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REGULAR PAPER

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The effect of exogenous dihydroxyacetone and methylglyoxal on growth, anthocyanin accumulation, and the glyoxalase system in Arabidopsis

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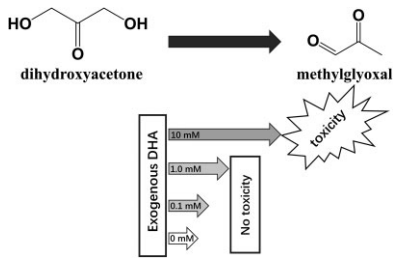
ABSTRACT

Dihydroxyacetone (DHA) occurs in wide-ranging organisms, including plants, and can undergo spontaneous conversion to methylglyoxal (MG). While the toxicity of MG to plants is well-known, the toxicity of DHA to plants remains to be elucidated. We investigated the effects of DHA and MG on Arabidopsis. Exogenous DHA at up to 10 mM did not affect the radicle emergence, the expansion of green cotyledons, the seedling growth, or the activity of glyoxalase II, while DHA at 10 mM inhibited the root elongation and increased the activity of glyoxalase I. Exogenous MG at 1.0 mM inhibited these physiological responses and increased both activities. Dihydroxyacetone at 10 mM increased the MG content in the roots. These results indicate that DHA is not so toxic as MG in Arabidopsis seeds and seedlings and suggest that the toxic effect of DHA at high concentrations is attributed to MG accumulation by the conversion to MG.

Graphical Abstract



Is dihydroxyacetone toxic in Arabidopsis?

Keywords: dihydroxyacetone, methylglyoxal, growth, anthocyanin, glyoxalase system

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Dihydroxyacetone (1,3-dihydroxy-2-propanone, DHA) is a natural product found in wide-ranging organisms, including plants: *Arabidopsis thaliana* (Schad *et al.* 2005), *Agave americana* (Abraham *et al.* 2016), and *Glycine max* (Liu *et al.* 2020). Dihydroxyacetone is a substance commercially refined from sugar beet and sugarcane (Stasiak-Różańska *et al.* 2009). The floral nectaries contain abundant DHA derived from dihydroxyacetone phosphate (DHAP) in *Leptospermum scoparium* (mānuka), which is produced as an intermediate in the metabolism of photosynthetic nectary parenchyma (Clearwater *et al.* 2021).

In plants, DHAP and glyceraldehyde-3-phosphate (GAP) are intermediates in glycolysis and photosynthesis and can be nonenzymatically converted to methylglyoxal (2-oxopropanal, MG) (Kaur *et al.* 2014a,b; Hossain *et al.* 2016). Methylglyoxal is toxic to plants at high concentrations, while MG is likely to function as a signaling agent at low concentrations in plants (Hoque *et al.* 2017). Methylglyoxal is detoxified by the glyoxalase system, which consists of 2 enzymes, glyoxalase I (Gly I; lactoylglutathione lyase; EC 4.4.1.5) and glyoxalase II (Gly II; hydroxyacylglutathione hydrolase; EC 3.1.2.6) in plants (Dakin and Dudley 1913). The activities of Gly I and Gly II were elevated in plants under a variety of stresses (Hasanuzzaman *et al.* 2017). Moreover, accumulation of anthocyanins is one of the typical stress responses of plants because anthocyanins play a role in scavenging reactive oxygen species (ROS), protecting plant cells from the oxidative damage (Naing and Kim 2021).

The effects of DHA on plants have yet to be investigated. In this study, we aimed to elucidate the effects of DHA and MG on the growth, anthocyanin accumulation, and the glyoxalase system using *Arabidopsis thaliana*. Furthermore, to understand the potential of DHA to accumulate MG in plants, we measured the contents of DHA and MG in the roots of seedlings.

Materials and methods

Plant material and culture conditions

Arabidopsis thaliana plants (ecotype Columbia-0) were used in this study. Seeds were surface sterilized by washing with 70% (v/v) ethanol 5 times, immersing in 5% (v/v) sodium hydrochloride solution for 5 min, and rinsing with sterile water 5 times. The surface-sterilized seeds were then sown onto one-quarter Murashige and Skoog (MS) medium containing 1.15 g L^{-1} MS salt mixture, 2% sucrose, 3 mg L^{-1} thiamine hydrochloride, 0.5 mg L^{-1} pyridoxine hydrochloride, and 5 mg L^{-1} nicotinic acid, which was solidified with 0.8% agar. The pH of the medium was adjusted to 5.85 with KOH. The seeds sown on agar plates were incubated at 4°C in darkness for 3 d before being transferred to a growth chamber with continuous light at 25°C, 80 µmol m^{-2} s^{-1} light intensity, and 70% relative humidity. The seeds were grown on the agar plate vertically or horizontally placed for the following experiments.

Germination and seedling growth assays

Radicle emergence of seeds

The seeds were grown on the agar plate horizontally placed after the transfer to the growth chamber. The agar plates contained DHA or MG at 0, 0.1, 1.0, and 10 mM. The number of radicles emerging from the seed was counted every 24 h for 7 d.

Expansion of green cotyledons

The seeds were grown on the agar plate horizontally placed in the growth chamber. The agar plates contained DHA or MG at 0, 0.1, 1.0, and 10 mM. After the transfer to the growth chamber, the number of green expanded cotyledons was counted at 2, 3, 4, and 5 d.

Root growth

The seeds were grown in the absence of DHA and MG on the vertically placed agar plate for 7 d. The seedlings were transferred to fresh vertically placed agar plates containing DHA or MG at 0, 0.1, 1.0, and 10 mM. The length of the primary root was measured every day for 7 d.

Fresh weight

The seeds were grown in the absence of DHA and MG on the vertically placed agar plate for 7 d. The seedlings were transferred to fresh vertically placed agar plates containing DHA or MG at 0, 0.1, 1.0, and 10 mM and were grown for another 7 d and then the fresh weight (FW) of 12 seedlings was measured.

Measurement of anthocyanin accumulation

The seeds were grown in the absence of DHA and MG on the vertically placed agar plate for 7 d. The seedlings were transferred to fresh vertically placed agar plates containing DHA or MG at 0, 0.1, 1.0, and 10 mM, and were grown for another 7 d. Anthocyanins were extracted from the shoots of 14-d-old seedlings as previously described (Hoque *et al.* 2016). In brief, 0.5 g of shoots were ground using a pre-cooled mortar pestle into a fine powder in liquid nitrogen and suspended in 2 mL of methanol/concentrated HCl (99/1, v/v), followed by centrifugation at 1300 × *g* for 15 min. The supernatant was collected and was diluted by adding methanol/concentrated HCl. The absorbance of the diluted solution was measured at 535 nm using a spectrophotometer (UV-160A, Shimadzu, Kyoto, Japan). The calculation of total anthocyanin content (TAC) was conducted based on the method described by Fuleki and Francis (1968). Total anthocyanin content was calculated using the following equation:

$$\mathrm{TAC} = \frac{A_{535\,\mathrm{nm}}\, V\, F}{98.2\, m},$$

where $A_{535\,\mathrm{nm}}$ is absorption at 535 nm, V is the final volume (mL), F is the dilution factor, 98.2 is the extinction coefficient (mM $^{-1}$ cm^{-1}) at 535 nm, and m is the mass of the sample (g).

Glyoxalase I (Gly I, EC: 4.4.1.5) and glyoxalase II (Gly II, EC: 3.1.2.6) activities

The seeds were grown in the absence of DHA and MG on the vertically placed agar plate for 7 d. The seedlings were transferred to fresh vertically placed agar plates containing DHA or MG at 0, 0.1, 1.0, and 10 mM, and were grown for another 7 d. The crude extract of Gly I and Gly II was obtained according to Hoque *et al.* (2017). Shoots (0.5 g) were ground using a pre-cooled mortar pestle into a fine powder in liquid nitrogen and homogenized in 100 mM ice-cold sodium phosphate buffer (pH 7.2) containing 1.0 mM phenylmethylsulfonyl fluoride and 1% (w/v) polyvinylpyrrolidone (PVP40). The homogenate was centrifuged at 10 000 × *g* at 4°C for 10 min. The supernatant was used to measure the activities of Gly I and Gly II. The protein content in the crude extract was determined by Bradford assay.

The Gly I and the Gly II activities in the crude extract were measured according to Hasanuzzama and Fujita (2013). The

Gly I and Gly II activities were calculated based on a change in absorbance at 240 and 412 nm between 0 and 15 s, respectively. The extinction coefficients are 3.37 mM^{-1} cm^{-1} for Gly I and 13.6 mM^{-1} cm^{-1} for Gly II.

Quantification of DHA and MG

The contents of DHA and MG in roots were quantified according to Mano and Biswas (2018) with a modification. In this study, authentic MG-bis-2,4-dinitrophenylhydrazone was obtained from Toronto Research Chemicals (Toronto, Canada). DHA-bis-2,4-dinitrophenylhydrazone and MG-bis-2,4-dinitrophenylhydrazone were synthesized for subsequent high-performance liquid chromatography (HPLC) analysis to determine the retention time.

The seeds were grown in the absence of DHA and MG on the vertically placed agar plate for 7 d. The seedlings were transferred to fresh vertically placed agar plates containing DHA or MG at 0, 0.1, 1.0, and 10 mM, and were grown for another 7 d. Dihydroxyacetone and MG in the roots and in the agar were derivatized with 2,4-dinitrophenylhydrazine and the derivatives were extracted and analyzed using HPLC instruments according to Mano and Biswas (2018). The identification of carbonyl derivatives was based on the retention time and the contents were determined using a calibration curve. Five calibration standards at 1 , 10 , 100 , 200 , and 500 μM were prepared for each analyte.

Reversed-phase chromatography was used for the HPLC analysis. The C18 column (4.6 mm i.d. x 150 mm, GL Sciences, Tokyo, Japan) was operated at 40°C. The eluents were water with 0.1% formic acid (eluent A) and methanol with 0.1% formic acid (eluent B). The eluent consisted of 80% of eluent A (0.1% formic acid in water) and 20% of eluent B (0.1% formic acid in methanol). The injection volume was 10 μL.

Statistical analysis

The significance of differences between data sets was assessed by ANOVA, followed by Dunnett's multiple comparisons *post-hoc* test. Differences at $P < .05$ (vs 0 mM) were considered significant.

Results

Effects of DHA and MG on radicle emergence, expansion of green cotyledons, elongation of the primary root, and fresh weight of Arabidopsis seedlings

We examined the effects of DHA and MG at 0, 0.1, 1.0, and 10 mM on the radicle emergence for 7 d. When the seeds were untreated with DHA or MG, radicle emergence was observed in 100% of the seeds (Figure 1a and b). Exogenous DHA at 0.1, 1.0, and 10 mM did not affect the radicle emergence rate (Figure 1a). Methylglyoxal at 0.1 mM did not affect the radicle emergence rate after 4 d but significantly delayed the radicle emergence in the first 3 days (Figure 1b). At 7th d, MG at 1.0 and 10 mM decreased the radicle emergence rate by 10% ($P < .05$) and by 77% ($P < .05$), respectively (Figure 1b). The results for MG are in agreement with previous results (Hoque *et al*. 2012).

We examined the effects of DHA and MG at 0, 0.1, 1.0, and 10 mM on the expansion of green cotyledons. When the seeds were untreated with DHA or MG, the expansion rate of green cotyledons was observed in 53% of the seeds at 5 d (Figure 1c and d). There were no significant differences in a rate of expansion of green cotyledons between the seeds untreated and treated with 0.1, 1.0, or 10 mM DHA except for the seeds treated with 10 mM DHA at 2 d (Figure 1c). Methylglyoxal at 0.1 mM significantly suppressed expansion of green cotyledons at 2-5 d and MG at 1.0 mM suppressed expansion of green cotyledons at 2 and 3 d ($P < .05$) but not at 4 or 5 d (Figure 1d). Methylglyoxal at 10 mM completely inhibited the expansion ($P < .05$) (Figure 1d). These results indicate that DHA at 10 mM and MG at higher than 0.1 mM suppress the expansion of green cotyledons.

There were no significant differences in the elongation of the primary root between the seedlings untreated and treated with DHA at 0.1 and 1.0 mM. Exogenous DHA at 10 mM completely inhibited the elongation of the primary root ($P < .05$) (Figure 2a). Exogenous MG at 0.1 mM did not inhibit the elongation of the primary root, while MG at 1.0 and 10 mM completely inhibited the elongation of the primary root ($P < .05$) (Figure 2b). These results indicate that DHA at lower than 1.0 mM does not inhibit the elongation of primary roots but that DHA at 10 mM and MG at higher than 1.0 mM inhibit the elongation.

There were no significant differences in the fresh weight (FW) between the seedlings untreated and those treated with DHA at 0.1, 1.0, and 10 mM (Figure 2c). Exogenous MG at 0.1 mM did not significantly decrease FW, whereas MG at 1.0 and 10 mM significantly decreased FW by 70% ($P < .05$) and by 90% ($P < .05$), respectively (Figure 2d). The formation of lateral roots was observed in the seedlings both untreated and treated with DHA at up to 10 mM and with MG at 0.1 mM for 7 d (Figure 2e and f). When the seedlings were treated with MG at higher than 1.0 mM for 7 d, the formation of lateral roots was not observed (Figure 2f). No chlorosis was observed in the seedlings treated with DHA at up to 10 mM for 7 d (Figure 2e), while chlorosis was observed in the seedlings treated with MG at 1.0 mM and 10 mM for 7 d (Figure 2f). These results indicate that DHA is not as effective in the formation of lateral roots or induction of chlorosis of seedlings as MG.

Effect of DHA and MG on anthocyanin accumulation in Arabidopsis shoots

We examined the effect of DHA and MG on anthocyanin accumulation in Arabidopsis shoots. The shoots of seedlings untreated with DHA or MG contained 3.3 μg g^{-1}-FW of anthocyanin. Dihydroxyacetone at 0.1, 1.0, and 10 mM did not significantly affect the anthocyanin accumulation (Figure 3a). Methylglyoxal at 0.1 mM did not affect the anthocyanin accumulation but MG at 1.0 mM significantly increased anthocyanin accumulation to 24.1 μg g^{-1}-FW ($P < .05$) (Figure 3b), in agreement with previous results (Hoque *et al*. 2017). The anthocyanin content of seedlings treated with 10 mM was not measured because the growth of seedlings was completely inhibited by 10 mM MG. These results indicate that neither DHA at up to 10 mM nor MG at 0.1 mM does not affect anthocyanin accumulation in the shoots but MG at 1.0 mM increases the accumulation. These results indicate that DHA does not affect anthocyanin accumulation in Arabidopsis seedlings.

Effects of DHA and MG on activities of Gly I and II in Arabidopsis shoots

We examined the effects of DHA and MG on the activities of Gly I and Gly II in Arabidopsis shoots (Figure 4). When the seedlings were untreated with DHA or MG, activities of Gly I and Gly II of the shoots were 0.008 μmol sec^{-1} mg^{-1} (Figure 4). Dihydroxyacetone at 0.1 and 1.0 mM did not affect Gly I activities but DHA at 10 mM significantly increased Gly I activity ($P < .05$) (Figure 4a).

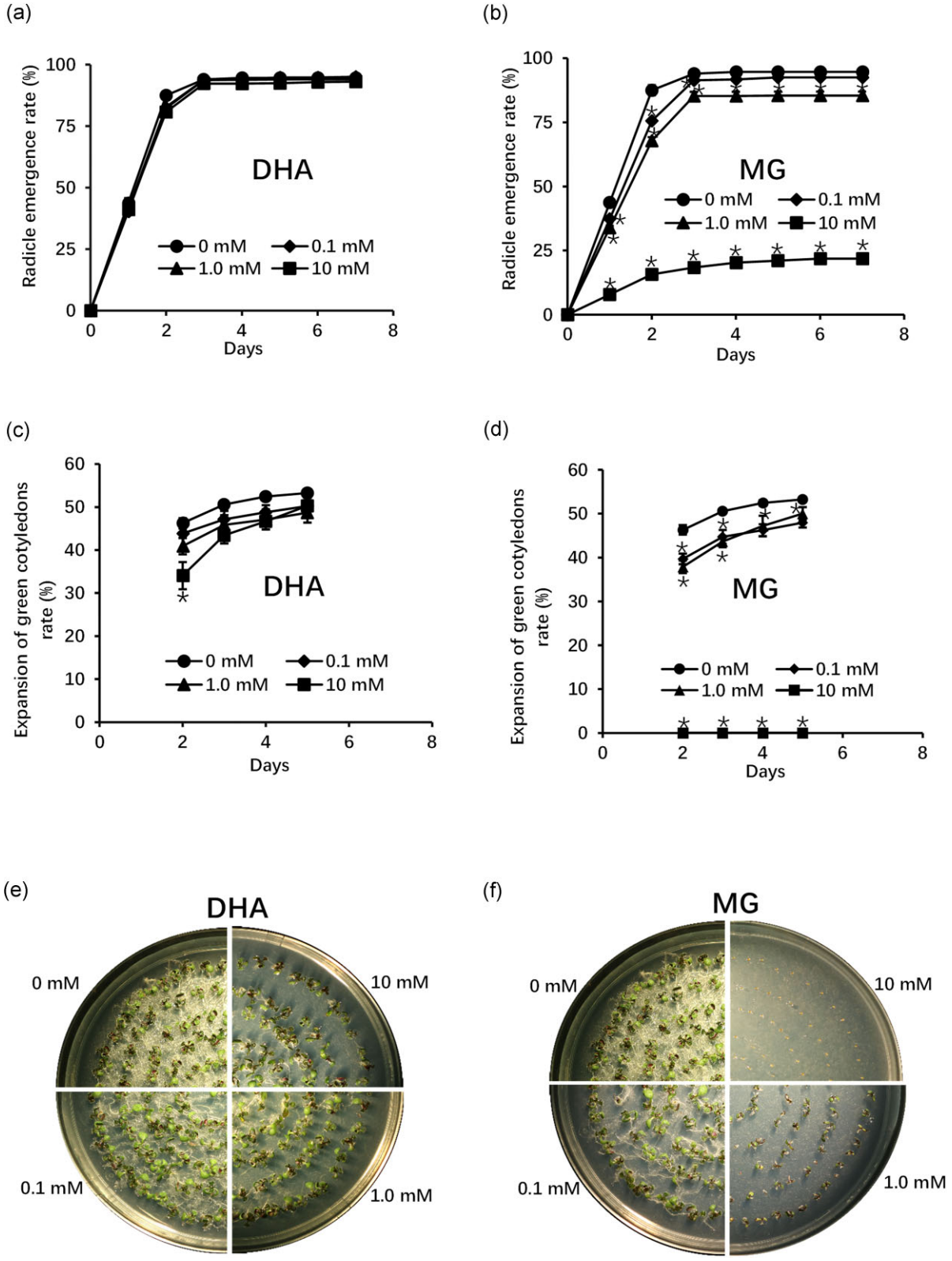

Figure 1. Effects of DHA and MG on radicle emergence and expansion of green cotyledons of Arabidopsis seeds. Approximately 200 seeds were sown on each agar plate containing DHA or MG at 0, 0.1, 1.0, and 10 mM as indicated. The seeds were grown on the agar plate horizontally placed after the transfer to the growth chamber for 7 d. (a, b) Radicle emergence rate (number of radicles emerging from the seed/total number of seeds). (c, d) Rate of expansion of green cotyledons (number of green expended cotyledons/total number of radicles that emerged from the seed). (e, f) Representative photographs of 7-d-old germinated Arabidopsis seeds grown on agar plates contained DHA or MG at 0, 0.1, 1.0, and 10 mM. The 9.0 cm round plates were used with the same sown lines of seeds. The data of radicle emergence for 0 mM DHA shown in panel (b) are the same as those for 0 mM MG shown in panel (a), the data of expansion of green cotyledons for 0 mM DHA shown in panel (d) are the same as those for 0 mM MG shown in panel (c), and the picture for 0 mM DHA shown in panel (f) is the same as the one for 0 mM MG shown in panel (e). Values represent means ± standard error ($n = 3$). The asterisk indicates significant difference $P < .05$ (vs 0 mM).

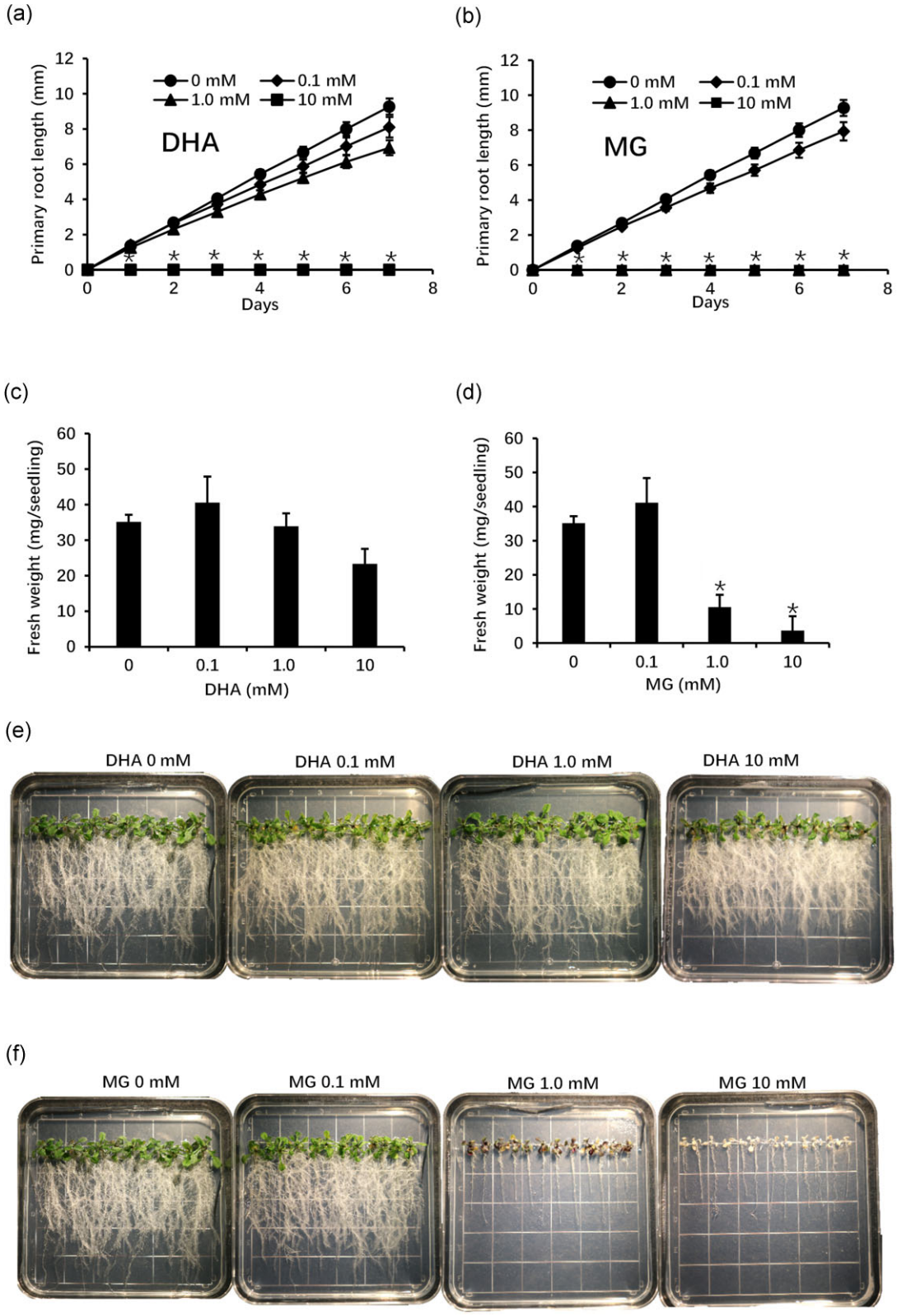

Figure 2. Effects of DHA and MG on elongation of primary root and FW of Arabidopsis seedlings. For DHA and MG treatments, 7-d-old seedlings grown in the agar plates in the absence of DHA or MG were transferred to and incubated in the presence of 0, 0.1, 1.0, and 10 mM DHA or MG for another 7 d. (a, b) Elongation of the primary root in the 14-d-old seedlings. (c, d) Fresh weight of 14-d-old seedlings. The data are presented as averages per seedling. (e, f) Representative photographs of 14-d-old Arabidopsis seedlings grown in the agar plates contained DHA or MG at 0, 0.1, 1.0, and 10 mM. (e, f) Square plates 9.6 cm in length were used with a grid size of 13 × 13 mm. The data of primary root length for 0 mM DHA shown in (b) are the same as those for 0 mM MG shown in (a), the data of fresh weight for 0 mM DHA shown in (d) are the same as those for 0 mM MG shown in (c), and the picture for 0 mM DHA shown in (f) are the same as those for 0 mM MG shown in (e). Values represent means ± standard error ($n = 3$). The asterisk indicates significant difference $P < .05$ (vs 0 mM).

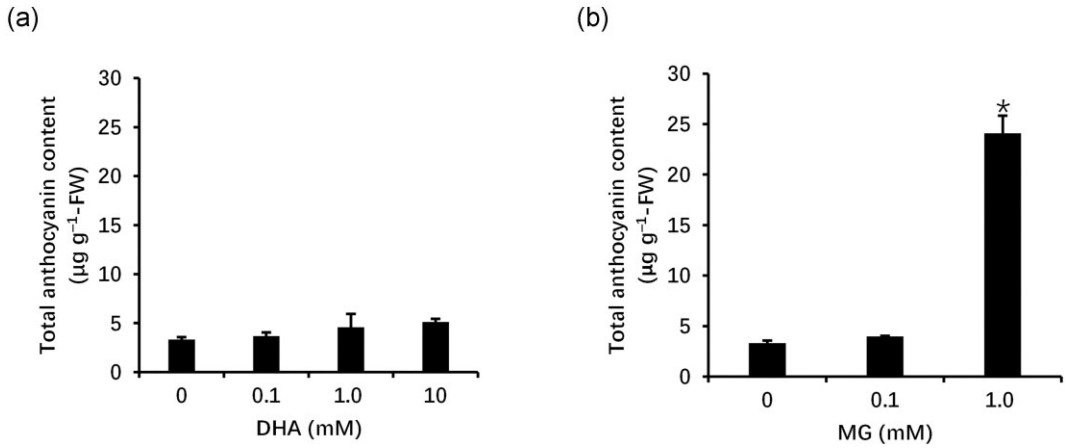

Figure 3. Effect of DHA and MG on anthocyanin accumulation in Arabidopsis shoots. Seven-day-old seedlings grown in the agar plates in the absence of DHA or MG were transferred to and incubated in the plates in the presence of 0, 0.1, 1.0, and 10 mM DHA or MG for another 7 d. Total anthocyanin content in shoots treated with DHA (a) and MG (b). The data of total anthocyanin content for 0 mM DHA shown in (b) are the same as those for 0 mM MG shown in (a). Values represent means ± standard error ($n = 3$). The asterisk indicates significant difference $P < .05$ (vs 0 mM).

Methylglyoxal at 0.1 mM did not affect Gly I activity but MG at 1.0 mM increased Gly I activity by 116% ($P < .05$) (Figure 4b), in agreement with previous results (Hoque *et al.* 2017). Neither DHA at up to 10 mM nor MG at 0.1 mM significantly increased Gly II activity but MG at 1.0 mM significantly increased Gly II activity by 109% ($P < .05$) (Figure 4c and d). These results indicate that DHA is not as effective in the Arabidopsis Gly system as MG.

Quantification of contents of DHA and MG in Arabidopsis roots

The roots untreated with DHA or MG contained 147.9 nmol g^{-1}-FW of DHA. When the seedlings were treated with DHA at 0.1, 1.0, or 10 mM for 7 d, the DHA content in the roots was not significantly changed (Figure 5a). Methylglyoxal at 0.1 mM also did not significantly affect the DHA content in the roots (Figure 5b).

The roots untreated with DHA or MG contained 28.7 nmol g^{-1}-FW of MG. There are no significant differences in MG contents between the untreated roots and the roots treated with DHA at 0.1 mM and 1.0 mM for 7 d. When treated with 10 mM, the roots contained 37.3 nmol g^{-1}-FW of MG, which was significantly higher than the MG content in untreated roots ($P < .05$) (Figure 5c). Methylglyoxal at 0.1 mM slightly but not significantly increased the contents of MG in the roots (Figure 5d) and Tukey test showed that there is no significant difference in MG contents between at 10 mM DHA and at 0.1 mM MG. Note that contents of DHA and MG were not measured in the roots treated with 1.0 mM or 10 mM MG because the seedlings were not grown when treated with 1.0 or 10 mM MG.

Furthermore, when the roots were treated with DHA at 50 and at 100 mM increased for 2 d, DHA contents were 4.1 and 6.0 μmol g^{-1}-FW, respectively (Figure 6a), and MG contents were 200 and 290 nmol g^{-1}-FW, respectively (Figure 6b). Exogenous DHA at higher concentrations induced higher levels of DHA and MG accumulation in the roots. These results suggest that exogenous DHA at higher concentrations induces uptake of DHA to lead to higher levels of MG accumulation due to the conversion of DHA to MG in the roots. Note that contents of DHA and MG were not measured in the roots treated with 50 or 100 mM DHA for 7 d because the growth of seedlings were impaired when treated with 50 and 100 mM DHA.

Discussion

Dihydroxyacetone occurs in plants but the effects of DHA on plants remain to be clarified. In this study, Arabidopsis roots contained around 150 nmol g^{-1}-FW of DHA regardless of the presence or absence of up to 10 mM DHA in the medium (Figure 5a), which compares with DHA contents (approximately 0.1 mM) of *Agave americana* (around 4 μg g^{-1}-FW) (Abraham *et al.* 2016) and *Dunaliella* (100 nmol g^{-1}-FW) (Lerner *et al.* 1977) and is much lower than DHA contents of mānuka nectar (approximately 10 mg g^{-1}) (Millner *et al.* 2016). On the other hand, methylglyoxal concentrations range from 30 to 90 μM in a variety of plants under a normal condition (Yadav *et al.* 2005). In this study, Arabidopsis roots contained 28.7 nmol g^{-1}-FW of MG (Figure 5c), which is likely to be within the same range (Yadav *et al.* 2005) if cellular compartmentation of MG is considered.

In this study, DHA at 0.1 and 1.0 mM did not affect radicle emergence, expansion of green cotyledons, elongation of the primary root, and seedling growth, while MG even at 0.1 mM inhibited radicle emergence and expansion of green cotyledons (Figures 1 and 2). Unlike in the case of DHA at 0.1 and 1.0 mM, DHA at 10 mM delayed expansion of green cotyledons and inhibited elongation of the primary root (Figures 1c and 2a and e). In addition, MG at 1.0 mM inhibited root elongation and induced chlorosis (Figure 2b and f), which is in agreement with previous results (Hoque *et al.* 2012). These results indicate that DHA at 10 mM induces toxicity to Arabidopsis and that DHA is not as toxic as MG to Arabidopsis.

It is considered that DHA is a nontoxic compound at the physiological concentrations. In this study, DHA at up to 1.0 mM did not either affect radicle emergence, expansion of green cotyledons, elongation of the primary root, and seedling growth (Figures 1a and c, 2a and d) or induce anthocyanin accumulation (Figure 3a) and chlorosis (Figure 2e), supporting the notion that DHA is a nontoxic compound.

However, DHA at 10 mM delayed expansion of green cotyledons and inhibited elongation of primary root (Figures 1c and 2a). It is well-known that DHA is spontaneously converted to MG but not enzymatically. The quantification of DHA and MG in the roots shows that exogenous DHA at 0.1 and at 1.0 mM did not increase DHA or MG content in the roots (Figure 5a and c) and exogenous DHA at 10 mM did not increase DHA content but

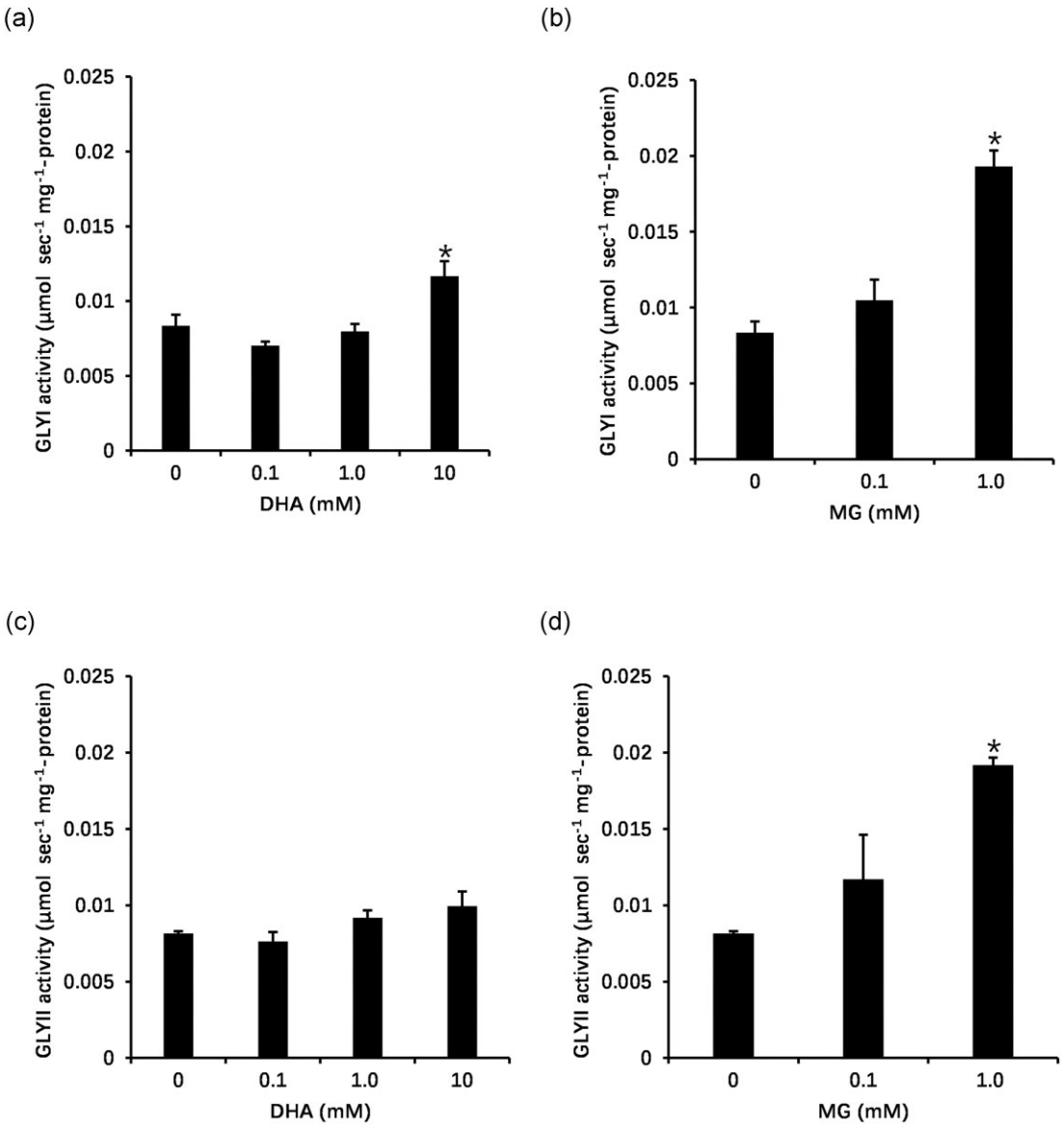

Figure 4. Effect of DHA and MG on activities of Gly I and Gly II in Arabidopsis shoots. For DHA and MG treatments, 7-d-old seedlings grown in the agar plates in the absence of DHA or MG were transferred to and incubated in the presence of 0, 0.1, 1.0, and 10 mM DHA or MG for another 7 d. (a, b) Glyoxalase I activity. (c, d) Glyoxalase II activity. The data of Gly I activity for 0 mM DHA shown in (b) are the same as those for 0 mM MG shown in (a) and the data of Gly II activity for 0 mM DHA shown in (d) are the same as those for 0 mM MG shown in (c). Values represent means ± standard error ($n = 3$). The asterisk indicates significant difference $P < .05$ (vs 0 mM).

significantly increased MG content (Figure 5a and c) to the same extent as MG at 0.1 mM did (Figure 5a and c). These results suggest that the accumulation of MG induced by 10 mM DHA leads to the delay of expansion of green cotyledons and the inhibition of elongation of the primary root.

Furthermore, Brouquisse *et al.* (2007) reported that 100 mM DHA increased DHA contents to approximately 3 mM in maize root tip and inhibited growth. Although Brouquisse *et al.* (2007) did not discuss conversion of DHA to MG, the inhibition of root tip growth by 100 mM DHA reported by Brouquisse *et al.* (2007) can be caused by MG accumulation induced by DHA.

In addition, exogenous DHA at up to 10 mM did not increase DHA contents in the roots (Figure 5a). The DHA taken up by cells can be rapidly phosphorylated into DHAP (Lerner *et al.* 1977), which is an intermediate in glycolysis. Hence, the rapid conversion of DHA to DHAP and the following metabolism might not elevate DHA contents in the DHA-treated seedlings.

Furthermore, in order to exclude a possibility that the conversion of DHA to MG in the agar medium delays expansion of green cotyledons and inhibits the elongation of primary root (Figures 1c and 2a), we measured MG contents in the medium. In the agar medium containing 10 mM DHA, MG was lower than 70 nmol g^{-1} for 7 d, suggesting that DHA is converted to MG in the agar medium. However, given 1 g of agar medium is 1 g of H_2O, MG content in the agar is estimated at lower than 70 µM, suggesting that the conversion of DHA to MG in the medium does not result in the delay of expansion or the inhibition of elongation. Consequently, the delay of expansion and the inhibition of elongation are likely to be attributed to the MG accumulation in plants. Taken together, it is concluded that DHA is not as toxic as MG to Arabidopsis seeds and seedlings.

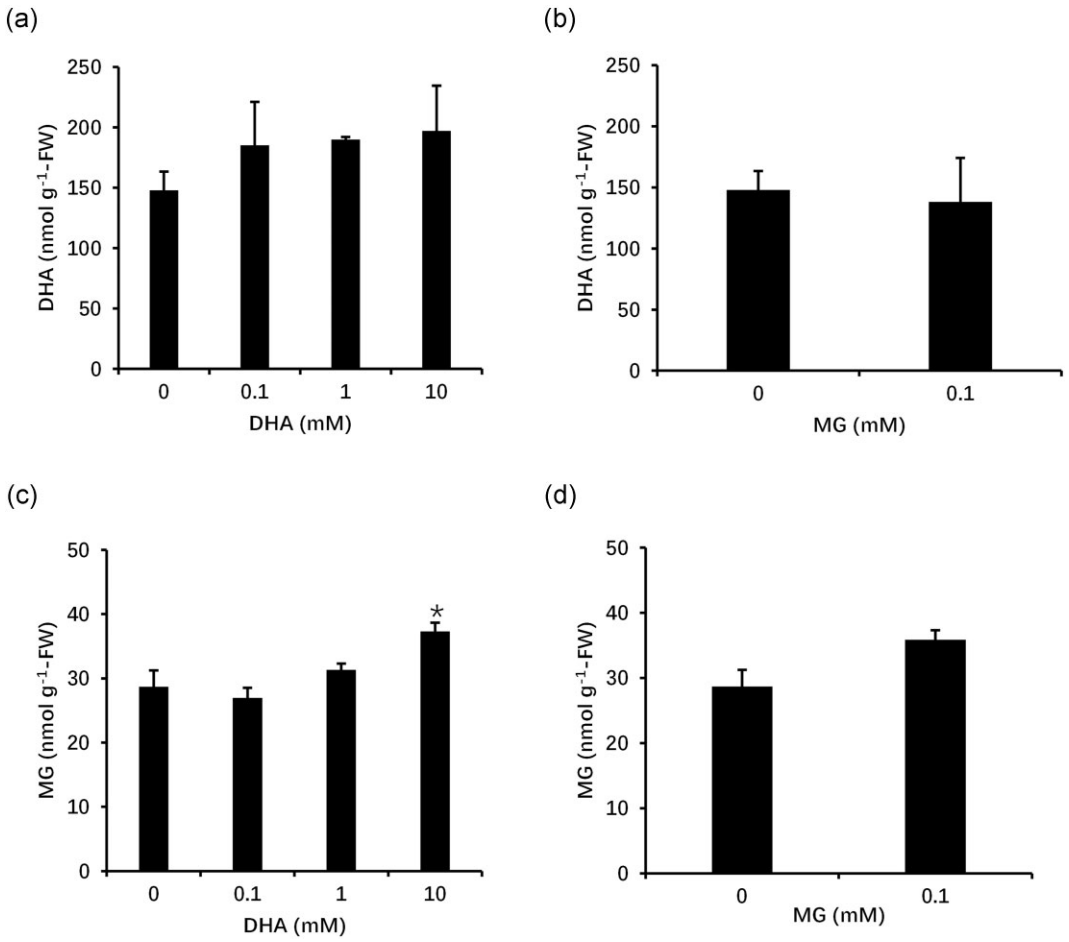

Figure 5. Contents of DHA and MG in Arabidopsis roots. Seven-day-old seedlings grown in the agar plates in the absence of DHA or MG were transferred to and incubated in the agar plates containing 0, 0.1, 1.0, and 10 mM DHA or MG for another 7 d. Contents of DHA (a, b) and MG (c, d) were expressed in nmol g^{-1}-FW. The data of DHA content for 0 mM DHA shown in (b) are the same as those for 0 mM MG shown in (a) and the data of MG content for 0 mM DHA shown in (d) are the same as those for 0 mM MG shown in (c). Values represent means ± standard error ($n = 3$). The asterisk indicates significant difference $P < .05$ (vs 0 mM).

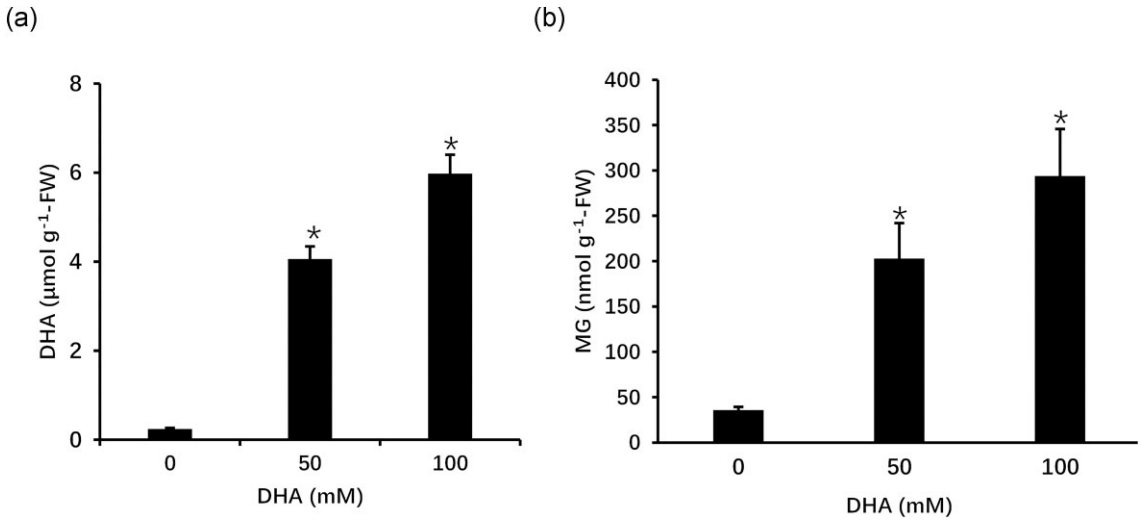

Figure 6. Contents of DHA and MG in Arabidopsis roots. Seven-day-old seedlings grown in the agar plates in the absence of DHA were transferred to and incubated in the agar plates containing 50 and 100 mM DHA for another 2 d. Contents of DHA (a) were expressed in μmol g^{-1}-FW. Contents of MG (b) were expressed in nmol g^{-1}-FW. Values represent means ± standard error ($n = 3$). The asterisk indicates significant difference $P < .05$ (vs 0 mM).

The glyoxalase system is the 2-step pathway that detoxifies ubiquitously present cytotoxic metabolite MG in plants (Kaur *et al.* 2014a,b). The glyoxalase activity is considered as a marker for various stresses because the stresses drastically increased the activity (Kaur *et al.* 2014b). The application of MG elevated Gly I and Gly II activities in the shoots of Arabidopsis seedlings (Hoque *et al.* 2017). In this study, the application of 1.0 mM MG increased Gly I and Gly II activities in the shoots (Figure 4b and d). Furthermore, in this study, the application of 10 mM DHA also significantly increased Gly I activity ($P < .05$) (Figure 4a) but not Gly II activity (Figure 4c) in the shoots of Arabidopsis seedlings, which can be induced by the conversion of DHA to MG.

In conclusion, DHA is not toxic at the physiological concentrations, but the conversion of DHA to MG is responsible for the toxicity of DHA at high concentrations in Arabidopsis.

Data availability

The analyzed data sets generated during the study are available from the corresponding author upon reasonable request.

Author contribution

M.Z., S.M., and Y.M. designed the research. M.Z. performed all experiments with support of I.C.M. and T.N. M.Z. analyzed data. I.C.M., T.N., and Y.N. provided suggestions. M.Z. and Y.M. wrote the manuscript.

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Disclosure statement

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References

Abraham PE, Yin H, Borland AM *et al.* Transcript, protein, and metabolite temporal dynamics in the CAM plant *Agave*. *Nat Plants* 2016;**2**:16178.

Brouquisse R, Rolin D, Cortès S *et al.* A metabolic study of the regulation of proteolysis by sugars in maize root tips: effects of glycerol and dihydroxyacetone. *Planta* 2007;**225**:693-709.

Clearwater MJ, Noe ST, Manley-Harris M *et al.* Nectary photosynthesis contributes to the production of mānuka (*Leptospermum scoparium*) floral nectar. *New Phytol* 2021;**232**:1703-17.

Dakin HD, Dudley HW. An enzyme concerned with the formation of hydroxy acids from ketonic aldehydes. *J Biol Chem* 1913;**14**:155-7.

Fuleki T, Francis FJ. Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanins in cranberries. *J Food Sci* 1968;**33**:72-7.

Hasanuzzaman M, Fujita M. Exogenous sodium nitroprusside alleviates arsenic-induced oxidative stress in wheat (*Triticum aestivum* L.) seedlings by enhancing antioxidant defense and glyoxalase system. *Ecotoxicology* 2013;**22**:584-96.

Hasanuzzaman M, Nahar K, Anee TI *et al.* Glutathione in plants: biosynthesis and physiological role in environmental stress tolerance. *Physiol Mol Biol Plants* 2017;**23**:249-68.

Hoque TS, Hossain MA, Mostofa MG *et al.* Methylglyoxal: an emerging signaling molecule in plant abiotic stress responses and tolerance. *Front Plant Sci* 2016;**7**:1341.

Hoque TS, Uraji M, Hoque MA *et al.* Methylglyoxal induces inhibition of growth, accumulation of anthocyanin, and activation of glyoxalase I and II in *Arabidopsis thaliana*. *J Biochem Mol Toxicol* 2017;**31**:e21901.

Hoque TS, Uraji M, Tuya A *et al.* Methylglyoxal inhibits seed germination and root elongation and up-regulates transcription of stress-responsive genes in ABA-dependent pathway in *Arabidopsis*. *Plant Biol (Stuttg)* 2012;**14**:854-8.

Hossain MA, Burritt DJ, Fujita M. Cross-stress tolerance in plants: molecular mechanisms and possible involvement of reactive oxygen species and methylglyoxal detoxification systems. In: Tuteja N Gill SS (eds). *Abiotic Stress Response in Plants*. Weinheim: John Wiley & Sons, 2016, 327-80.

Kaur C, Ghosh A, Pareek A *et al.* Glyoxalases and stress tolerance in plants. *Biochem Soc Trans* 2014a;**42**:485-90.

Kaur C, Singla-Paree SL, Sopory SK. Glyoxalase and methylglyoxal as biomarkers for plant stress tolerance. *Crit Rev Plant Sci* 2014b;**33**:429-56.

Lerner HR, Avron M. Dihydroxyacetone kinase activity in *Dunaliella parva*. *Plant Physiol* 1977;**59**:15-7.

Liu D, Li M, Liu Y *et al.* Integration of the metabolome and transcriptome reveals the resistance mechanism to low nitrogen in wild soybean seedling roots. *Environ Exp Bot* 2020;**175**:104043.

Mano J, Biswas MS. Analysis of reactive carbonyl species generated under oxidative stress. *Methods Mol Biol* 2018;**1743**:117-24.

Millner JP, Hamilton G, Ritchie C *et al.* High UMFÂ® honey production from manuka plantations. *Hill Ctry Grassl Res Pract Ser* 2016;**16**:113-8.

Naing AH, Kim CK. Abiotic stress-induced anthocyanins in plants: their role in tolerance to abiotic stresses. *Physiol Plant* 2021;**172**:1711-23.

Schad M, Mungur R, Fiehn O *et al.* Metabolic profiling of laser microdissected vascular bundles of *Arabidopsis thaliana*. *Plant Methods* 2005;**1**:2.

Stasiak-Różańska L, Błażejak S. Acetic acid bacteria—perspectives of application in biotechnology—a review. *Polish J Food Nutr Sci* 2009;**59**:17-23.

Yadav SK, Singla-Pareek SL, Reddy MK *et al.* Transgenic tobacco plants overexpressing glyoxalase enzymes resist an increase in methylglyoxal and maintain higher reduced glutathione levels under salinity stress. *FEBS Lett* 2005;**579**:6265-71.

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