-0599. E-mail: azwfu2003@yahoo.com.cn.

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Notes

The authors declare no competing financial interest.

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