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# Responses of the chloroplast glyoxalase system to high CO2 concentrations

Shimakawa, Ginga ; Ifuku, Kentaro ; Suzuki, Yuji ; Makino, Amane ; Ishizaki, Kimitsune ; Fukayama, Hiroshi ; Morita, Ryutaro ; Sakamoto,…

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 chloroplasts during photosynthesis.

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- 6 \*Corresponding Author: Ginga Shimakawa

7 Department of Biological and Environmental Science, Faculty of Agriculture, Graduate
8 School of Agricultural Science, Kobe University, 1-1 Rokkodai, Nada, Kobe 657-8501, Japan
9 Fax: +81-78-803-5851; e-mail: ginshimakawa@gmail.com

#Present address: Institute for Integrative Biology of the Cell, Centre National de la
Recherche Scientifique, Comissariat à lEnergie Atomique et aux Energies Alternatives Saclay,
Institut de Biologie et de Technologie de Saclay, Université Paris-Sud, Gif-sur-Yvette, France

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26 Title: Responses of the chloroplast glyoxalase system to high CO<sub>2</sub> concentrations

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Authors: Ginga Shimakawa<sup>1,#,\*</sup>, Kentaro Ifuku<sup>2,3</sup>, Yuji Suzuki<sup>3,4,5</sup>, Amane Makino<sup>4</sup>,
Kimitsune Ishizaki<sup>6</sup>, Hiroshi Fukayama<sup>1</sup>, Ryutaro Morita<sup>1</sup>, Katsuhiko Sakamoto<sup>1</sup>, Akiko
Nishi<sup>1</sup>, and Chikahiro Miyake<sup>1,3</sup>

- 31
- <sup>1</sup>Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodai, Nada, Kobe
  657-8501 Japan
- <sup>2</sup>Division of Integrated Life Science, Graduate School of Biostudies, Kyoto University, Sakyo,
- 35 Kyoto 606-8502 Japan
- <sup>3</sup>Core Research for Environmental Science and Technology, Japan Science and Technology
- 37 Agency, 7 Goban, Chiyoda, Tokyo 102-0076 Japan
- <sup>4</sup>Graduate School of Agricultural Science, Tohoku University, Tsutsumidori-Amamiya, Aoba,
- 39 Sendai 981-8555 Japan
- 40 <sup>5</sup>Faculty of Agriculture, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550 Japan
- <sup>6</sup>Graduate School of Science, Kobe University, 1-1 Rokkodai, Nada, Kobe 657-8501 Japan
  42
- 43 **\*Present address:** Institute for Integrative Biology of the Cell, Centre National de la
  44 Recherche Scientifique, Comissariat à lEnergie Atomique et aux Energies Alternativ es Saclay,
- 45 Institut de Biologie et de Technologie de Saclay, Université Paris-Sud, Gif-sur-Yvette, France

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47 **Keywords:** dicarbonyls; methylglyoxal; glyoxalase system; photosynthesis; high [CO<sub>2</sub>]

48 Abstract

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Sugar metabolism pathways such as photosynthesis produce dicarbonyls, e.g. methylglyoxal 50 51 (MG), which can cause cellular damage. The glyoxalase (GLX) system comprises two enzymes GLX1 and GLX2, and detoxifies MG; however, this system is poorly understood in 52 53 the chloroplast, compared with the cytosol. In the present study, we determined GLX1 and 54 GLX2 activities in spinach chloroplasts, which constituted 40% and 10%, respectively, of the 55 total leaf glyoxalase activity. In Arabidopsis thaliana, five GFP-fusion GLXs were present in 56 the chloroplasts. Under high CO<sub>2</sub> concentrations, where increased photosynthesis promotes 57 the MG production, GLX1 and GLX2 activities in A. thaliana increased and the expression of 58 AtGLX1-2 and AtGLX2-5 was enhanced. On the basis of these findings and the phylogeny of 59 GLX in oxygenic phototrophs, we propose that the GLX system scavenges MG produced in 60 chloroplasts during photosynthesis.

61 Introduction

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The substantial increase in  $CO_2$  partial pressure in the atmosphere can cause a variety of global environmental changes. One possible effect of elevated  $CO_2$  concentrations ([ $CO_2$ ]) is increased photosynthetic  $CO_2$  assimilation in plants, which may appear favourable for agricultural productivity in the near future. However, acceleration of photosynthesis by elevated [ $CO_2$ ] has the potential to cause dicarbonyl stress in plants.

68 Dicarbonyls ( $\alpha$ -oxoaldehydes) include methylglyoxal (MG), glyoxal, and 69 3-deoxyglucosone, which are generated as by-products in sugar metabolism pathways in 70 plants, including photosynthesis and respiration. Dicarbonyl MG is inevitably produced 71 during the equilibrium reaction between glyceraldehyde 3-phosphate and dihydroxyacetone 72 phosphate, which is catalysed by triosephosphate isomerase in both glycolysis and 73 Calvin-Benson cycle [1,2]. Accumulated dicarbonyls react with amino acid residues (lysine 74 and arginine) of proteins and produce advanced glycation end products (AGEs) and cause 75 protein inactivation [3]. Thus, both photosynthesis and respiration can be associated with dicarbonyl stress. 76

Elevated  $[CO_2]$  can enhance the risk of dicarbonyl stress due to increased photosynthetic activity. In intact plant leaves, accelerated photosynthesis by elevated  $[CO_2]$ causes accumulation of sugars in the cells [4] and increase in MG production [2]. In addition, the amount of carbonylated proteins increases under high CO<sub>2</sub> conditions in *Arabidopsis thaliana* and soybean leaves [5]. Overall, plants are exposed to cellular damage from respiratory and photosynthetic sugar metabolism pathways, which is more profound under high  $[CO_2]$ .

84 Photosynthetic organisms have detoxifying systems that target MG similar to the 85 corresponding mechanisms in vertebrates. These detoxifying systems are the aldo-keto

86 reductase (AKR) and glyoxalase (GLX) systems. AKR reduces MG to acetol with NAD(P)H 87 as an electron donor [6]. In the C<sub>3</sub> plant A. thaliana, several AKRs that belong to the AKR4C 88 subfamily [6], e.g., AKR4C9, has been found in chloroplasts [7]. Additionally, gene 89 expression of the AKR4C subfamily members is enhanced under high light and high [CO<sub>2</sub>] 90 conditions [8]. In contrast, MG is non-enzymatically scavenged by reduced glutathione 91 (GSH) to hemithioacetal (HA), and the GLX system converts HA to D-lactate. The GLX 92 system is comprised of two enzymes, GLX1 and GLX2 [9]. GLX1 is a divalent metal-ion 93 dependent lyase that catalyses the isomerisation of HA to S-D-lactoylglutathione (SLG). 94 GLX2 is a thiolesterase that catalyses the hydrolysis of SLG to D-lactate and GSH. The major physiological substrate of GLX1 is MG, although other dicarbonyls such as glyoxal, 95 96 hydroxypyruvate, and 4,5-doxovalerate are also catalysed by GLX1 [9]. The GLX system has 97 been identified as a scavenging system for MG that is produced during glycolysis in the 98 cytosol [9]. However, the production of MG was observed in isolated chloroplasts in a 99 light-dependent manner [2], which suggests that the GLX system is used in chloroplasts to 100 scavenge MG produced in photosynthesis.

101 We investigated the physiological significance of the GLX system in the chloroplasts 102 of plant leaves on the basis of the above-mentioned hypothesis. We determined GLX1 and 103 GLX2 activities in chloroplasts isolated from spinach leaves. To examine the molecular 104 mechanisms of the chloroplast GLX system in plant leaves, we selected three GLX1 105 (AtGLX1-1, -2, and -3) and two GLX2 candidate genes (AtGLX2-4 and -5) based on the 106 presence of transit peptides in the model C<sub>3</sub> plant A. thaliana. We observed the expression of 107 GFP-AtGLX fusion proteins in chloroplasts. Additionally, we found that both activity and 108 gene expression of the components of the GLX system in A. thaliana leaves were enhanced 109 under high [CO<sub>2</sub>], which in turn accelerated photosynthesis and dicarbonyl production.

110 Materials and Methods

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112 Plant materials

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114 Spinach was obtained from a vegetable store in Kobe, Japan. The wild-type A. thaliana ecotype Columbia gl1 was grown under long-day conditions (16 h light, 23 °C, 100 µmol 115 photons m<sup>-2</sup> s<sup>-1</sup>/8 h dark, 21 °C), under ambient (400 ppm), or high (2000 ppm) [CO<sub>2</sub>] 116 117 conditions. Partial CO<sub>2</sub> pressure was regulated using a controller device purchased from Nippon Medical and Chemical Instruments Co., LTD. (Osaka, Japan) in a closed phytotron 118 119 (Biotron nc350, Nippon Medical and Chemical Instruments Co., LTD, Osaka, Japan; 120 FLI-2000H, EYELA, Tokyo, Japan). Seeds were planted in pots filled with Metro-Mix 350 121 (Sun Gro Horticulture, Agawam, MA, USA), and watered with a Hyponex solution (Hyponex, 122 Osaka, Japan). Rosette leaves were collected four weeks after germination for experimental 123 use.

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#### 125 Preparation of stromal fractions from leaf chloroplasts

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127 Intact chloroplasts were isolated from spinach and A. thaliana leaves using the commonly 128 employed Percoll density gradient [10,2]. Chloroplasts were harvested by gentle centrifugation, and subsequently ruptured osmotically in extraction buffer (50 mM 129 130 HEPES-KOH, 1 mM MgCl<sub>2</sub>, 10 mM NaCl, 0.5 mM KH<sub>2</sub>PO<sub>4</sub>, 2 mM EDTA, pH 7.6). After 30 131 min of centrifugation at  $10,000 \times g$ , the supernatant containing the stromal fraction was 132 collected. Protein concentration was measured using a Pierce 660 nm Protein Assay (Thermo 133 Scientific, Waltham, MA, USA). Total chlorophyll (Chl) content of the chloroplast was 134 spectroscopically measured in 80% (v/v) acetone, following the method of Arnon (1949) [11].

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#### 136 **Preparation of total soluble proteins from plant leaves**

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Spinach and *A. thaliana* leaves were ground in a mortar with extraction buffer (described above) containing a protease inhibitor cocktail tablet (Complete Mini, Roche, Basel, Switzerland). After centrifugation at  $10,000 \times g$  for 30 min, the supernatant containing the total soluble fraction was collected. Protein concentration was measured using a Pierce 660 m Protein Assay (Thermo Scientific, Waltham, MA, USA). Other leaf subsamples were ground in a mortar in 80% (v/v) acetone, and total Chl was spectroscopically determined following the method of Arnon (1949) [11].

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#### 146 Enzyme assays

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GLX1 activity was measured in a 1 mL reaction mixture containing 50 mM sodium phosphate (pH 7.0), various amounts of protein (see figure and table legends), and concentrations of HA non-enzymatically formed from MG and GSH. Enzymatic activity was assessed by measuring the initial rate of SLG formation, indicated by increased absorbance at 240 nm. An  $\varepsilon_{240}$  of 2.86 mM<sup>-1</sup> cm<sup>-1</sup> was used to calculate GLX1 activity [12]. To measure the activity of the recombinant S6803GLX1 protein, we added 10 M of Ni<sup>2+</sup> as NiSO<sub>4</sub> per mol protein to the reaction mixture.

155 GLX2 activity was measured in a 1 mL reaction mixture containing 50 mM 156 Tris-HCl (pH 7.4), protein (see the figure and table legends), and various concentrations of 157 SLG. Measurement of absorbance at 240 nm was used to assess hydrolytic activity. An  $\varepsilon_{240}$  of 158 3.1 mM<sup>-1</sup> cm<sup>-1</sup> was used to calculate GLX2 activity [12].

160 Preparation of recombinant AtGLX2-4 protein

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162 The coding region was obtained from total RNA of A. thaliana using the KOD-plus Neo 163 (Toyobo, Osaka, Japan) and was subcloned into the Bam HI site of a pGEX4T-3 vector (GE 164 Healthcare, Little Chalfont, UK), using an In-Fusion HD Cloning Kit (Takara, Shiga, Japan). 165 We removed the region encoding an extension of the N-terminus of AtGLX2-4, according to a 166 comparison with the respective sequence of AtGLX2-5 [13]. The primers used are shown in 167 the Supplemental Table S1. BL21 was used as the host cell line and the proteins were 168 expressed at 15 °C for 16 h in Luria-Bertani broth containing 0.1 mМ 169 isopropyl- $\beta$ -D-thiogalactopyranoside.

170 The harvested cells were resuspended in binding buffer (140 mM NaCl, 2.7 mM KCl, 171 10 mM Na<sub>3</sub>PO<sub>4</sub>, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, 1 mM phenylmethylsulfonyl fluoride, pH 7.3). The cells 172 were mildly sonicated, and the crude extract was centrifuged at  $13,000 \times g$  at 4 °C for 30 min. 173 The lysate was loaded onto GSTrap FF columns (GE Healthcare, Little Chalfont, UK) 174 equilibrated with binding buffer. Unbound protein was removed by washing the columns with 175 binding buffer, and GST-fusion proteins were eluted with elution buffer (50 mM Tris-HCl, 10 176 mM GSH, 1 mM phenylmethylsulfonyl fluoride, pH 8.0). To remove GSH, the eluted solution 177 was loaded onto a PD-10 column (GE Healthcare, Little Chalfont, UK) in GSH-free elution 178 buffer. The concentration of eluted proteins was measured using a Pierce 660 nm Protein 179 Assay (Thermo Scientific, Waltham, MA, USA).

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### 181 Transient expression in *A. thaliana* mesophyll protoplasts

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183 Preparation of protoplasts from A. thaliana rosette leaves and PEG-calcium transfection were

184 performed following the methods described previously [14]. The coding regions of *AtGLX1-1*,

185 AtGLX1-2, and AtGLX1-3 in both transit (AtGLX1-1 1, AtGLX1-2 6, and AtGLX1-3 4; 186 Supplemental Fig. S1) and non-transit forms (AtGLX1-1 2, AtGLX1-2 2, and AtGLX1-3 2; 187 Supplemental Fig. S1) were obtained by PCR using the KOD-FX Neo (Toyobo, Osaka, 188 Japan) and were then subcloned into the SalI and NcoI sites of a pCaMV35S GFP vector 189 using the In-Fusion HD Cloning Kit (Takara, Shiga, Japan). For GLX2, the coding regions of 190 GLX2-4 and GLX2-5 were subcloned into the Sal I and Nco I sites of a pCaMV35S GFP 191 vector, similar to the GLX1 isozymes. The primers used for cloning are shown in 192 Supplemental Table S1. For transformation, 10 µg DNA of each plasmid were transfected into  $2 \times 10^4$  protoplasts. GFP and Chl fluorescence was observed using a fluorescence microscope 193 194 (BZ-8000, KEYENCE, Japan). The field of cells was excited at 480 nm and fluorescence 195 emission was measured at 510 nm.

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#### 197 **Real-time PCR**

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199 Quantitative real time-PCR was performed using SYBR Premix Ex Taq (Takara, Shiga, 200 Japan) and a LightCycler 1.5 (Roche, Basel, Switzerland). A comparative threshold cycle 201 method was applied to determine relative concentrations of mRNA. The primers used are 202 shown in Supplemental Table S1. We used the Arabidopsis actin gene, *actin2*, as a reference 203 gene, and the obtained data were normalised to *actin2* using the  $\Delta\Delta$ CT method [15].

204

#### 205 Phylogenetic analysis

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207 Sequences were aligned using ClustalW. The sequence comparisons were performed only in

208 the Glyoxalase domain in the N-terminus of GLX1 and the hydroxyacylglutathione hydrolase

209 domain in the C-terminus of GLX2, both of which were predicted from the Pfam database.

210 The amino acid sequences of GLX1 and GLX2 were obtained from CyanoBase (Slr0381 for 211 S6803GLX1; Sll1019 for S6803GLX2), the National Center for Biotechnology Information (XP 005849034 for CvGLX1-1; XP 005847305 for CvGLX1-2; XP 005535468 for 212 213 CmGLX1; XP 002180432 for PtGLX1; WP 029404302 for EcGLX1; ONH72282 for ScGLX1; XP 005845456 for CvGLX2; XP 005539165 for CmGLX2; XP 002185216 for 214 215 PtGLX2; WP 063077511 for EcGLX2; KZV12511 for ScGLX2), the MarpolBase (Mapoly0020s0109 for MpGLX1-1; Mapoly0033s0085 for MpGLX1-2; Mapoly0033s0083 216 217 for MpGLX1-3; Mapoly0204s0005 for MpGLX2-1; Mapoly0204s0009 for MpGLX2-2), 218 Phytozome (402441 for SmGLX1-1; 270490 for SmGLX1-2; 164521 for SmGLX2-1; 219 149526 for SmGLX2-2; Os02g17920 for OsGLX1-1; Os08g09250 for OsGLX1-2; 220 Os05g14194 for OsGLX1-3; Os05g22970 for OsGLX1-4; Os09g34100 for OsGLX2-1; Os03g21460 for OsGLX2-2; Zm00008a021415 for ZmGLX1-1; Zm00008a015465 for 221 222 ZmGLX1-2; Zm00008a039630 for ZmGLX1-3; Zm00008a025479 for ZmGLX1-4; 223 Zm00008a007714 for ZmGLX1-5; Zm00008a028591 for ZmGLX2-1; Zm00008a001400 for 224 ZmGLX2-2), and TAIR (Table 2).

225 Phylogenetic trees for GLX1 and GLX2 were constructed using MEGA7, based on 226 the sequence alignments in Supplemental Fig. S3 and S4 [16]. The evolutionary history was 227 inferred using a neighbour-joining method. The percentages of replicate trees in which the 228 associated taxa clustered together in the bootstrap test are indicated near the respective 229 branches. The tree was drawn to scale, with branch lengths in the same units as those of the 230 evolutionary distances used to infer the phylogenetic tree. Evolutionary distances were 231 computed using the Poisson correction method based on the number of amino acid 232 substitutions per site.

- 233 Results
- 234

#### 235 GLX1 and GLX2 activity in spinach chloroplasts

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237 We measured GLX1 and GLX2 activities in the stromal fraction of spinach chloroplasts and 238 compared these activities with the respective enzymatic activity in the total soluble fraction of 239 spinach leaves. In the total soluble fraction extracted from spinach leaves, we found similar 240 maximum activity of GLX1 and GLX2 per total soluble protein content (Fig. 1A and B). 241 However, in the spinach chloroplast stromal fraction, the activity of GLX1 per total stromal 242 protein was larger than that of GLX2 (Fig. 1A and B). No enzymatic activity of both GLX1 243 and GLX2 was measurable after 10 min of boiling. These results indicate that GLX1 and 244 GLXs are active in the stroma extracts, and that GLX1 activity is higher than GLX2 activity.

245 Subsequently, we calculated GLX1 and GLX2 activity in the total soluble and 246 stromal fractions as values per total Chl content, based on the ratios of leaf soluble protein to Chl (3.5  $\pm$  0.4 mg leaf soluble protein mg<sup>-1</sup> Chl, n = 3) and stromal protein to Chl (1.89  $\pm$  0.08 247 mg stromal protein mg<sup>-1</sup> Chl, n = 3), in spinach leaves. For this, we evaluated GLX activity in 248 249 both fractions based on the same parameter, i.e., total Chl content (Fig. 1C and D) as all Chl in spinach leaves should originate from chloroplasts. The maximum activities  $(V_{max})$  of GLX1 250 and GLX2 in the leaf soluble fraction were  $45 \pm 5$  and  $60 \pm 8 \mu mol SLG (mg Chl)^{-1} h^{-1} (n =$ 251 252 3), respectively (Table 1). Additionally, the  $K_{\rm m}$  for the HA or SLG substrates were 0.046  $\pm$ 253 0.014 and 0.21  $\pm$  0.06 mM (n = 3) in the leaf soluble fraction, respectively (Table 1). In contrast, in the chloroplast extracts,  $V_{\text{max}}$  and  $K_{\text{m}}$  of the GLX1 reaction were  $16 \pm 2 \mu \text{mol SLG}$ 254  $(\text{mg Chl})^{-1}$  h<sup>-1</sup> and  $0.072 \pm 0.017$  mM (n = 3), and those of the GLX2 reaction were  $4.8 \pm 0.4$ 255  $\mu$ mol SLG (mg Chl)<sup>-1</sup> h<sup>-1</sup> and 0.12  $\pm$  0.03 mM (n = 3), respectively (Table 1). These results 256 257 indicate that approximately 40% of GLX1 and 10% of GLX2 activity in spinach leaves 258 originated from the chloroplasts.

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#### 260 Characteristics of GLX1 and GLX2 in A. thaliana

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262 To investigate the molecular mechanism of the chloroplast GLX system in leaves, we used the 263 model C<sub>3</sub> plant A. thaliana, which has three isozymes of GLX1 (Table 2). Based on the 264 primary structure, these three GLX1 isozymes are categorised into two metal-dependent classes, Ni<sup>2+</sup>-dependent (AtGLX1-1 and -2) and Zn<sup>2+</sup>-dependent enzymes (AtGLX1-3) [17]. 265 266 Furthermore, all three GLX1 isozymes have splice variants with transit peptides 267 (AtGLX1-1 1, AtGLX1-2 6, and AtGLX1-3 4; Supplemental Fig. S1) that are predicted to 268 be localised in chloroplasts [17]. Moreover, these AtGLX1s show isomerisation activity for 269 HA to SLG (Table 2) [18].

270 There are five GLX2 isozymes (AtGLX2-1, -2, -3, -4, and -5) that are annotated 271 based on the primary structure in A. thaliana (Table 2). All isozymes, except GLX2-2, have 272 putative transit peptides [18]. It has previously been reported that recombinant AtGLX2-2 and 273 -5 proteins show GLX2 activity (Table 2) [13,19,20], and that AtGLX2-1 and -3 possess no 274 SLG hydrolysis activity and do not function in the GLX system [20,21]. To the best of our 275 knowledge, the present study is the first to determine GLX2 activity of the glutathione S-transferase (GST)-tagged AtGLX2-4 recombinant protein.  $K_{\rm m}$  was 0.24 ± 0.02 mM and  $k_{\rm cat}$ 276 was  $19000 \pm 5000 \text{ min}^{-1}$  (n = 3; Table 2). Taken together, our results show that AtGLX2-4 277 278 possesses GLX2 activity as well as AtGLX2-2 and -5.

279

#### 280 Subcellular localisation of GLX1 and GLX2 in A. thaliana

<sup>282</sup> We examined subcellular localisation of five GLX isozymes with transit peptides, including

AtGLX1-1, -2, and -3 (i.e., AtGLX1-1\_1, AtGLX1-2\_6, and AtGLX1-3\_4; Supplemental Fig. S1), and AtGLX2-4 and -5 in *A. thaliana*. These five GLXs were transiently expressed in *A. thaliana* mesophyll protoplasts as GFP-fusion proteins. The GFP fluorescence from all five GLX constructs was exclusively detected in chloroplasts, which were identified by the red auto-fluorescence of Chl (Fig. 2). These results suggest that the three GLX1 isozymes (AtGLX1-1, -2, and -3) and the two GLX2 isozymes (AtGLX2-4 and -5) are located in the chloroplasts of *A. thaliana* mesophyll cells.

290 All three GLX1 isozymes in A. thaliana have splice variants that have no N-terminal 291 transit peptide (Supplemental Fig. S1). Thus, AtGLX1-1, -2, and -3 were assumed to be 292 expressed not only with transit peptides but also without transit peptides. Furthermore, we 293 investigated the subcellular localisation by transiently expressing GFP-fusion constructs of 294 AtGLX1-1, -2, and -3, which lacked the transit peptide (AtGLX1-1 2, AtGLX1-2 2, and 295 AtGLX1-3 2), designated as AtGLX1-1w/oS, AtGLX1-2w/oS, and AtGLX1-3w/oS, 296 respectively. We detected GFP fluorescence around chloroplasts (Supplemental Fig. S2). 297 These results suggest that splice variants of these GLX1 isozymes exist in the cytosol of A. 298 thaliana mesophyll cells.

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#### 300 Responses of GLX1 and GLX2 to high [CO2] in A. thaliana

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To investigate the responses of the chloroplast GLX system to high  $[CO_2]$ , we measured GLX1 and GLX2 activity in *A. thaliana* leaves grown under atmospheric and high  $[CO_2]$ conditions. Both GLX1 and GLX2 activities in the soluble fraction of the *A. thaliana* leaves were significantly higher in plants grown under high  $[CO_2]$  than in plants grown under atmospheric  $[CO_2]$  (Fig. 3A).

307 Furthermore, we investigated the expression of the genes encoding AtGLXs. The

expression of *AtGLX1-2* and *AtGLX2-5* was significantly increased under high [CO<sub>2</sub>] growth
conditions (Fig. 3B). The gene for *AtGLX1-2* showed the highest expression levels under high
[CO<sub>2</sub>], which was approximately four-fold that measured under atmospheric conditions (Fig. 3B).
310 [CO<sub>2</sub>], which was approximately four-fold that measured under atmospheric conditions (Fig. 3B).

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#### 313 Phylogeny of GLX1 and GLX2 in oxygenic phototrophs

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315 The production of MG derived from photosynthesis [2] implies that oxygenic phototrophs 316 require the GLX system in chloroplasts. In fact, GLX1 and GLX2 activities were observed in 317 the stromal fraction of spinach chloroplasts (Fig. 1 and Table 1). Furthermore, three GLX1 318 isozymes, AtGLX1-1, -2, and -3, and two GLX2 isozymes, AtGLX2-4 and -5, were detected 319 in the chloroplasts of A. thaliana (Fig. 2). Reportedly, AtGLX2-2, which does not possess a 320 transit peptide, is exclusively located in the cytosol [18, 20, 21]. Previously, we characterised 321 the GLX system in the cyanobacterium Synechocystis sp. PCC 6803 (S. 6803) [22], 322 suggesting that the chloroplast GLX system may have originated from cyanobacteria, which 323 are the ancestors of chloroplasts of plants. In the present study, we compared the primary 324 structures of GLX1 and GLX2 between the heterotrophic prokaryote Escherichia coli, eukaryote Saccharomyces cerevisiae, and a variety of oxygenic phototrophs, including S. 325 326 6803, the green alga Chlorella variabilis, the liverwort Marchantia polymorpha, the fern 327 Selaginella moellendorffii, A. thaliana, the C<sub>3</sub> plant Oryza sativa, the C<sub>4</sub> plant Zea mays, the 328 red alga Cyanidioschyzon merolae, and the diatom Phaeodactylum tricornutum (Supplemental 329 Figs. S3 and S4) to evaluate the phylogeny of the GLX system in oxygenic phototrophs. The phylogenetic tree of GLX1 clearly showed the division of two groups with Ni<sup>2+</sup>- and 330  $Zn^{2+}$ -dependent GLX1s (Fig. 4). The activity of cyanobacterial GLX1 was biochemically 331 characterised to be a Ni<sup>2+</sup>-dependent type [16,22] (Supplemental Table S2). Both GLX1s in S. 332

6803 and E. coli were separate from the other Ni<sup>2+</sup>-dependent GLX1 in the phylogenetic tree 333 334 (Fig. 4); this suggests that the primary structure of GLX1 has largely varied in the evolution of these eukaryotes. Compared with Ni<sup>2+</sup>-dependent GLX1, the phylogeny of Zn<sup>2+</sup>-dependent 335 336 GLX1 is likely to follow the evolutionary lineage of oxygenic phototrophs from cyanobacteria to angiosperms (Fig. 4). The green alga C. variabilis and land plants have both 337  $Ni^{2+}$ - and  $Zn^{2+}$ -dependent GLX1 isozymes, whereas the red alga *C. merolae* and the diatom *P.* 338 *tricornutum* have only the  $Zn^{2+}$ -dependent isoform, similarly to heterotrophs, *E. coli* and *S.* 339 340 cerevisiae (Fig. 4). In contrast, the phylogenetic tree of GLX2 showed a distinct division of 341 AtGLX2 into two groups. Both AtGLX2-4 and -5 detected in chloroplasts (Fig. 2); and 342 AtGLX2-2 located in the cytoplasm [18]. Interestingly, the C<sub>3</sub> plant O. sativa and the C<sub>4</sub> plant Z. mays possess GLX2 isozymes similar to AtGLX2-4 or -5 (OsGLX2-1 and ZmGLX2-1, 343 344 respectively; Fig. 5), which are presumably located in chloroplasts. Moreover, both O. sativa 345 and Z. mays show GLX2 (OsGLX2-2 and ZmGLX2-2) to be homologous to AtGLX2-2 (Fig. 346 5). These results suggest a categorisation into chloroplastic and cytoplasmatic GLX2 347 isozymes, which may occur in all angiosperms. The liverwort *M. polymorpha* and the fern *S.* 348 moellendorffii have two isozymes of GLX2, whereas the genomes of the eukaryotic algae C. 349 variabilis, C. merolae, and P. tricornutum contain only one gene encoding GLX2 (Fig. 5), 350 suggesting that GLX2 is used only in the cytosol in these algae unless GLX2 is expressed in 351 various forms and exists both in cytosol and chloroplasts, similar to AtGLX1-1, -2, and -3 352 (Fig. 2 and Supplemental Fig. 2).

354

355 In the present study, we characterised the chloroplast-localised GLX system and investigated 356 its response to high [CO<sub>2</sub>] conditions. GLX1 and GLX2 activities in the stromal fraction were 357 estimated at approximately 40% and 10%, respectively, of that measured in the total soluble 358 fraction in spinach leaves (Fig. 1 and Table 1). This indicates that the chloroplast GLX system 359 participates in the dynamic cellular metabolism of MG that is probably produced during 360 photosynthesis. In A. thaliana, three AtGLX1s (AtGLX1-1, -2, and -3) fused with GFP were 361 found in the chloroplasts of mesophyll protoplasts of A. thaliana (Fig. 2). The GFP-fusion 362 constructs of AtGLX2-4 and -5 observed in chloroplasts (Fig. 2) were different from the 363 cytosolic AtGLX2-2 isozyme. From these data, we summarised the metabolic pathway of MG 364 in the GLX system in A. thaliana (Fig. 6). The GLX system has been known to be present in 365 the cytosol where it scavenges MG produced during glycolysis [9]. However, photosynthetic 366 CO<sub>2</sub> assimilation stimulates MG production [2]. Furthermore, proteome analysis using mass 367 spectrometry showed AtGLX1-1 and -3 in the chloroplasts of A. thaliana [23]. These results 368 support the hypothesis of the present study. Recently, Schmitz et al. (2017) measured 369 YFP-GLX isozyme fusion constructs expressed in tobacco leaves by Agrobacterium leaf infiltration to show that three AtGLXs (i.e., AtGLX1-1 and -3, and AtGLX2-5) are present in 370 371 chloroplasts. In general, our results are consistent with this study. However, Schmitz et al. 372 (2017) concluded that AtGLX1-2 and AtGLX2-4 are present in the endoplasmic reticulum 373 and mitochondria, respectively. This discrepancy might be attributed to the different 374 transformation methods used. Furthermore, we used the protoplast of A. thaliana as the host 375 cell to express AtGLXs fused with GFP, which differs from that of tobacco in the 376 above-mentioned study [18]. In spinach chloroplasts, GLX1 activity was higher than that of 377 GLX2 (Fig. 1 and Table 1), suggesting that a part of SLG (with GSH) may be exported from

chloroplasts into the cytosol. In addition, GLX2 activity has been detected in the mitochondrial matrix of spinach leaves, and furthermore, the final product of the GLX system, D-lactate, is metabolised to pyruvate by D-lactate dehydrogenase in the mitochondria [24]. The coordinate metabolism of chloroplasts, the cytosol, and mitochondria for scavenging MG likely include dynamic redox signalling across these organelles. Taken together, the GLX system in plant leaves occurs in chloroplasts and is up-regulated to alleviate dicarbonyl stress under high [CO<sub>2</sub>].

385 Alternative splicing may regulate the compartmentalisation of GLX1 activity 386 between chloroplasts and the cytosol. It has been reported that approximately 60% of the A. 387 thaliana genes are alternatively spliced and one third of those are functional [25]. Three 388 GLX1 isozymes in A. thaliana have splice variants located in the cytosol (Supplemental Fig. 389 S2), as well as variants that include transit peptides for their expression in chloroplasts (Fig. 390 2). These data are consistent with the findings of Schmitz et al. (2017). At night, 391 photosynthetic CO<sub>2</sub> assimilation ceases and the concentration of triose phosphates such as 392 glyceraldehyde 3-phosphate and dihydroxyacetone phosphate in chloroplasts is lower than 393 that under light conditions [26]. Therefore, the chloroplast GLX system is presumably 394 required during daytime, but not during night time. Further research is needed to elucidate the 395 dependence of the modulation of GLX1 activity on photosynthetic activity between the 396 organelles.

An increase in [CO<sub>2</sub>] can accelerate dicarbonyl stress despite enhanced GLX system activity. Atmospheric [CO<sub>2</sub>] has been increasing and thus affecting plants. Under high [CO<sub>2</sub>] soluble sugars such as D-glucose accumulate in plant cells [4], which promotes the production of MG [2]. We found that both GLX1 and GLX2 activities were enhanced (Fig. 3A) and the genes encoding AtGLX1-2 and AtGLX2-5 were highly expressed (Fig. 3B) under high [CO<sub>2</sub>] conditions. These data are consistent with the increase of expression of genes associated with 403 the GLX system in response to high levels of sugar [18] and MG [27]. Overall, the GLX 404 system is supposed to prevent the accumulation of MG in cells under high [CO<sub>2</sub>] conditions. 405 Nevertheless, this carbonylated protein can accumulate under high [CO<sub>2</sub>] even in wild-type 406 plants [5], suggesting that plant leaves are exposed to dicarbonyl stress under high [CO<sub>2</sub>], 407 even if the GLX system is upregulated. Further enhancement of the GLX system through 408 genetic modification and additive agents might facilitate avoidance of disadvantageous 409 accumulation of MG under high [CO<sub>2</sub>] conditions [27]. In the present study, no significant 410 differences were found in the expression levels of AtGLX1-1, AtGLX1-3, or AtGLX2-4 genes 411 between atmospheric and high [CO<sub>2</sub>] (Fig. 3B), which may suggest that these GLX isozymes 412 respond to variations in other natural environmental factors, such as high light intensity [18].

There are diverse scavenging systems for MG in chloroplasts. One of the most 413 414 prominent mechanisms is AKR. Previously, Saito et al. (2011) verified the 415 NADPH-dependent reducing activity of MG in the stromal fraction of spinach chloroplasts. The  $K_{\rm m}$  and  $V_{\rm max}$  values for MG were 6.5 mM and 3.3 µmol MG (mg Chl)<sup>-1</sup> h<sup>-1</sup>, respectively. 416 417 In A. thaliana, AKR4C9, a member of the AKR4C subfamily, had a  $k_{cat}/K_m$  value of approximately  $10^2-10^3$  mM<sup>-1</sup> min<sup>-1</sup> for scavenging MG [6,8] and was located in the 418 419 chloroplasts [7]. The expression of the AKR4C subfamily was enhanced in response to high 420 light and high CO<sub>2</sub> [8]. This indicates that AKR may detoxify MG produced during 421 photosynthesis. In contrast, the short- and medium-chain dehydrogenases/reductases SDR and 422 MDR, respectively, can reduce MG to acetol with NADPH as the electron donor, similarly to 423 AKR [7,22]. Both SDR and MDR are broadly conserved in oxygenic phototrophs; however, 424 the NADPH-dependent MG reducing activities of the recombinant proteins are significantly 425 lower in plant leaves than in cyanobacteria [22]. In the present study, GLX1 and GLX2 426 activities in the chloroplasts of spinach leaves (Fig. 1 and Table 1) had lower  $K_m$  and higher 427  $V_{\text{max}}$  than the NADPH-dependent MG reducing reaction. In chloroplasts, MG can rapidly

accumulate to a high level when the detoxification efficiency is low because the production
rate of MG during photosynthesis is approximately 3% of the photosynthetic O<sub>2</sub> evolution
rate [2]. High cellular accumulation of MG (e.g. mM-order) inhibits plant growth [28].
However, the lack of a GLX isozyme does not inhibit plant growth [18], most likely because
oxygenic phototrophs have developed a variety of complementary systems for controlling
MG accumulation.

434 The chloroplast GLX system might have developed during the evolutionary history of oxygenic phototrophs. In the present study, we phylogenetically analysed GLX1 and GLX2 435 436 of different oxygenic phototrophs, including S. 6803, C. variabilis, M. polymorpha, S. 437 moellendorffii, A. thaliana, O. sativa, Z. mays, C. merolae, and P. tricornutum. Cyanobacteria 438 are the progenitors of oxygenic photosynthesis and contain the GLX system (Fig. 4 and 5) 439 [22], which implies that photosynthetic organisms developed the GLX system for scavenging 440 MG originating from the Calvin-Benson cycle as oxygenic phototrophs and conserved and 441 diversified the GLX system over their evolutionary history (Fig. 4 and 5). Generally, green 442 algae, red algae, and land plants, termed Archaeplastida, are assigned to one group, which has experienced a single endosymbiotic event. Nevertheless, the red alga C. merolae and P. 443 *tricornutum* only have a  $Zn^{2+}$ -dependent GLX1 (Fig. 4). These oxygenic phototrophs can be 444 445 also termed as red plastid lineage and known to have evolved differently from green algae and land plants (so-called green plastid lineage) [29]. Possibly, Ni<sup>2+</sup>-dependent GLX1 had been 446 447 inherited from cyanobacteria selectively to the oxygenic phototrophs in green plastid lineage. 448 The eukaryotic algae C. variabilis, C. merolae, and P. tricornutum have only one GLX2, 449 whereas land plants produce more than two isozymes of GLX2 (Fig. 5), indicating that the 450 chloroplastic GLX system may have been established in the evolutionary process from algae 451 to land plants. Adaption to terrestrial life may have required oxygenic phototrophs to enhance 452 their cellular MG scavenging system, because excretion of dicarbonyls is presumably more difficult in the terrestrial environment than in the aquatic environment. However, notably, CO<sub>2</sub>
availability differs between the atmosphere, fresh water, and sea water. Thus, C<sub>4</sub> plants and
many algae developed CO<sub>2</sub>-concentrating mechanisms to increase fixation efficiency [30].
This again might affect the scavenging systems for dicarbonyls such as the GLX system.
Enzyme activity, subcellular localisation, and physiological significance of the GLX system in
diverse oxygenic phototrophs should be assessed simultaneously with their photosynthetic
physiology in future studies.

460 As a trade-off for the benefits derived from photosynthesis, oxygenic phototrophs are 461 exposed to a variety of risks from oxidative stress. The dicarbonyl MG is produced in the 462 Calvin-Benson cycle [2], which accepts electrons from the photosynthetic electron transport 463 system of photosystem I (PSI) on the thylakoid membrane to form MG radicals, finally 464 reducing  $O_2$  to a superoxide anion radical  $(O_2^{-})$  [31]. Thus, MG accumulates in the 465 chloroplasts and elicits the production of reactive oxygen species (ROS), which can cause 466 oxidative damage in photosynthetic cells. In chloroplasts, ROS can also be generated due to 467 the excitation energy in photosystems I and II, which can then impair the growth of oxygenic 468 phototrophs [32,33]. Further, ROS can react with membrane lipids and free fatty acids to 469 produce  $\alpha,\beta$ -unsaturated carbonyls such as acrolein [34], which can also inhibit growth of 470 oxygenic phototrophs [35]. In general, oxygenic phototrophs developed defence mechanisms 471 against the effects of ROS and dicarbonyl stress. The large amount of GSH (3.5 mM) in 472 chloroplasts [36] may play an essential role for the detoxification mechanisms of not only 473 ROS but also MG in chloroplasts. Nevertheless, predicted increases in  $[CO_2]$  and global 474 warming potentially threaten plant growth, and may cause dicarbonyl stress, known as "plant 475 diabetes" [37, 38]. The physiological significance of scavenging systems of dicarbonyls in 476 chloroplasts thus provides insight into the acclimation of plants to high [CO<sub>2</sub>] conditions and 477 will aid in developing future agricultural and biotechnological strategies.

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479	
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481	
482	Conflict of interest
483	
484	The authors have no conflict of interest to declare.

- 485 Supporting information
- 486
- 487 Supplemental Table S1. Primers used in this study
- 488 Supplemental Table S2. Kinetic parameters of the recombinant S6803GLX1
- 489 Supplemental Fig. S1. Amino acid sequences of each splice variant of AtGLX1s
- 490 Supplemental Fig. S2. Subcellular localisation of AtGLX1w/oS-GFP fusion proteins in A.
- 491 *thaliana* mesophyll protoplasts
- 492 Supplemental Fig. S3. Sequence comparison of the glyoxalase domain in the N-terminus of
- 493 glyoxalase I
- 494 Supplemental Fig. S4. Sequence comparison of the hydroxyacylglutathione hydrolase
- 495 domain in the C-terminus of glyoxalase II

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## 603 Tables

	K <sub>m</sub> (mM)	$V_{max}$ (µmol SLG [mg chlorophyll] <sup>-1</sup> h <sup>-1</sup> )
GLX1		
Leaves	0.046 ± 0.014	45 ± 5
Chloroplasts	0.072 ± 0.017	16 ± 2
GLX2		
Leaves	0.21 ± 0.06	60 ± 8
Chloroplasts	0.12 ± 0.03	$4.8 \pm 0.4$

Table 1. Comparison of glyoxalase (GLX) 1 and 2 activity between spinach leaves and chloroplasts

For the measurements, hemithioacetal (HA) and S-D-lactoylglutathione (SLG) were used as the substrates for

GLX1 and GLX2, respectively. Data are means  $\pm$  SD from three independent experiments.

Nama	Loous	Transit	Kinetic parameters of the recombinant protein			
Name	Locus	peptide	K <sub>m</sub> (mM)	k <sub>cat</sub> (min⁻¹)	k <sub>cat</sub> /K <sub>m</sub> (mM⁻¹ min⁻¹)	
GLX1						
AtGLX1-1	At1g67280	+	<sup>a</sup> 0.279 ± 0.004	<sup>a</sup> 530 ± 30	<sup>a</sup> 1900	
AtGLX1-2	At1g11840	+	°0.330 ± 0.010	<sup>a</sup> 550 ± 40	ª2100	
AtGLX1-3	At1g08110	+	°0.476 ± 0.125	<sup>a</sup> 10500 ± 1800	ª22100	
GLX2						
AtGLX2-1	At2g43430	+		No GLX2 activity		
AtGLX2-2	At3g10850	-	°0.098 ± 0.007	°28000 ± 500	°282000	
AtGLX2-3	At1g53580	+		No GLX2 activity		
AtGLX2-4	At1g06130	+	0.24 ± 0.02	19000 ± 5000	80000	
AtGLX2-5	At2g31350	+	<sup>e</sup> 0.27 ± 0.06	°10800 ± 1200	<sup>e</sup> 40000	

Table 2. Characteristics of glyoxalase (GLX) 1 and 2 in A. thaliana

For the measurement of the recombinant AtGLX2-4 protein (0.2 μg mL<sup>-1</sup>), S-D-lactoylglutathione was used as the substrate. Data are means ± SD from three independent experiments. Notes: <sup>a</sup>Schmitz et al. (2017) [18]; <sup>b</sup>Ridderström and Mannervik (1997) [19]; and <sup>c</sup>Limphong et al. (2010) [20].

606 Figure legends

607

**Fig. 1.** Dependence of the substrates in the soluble extracts (20  $\mu$ g protein mL<sup>-1</sup>) from spinach leaves (grey circles) and chloroplasts (green triangles) on glyoxalase (GLX) 1 (A, C) and 2 (B, D) activities. The activity was estimated based on the amounts of protein (A, B) or chlorophyll (Chl) (C, D). The substrates hemithioacetal (HA) and *S*-D-lactoylglutathione (SLG) were used; the production and consumption rates of SLG were measured for GLX1 and GLX2 activity, respectively. Data are presented as means  $\pm$  SD from three independent experiments.

615

616 **Fig. 2.** Subcellular localisation of the GFP-fused glyoxalase (GLX) 1 and 2 proteins 617 expressed in transiently transformed *A. thaliana* mesophyll protoplasts. Fluorescent images of 618 GFP ( $F_{GFP}$ ), chlorophyll autofluorescence ( $F_{Chl}$ ), and bright field (BF) are shown. Free GFP 619 protein was used as a control. Black bars = 10 µm.

620

621 Fig. 3. Activity (A) and expression (B) of glyoxalase (GLX) 1 and 2 in A. thaliana leaves in 622 response to the increase in ambient [CO<sub>2</sub>]. Wild-type plants were grown at atmospheric (400 623 ppm, grey bars) and high (2000 ppm, dark grey bars) [CO<sub>2</sub>]. (A) The GLX1 and GLX2 624 activity was measured in the total soluble extracts (20  $\mu$ g protein mL<sup>-1</sup>) from plant leaves. (B) 625 The transcript abundance was calculated relative to the expression level of the reference gene 626 (actin2). Data are presented as means  $\pm$  SD from three independent experiments. Differences 627 between the plants grown under atmospheric and high [CO<sub>2</sub>] were analysed with a Student's 628 *t*-test. Asterisks indicate statistically significant differences at p < 0.05.

629

630 Fig. 4. Phylogenetic tree of the evolutionary relationship between glyoxalase I (GLX1) in

oxygenic phototrophs. Branch lengths correspond to the evolutionary distances. Organisms
included in this phylogenetic analysis are S6803, cyanobacterium *Synechocystis* sp. PCC
6803; Cv, green alga *Chlorella variabilis*; Mp, liverwort *Marchantia polymorpha*; Sm, fern *Selaginella moellendorffii*; At, C<sub>3</sub> plant *Arabidopsis thaliana*; Os, C<sub>3</sub> plant *Oryza sativa*; Zm,
C<sub>4</sub> plant *Zea mays*; Cm, red alga *Cyanidioschyzon merolae*; Pt, diatom *Phaeodactylum tricornutum*; Ec, heterotrophic prokaryote *Escherichia coli*; and Sc, heterotrophic eukaryote *Saccharomyces cerevisiae*.

638

Fig. 5. Phylogenetic tree of the evolutionary relationship among glyoxalase II (GLX2) 639 640 expressed in oxygenic phototrophs. Branch lengths correspond to the evolutionary distances. 641 Organisms included in this phylogenetic analysis are S6803, cyanobacterium Synechocystis sp. 642 PCC 6803; Cv, green alga Chlorella variabilis; Mp, liverwort Marchantia polymorpha; Sm, 643 fern Selaginella moellendorffii; At, C<sub>3</sub> plant Arabidopsis thaliana; Os, C<sub>3</sub> plant Orvza sativa; 644 Zm, C4 plant Zea mays; Cm, red alga Cyanidioschyzon merolae; Pt, diatom Phaeodactylum 645 tricornutum; Ec, heterotrophic prokaryote Escherichia coli; and Sc, heterotrophic eukaryote 646 Saccharomyces cerevisiae.

647

Fig. 6. Proposed metabolic model of the glyoxalase (GLX) system in plant leaves.
Abbreviations used: MG, methylglyoxal; TPs, triose phosphates: i.e., glyceraldehyde
3-phosphate and dihydroxyacetone; GSH, reduced glutathione; HA, hemithioacetal; and SLG,
S-p-lactoylglutathione.



**Fig. 1.** Dependence of the substrates in the soluble extracts (20  $\mu$ g protein mL<sup>-1</sup>) from spinach leaves (grey circles) and chloroplasts (green triangles) on glyoxalase (GLX) 1 (A, C) and 2 (B, D) activities. The activity was estimated based on the amounts of protein (A, B) or chlorophyll (Chl) (C, D). The substrates hemithioacetal (HA) and *S*-D-lactoylglutathione (SLG) were used; the production and consumption rates of SLG were measured for GLX1 and GLX2 activity, respectively. Data are presented as means  $\pm$  SD from three independent experiments.



**Fig. 2.** Subcellular localisation of the GFP-fused glyoxalase (GLX) 1 and 2 proteins expressed in transiently transformed *A. thaliana* mesophyll protoplasts. Fluorescent images of GFP ( $F_{GFP}$ ), chlorophyll autofluorescence ( $F_{Chl}$ ), and bright field (BF) are shown. Free GFP protein was used as a control. Black bars = 10 µm.

Figure 2. Shimakawa et al.







**Fig. 4.** Phylogenetic tree of the evolutionary relationship between glyoxalase I (GLX1) in oxygenic phototrophs. Branch lengths correspond to the evolutionary distances. Organisms included in this phylogenetic analysis are S6803, cyanobacterium *Synechocystis* sp. PCC 6803; Cv, green alga *Chlorella variabilis*; Mp, liverwort *Marchantia polymorpha*; Sm, fern *Selaginella moellendorffii*; At, C<sub>3</sub> plant *Arabidopsis thaliana*; Os, C<sub>3</sub> plant *Oryza sativa*; Zm, C<sub>4</sub> plant *Zea mays*; Cm, red alga *Cyanidioschyzon merolae*; Pt, diatom *Phaeodactylum tricornutum*; Ec, heterotrophic prokaryote *Escherichia coli*; and Sc, heterotrophic eukaryote *Saccharomyces cerevisiae*.



**Fig. 5.** Phylogenetic tree of the evolutionary relationship between glyoxalase II (GLX2) expressed in oxygenic phototrophs. Branch lengths correspond to the evolutionary distances. Organisms included in this phylogenetic analysis are S6803, cyanobacterium *Synechocystis* sp. PCC 6803; Cv, green alga *Chlorella variabilis*; Mp, liverwort *Marchantia polymorpha*; Sm, fern *Selaginella moellendorffii*; At, C<sub>3</sub> plant *Arabidopsis thaliana*; Os, C<sub>3</sub> plant *Oryza sativa*; Zm, C<sub>4</sub> plant *Zea mays*; Cm, red alga *Cyanidioschyzon merolae*; Pt, diatom *Phaeodactylum tricornutum*; Ec, heterotrophic prokaryote *Escherichia coli*; and Sc, heterotrophic eukaryote *Saccharomyces cerevisiae*.



**Fig. 6.** Proposed metabolic model of the glyoxalase (GLX) system in plant leaves. Abbreviations used: MG, methylglyoxal; TPs, triose phosphates: i.e., glyceraldehyde 3-phosphate and dihydroxyacetone; GSH, reduced glutathione; HA, hemithioacetal; and SLG, *S*-D-lactoylglutathione.

## Supplemental Table S1. Primers used in this study

P1: GstGLX2-4 fGGTTCCGCGTGGATCCATGCAAATTGAACTGGTGCCATGTCP2: GstGLX2-4 rGGGAATTCGGGGATCCTAAGCTTGAAATTGTCCTTTGP3: sGLX1-1Gfp fTACAATTACAGTCGACATGAGTTCGTACTCAATAGCAAGTP4: GLX1-1Gfp fTACAATTACAGTCGACATGGCGTCGGAAGCGAGGP5: GLX1-2Gfp fTACAATTACAGTCGACATGGCTGCGGTTACGGTAGTAP6: sGLX1-2Gfp fTACAATTACAGTCGACATGGCTGCAGTGCGTTGCGTCTCGATTTGTTP8: GLX1-2Gfp fTACAATTACAGTCGACATGGCTTCCAGTCTGAGTTGTTP9: sGLX1-3Gfp fTACAATTACAGTCGACATGGCTTCCAGTTCTGATTTGTTP9: sGLX1-3Gfp fTACAATTACAGTCGACATGGCTTCATGTTGTTTATCGTGTP10: GLX1-3Gfp fTACAATTACAGTCGACATGGCAGCATCCTCAAGGCTP11: GLX1-3Gfp fCCCTTGCTCACCATGGCACATGCTAAGGCAAAGTCAAP12: GLX2-4Gfp fCCCTTGCTCACCATGGCACATGCCAAAGGTCAAP13: GLX2-4Gfp fCCCTTGCTCACCATGCCAAGCCATCCCAAAGTTP13: GLX2-4Gfp fCCCTTGCTCACCATGGCAACCATCCCAAAGGTTP14: GLX2-5Gfp fTACAATTACAGTCGACATGCAAACCATCCGAAAGCTTCP15: GLX2-5Gfp fCCCTTGCTCACCATGGCGAAATCATCCTTTGCTP16: qActin2 fCCCTTGCTCACCATGGCGAAAGCATGCTATGTCCP17: qActin2 rCCGTACGAGTGCTAACGCGAAGGAAP20: qGLX1-1 fATGGCGTCGGAAGCGAGGGAP20: qGLX1-2 fTGCGTGCTATGTGTGTGTGGCP21: qGLX1-3 fGAGGAGAGGCCTTGTTGTGTGTGAAP22: qGLX1-3 fGAGGAGAGGCCTTGTTAATCTGTAAP22: qGLX1-3 fGAGGAGAGGCCTTGTTAATCTGTAAP25: qGLX2-4 fCCGAGCCACACTAAGGCAGGAGGAGGCP26: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTTGAGGAC	Name	Sequences (5'-3')
P2: GstGLX2-4 rGGGAATTCGGGGATCCTAAGCTTTGAAATTGTCCTTTGP3: sGLX1-1Gfp fTACAATTACAGTCGACATGAGTTCGTACTCAATAGCAAGTP4: GLX1-1Gfp fTACAATTACAGTCGACATGGCGTCGGAAGCGAGGP5: GLX1-1Gfp rCCCTTGCTCACCATGGCAGCGTGCGGTTACGGTAGTAP6: sGLX1-2Gfp fTACAATTACAGTCGACATGACTGAGGCTTCGATTGTTP8: GLX1-2Gfp fTACAATTACAGTCGACATGGCTGAGGCTTCGATTGTTP9: sGLX1-3Gfp fTACAATTACAGTCGACATGGCTGAGGATCATTGTGTP9: sGLX1-3Gfp fTACAATTACAGTCGACATGGCTCCAGTTCCTAGGGTP10: GLX1-3Gfp fTACAATTACAGTCGACATGGCTGAGGATCATTCCTATGGCP11: GLX1-3Gfp rCCCTTGCTCACCATGGCCACAGTCTCCAAAGTTAP12: GLX2-4Gfp fTACAATTACAGTCGACATGCAAGCCATCTCCAAAGTTP13: GLX2-4Gfp fTACAATTACAGTCGACATGCAAACCATCCTGAAAGCTACP14: GLX2-5Gfp fTACAATTACAGTCGACATGCAAAACCATCCTGAAAGCTTCP15: GLX2-5Gfp fCCCTTGCTCACCATGGCGAAATCATCCTTTGCTTCCGP16: qActin2 fTCCAGATGCCCAGAGCGAGGGAP19: qGLX1-1 fATGGCGTCGGAAAGCAAGCAAP20: qGLX1-2 fTGTCGTGCTAGTGTGTGTGCP21: qGLX1-2 fGAGGAGAGGCCTTGTTGTGGGAAAP22: qGLX1-3 fGAGGAGAGGCCTTGTTGTGTGTGGP22: qGLX1-3 fGAGGAGAGGCCTTGTTGTGGAAP22: qGLX1-3 fGAGGAAGGGCTTGTTAATCTGTAAP23: qGLX1-3 rGACGCAACATAGGCTTTAATCTGTAAP25: qGLX2-4 fCCAGGGATCCTGGTTTAATCTGTAAP25: qGLX2-4 fGCATCCTGGTTTAATCTGTAAP26: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTTGAGGAC	P1: GstGLX2-4 f	GGTTCCGCGTGGATCCATGCAAATTGAACTGGTGCCATGTC
P3: sGLX1-1Gfp fTACAATTACAGTCGACATGAGTTCGTACTCAATAGCAAGTP4: GLX1-1Gfp fTACAATTACAGTCGACATGGCGCGCGGAAGCGAGGP5: GLX1-1Gfp rCCCTTGCTCACCATGGCAGCTGCGTTTACGGTAGTAP6: sGLX1-2Gfp fTACAATTACAGTCGACATGACATGAGATTGCTTCCGCCTCP7: GLX1-2Gfp fTACAATTACAGTCGACATGGCTGCAGGCTTCTGATTTGTTP8: SGLX1-2Gfp rCCCTTGCTCACCATGGCTTCCAGTTCTTATCGTGTP9: sGLX1-3Gfp fTACAATTACAGTCGACATGGTGAGGATCATTCCTATGGCP10: GLX1-3Gfp fTACAATTACAGTCGACATGGTGAGGATCATTCCTATGGCP11: GLX1-3Gfp rCCCTTGCTCACCATGGCCACCAGTGCTAGAGAAAGTCAAP12: GLX2-4Gfp fTACAATTACAGTCGACATGCAAAGCCATCTCCAAAGTTP13: GLX2-4Gfp rCCCTTGCTCACCATGGCAGATTGCAAAGCTTCP14: GLX2-5Gfp fTACAATTACAGTCGACATGCAAACCATCTCGAAAGCTTCP15: GLX2-5Gfp fTACAATTACAGTCGACATGCAAACCATCTGGAAAGCTTCP16: qActin2 fTCCGTTGCTCACCATGGCGAAATCATCCTTTGCTTCCP17: qActin2 rCCGTGCGTACGGAAGCGAGGGAP19: qGLX1-1 fATGGCGTCGGAAGCGAGGGAAP20: qGLX1-2 fTGTCGTGCTATGTGTGTGTGCP21: qGLX1-2 fGAGGAGAGGCCTTGTTGTGAAP22: qGLX1-3 fGAGGAGAGGCCTTGTTGTGGAAP22: qGLX1-3 rGACGCAACATAGGCTTTATACTGP24: qGLX2-4 fGCGATCCTGGTTTAATCTGTAAP25: qGLX2-4 rCGAGGGTTCATCAAAGTAGAAACP26: qGLX2-5 rGATCCCAGAGCCGCGAGATGP27: qGLX2-5 rGATCCACATCATTGAGAC	P2: GstGLX2-4 r	GGGAATTCGGGGATCCTAAGCTTTGAAATTGTCCTTTG
P4: GLX1-1Gfp fTACAATTACAGTCGACATGGCGTCGGAAGCGAGGP5: GLX1-1Gfp rCCCTTGCTCACCATGGCAGCTGCGTTTACGGTAGTAP6: sGLX1-2Gfp fTACAATTACAGTCGACATGACTGAGGCTTCCGACTTGTTTGT	P3: sGLX1-1Gfp f	TACAATTACAGTCGACATGAGTTCGTACTCAATAGCAAGT
P5: GLX1-1Gfp rCCCTTGCTCACCATGGCAGCTGCGTTTACGGTAGTAP6: sGLX1-2Gfp fTACAATTACAGTCGACATGAATGAGATTGCTTCCGCCTCP7: GLX1-2Gfp fTACAATTACAGTCGACATGGCTGAGGCTTCGATTTGTTP8: GLX1-3Gfp fTACAATTACAGTCGACATGGCTCCAGTTCCTTGAGAAAATCTTP9: sGLX1-3Gfp fTACAATTACAGTCGACATGGTGAGGATCATTCCTATGGCP10: GLX1-3Gfp fTACAATTACAGTCGACATGGCAGGGATCATTCCTATGGCP11: GLX1-3Gfp rCCCTTGCTCACCATGGCACATGCAAGCCATTCCAAAGTTAP12: GLX2-4Gfp fTACAATTACAGTCGACATGCAAAGCCATCTCCAAAGTTP13: GLX2-4Gfp rCCCTTGCTCACCATGGCACATGCAAACCATCTGGAAAGCTCCP14: GLX2-5Gfp fTACAATTACAGTCGACATGCAAACCATCTGGAAAGCTTCP15: GLX2-5Gfp rCCCTTGCTCACCATGGCGAAATCATCCTTTGCTTTCCP16: qActin2 fTCAGATGCCCAGAAGTCCTTGCTGATATCCP19: qGLX1-1 fATGGCGTCGGAAGCGAGGGAP20: qGLX1-2 fTGTCGTGCTATGTGTGTGTGGCP21: qGLX1-3 fGAGGAGAGCCTTGTTGTGAAP22: qGLX1-3 fGACGCAACATAGGCTTAACCGCAAAP23: qGLX2-4 fCCGAGGGTTCATCAAGTAGAAACP26: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTTGAGGAC	P4: GLX1-1Gfp f	TACAATTACAGTCGACATGGCGTCGGAAGCGAGG
P6: sGLX1-2Gfp fTACAATTACAGTCGACATGAATGAGATTGCTTCCGCCTCP7: GLX1-2Gfp fTACAATTACAGTCGACATGGCTGAGGCTTCGATTTGTTP8: GLX1-2Gfp rCCCTTGCTCACCATGGCTTCCAGTTCCTTGAGAAAATCTTP9: sGLX1-3Gfp fTACAATTACAGTCGACATGGTGAGGATCATTCCTATGGCP10: GLX1-3Gfp rCCCTTGCTCACCATGGCCTCCAGTTCTTGAGAAAAGTCAAP12: GLX2-4Gfp fTACAATTACAGTCGACATGCAAGCCATCTCCAAGGTTP13: GLX2-4Gfp rCCCTTGCTCACCATGGCGACATGCAAACCATCTCGAAAGCTTCP14: GLX2-5Gfp fTACAATTACAGTCGACATGCAAACCATCTCGAAAGCTTCP15: GLX2-5Gfp rCCCTTGCTCACCATGGCGAAGTCTTGTTCCP16: qActin2 fTCCAGATGCCCAGAAGTCTTGTTCCP17: qActin2 rCCCGTACGACAGTGCAAGCGAGGAP19: qGLX1-1 fATGGCGTCGGAAGCGAGGGAP20: qGLX1-2 fTGTCGTGCTATGTGTGTGGCP21: qGLX1-2 fGAGGAGAGGCCTTGTTAACGGCAAAP22: qGLX1-3 fGAGGAGAGGCCTTGTTAACTGTAAP22: qGLX1-3 fGACGCAACATAGGCTTTAATCTGTAAP25: qGLX2-4 fCCAGGGGTTCATCAAGTAGAAACP26: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTTGAGGAC	P5: GLX1-1Gfp r	CCCTTGCTCACCATGGCAGCTGCGTTTACGGTAGTA
P7: GLX1-2Gfp fTACAATTACAGTCGACATGGCTGAGGCTTCTGATTTGTTP8: GLX1-2Gfp rCCCTTGCTCACCATGGCTTCCAGTTCTTGAGAAAATCTTP9: sGLX1-3Gfp fTACAATTACAGTCGACATGCTTCATGTTGTTTATCGTGTP10: GLX1-3Gfp rCCCTTGCTCACCATGGCCTCCAGTTCTTGAGAAAGTCAAP12: GLX2-4Gfp fTACAATTACAGTCGACATGCAAGCCATCCCAAAGTTP13: GLX2-4Gfp fCCCTTGCTCACCATGGCAGCATCCCAAAGCTTCP14: GLX2-5Gfp fTACAATTACAGTCGACATGCAAACCATCTCGAAAGCTTCP15: GLX2-5Gfp fCCCTTGCTCACCATGGCGAAATCATCCTTTGCTTTCCGP16: qActin2 fCCCTTGCTCACCATGGCGAAAGTCTTGTTCCP19: qGLX1-1 fCCAGCACACGTGGAAAGCCAACCAP20: qGLX1-2 fTGCCGTGCTCACCATGGCGAAAGTCAP21: qGLX1-3 fGAAGGAGAGGCCTTGTGTGGCP21: qGLX1-3 rGAACGCAACATAGGCTTTAAACTGP22: qGLX1-3 rGACGCAACATAGGCTTTAATCTGTAAP25: qGLX2-4 rCCAGGGGTTCATCAAAGTGAAACCP26: qGLX2-5 rGATGCACACTCATTTGAGAACCP27: qGLX2-5 rGATGCACACTCATTTGAGGAC	P6: sGLX1-2Gfp f	TACAATTACAGTCGACATGAATGAGATTGCTTCCGCCTC
P8: GLX1-2Gfp rCCCTTGCTCACCATGGCTTCCAGTTCCTTGAGAAAATCTTP9: sGLX1-3Gfp fTACAATTACAGTCGACATGCTTCATGTGTTTATCGTGTP10: GLX1-3Gfp fTACAATTACAGTCGACATGGTGAGGATCATTCCTATGGCP11: GLX1-3Gfp rCCCTTGCTCACCATGGCCTCCAGTTCTTGAGAAAGTCAAP12: GLX2-4Gfp fTACAATTACAGTCGACATGCAAGCCATCTCCAAAGTTP13: GLX2-4Gfp rCCCTTGCTCACCATGGCAGCTTTGAAATTGTCCTTGCTCTP14: GLX2-5Gfp fTACAATTACAGTCGACATGCAAACCATCTCGAAAGCTTCP15: GLX2-5Gfp rCCCTTGCTCACCATGGCGAAATCATCCTTTGCTTTCCGP17: qActin2 rCCGTACAGATCCTTCCTGATATCCP18: qGLX1-1 fATGGCGTCGGAAGCGAGGGAP20: qGLX1-2 fTGTCGTGCTATGTGTGTGCP21: qGLX1-2 fGAGGAGAGGCCTTGTTGTGGCP22: qGLX1-3 fGAGGAGAGGCCTTGTTGTGGAAP23: qGLX1-3 rGACGCAACATAGGCTTTATACTGP24: qGLX2-4 fCCGAGGGTCATAGGTGTAAAACCP25: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTTGAGGAC	P7: GLX1-2Gfp f	TACAATTACAGTCGACATGGCTGAGGCTTCTGATTTGTT
P9: sGLX1-3Gfp fTACAATTACAGTCGACATGCTTCATGTTGTTATCGTGTP10: GLX1-3Gfp fTACAATTACAGTCGACATGGTGAGGATCATTCCTATGGCP11: GLX1-3Gfp rCCCTTGCTCACCATGGCCTCCAGTTCTTGAGAAAGTCAAP12: GLX2-4Gfp fTACAATTACAGTCGACATGCAAGCCATCTCCAAAGTTP13: GLX2-4Gfp rCCCTTGCTCACCATGGCAGCTTTGAAATTGTCCTTTGCTCTP14: GLX2-5Gfp fTACAATTACAGTCGACATGCAAACCATCTCGAAAGCTTCP15: GLX2-5Gfp rCCCTTGCTCACCATGGCGAAATCATCCTTTGCTTTCCGP16: qActin2 fTCAGATGCCCAGAATCCTTCTGATATCCP18: qGLX1-1 fATGGCGTCGGAAGCGAGGGAP20: qGLX1-2 fTGCGTGCTATGTGTGTGTGGTGGCP21: qGLX1-2 rAAACCGTCCATAACCGCAAAP22: qGLX1-3 fGAGGAGAGGCCTTGTTGTGAAP23: qGLX1-3 rGACGCAACATAGGCTTTATACTGP24: qGLX2-4 fGCGATCCTGGTTAATCTGTAAP25: qGLX2-5 fCATTCCAGAGGCCGCAGATGP26: qGLX2-5 rGGTGCACACTCATTGAGGAC	P8: GLX1-2Gfp r	CCCTTGCTCACCATGGCTTCCAGTTCCTTGAGAAAATCTT
P10: GLX1-3Gfp fTACAATTACAGTCGACATGGTGAGGATCATTCCTATGGCP11: GLX1-3Gfp rCCCTTGCTCACCATGGCCTCCAGTTCTTGAGAAAGTCAAP12: GLX2-4Gfp fTACAATTACAGTCGACATGCAAGCCATCTCCAAAGTTP13: GLX2-4Gfp rCCCTTGCTCACCATGGCAGCTTTGAAATTGTCCTTTGCTCTP14: GLX2-5Gfp fTACAATTACAGTCGACATGCAAACCATCTCGAAAGCTTCP15: GLX2-5Gfp rCCCTTGCTCACCATGGCGAAATCATCCTTTGCTTCCGP16: qActin2 fTCAGATGCCCAGAAGCCTTCCTGATATCCP18: qGLX1-1 fATGGCGTCGGAAGCGAGGGAP19: qGLX1-2 fTCCCAGCACACGTGAGTAAAAGTCAP20: qGLX1-2 fTGTCGTGCTATGTGTGTGGCP21: qGLX1-2 fGAGGAGAGGCCTTGTTGTGAAP22: qGLX1-3 fGAGGAGAGGCCTTGTTGTGAAP23: qGLX1-3 rGACGCAACATAGGCTTTATACTGP24: qGLX2-4 fGCGATCCTGGTTTAATCTGTAAP25: qGLX2-4 rCGAGGGTTCATCAAAGTAGAAACP26: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTTGAGGAC	P9: sGLX1-3Gfp f	TACAATTACAGTCGACATGCTTCATGTTGTTTATCGTGT
P11: GLX1-3Gfp rCCCTTGCTCACCATGGCCTCCAGTTCTTGAGAAAGTCAAP12: GLX2-4Gfp fTACAATTACAGTCGACATGCAAGCCATCCCAAAGTTP13: GLX2-4Gfp rCCCTTGCTCACCATGGCAGCTTTGAAATTGTCCTTTGCTCTP14: GLX2-5Gfp fTACAATTACAGTCGACATGCAAACCATCTCGAAAGCTTCP15: GLX2-5Gfp rCCCTTGCTCACCATGGCGAAATCATCCTTTGCTTTCCGP16: qActin2 fTCAGATGCCCAGAAGTCTTGTTCCP18: qGLX1-1 fATGGCGTCGGAAGCGAGGGAP19: qGLX1-2 fTGTCGTGCTATGTGTGTGTGCP20: qGLX1-2 fTGTCGTGCTATGTGTGTGTGCP21: qGLX1-2 rAAACCGTCCATAACCGCAAAP22: qGLX1-3 fGAGGAGAGGCCTTGTTATACTGP24: qGLX2-4 fGCGATCCTGGTTTAATCTGTAAP25: qGLX2-4 rCGAGGGTTCATCAAAGTAGAAACP26: qGLX2-5 rGGTGCACACTCATTTGAGGAC	P10: GLX1-3Gfp f	TACAATTACAGTCGACATGGTGAGGATCATTCCTATGGC
P12: GLX2-4Gfp fTACAATTACAGTCGACATGCAAGCCATCTCCAAAGTTP13: GLX2-4Gfp rCCCTTGCTCACCATGGCAGCTTTGAAATTGTCCTTTGCTCTP14: GLX2-5Gfp fTACAATTACAGTCGACATGCAAACCATCTCGAAAGCTTCP15: GLX2-5Gfp rCCCTTGCTCACCATGGCGAAATCATCCTTTGCTTTCCGP16: qActin2 fTCAGATGCCCAGAAGTCTTGTTCCP17: qActin2 rCCGTACAGATCCTTCCTGATATCCP18: qGLX1-1 fATGGCGTCGGAAGCGAGGGAP20: qGLX1-2 fTGTCGTGCTATGTGTGTGCP21: qGLX1-2 rAAACCGTCCATAACCGCAAAP22: qGLX1-3 fGAGGAGAGGCCTTGTTGTGAAP23: qGLX1-3 rGACGCAACATAGGCTTTAACTGP24: qGLX2-4 fCCGAGGGTTCATCAAGTAGAAACP26: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTTGAGGAC	P11: GLX1-3Gfp r	CCCTTGCTCACCATGGCCTCCAGTTCTTGAGAAAGTCAA
P13: GLX2-4Gfp rCCCTTGCTCACCATGGCAGCTTTGAAATTGTCCTTTGCTCTP14: GLX2-5Gfp fTACAATTACAGTCGACATGCAAACCATCTCGAAAGCTTCP15: GLX2-5Gfp rCCCTTGCTCACCATGGCGAAATCATCCTTTGCTTTCCGP16: qActin2 fTCAGATGCCCAGAAGTCTTGTTCCP17: qActin2 rCCGTACAGATCCTTCCTGATATCCP18: qGLX1-1 fATGGCGTCGGAAGCGAGGGAP19: qGLX1-2 fTGTCGTGCTATGTGTGTGCP20: qGLX1-2 fTGTCGTGCTATGTGTGTGCP21: qGLX1-2 rAAACCGTCCATAACCGCAAAP22: qGLX1-3 fGAGGAGAGGCCTTGTTGTGAAP23: qGLX1-3 rGACGCAACATAGGCTTTATACTGP24: qGLX2-4 fCCAGGGTTCATCAAAGTAGAAACP25: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTGAGGAC	P12: GLX2-4Gfp f	TACAATTACAGTCGACATGCAAGCCATCTCCAAAGTT
P14: GLX2-5Gfp fTACAATTACAGTCGACATGCAAACCATCTCGAAAGCTTCP15: GLX2-5Gfp rCCCTTGCTCACCATGGCGAAATCATCCTTGCTTCCGP16: qActin2 fTCAGATGCCCAGAAGTCTTGTTCCP17: qActin2 rCCGTACAGATCCTTCCTGATATCCP18: qGLX1-1 fATGGCGTCGGAAGCGAGGGAP19: qGLX1-2 fTGTCGTGCTATGTGTGTGCP20: qGLX1-2 fTGTCGTGCTATGTGTGTGCP21: qGLX1-3 fGAGGAGAGGCCTTGTTGTGAAP23: qGLX1-3 rGACGCAACATAGGCTTTATACTGP24: qGLX2-4 fGCGATCCTGGTTTAATCTGTAAP25: qGLX2-4 rCGAGGGTTCATCAAGTAGAAACP26: qGLX2-5 rGATTCCAGAGGCCGCAGATG	P13: GLX2-4Gfp r	CCCTTGCTCACCATGGCAGCTTTGAAATTGTCCTTTGCTCT
P15: GLX2-5Gfp rCCCTTGCTCACCATGGCGAAATCATCCTTTGCTTTCCGP16: qActin2 fTCAGATGCCCAGAAGTCTTGTTCCP17: qActin2 rCCGTACAGATCCTTCCTGATATCCP18: qGLX1-1 fATGGCGTCGGAAGCGAGGGAP19: qGLX1-2 fTGTCGTGCTATGTGTGTGCP20: qGLX1-2 fGAGGAGAGGCCTTGTTGTGAAP22: qGLX1-3 fGAGGAGAGGCCTTGTTGTGAAP23: qGLX1-3 rGACGCAACATAGGCTTTTATACTGP24: qGLX2-4 fGCGATCCTGGTTTAATCTGTAAP25: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTTGAGGAC	P14: GLX2-5Gfp f	TACAATTACAGTCGACATGCAAACCATCTCGAAAGCTTC
P16: qActin2 fTCAGATGCCCAGAAGTCTTGTTCCP17: qActin2 rCCGTACAGATCCTTCCTGATATCCP18: qGLX1-1 fATGGCGTCGGAAGCGAGGGAP19: qGLX1-2 fCCAGCACACGTGAGTAAAAGTCAP20: qGLX1-2 fTGTCGTGCTATGTGTGTGCP21: qGLX1-3 fGAGGAGAGGCCTTGTTGTGAAP23: qGLX1-3 rGACGCAACATAGGCTTTATACTGP24: qGLX2-4 fGCGATCCTGGTTTAATCTGTAAP25: qGLX2-4 rCGAGGGTCCAGAGAGAAACP26: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTTGAGGAC	P15: GLX2-5Gfp r	CCCTTGCTCACCATGGCGAAATCATCCTTTGCTTTCCG
P17: qActin2 rCCGTACAGATCCTTCCTGATATCCP18: qGLX1-1 fATGGCGTCGGAAGCGAGGGAP19: qGLX1-1 rCCAGCACACGTGAGTAAAAGTCAP20: qGLX1-2 fTGTCGTGCTATGTGTGTGCP21: qGLX1-2 rAAACCGTCCATAACCGCAAAP22: qGLX1-3 fGAGGAGAGGCCTTGTTGTGAAP23: qGLX1-3 rGACGCAACATAGGCTTTATACTGP24: qGLX2-4 fGCGATCCTGGTTTAATCTGTAAP25: qGLX2-4 rCGAGGGTTCATCAAAGTAGAAACP26: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTTGAGGAC	P16: qActin2 f	TCAGATGCCCAGAAGTCTTGTTCC
P18: qGLX1-1 fATGGCGTCGGAAGCGAGGGAP19: qGLX1-1 rCCAGCACACGTGAGTAAAAGTCAP20: qGLX1-2 fTGTCGTGCTATGTGTGTGCP21: qGLX1-2 rAAACCGTCCATAACCGCAAAP22: qGLX1-3 fGAGGAGAGGCCTTGTTGTGAAP23: qGLX1-3 rGACGCAACATAGGCTTTTATACTGP24: qGLX2-4 fGCGATCCTGGTTTAATCTGTAAP25: qGLX2-4 rCGAGGGTTCATCAAAGTAGAAACP26: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTTGAGGAC	P17: qActin2 r	CCGTACAGATCCTTCCTGATATCC
P19: qGLX1-1 rCCAGCACACGTGAGTAAAAGTCAP20: qGLX1-2 fTGTCGTGCTATGTGTGTGCP21: qGLX1-2 rAAACCGTCCATAACCGCAAAP22: qGLX1-3 fGAGGAGAGGCCTTGTTGTGAAP23: qGLX1-3 rGACGCAACATAGGCTTTTATACTGP24: qGLX2-4 fGCGATCCTGGTTTAATCTGTAAP25: qGLX2-4 rCGAGGGTTCATCAAAGTAGAAACP26: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTTGAGGAC	P18: qGLX1-1 f	ATGGCGTCGGAAGCGAGGGA
P20: qGLX1-2 fTGTCGTGCTATGTGTGTGCP21: qGLX1-2 rAAACCGTCCATAACCGCAAAP22: qGLX1-3 fGAGGAGAGGCCTTGTTGTGAAP23: qGLX1-3 rGACGCAACATAGGCTTTTATACTGP24: qGLX2-4 fGCGATCCTGGTTTAATCTGTAAP25: qGLX2-4 rCGAGGGTTCATCAAAGTAGAAACP26: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTTGAGGAC	P19: qGLX1-1 r	CCAGCACACGTGAGTAAAAGTCA
P21: qGLX1-2 rAAACCGTCCATAACCGCAAAP22: qGLX1-3 fGAGGAGAGGCCTTGTTGTGAAP23: qGLX1-3 rGACGCAACATAGGCTTTTATACTGP24: qGLX2-4 fGCGATCCTGGTTTAATCTGTAAP25: qGLX2-4 rCGAGGGTTCATCAAAGTAGAAACP26: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTTGAGGAC	P20: qGLX1-2 f	TGTCGTGCTATGTGTGTGC
P22: qGLX1-3 fGAGGAGAGGCCTTGTTGTGAAP23: qGLX1-3 rGACGCAACATAGGCTTTTATACTGP24: qGLX2-4 fGCGATCCTGGTTTAATCTGTAAP25: qGLX2-4 rCGAGGGTTCATCAAAGTAGAAACP26: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTTGAGGAC	P21: qGLX1-2 r	AAACCGTCCATAACCGCAAA
P23: qGLX1-3 rGACGCAACATAGGCTTTTATACTGP24: qGLX2-4 fGCGATCCTGGTTTAATCTGTAAP25: qGLX2-4 rCGAGGGTTCATCAAAGTAGAAACP26: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTTGAGGAC	P22: qGLX1-3 f	GAGGAGAGGCCTTGTTGTGAA
P24: qGLX2-4 fGCGATCCTGGTTTAATCTGTAAP25: qGLX2-4 rCGAGGGTTCATCAAAGTAGAAACP26: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTTGAGGAC	P23: qGLX1-3 r	GACGCAACATAGGCTTTTATACTG
P25: qGLX2-4 rCGAGGGTTCATCAAAGTAGAAACP26: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTTGAGGAC	P24: qGLX2-4 f	GCGATCCTGGTTTAATCTGTAA
P26: qGLX2-5 fCATTCCAGAGGCCGCAGATGP27: qGLX2-5 rGGTGCACACTCATTTGAGGAC	P25: qGLX2-4 r	CGAGGGTTCATCAAAGTAGAAAC
P27: qGLX2-5 r GGTGCACACTCATTTGAGGAC	P26: qGLX2-5 f	CATTCCAGAGGCCGCAGATG
	P27: qGLX2-5 r	GGTGCACACTCATTTGAGGAC

	K <sub>m</sub> (mM)	$k_{\rm cat}$ (min <sup>-1</sup> )	$k_{\text{cat}}/K_{\text{m}} \text{ (mM}^{-1} \text{ min}^{-1}\text{)}$
S6803GLX1			
*In the absence of Ni <sup>2+</sup>	0.39 ± 0.10	350 ± 30	910
In the presence of Ni <sup>2+</sup>	0.018 ± 0.001	10500 ± 1500	590000

Supplemental Table S2. Kinetic parameter of the recombinant S6803GLX1

For the measurement of the recombinant S6803GLX1 protein (0.05  $\mu$ g mL<sup>-1</sup>), hemithioacetal was used as the substrate. Data are means ± SD for three independent experiments. \*Shimakawa et al. (2013).

#### GLX1-3

GLX1-3_1	MASEARESPA
GLX1-3_2	MASEARESPA
GLX1-3_3	MASEARESPA
GLX1-3_4	MSSYSIASAISRISPLIRFVKPYSTGFSFITCACNSTRRPKRFDQLCVFSMASEARESPA
	*******
GLX1-3_1	NNPGLSTNRDEATKGYIMQQTMFRIKDPKASLDFYSRVLGMSLLKRLDFSEMKFSLYFLG
GLX1-3_2	NNPGLSTNRDEATKGYIMQQTMFRIKDPKASLDFYSRVLGMSLLKRLDFSEMKFSLYFLG
GLX1-3_3	NNPGLSTNRDEATKGYIMQQTMFRIKDPKASLDFYSRVLGMSLLKRLDFSEMKFSLYFLG
GLX1-3_4	NNPGLSTNRDEATKGYIMQQTMFRIKDPKASLDFYSRVLGMSLLKRLDFSEMKFSLYFLG
	***************************************
GLX1-3_1	${\tt YEDTTTAPTDPTERTVWTFGQPATIELTHNWGTESDPEFKGYHNGNSEPRGFGHIGVTVD$
GLX1-3_2	YEDTTTAPTDPTERTVWTFGQPATIELTHNWGTESDPEFKGYHNGNSEPRGFGHIGVTVD
GLX1-3_3	YEDTTTAPTDPTERTVWTFGQPATIELTHNWGTESDPEFKGYHNGNSEPRGFGHIGVTVD
GLX1-3_4	${\tt YEDTTTAPTDPTERTVWTFGQPATIELTHNWGTESDPEFKGYHNGNSEPRGFGHIGVTVD$
	***************************************
GLX1-3_1	DVHKACERFEELGVEFAKKPNDGKMKNIAFIKDPDGYWIEIFDLKTIGTTTVNAA
GLX1-3_2	DVHKACERFEELGVEFAKKPNDGKMKNIAFIKDPDGYWIEIFDLKTIGTTTVNAA
GLX1-3_3	DVHKACERFEELGVEFAKKPNDGKMKNIAFIKDPDGYWIEIFDLKTIGTTTVNAA
GLX1-3 4	DVHKACERFEELGVEFAKKPNDGKMKNIAFIKDPDGYWIEIFDLKTIGTTTVNAA
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GLX1-2	
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GLX1-2 1	MAEASDLLEWPKKDNRRFLH
GLX1-22	MAEASDLLEWPKKDNRRFLH
GLX1-2 3	MAEASDLLEWPKKDNRRFLHV
GLX1-24	MAEASDLLEWPKKDNRRFLH
GLX1-25	MAEASDLLEWPKKDNRRFLHV
GLX1-2 6	MNEIASASMLRLCQCFISICNVHFVSMRAAESSFLLSRNMAEASDLLEWPKKDNRRFLHV
-	**********
GT Y1 0 1	
GLAI-2_I	VIRVGDLDRTIEFITEVFGMKLLRKRDIPEERISNAFLGFGPETSNFVVELTINIGVSS:
GLX1-2_2	VYRVGDLDRTIEFYTEVFGMRLLRRRDIPEEKYSNAFLGFGPETSNFVVELTYNYGVSS:
GLX1-2_3	VYRVGDLDRTIEFYTEVFGMKLLRKRDIPEEKYSNAFLGFGPETSNFVVELTYNYGVSS
GLX1-2_4	VYRVGDLDRTIEFYTEVFGMKLLRKRDIPEEKYSNAFLGFGPETSNFVVELTYNYGVSS
GLX1-2_5	VYRVGDLDRTIEFYTEVFGMKLLRKRDIPEEKYSNAFLGFGPETSNFVVELTYNYGVSS
GLX1-2_6	VYRVGDLDRTIEFYTEVFGMKLLRKRDIPEEKYSNAFLGFGPETSNFVVELTYNYGVSS
	***************************************
GLX1-2_1	DIGTGFGHFAISTQDVSKLVENVRAKGGNVTREPGPVKGGGSVIAFVKDPDGYTFELIQ
GLX1-22	DIGTGFGHFAISTQDVSKLVENVRAKGGNVTREPGPVKGGGSVIAFVKDPDGYTFELIQ
GLX1-23	DIGTGFGHFAISTQDVSKLVENVRAKGGNVTREPGPVKGGGSVIAFVKDPDGYTFELIQ
GLX1-2 4	DIGTGFGHFAISTQDVSKLVENVRAKGGNVTREPGPVKGGGSVIAFVKDPDGYTFELIQ
GLX1-2 5	DIGTGFGHFAISTQDVSKLVENVRAKGGNVTREPGPVKGGGSVIAFVKDPDGYTFELIQ
GLX1-2 6	DIGTGFGHFAISTODVSKLVENVRAKGGNVTREPGPVKGGGSVIAFVKDPDGYTFELIO
-	***********
CLV1-2 1	
GLX1-2_1 GLX1-2_2	GPTPEPPCQVMLKVGDLDKATKFTEKALGMKLLKKTEKPETKTTIGMGTALETESTVI GDTDEDECOUMT DUCDI DDATKEVEKAT CMDTI DKTEDDEVKVTTCMMCVAEPVECTUT
GLA1-2_2	GPTPEPCQVMLKVGDLDKATKTISKALGMKLLKKTEKPETKTTIGMMGTALETESTVE CDMDEDECOMMTDWCDIDDATKEVEVATCMDTTDKTEDDEVVVMTCMMCVAPEVECTUTT
GLX1-2_3	GPTPEPPCQVMLRVGDLDRATRTERALGMRDLRATERPETRTTIGMMGTALETESTVL GPTPEPCQVMLRVGDLDRATRTTERALGMRDLRATERPETRTTIGMMGTALETESTVL
CIV1-2 5	CONDEDECCIONI DUCCI DE LEVERAL CHELLER ENTERDEVENTI CHACTAREVECTUL
GLA1-2_5	GPTPEPCQVMLKVGDLDKATKFISKALGMKLLKKTEKPETKTTIGMGTALETESTVE
GLAI-2_0	**************************************
GLX1-2_1	LTYNYDVTEYTKGNAYAQIAIGTDDVYKSGEVIKIVNQELGGKITREAGPLPGLGTKIVS
GLX1-2_2	LTYNYDVTEYTKGNAYAQIAIGTDDVYKSGEVIKIVNQELGGKITREAGPLPGLGTKIV
GLX1-2_3	LTYNYDVTEYTKGNAYAQIAIGTDDVYKSGEVIKIVNQELGGKITREAGPLPGLGTKIV
GLX1-2_4	LTYNYDVTEYTKGNAYAQIAIGTDDVYKSGEVIKIVNQELGGKITREAGPLPGLGTKIVS
GLX1-2_5	LTYNYDVTEYTKGNAYAQAQMMCTKAVKLLR
GLX1-2_6	LTYNYDVTEYTKGNAYAQIAIGTDDVYKSGEVIKIVNQELGGKITREAGPLPGLGTKIV
	***************************************
GLX1-2 1	FLDPDGWKTVLVDNKDFLKELE
GLX1-2 2	FLDPDGWKTVLVDNKDFLKELE
GLX1-2 3	FLDPDGWKTVLVDNKDFLKELE
GLX1-2 4	FLDPDGWKTVLVDNKDFLKELE
GLX1-2 5	
GLX1-2 6	FLDPDGWKTVLVDNKDFLKELE
·•	

GLX1-1

GLX1-1_1	MVRIIPMAASSIRPSLACFSDSPRFPISLLSRNLSRTLHVPQSQLFGLTSHKLLRRSVNC
GLX1-1_2	
GLX1-1 1	LGVARSGKAAOATTODDLLTWVKNDKRRMLHVVYRVGDMDRTIKFYTECLGMKLLRKRDT
GLX1-1_2	MI.HVVYRVGDMDRTIKFYTECI.GMKI.LBKBDI
	********************
GLX1-1 1	PEEKYTNAFLGYGPEDSHFVIELTYNYGVDKYDIGAGFGHFGIAVDDVAKTVELVKAKGG
GLX1-1 2	PEEKYTNAFLGYGPEDSHFVIELTYNYGVDKYDIGAGFGHFGIAVDDVAKTVELVKAKGG
-	***************************************
$GLX1-1_1$	KVSREPGPVKGGKTVIAFIEDPDGYKFELLERGPTPEPLCQVMLRVGDLDRAIKFYEKAF
GLX1-1_2	KVSREPGPVKGGKTVIAFIEDPDGYKFELLERGPTPEPLCQVMLRVGDLDRAIKFYEKAF
	***************************************
GLX1-1 1	GMELLRTRDNPEYKYTIAMMGYGPEDKFPVLELTYNYGVTEYDKGNAYAOIAIGTDDVYK
GLX1-1 2	GMELLRTRDNPEYKYTIAMMGYGPEDKFPVLELTYNYGVTEYDKGNAYAOIAIGTDDVYK
	******
GLX1-1_1	TAEAIKLFGGKITREPGPLPGISTKITACLDPDGWKSVFVDNIDFLKELE
GLX1-1_2	TAEAIKLFGGKITREPGPLPGISTKITACLDPDGWKSVFVDNIDFLKELE
	*************

Supplemental Fig. S1. Amino acid sequences for each AtGLX1 splice variant.



**Supplemental Fig. S2.** Subcellular localisation of GLX1w/oS–GFP fusion proteins expressed in *A. thaliana* mesophyll protoplasts. Fluorescence images of GFP ( $F_{GFP}$ ), chlorophyll autofluorescence ( $F_{Chl}$ ), and the bright field (BF) are shown. Black bars = 10 µm.

#### Specific regions to Zn-type GLX1

S6803GLX1	LLHTMIRVGDLDKSLQFYCDILGMNLLRKKDYPSGEFTLAFVGYGK	ESENAVIELTHNWGTDK
EcGLX1	LLHTMLRVGDLQRSIDFYTKVLGMKLLRTSENPEYK <mark>Y</mark> SLAFVGYGP	ETEEAVIELTY <mark>N</mark> WGVDK
OsGLX1-1	LLHVVYRVGDIDRTIKFYTECLGMKLLRKRDIPEEK <mark>Y</mark> TNAFLGYGA	EDNHFVVELTYNYGVDK
ZmGLX1-1	LLHVVYRVGDLDKTIKFYTECLGMKLLRKRDIPEEKYSNAFLGYGP	EESHFVVELTYNYGVDK
AtGLX1-1	MLHVVYRVGDMDRTIKFYTECLGMKLLRKRDIPEEKYTNAFLGYGP	EDSHFVIELTY <mark>N</mark> YGVDK
MpGLX1-1	MLHVVYRVGDLDKTIKFYTECLGMKLLRKRDFPDEKYTNAFLGYGP	EDSHFVVELTYNYGVFS
SmGLX1-1	MLHVVYRVGDLDKTIKFYTECLGMKLLRKRDIPEERYTNAFLGYGP	EDSHFVVELTY <mark>N</mark> YGVDK
OsGLX1-3	MLHVVYRVGDLDKTIKFYTECLGMKLLRKRDIPEERYTNAFLGYGP	EDSHFVVELTY <mark>N</mark> YGVES
ZmGLX1-4	LLHVVYRVGDLDKTIKFYTECLGMKLLRKRDIPEERYTNAFLGYGP	EDSHFVVELTY <mark>N</mark> YGVES
CvGLX1-1	MLHAVYRVGDMDATIKYYQDCFGMKLLRFRDIKEEK <mark>Y</mark> SNAFLGYGP	EETHFAMELTY <mark>N</mark> YGVDS
OsGLX1-2	LLHAVYRVGDLDRTIKCYTECFGMKLLRKRDVPEEKYTNAFLGFGP	EDTNFALELTY <mark>N</mark> YGVDK
ZmGLX1-3	MLHAVYRVGDLDRTIKYYTECFGMKLLRKRDVPDEKYTNAFLGFGP	ENTNFAVELTY <mark>N</mark> YGVDK
ZmGLX1-2	MLHAVYRVGDLDRTIKYAHHLQLRVMLFVHYTECFGMKLLRKRDIPEEKYTNAFLGFGP	EDTNFAVELTY <mark>N</mark> YGVDK
AtGLX1-2	FL <mark>H</mark> VVY <mark>R</mark> VGDLDRTIEFYTEVFGMKLLRKRDIPEEK <mark>Y</mark> SNAFLGFGP	ETSNFVV <mark>E</mark> LTY <mark>N</mark> YGVSS
MpGLX1-2	IQQTMYRIKDPKVSLDFYTRILGMTLLKRLDFPEMKFSLYFVGYEDPAS	IPTDPAERMAYTFVI <mark>E</mark> LTH <mark>N</mark> WGTESDENFK
MpGLX1-3	IQQTMYRIKDPKVSLDFYTRILGMTLLKKLDFPEMKFSLYFVGYEDPAS	IPTDPAERMAYTFSSKATI <mark>E</mark> LTH <mark>N</mark> WGTESDENFK
SmGLX1-2	VQQTMYRIKDPKASLDFYSRVLGMTLLKRLDFPDSKFSLYFVGYEDSAE	APKDPIERVRWTFRKKATI <mark>E</mark> LTH <mark>N</mark> WGTETDPDFK
OsGLX1-4	MQQTMFRVKDPKVSLDFYSRVMGMSLLKRLDFPEMKFSLYFLGYEDVES	APTDPVKRTVWTFGQRATI <mark>E</mark> LTH <mark>N</mark> WGTENDPEFK
ZmGLX1-5	LQQTMLRVKDPKVSLDFYSRVMGMSLLKRLDFEEMKFSLYFLGYEDVTI	APDDHIKRTEWTFRQKATI <mark>E</mark> LTH <mark>N</mark> WGTENDPEFK
AtGLX1-3	MQQTMFRIKDPKASLDFYSRVLGMSLLKRLDFSEMKFSLYFLGYEDTTT	'APTDPTERTVWTFGQPATI <mark>E</mark> LTH <mark>N</mark> WGTESDPEFK
CvGLX1-2	FQQTMLRVKDPQPSLDFYTRVLGMTLLCKLDFADMKFSLYFLAYQSPED	VPADPVERAKWMFGLPACI <mark>E</mark> LTH <mark>N</mark> WGTESDPDFK
PtGLX1	FSQTMLRVKDPRKSLAFYKAMGMKLLSEKHFNDFSLYFLGSSNVAD	0GADTKTLFQPVI <mark>E</mark> LTH <mark>N</mark> HGTENDDDFR
CmGLX1	FAQTMIRIKDPSKSRQFYEALG-MNFLTRFDFPELSFSLYFFALEKDPTVPAE	DAPQPERAKWLFSRQYPTI <mark>E</mark> LTH <mark>N</mark> WGTEKDPNFK
ScGLX1	LNHTCLRVKDPARAVKFYTEHFGMKLLSRKDFEEAKFSLYFLSFPKD	DIPKNKNGEPDVFGAHGVL <mark>E</mark> LTH <mark>N</mark> WGTEKNPDYK
	<u> </u>	
S6803GLX1	YDLGNGFGHIALGVEDIYSTCDKIRDK-GGKVVREPGPMKHGTTVIAFVEDPDGYKIEL	
S6803GLX1 EcGLX1	YDLGNGFGHIALGVEDIYSTCDKIRDK-GGKVVREPGPMKHGTTVIAFVEDPDGYKIEL YELGTAYGHIALSVDNAAEACEKIRQN-GGNVTREAGPVKGGTTVIAFVEDPDGYKIEL	
S6803GLX1 EcGLX1 OsGLX1-1	YDLGNGFGHIALGVEDIYSTCDKIRDK-GGKVVREPGPMKHGTTVIAFVEDPDGYKIEL YELGTAYGHIALSVDNAAEACEKIRQN-GGNVTREAGPVKGGTTVIAFVEDPDGYKIEL YDIGAGFGHFGIAVDDVAKTVELIRAK-GGKVTREPGPVKGGKTVIAFVEDPDGYKFEI	
S6803GLX1 EcGLX1 OsGLX1-1 ZmGLX1-1	YDLGNGFGHIALGVEDIYSTCDKIRDK-GGKVVREPGPMKHGTTVIAFVEDPDGYKIEL YELGTAYGHIALSVDNAAEACEKIRQN-GGNVTREAGPVKGGTTVIAFVEDPDGYKIEL YDIGAGFGHFGIAVDDVAKTVELIRAK-GGKVTREPGPVKGGKTVIAFVEDPDGYKFEI YDIGEGFGHFGIAVEDVAKTVELIRAK-AGKVIREAGPVKGGETVIAFVEDPDGYKFEI	
S6803GLX1 EcGLX1 OsGLX1-1 ZmGLX1-1 AtGLX1-1	YDLGNGFGHIALGVEDIYSTCDKIRDK-GGKVVREPGPMKHGTTVIAFVEDPDGYKIEL YELGTAYGHIALSVDNAAEACEKIRQN-GGNVTREAGPVKGGTTVIAFVEDPDGYKIEL YDIGAGFGHFGIAVDDVAKTVELIRAK-GGKVTREPGPVKGGETVIAFVEDPDGYKFEI YDIGAGFGHFGIAVDDVAKTVELIRAK-AGKVIREAGPVKGGETVIAFVEDPDGYKFEI	
S6803GLX1 EcGLX1 OsGLX1-1 ZmGLX1-1 AtGLX1-1 MpGLX1-1	YDLGNGFGHIALGVEDIYSTCDKIRDK-GGKVVREPGPMKHGTTVIAFVEDPDGYKIEL YELGTAYGHIALSVDNAAEACEKIRQN-GGKVTREAGPVKGGTTVIAFVEDPDGYKIEL YDIGAGFGHFGIAVDDVAKTVELIRAK-GGKVTREPGPVKGGKTVIAFVEDPDGYKFEI YDIGAGFGHFGIAVEDVAKTVELIRAK-AGKVIREAGPVKGGKTVIAFIEDPDGYKFEI YDIGAGFGHFGIAVEDVQKVCDLVKAK-GGVVSREPGPVKGGKSVIAFVDDPDGYKFEI	
S6803GLX1 EcGLX1 OsGLX1-1 ZmGLX1-1 AtGLX1-1 MpGLX1-1 SmGLX1-1	YDLGNGFGHIALGVEDIYSTCDKIRDK-GGKVVREPGPMKHGTTVIARVEDPDGYKIEL YELGTAYGHIALSVDNAAEACEKIRQN-GGNVTREAGPVKGGTTVIARVEDPDGYKIEL YDIGAGFGHFGIAVDDVAKTVELIRAK-GGKVTREPGPVKGGKTVIARVEDPDGYKFEI YDIGAGFGHFGIAVEDVAKTVELIRAK-AGKVIREAGPVKGGKTVIARTEDPDGYKFEL YDIGTGFGHFGIAVEDVQKVCDLVKAK-GGVVSREPGPVKGGKSVIARVDDPDGYKFEL YDIGTGFGHFGIAVEDVYKTVDLVKAK-GGKVSREAGPVKGGSTVIAFVDDPDGYKFEL	
S6803GLX1 EcGLX1 OsGLX1-1 ZmGLX1-1 AtGLX1-1 MpGLX1-1 SmGLX1-1 OsGLX1-3	YDLGNGFGHIALGVEDIYSTCDKIRDK-GGKVVREPGPMKHGTTVIAFVEDPDGYKIEL YELGTAYGHIALSVDNAAEACEKIRQN-GGNVTREAGPVKGGTTVIAFVEDPDGYKIEL YDIGAGFGHFGIAVDDVAKTVELIRAK-GGKVTREPGPVKGGKTVIAFVEDPDGYKFEI YDIGAGFGHFGIAVDDVAKTVELIRAK-AGKVSREPGPVKGGKTVIAFIEDPDGYKFEL YDIGTGFGHFGIAVDDVAKTVELIVKAK-GGKVSREPGPVKGGKSVIAFIEDPDGYKFEL YDIGTGFGHFGIAVEDVQKVCDLVKAK-GGKVSREAGPVKGGSTVIAFVDDPDGYKFEL YDIGTGFGHFGIAVEDVXKTVDLIKAK-GGTVTREPGPVKGGKSVIAFIEDPDGYKFEL	
S6803GLX1 EcGLX1 OsGLX1-1 ZmGLX1-1 AtGLX1-1 MpGLX1-1 SmGLX1-1 OsGLX1-3 ZmGLX1-4	YDLGNGFGHIALGVEDIYSTCDKIRDK-GGKVVREPGPMKHGTTVIAFVEDPDGYKIEL YELGTAYGHIALSVDNAAEACEKIRQN-GGNVTREAGPVKGGTTVIAFVEDPDGYKIEL YDIGAGFGHFGIAVDDVAKTVELIRAK-AGKVIREAGPVKGGKTVIAFVEDPDGYKFEI YDIGAGFGHFGIAVDDVAKTVELVRAK-GGKVSREPGPVKGGKTVIAFIEDPDGYKFEL YDIGTGFGHFGIAVEDVQKVCDLVKAK-GGKVSREAGPVKGGKSVIAFUDDPDGYKFEL YDIGTGFGHFGIAVEDVYKTVDLVKAK-GGTVTREPGPVKGGKSVIAFUDDPDGYKFEL YDIGTGFGHFGIAVEDVAKTVDLIKAK-GGTVTREPGPVKGGKSVIAFUEDPDGYKFEL YDIGTAFGHFGIAVEDVAKTVLLIKAK-GGTVTREPGPVKGGKSVIAFIEDPDGYKFEL	
S6803GLX1 EcGLX1 OsGLX1-1 ZmGLX1-1 AtGLX1-1 MpGLX1-1 SmGLX1-1 OsGLX1-3 ZmGLX1-4 CvGLX1-1	YDLGNGFGHIALGVEDIYSTCDKIRDK-GGKVVREPGPMKHGTTVIAFVEDPDGYKIEL YELGTAYGHIALSVDNAAEACEKIRQN-GGNVTREAGPVKGGTTVIAFVEDPDGYKIEL YDIGAGFGHFGIAVDDVAKTVELIRAK-AGKVTREPGPVKGGETVIAFVEDPDGYKFEI YDIGEGFGHFGIAVDDVAKTVELIRAK-AGKVSREPGPVKGGKTVIAFIEDPDGYKFEL YDIGTGFGHFGIAVEDVQKVCDLVKAK-GGKVSREPGPVKGGKSVIAFVDDPDGYKFEL YDIGTGFGHFGIAVEDVYKTVDLIKAK-GGVVSREAGPVKGGSTVIAFVDDPDGYKFEL YDIGTGFGHFGIAVEDVXKTVDLIKAK-GGTVTREPGPVKGGKSVIAFIEDPDGYKFEL YDIGTGFGHFGIAVEDVAKTVELIKAK-GGTVTREPGPVKGGKSVIAFIEDPDGYKFEL	
S6803GLX1 EcGLX1 OSGLX1-1 ZmGLX1-1 AtGLX1-1 MpGLX1-1 SmGLX1-1 OSGLX1-3 ZmGLX1-4 CvGLX1-1 OSGLX1-2	YDLGNGFGHIALGVEDIYSTCDKIRDK-GGKVVREPGPMKHGTTVIAFVEDPDGYKIEL YDLGAGFGHFGIAVDDVAKTVELIRAK-GGKVTREAGPVKGGKTVIAFVEDPDGYKFEI YDIGAGFGHFGIAVDDVAKTVELIRAK-GGKVTREAGPVKGGKTVIAFVEDPDGYKFEI YDIGAGFGHFGIAVEDVAKTVELIRAK-GGKVSREAGPVKGGKTVIAFUEDPDGYKFEI YDIGTGFGHFGIAVEDVQKVCDLVKAK-GGKVSREAGPVKGGKTVIAFUDPDGYKFEI YDIGTGFGHFGIAVEDVYKTVDLIKAK-GGKVSREAGPVKGGSTVIAFVDDPDGYKFEI YDIGTGFGHFGIAVEDVXKTVDLIKAK-GGTVTREPGPVKGGSSTVIAFUDPDGYKFEI YDIGTGFGHFGIAVEDVXKTVDLIKAK-GGTVTREPGPVKGGSSTVIAFUEDPDGYKFEI YDIGTGFGHFGIAVEDVXKTVDLIKAK-GGTVTREPGPVKGGSSTVIAFUEDPDGYKFEI YDIGTAFGHFGIAVEDVXKTVDLIKAK-GGTVTREPGPVKGGSSTVIAFUEDPDGYKFEI YDIGTAFGHFGIAVEDVKIKAK-GGTVTREPGPVKGGSSTVIAFUEDPDGYKFEI	
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Supplemental Fig. S3. Sequence comparison of the Glyoxalase domain (Pfam, PF00903) in the N-terminus of glyoxalase I (GLX1) among oxygenic phototrophs. Blue shading represents the metal-binding sites; orange shading represents substratebinding sites. Navy arrows indicate the regions specific to Zn<sup>2+</sup>-dependent GLX1. S6803, cyanobacterium *Synechocystis* sp. PCC 6803; Cv, green alga Chlorella variabilis; Mp, liverwort Marchantia polymorpha; Sm, fern Selaginella moellendorffii; At, C<sub>3</sub> plant Arabidopsis thaliana; Os, C<sub>3</sub> plant Oryza sativa; Zm, C<sub>4</sub> plant Zea mays; Cm, red alga Cyanidioschyzon merolae; Pt, diatom Phaeodactylum tricornutum; Ec, heterotrophic prokaryote Escherichia coli; and Sc, heterotrophic eukaryote Saccharomyces cerevisiae.

EYTVKNLKFILTVEPDNEKVKQKLEWAQKQREANQPTIPSTIGEEFETNTFMRVDLPEIQAKFGAKSPVEALREVRKTKDNW
EYTVKNLKFMLTLEPENEKTKQKLEWAEKQREANQPTVPSTIGDEFEINTFMRVDLPEIQAKFSVNSPVEAMREVRKTKDNW
EYTVKNLEFALTVEPNNGKIQQKLAWARQQRQADLPTIPSTLEEELETNPFMRVDKPEIQEKLGCKSPIDTMREVRNKKDQW
EYTAKNLKFAMSVDPHNDALKQKVAWTEEQRRNDKPTVPSTIREELQTNPFMRVNVKEFQVHMGESDPVELLASLRAAKDQF
E <mark>Y</mark> TVANLAFALTVEPNNESIRNKLKEAERLRAENKATIPSTVGGEQQWNVFLRAADAQWMTEL <mark>R</mark> ER <mark>K</mark> NRF
E <mark>Y</mark> TLSNSKFALSIEPTNEVLQSYAAYVAELRDKKLPTIPTTMKMEKACNPFLRTENTDIRRALGIPETADEAEALGII <mark>R</mark> RA <mark>KD</mark> NF
E <mark>Y</mark> TLSNSKFALSLEPNNEVLQSYAAHVAELRSKKLPTIPTTVKMEKACNPFLRSSNTDIRRALRIPEAADEAEALGII <mark>R</mark> KA <mark>KD</mark> DF
E <mark>Y</mark> TLSNSKFALSIEPGNKDLQEYAANAADLRKRNTPTVPTTIGREKQCNPFLRTSSPEIKNTLSIPDHFDDARVLEVV <mark>R</mark> RA <mark>KD</mark> NF
E <mark>Y</mark> TLSNAKFALSVEPGNKALQEYAANAAELRNKNIPTVPTTIGREKECNPFLRTSNPEIKRTLSVPDHFDEDRVLGVV <mark>R</mark> RA <mark>KD</mark> NF
E <mark>Y</mark> TASNAKFAAHVDEGNEDLQRMKADIEAKRARGEPTVPSQLGDELKCNPFLRPGTLDSPAIRSKLGVPEGASNDVAFGAI <mark>R</mark> AA <mark>K</mark> DTF
E <mark>Y</mark> TSSNAKFALAIEPGNSALVSRAEEIKATRERGEPTVPSNLGVEKQTNPFLRCDMSAEIRQNIGVKISDSDADVFGRI <mark>R</mark> KA <mark>K</mark> DKF
E <mark>Y</mark> TLSNAKFAMSVEPQNEALSSRYEKVAELRRKGLPTVPTSLGEEKSFNPFLRPFSQELRKSVHLNSSASDVETFAAV <mark>R</mark> LA <mark>KD</mark> RY
E <mark>Y</mark> TLSNAKFAMTIEPNNPALNSHFEKVKQLRDSGLATIPSSVGEEKKFNPFLRPASREIRRSLNLSDDASDSDVFAAV <mark>R</mark> KA <mark>KD</mark> RA
E <mark>Y</mark> TASNIRFAVTVEPHNEDLLSQRQLVEQLRQKGQPTIPTTIGQENSFNPFLRPFVESVRQSLNKSASENNVEVFTAL <mark>R</mark> LA <mark>K</mark> DKF
E <mark>Y</mark> TLGNLKFALTVDPSNKDLQERFQTVQGDRQRGQATIPSWLGTEKRTNPFLRWDNPAIQARVGMTEPARVFGKL <mark>R</mark> GM <mark>KD</mark> NF
EYTLSNMKFALSILPHDLSINDYYRKVKELRAKNQITLPVILKNERQINVFLRTEDIDLINVINEETLLQQPEERFAWL <mark>R</mark> SKKDRF
EYTSDNVKFVRKIYPQVGENKALDELEQFCSKHEVTAGRFTLKDEVEFNPFMRLEDPKVQKAAGDTNNSWDRAQIMDKLRAMKNRM

**Supplemental Fig. S4.** Sequence comparison of the hydroxyacylglutathione hydrolase domain (Pfam, PF16123) in the C-terminus of glyoxalase II (GLX2) among oxygenic phototrophs. Orange shading indicates substrate-binding sites. S6803, cyanobacterium *Synechocystis* sp. PCC 6803; Cv, green alga *Chlorella variabilis*; Mp, liverwort *Marchantia polymorpha*; Sm, fern *Selaginella moellendorffii*; At, C<sub>3</sub> plant *Arabidopsis thaliana*; Os, C<sub>3</sub> plant *Oryza sativa*; Zm, C<sub>4</sub> plant *Zea mays*; Cm, red alga *Cyanidioschyzon merolae*; Pt, diatom *Phaeodactylum tricornutum*; Ec, heterotrophic prokaryote *Escherichia coli*; and Sc, heterotrophic eukaryote *Saccharomyces cerevisiae*.