



Research article

Methylglyoxal triggers the heat tolerance in maize seedlings by driving AsA-GSH cycle and reactive oxygen species-/methylglyoxal-scavenging system

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ABSTRACT

Traditionally, methylglyoxal (MG) was looked upon as a toxic byproduct of cellular metabolism. Nowadays, MG has been found to be a novel signaling molecule. However, whether MG can trigger the heat tolerance in maize seedlings and the underlying mechanisms is still elusive. In this study, the maize seedlings irrigated with MG increased the survival percentage of seedlings under heat stress (HS), remitted a decrease in tissue vitality and an increase in electrolyte leakage, and reduced membrane lipid peroxidation, implying MG could trigger the heat tolerance of maize seedlings. The further experiments showed that MG drove the ascorbic acid (AsA)-glutathione (GSH) cycle by activating enzymes (glutathione reductase, monodehydroascorbate reductase, dehydroascorbate reductase, and ascorbate peroxidase) and increasing the contents of antioxidants (AsA and GSH) and the ratio of GSH/(GSH + oxidized glutathione) and AsA/(AsA + dehydroascorbate) under both non-HS and HS. Also, the reactive oxygen species (ROS)-scavenger system (catalase, guaiacol peroxidase, carotenoid, total phenols, and flavonoids) and MG-scavenger system (glyoxalase I and glyoxalase II) also were up-regulated in maize seedlings pretreated with MG under non-HS and HS. This work for the first time reported that MG could trigger the heat tolerance of maize seedlings by driving the AsA-GSH cycle and ROS-/MG-scavenging system.

1. Introduction

Historically, methylglyoxal (MG) is looked up as a toxic byproduct of cellular metabolism, especially glycolysis and photosynthesis (Kaur et al., 2016; Hoque et al., 2016; Li, 2016; Mostofa et al., 2018). Excessive accumulation of MG in plants can lead to carbonyl stress (also known as MG stress), namely MG can rapidly react with amino acid residues (e.g. arginine, lysine, and cysteine) of proteins, bases of nucleic acids (e.g. guanine), and membrane phospholipids, producing advanced glycation end products (AGEs). The glycation of proteins, nucleotides, and phospholipids results in the misfolding of proteins, mutagenesis, apoptosis, and membrane lipid bilayer damage, respectively (Wahid et al., 2007; Mittler et al., 2012; Bitá and Gerats, 2013; Hasanuzzaman et al., 2013; Asthir, 2015; Hemmati et al., 2015; Kollist et al., 2019).

Nowadays, MG, similar to Ca²⁺, hydrogen peroxide (H₂O₂), nitric oxide (NO), and hydrogen sulphide (H₂S), has been found to be a novel signaling molecule with multifunction in plants, participating in cellular metabolism, plant growth, development, reproduction, and

response and adaptation to environmental stress (Kaur et al., 2016; Hoque et al., 2016; Li, 2016; Mostofa et al., 2018). In general, MG, as a signaling molecule, can be maintained homeostasis in plant cells by the coordinated action of MG-generating (monoamine oxidase, cytochrome P450 oxidase, MG synthase, and the conversation between dihydroxyacetone phosphate and glyceraldehyde-3-phosphate) and scavenging systems (glyoxalase I, glyoxalase II, and glyoxalase III) (Kaur et al., 2016; Hoque et al., 2016; Li, 2016; Mostofa et al., 2018). Recently, the positive effect of MG was first reported by Bless et al. (2017), who found that in *Brassica rapa* seedling, MG pretreatment could improve the seed germination and seedling growth under zirconium stress. Afterwards, MG has been found to involve in granule formation and starch synthesis in rice endosperm (You et al., 2019), response to magnesium-deficiency in *Citrus sinensis* (Cai et al.), and the enhancement of somatic embryogenesis in sugarcane (Mahlanza et al., 2019). Our previous findings also showed that MG pretreatment increased the survival of maize seedlings under heat stress (HS), this increase was closely associated with signaling molecule H₂S (Li et al., 2018a). These data further support the signaling role of MG in plants. However, the

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underlying mechanism of MG-induced heat tolerance in maize seedlings is still elusive.

HS is a major stress factor that impacts on cellular metabolism, plant growth, development, and yield. HS commonly causes direct and indirect injury, the former is involved in the loss of biomembrane integrity and cellular compartmentation as well as the denaturation of proteins; the latter refers to the secondary injury, namely oxidative stress induced by overproduction of reactive oxygen species (ROS), MG stress triggered by overaccumulation of MG, osmotic stress caused by the decline in osmotic potential and water potential in plant cells, and so forth (Wahid et al., 2007; Hasanuzzaman et al., 2013; Asthir, 2015; Hemmati et al., 2015; Ohama et al., 2017; Lawas et al., 2018; Sami et al., 2019). To cope with these injuries, numerous adaptation and tolerance strategies are developed during plant evolution. These strategies include the recovery and remodelling of biomembrane and cellular compartmentation by modifying components and saturation of membrane lipids, synthesis of stress proteins (such as heat shock proteins: HSPs), stimulation of ROS/MG-scavenging system (including antioxidant system and glyoxalase system), and accumulation of osmolytes (such as proline, glycine betaine, trehalose, and soluble sugar), and so on (Wahid et al., 2007; Hasanuzzaman et al., 2013; Asthir, 2015; Hemmati et al., 2015; Ohama et al., 2017; Lawas et al., 2018; Sami et al., 2019; Zhang et al., 2019).

Maize not only is a main crop plant, but also a new model plant, its production is largely affected by HS (Leipner and Stamp, 2009; Strable and Scanlon, 2009). Seeking the efficient approaches to improve plant heat tolerance and understanding its possible mechanism is still a hot topic in plant stress biology. Nevertheless, whether MG pretreatment could trigger the heat tolerance and its relation to ascorbate-glutathione (AsA-GSH) cycle and ROS/MG-scavenging system still remains unclear. In this study, the effect of MG pretreatment on the heat tolerance of maize seedlings and the underlying mechanisms are investigated, which aims to illustrate the joint action of AsA-GSH cycle and ROS/MG-scavenging system in MG-triggered heat tolerance in plants.

2. Materials and methods

2.1. Plant material culture and methylglyoxal pretreatment

Maize (*Zea mays* L., Ludan No. 8) seeds were purchased from Yiliang Seed Company, China. The seeds were sterilized in 0.1% HgCl₂ solution for 10 min and then washed thoroughly with sterile water. The sterilized seeds were imbibed in distilled water and then sowed on the six-layer filter papers in trays (approximately 250 seeds per tray) with covers. Afterwards, the seeds were germinated at 26 °C for 2.5 d (60 h) in the dark (the germination rate was above 90%). The uniform seedlings (about 230 seedlings per tray) were classified into four groups and irrigated with 0 (control), 50, 100, and 150 μM methylglyoxal (MG) for 6 h (MG pretreatment had no significant effect on seedling growth, indicating MG had no toxic effect on maize seedlings at these experiment concentrations). After irrigation, the roots of maize seedlings were washed five times with distilled water to remove residue MG on the root surface. The control and pretreated maize seedlings were subjected to HS at 46 °C for 16 h (the suitable parameters were screened from preliminary experiments) and then recovered in a climate chamber (26 °C, 100 μmol m⁻²s⁻¹, and 12 h photoperiod), as well as supplied nutrients with 1/2 Hoagland solution.

2.2. Measurement of survival percentage, lipid peroxidation, tissue vitality, and electrolyte leakage in maize seedlings

After recovery, namely on the seventh day, the survival percentage of maize seedlings was scored according to the formula: survival percentage (%) = survived seedlings/total seedlings × 100%. Meanwhile, photographs were taken.

To further explore the effect of MG pretreatment on lipid peroxidation (reflected in malondialdehyde: MDA), tissue vitality (the reduction of triphenyl tetrazolium chloride: TTC), and electrolyte leakage of maize seedlings under HS at 46 °C for 16 h, they were determined after HS according to the methods of Li et al. (2012). The MDA content, tissue vitality, and electrolyte leakage were expressed in μmol·g⁻¹ fresh weight (FW), A₄₈₅, and %, respectively.

2.3. Determination of antioxidant enzyme activity in maize seedlings

At 6-h MG-pretreatment and 16-h HS, antioxidant enzymes glutathione reductase (GR), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and superoxide dismutase (SOD) in maize seedlings were extracted and determined as per Li et al. (2014). The change in absorbance of GR, CAT, APX, GPX, and SOD was separately recorded at 340, 240, 290, 470, and 560 nm, their activities were expressed as μmol g⁻¹ FW min⁻¹ and U g⁻¹ FW, respectively.

In addition, monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione-S-transferase (GST) in maize seedlings were extracted and measured on the basis of Rahman et al. (2016). The change in optical density of MDHAR, DHAR, and GST was observed at 265 and 340 nm, respectively, their activities were calculated using extinction coefficient of 6.2, 14, and 9.6 mM⁻¹ cm⁻¹, and expressed in μmol g⁻¹ FW min⁻¹.

Similarly, phenylalanine ammonia-lyase (PAL) in maize seedlings was extracted and estimated based on the procedure described by Nadernejad et al. (2013). An increase in the optical density was recorded at 290 nm due to the production of cinnamic acid. The activity of PAL was expressed as μmol g⁻¹ FW min⁻¹.

2.4. Water-soluble and lipid-soluble antioxidant assay in maize seedlings

Similarly, at 6-h MG-pretreatment and 16-h HS, water-soluble antioxidants glutathione (reduced glutathione: GSH and oxidized GSH: GSSG), ascorbate (reduced ascorbate: AsA and oxidized AsA: DHA), total phenols (TP), and flavonoids (FLA), as well as lipid-soluble antioxidant carotenoids (CAR) in maize seedlings were extracted and assayed. GSH, GSSG, GSH/GSH + GSSG, AsA, DHA, and AsA/AsA + DHA were estimated in line with the procedure described by Li et al. (2014); TP, FLA, and CAR contents were assayed in the light of Rahman et al. (2016). The contents of GSH, GSSG, AsA, and DHA were expressed as μmol g⁻¹ FW, while TP, FLA, and CAR as μg g⁻¹ FW.

2.5. Estimate of glyoxalase system and methylglyoxal in maize seedlings

Meanwhile, at 6-h MG-pretreatment and 16-h HS, glyoxalase system activity (Gly I and Gly II) and exogenous methylglyoxal (MG) content in maize seedlings were estimated using the previously described methods (Li et al., 2018). The activities of Gly I and Gly II were separately calculated using extinction coefficient of 3.37 mM⁻¹ cm⁻¹, and expressed in μmol g⁻¹ FW min⁻¹, whereas MG content was expressed as μmol g⁻¹ FW.

2.6. Statistical analysis

Experiments were completely randomized design and repeated at least three times and two replications in each. The data were carried statistically out using one-way analysis of variance (ANOVA). The figures were plotted using sigmaplot 12.5. The significant difference was estimated using the least significant difference (LSD) test, asterisk (*) and double asterisks (**) in figures indicated significant differences ($P < 0.05$) and very significant differences ($P < 0.01$) compared with the control, respectively. The data in figures were expressed in means ± standard error (SE).

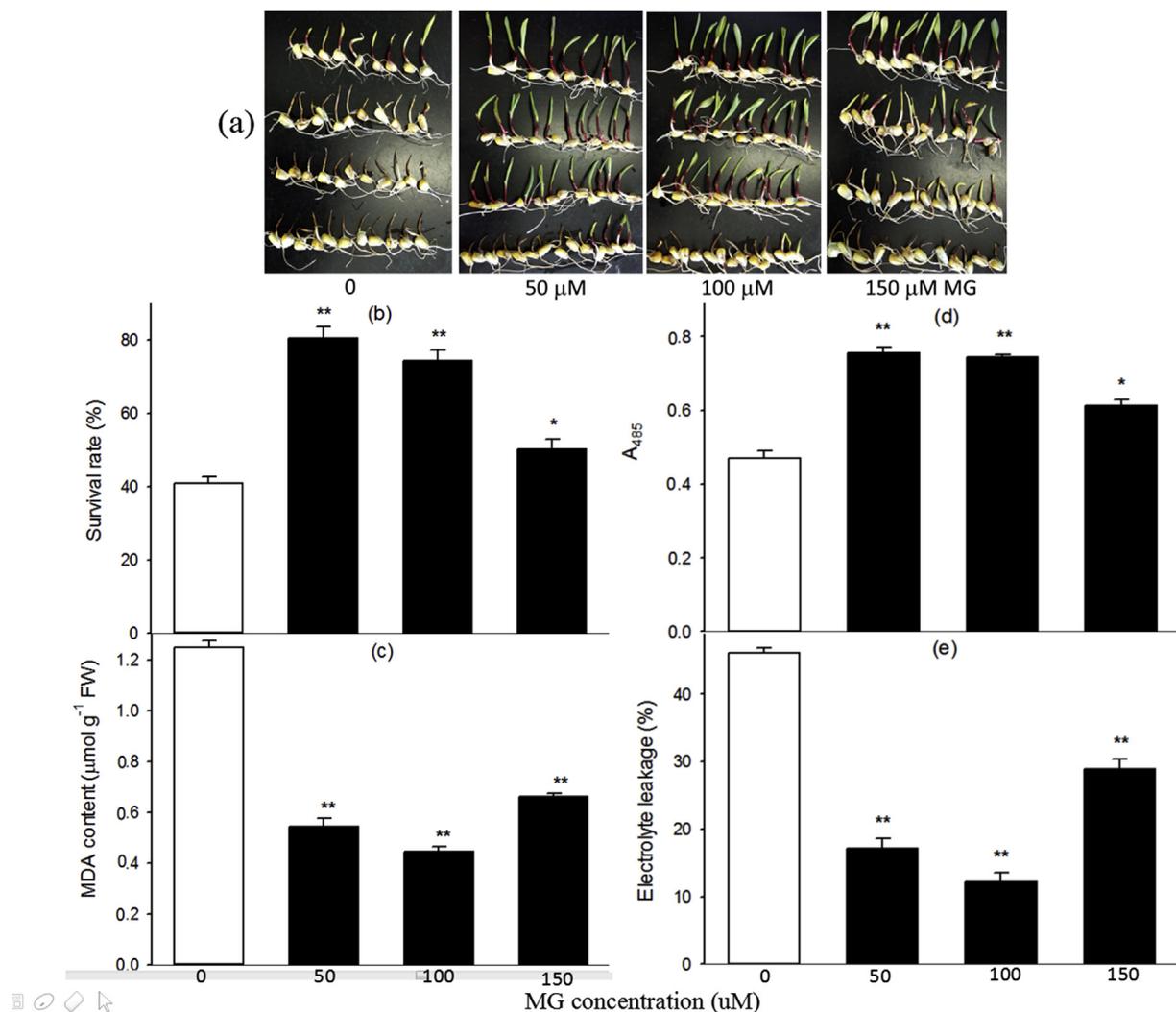


Fig. 1. Effect of irrigation of maize seedlings with different concentrations of methylglyoxal (MG) on the survival percentage (A, B), malondialdehyde (MDA) content (C), tissue vitality (D), and biomembrane integrity (E) under heat stress (HS). The 2.5-day-old maize seedlings were irrigated with different concentrations of MG for 6 h, and then exposed to HS at 46 °C for 16 h. After HS, the seedlings were recovered at 26 °C for 7 d. After HS, MDA content, tissue vitality, and biomembrane integrity were determined; after recovery, the survival percentage was counted and the photographs were taken. In figures, the data represented means \pm standard error (SE), and asterisk (*) and double asterisks (**) indicated significant differences ($P < 0.05$) and very significant differences ($P < 0.01$) compared with the control, respectively.

3. Results

3.1. MG triggers the heat tolerance in maize seedlings

The 2.5-day-old maize seedlings were irrigated with the different concentrations of methylglyoxal (MG), and then exposed to HS at 46 °C for 16 h. The data as shown in Fig. 1A and B, as expected, MG pretreatment increased the survival percentage of maize seedlings and improved the seedling growth under HS compared with the control without MG pretreatment in a concentration-dependent manner, the pretreatment with 50, 100, and 150 μM MG reached very significant ($P < 0.01$) and significant difference ($P < 0.05$), indicating 50 μM MG is the most effective concentration. Similarly, in comparison to the control, MG pretreatment enhanced the tissue vitality of maize seedlings, and the significance is similar to that of the survival percentage (Fig. 1D). In addition, MG alleviated the membrane lipid peroxidation and loss of biomembrane integrity, as reflected in a lower accumulation of malondialdehyde (MDA) and less electrolyte leakage compared with the control, the alleviation reached very significant difference, respectively ($P < 0.01$, Fig. 1C, E). These data showed that MG pretreatment could trigger the heat tolerance of maize seedlings in a dose-dependent

manner.

3.2. MG drives the AsA-GSH cycle in maize seedlings under non-HS and HS conditions

To explore the effect of MG pretreatment on AsA-GSH cycle in maize seedlings under non-HS and HS conditions, GR, GSH, GSSG, GSH/(GSH + GSSG), MDHAR, DHAR, AsA, DHA, and AsA/(AsA + DHA) were determined. Under non-HS conditions, compared with the control, MG pretreatment activated GR activity ($P < 0.01$ at 50, 100, and 150 μM, Fig. 2A), increased GSH content ($P < 0.01$ at 50 and 100 μM, Fig. 2B) and GSH/(GSH + GSSG) ratio ($P < 0.01$ at 50, 100, and 150 μM, Fig. 2D), but decreased GSSG level ($P < 0.01$ at 100 and 150 μM) in maize seedlings (Fig. 2C). Under HS conditions, compared with the non-HS seedlings, GR, GSH, and GSSG all declined, while GSH/(GSH + GSSG) ratio increased; but the seedlings pretreated with MG maintained a higher GR activity ($P < 0.01$ at 50, 100, and 150 μM, Fig. 2A), GSH ($P < 0.01$ at 50 and 100 μM, Fig. 2B) and GSSG ($P < 0.01$ at 50 and 100 μM; $P < 0.05$ at 150 μM, Fig. 2C) contents, whereas a lower GSH/(GSH + GSSG) ratio ($P < 0.01$ at 50, 100, and 150 μM, Fig. 2D) compared with the control.

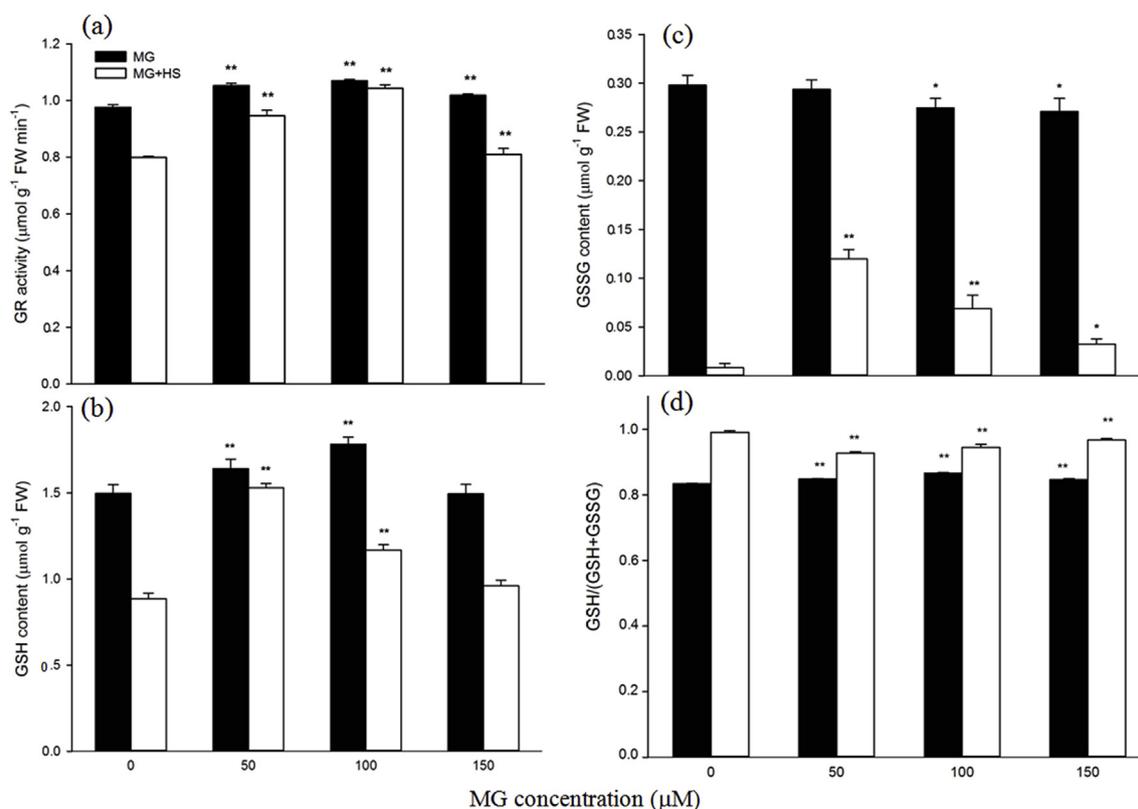


Fig. 2. Effect of irrigation of maize seedlings with different concentrations of methylglyoxal (MG) on glutathione reductase (GR, A) activity, reduced glutathione (GSH, B) and oxidized glutathione (GSSG, C) contents, and GSH/GSH + GSSG ratio (D) under non-HS and HS conditions. The 2.5-day-old maize seedlings were irrigated with different concentrations of MG for 6 h, and then exposed to HS at 46 °C for 16 h. After MG pretreatment and HS, GR, GSH, GSSG, and GSH/GSH + GSSG were assayed. In figures, the data represented means \pm standard error (SE), and asterisk (*) and double asterisks (**) indicated significant differences ($P < 0.05$) and very significant differences ($P < 0.01$) compared with the control, respectively.

For MDHAR, DHAR, AsA, DHA, and AsA/(AsA + DHA), under non-HS conditions, MG increased MDHAR activity ($P < 0.01$ at 50 μM, Fig. 3A), AsA content ($P < 0.01$ at 50 and 100 μM, Fig. 3C), and AsA/(AsA + DHA) ratio ($P < 0.01$ at 50 and 100 μM, Fig. 3E); decreased DHAR activity ($P < 0.05$ at 50 and 100 μM, $P < 0.01$ at 150 μM, Fig. 3B) and DHA content ($P < 0.01$ at 50 and 100 μM, $P < 0.05$ at 150 μM, Fig. 3D) in comparison to the control. In addition, under HS, MDHAR, DHAR, AsA, and DHA all descended, but AsA/(AsA + DHA) ratio ascended in comparison to the non-HS conditions (Fig. 3); in comparison with the control, the MG-pretreated maize seedlings had higher MDHAR ($P < 0.01$ at 50, 100, 150 μM, Fig. 3A) and DHAR ($P < 0.01$ at 50, 100, and 150 μM, Fig. 3B) activities, AsA content ($P < 0.01$ at 50 and 100 μM, $P < 0.05$ at 150 μM, Fig. 3C), and AsA/(AsA + DHA) ratio ($P < 0.01$ at 50 and 100 μM, $P < 0.05$ at 150 μM, Fig. 3E); but a lower DHA level ($P < 0.01$ at 50 and 100 μM, Fig. 3D).

This section data indicated that MG pretreatment could drive the AsA-GSH cycle by activating enzyme activity (GR, MDHAR, and DHAR) and modifying relevant components (GSH, GSSG, AsA, and DHA), as well as the ratio of GSH/(GSH + GSSG) and AsA/(AsA + DHA) in maize seedlings under both non-HS and HS conditions.

3.3. MG stimulates antioxidant system in maize seedlings under non-HS and HS conditions

In addition to AsA-GSH cycle, other antioxidant enzymes and non-enzymatic antioxidants in maize seedlings under non-HS and HS conditions also were assayed. The results as indicated in Fig. 4, under non-HS conditions, compared with the control, MG pretreatment stimulated CAT activity ($P < 0.01$ at 50, 100, and 150 μM, Fig. 4A), but lowered APX ($P < 0.01$ at 50 and 100 μM, $P < 0.05$ at 150 μM, Fig. 4B) and GPX ($P < 0.01$ at 100 and 150 μM, Fig. 4C) activities, while the change

in the activities of GST, PAL, and SOD was not significant (Fig. 4D, E, F). Under HS, compared with the non-HS seedlings, CAT, GPX, GST, and PAL activities descended, whereas APX activity ascended, but the significant difference was not observed in SOD activity (Fig. 4); in comparison to the control, MG pretreatment increased the activities of GPX ($P < 0.01$ at 50, 100, and 150 μM, Fig. 4C), GST ($P < 0.01$ at 50 and 100 μM, Fig. 4D), and PAL ($P < 0.01$ at 50 and 100 μM, Fig. 4E); but reduced APX activity (but higher than that of non-HS seedlings, $P < 0.01$ at 50 and 100 μM, $P < 0.05$ at 150 μM, Fig. 4B), while the change in the activities of CAT and SOD had no significance (Fig. 4A, F).

For other non-enzyme antioxidants FLA, CAR, and TP, under non-HS conditions, MG pretreatment raised FLA ($P < 0.01$ at 50 μM, but declined at 150 μM, $P < 0.01$; Fig. 5A) and CAR ($P < 0.05$ at 50, 100, and 150 μM, Fig. 5B) contents, but no significant effect on TP content (Fig. 5C). Under HS, FLA and TP contents declined, while CAR content increased compared with the non-HS seedlings (Fig. 5); in comparison to the control, the MG-pretreated seedlings maintained higher levels of FLA ($P < 0.01$ at 50 and 100 μM, Fig. 5A), CAR ($P < 0.01$ at 50 and 100 μM, Fig. 5B), and TP ($P < 0.01$ at 50 and 100 μM, Fig. 5C).

This section results suggested that MG pretreatment was able to stimulate antioxidant enzymes (CAT, APX, GPX, GST, PAL, and SOD) and non-enzymatic antioxidants (FLA, CAR, and TP) in maize seedlings under both non-HS and HS conditions.

3.4. Exogenous MG modifies glyoxalase system and endogenous MG level in maize seedlings under non-HS and HS conditions

In addition, to further study the effect of exogenous MG pretreatment on glyoxalase system and endogenous MG level under non-HS and HS conditions, Gly I and Gly II activities and MG content in maize seedlings were tested. The data indicated that under non-HS conditions,

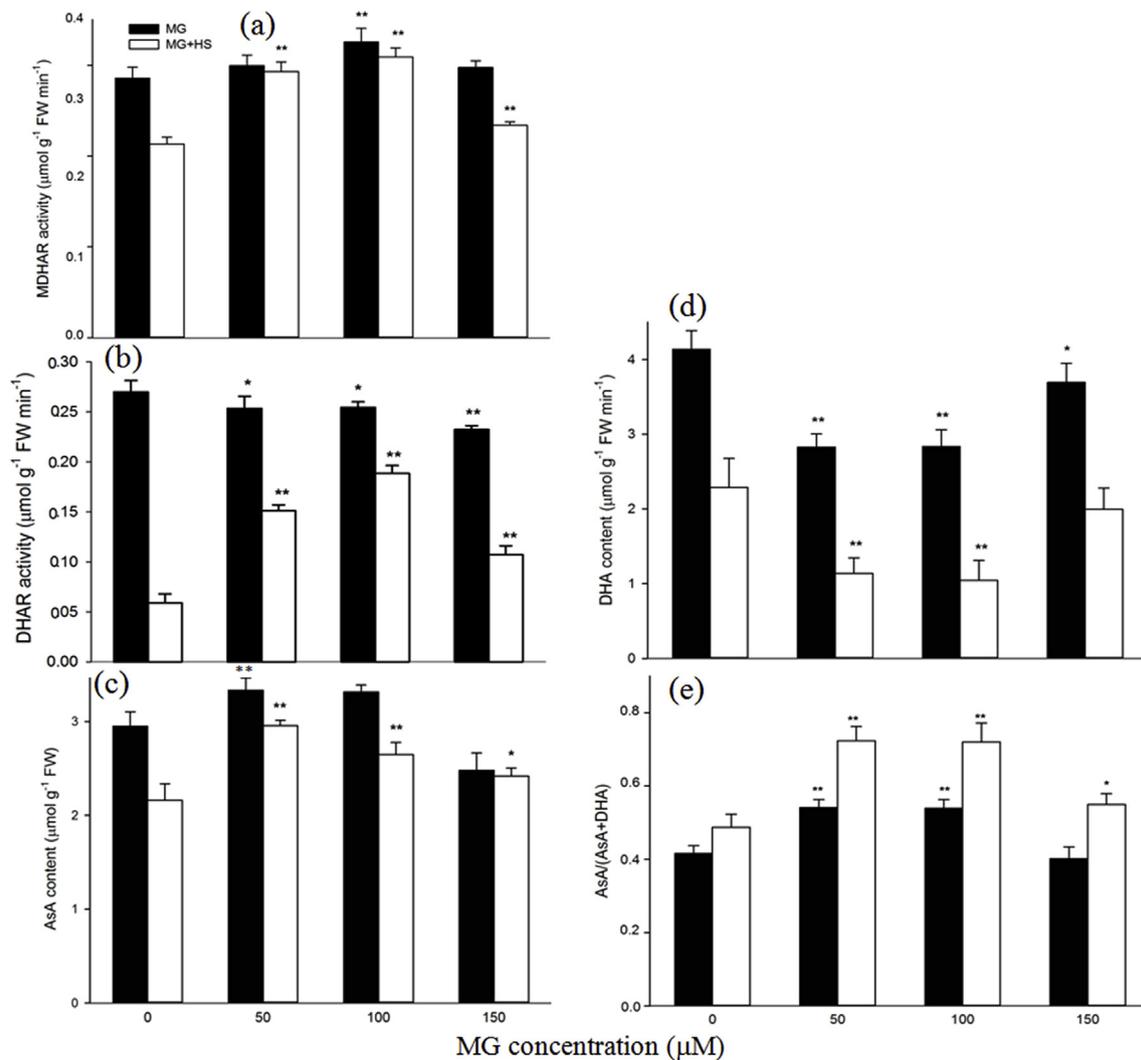


Fig. 3. Effect of irrigation of maize seedlings with different concentrations of methylglyoxal (MG) on monodehydroascorbate reductase (MDHAR, A) and dehydroascorbate reductase (DHAR, B) activities, reduced ascorbate (AsA, C) and dehydroascorbate (DHA, D) contents, and AsA/AsA + DHA ratio (E) under non-HS and HS conditions. The 2.5-day-old maize seedlings were irrigated with different concentrations of MG for 6 h, and then exposed to HS at 46 °C for 16 h. After MG pretreatment and HS, MDHAR, DHAR, AsA, DHA, AsA/AsA + DHA were measured. In figures, the data represented means \pm standard error (SE), and asterisk (*) and double asterisks (**) indicated significant differences ($P < 0.05$) and very significant differences ($P < 0.01$) compared with the control, respectively.

compared with the control, exogenous MG pretreatment activated the activities of Gly I ($P < 0.05$ at 50 μM, Fig. 6A) and Gly II ($P < 0.01$ at 50 and 100 μM, but declined at 150 μM, $P < 0.01$; Fig. 6B) as well as the contents of endogenous MG ($P < 0.01$ at 50 and 100 μM, Fig. 6C). Under HS, Gly I and endogenous MG content increased, but Gly II activity decreased (Fig. 6) compared with the non-HS seedlings; in comparison to the control, the MG-pretreated seedlings showed higher Gly I ($P < 0.01$ at 50 and 100 μM, Fig. 6A) and Gly II ($P < 0.01$ at 50 and 100 μM, $P < 0.05$ at 150 μM, Fig. 6B) activities as well as endogenous MG level ($P < 0.01$ at 50, 100, and 100 μM, Fig. 6C).

The data in this section implied that exogenous MG pretreatment was capable to increase endogenous MG level, as signaling molecule, which in turn activated Gly I and Gly II in maize seedlings under both non-HS and HS conditions.

4. Discussion

Increasing evidences illustrate that MG as signaling molecule is involved in response and adaptation to environmental stress (Kaur et al., 2016; Hoque et al., 2016; Li, 2016; Hasanuzzaman et al., 2018; Mostofa et al., 2018; Nguyen et al., 2019). However, whether MG pretreatment

can trigger the heat tolerance in plants is not clear. The seedling stage is the most important phase in the life cycle of plants and fragile to environmental stress including heat stress. Therefore, the heat tolerance research on seedling stage is of great importance to agricultural production and ecosystem (Fenner and Thompson, 2005). In general, the survival percentage, MDA content, tissue vitality, and electrolyte leakage are used to evaluate stress tolerance including heat tolerance at cell, tissue, and the whole-plant levels and the survival percentage is a robust and comprehensive evidence (Li et al., 2012; Jambunathan, 2010). In the present study showed that pretreatment with different concentrations (50, 100, and 150 μM) of MG was able to alleviate the loss of biomembrane integrity and cellular compartmentation (as indicated in MDA content and electrolyte leakage, Fig. 1C, E) and a decrease in tissue vitality (reflected in the reduction of triphenyl tetrazolium chloride, Fig. 1D), followed by increase in the survival percentage of maize seedlings under HS (Fig. 1A and B), but the effect of a higher concentration (150 μM) was lower than that of the concentrations at 50 and 100 μM, indicating that MG pretreatment was capable to trigger the heat tolerance of maize seedlings in a dose-dependent manner. That is to say, MG at suitable concentrations (50 and 100 μM) could trigger the heat tolerance, but a higher concentration

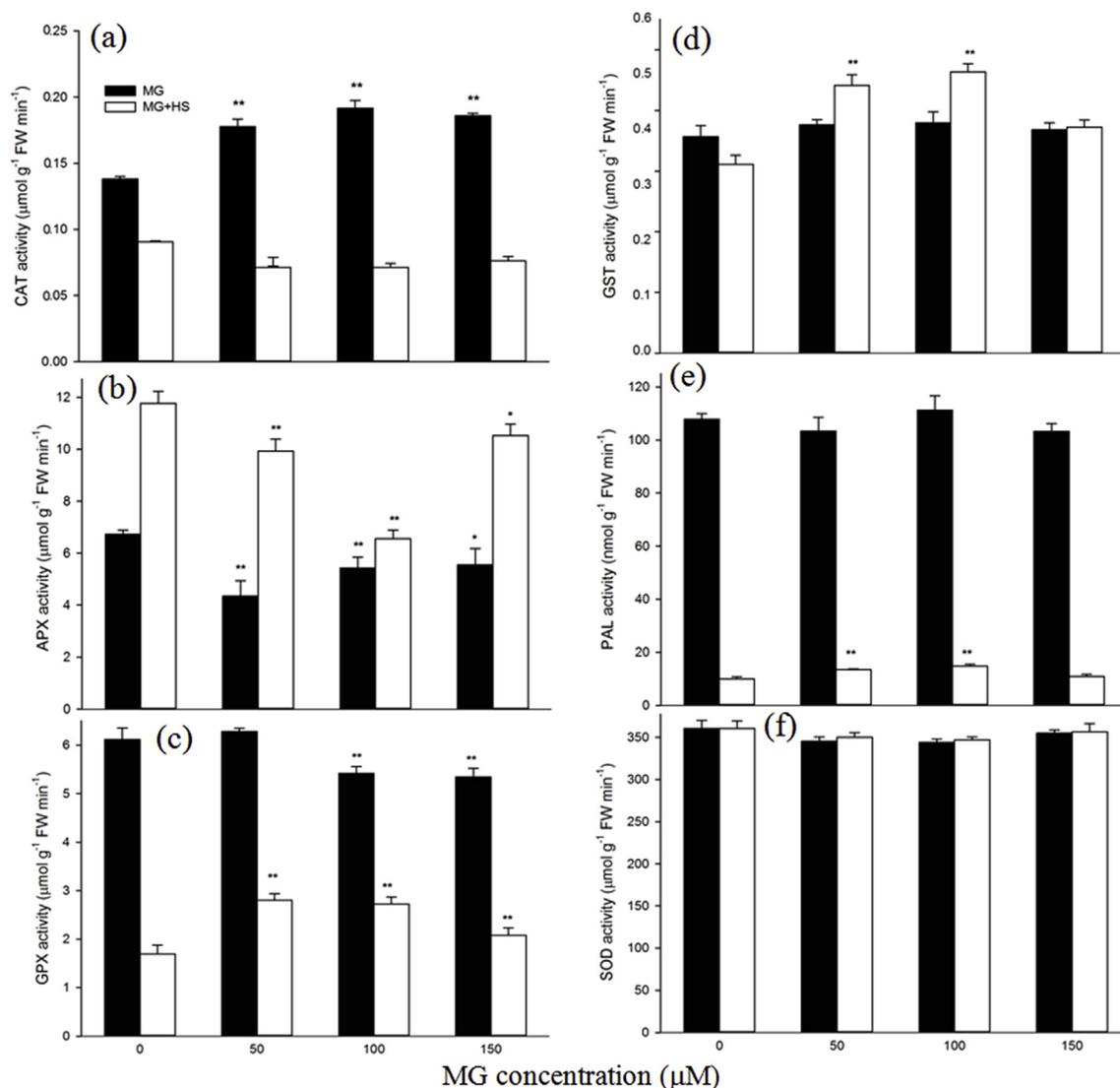


Fig. 4. Effect of irrigation of maize seedlings with different concentrations of methylglyoxal (MG) on the activities of catalase (CAT, A), ascorbate peroxidase (APX, B), guaiacol peroxidase (GPX, C), glutathione-S-transferase (GST, D), phenylalanine ammonia-lyase (PAL, E), and superoxide dismutase (SOD, F) under non-HS and HS conditions. The 2.5-day-old maize seedlings were irrigated with different concentrations of MG for 6 h, and then exposed to HS at 46 °C for 16 h. After MG pretreatment and HS, CAT, APX, GPX, GST, PAL, and SOD were estimated. In figures, the data represented means \pm standard error (SE), and asterisk (*) and double asterisks (**) indicated significant differences ($P < 0.05$) and very significant differences ($P < 0.01$) compared with the control, respectively.

(150 μM) showed up a toxic effect, further supporting the signaling role of MG in plants. Among experiment concentrations, the 50 μM MG is the most effective to trigger heat tolerance in maize seedlings.

AsA-GSH cycle is a major regulator of cellular redox by the joint action of antioxidant enzymes (GR, MDHAR, and DHAR) and non-enzymatic antioxidants (AsA and GSH) (Anjum et al., 2010; Hossain et al., 2018; Shan et al., 2018; Singh et al., 2019). GR catalyzes the conversion of GSSG into GSH using NADPH as hydrogen donor, MDHAR converts monodehydroascorbate (MDHA) into AsA; similarly, DHAR reduces DHA to AsA using GSH as hydrogen donor (Anjum et al., 2010; Hossain et al., 2018; Shan et al., 2018). In model plant Arabidopsis, the transgenic plants with overexpression transcription factor GmNAC085 (from soybean) could resist to drought stress by regulating GSH biosynthesis, redox balance, and GSH-dependent detoxification of ROS and MG (Nguyen et al., 2019). In this study, compared with the control without MG pretreatment, MG pretreatment stimulated the activity of GR in maize seedlings under non-HS conditions, which in turn increased the content of GSH and lowered GSSG level, followed by enhancing the ratio of GSH to total glutathione (GSH + GSSG) (Fig. 2), thus heightening the antioxidant capacity of maize seedlings. Under HS conditions,

compared with the control seedlings, the activities of GR, GSH, and GSSG all dropped (Fig. 2A, B, C), but the GSH/GSH + GSSG ratio increased (Fig. 2D); however, the MG-pretreated maize seedlings maintained a higher GR activity compared with the control, followed by increase in GSH level and GSH/GSH + GSSG ratio (Fig. 2). In addition, under HS conditions, in comparison to the control, the MG-pretreated maize seedlings had a higher GSSG level (but lower than that of non-HS seedlings) (Fig. 2C), suggesting that the combination of MG and HS might trigger oxidative stress, changing the redox status (also serving as a signaling) in plant cells, the reason of which might be MG can induce ROS production by triggering AGEs and/or activating peroxidase (Hoque et al., 2012, 2016; Mostofa et al., 2018). In addition, an over-accumulation of endogenous MG induced by exogenous MG (Fig. 6C) needs to be scavenged by Gly I (using GSH as cofactor) to maintain MG homeostasis in plant cells (Hossain et al., 2018). Similarly, compared with the control, under non-HS conditions, MG increased the activities of MDHAR (Fig. 3A), which in turn raised the level of AsA and AsA/AsA + DHA ratio (Fig. 3C, E), as well as reduced the content of DHA (Fig. 3D), therefore maintaining a higher redox state in plant cells. Under HS conditions, in comparison to the control, the activities of

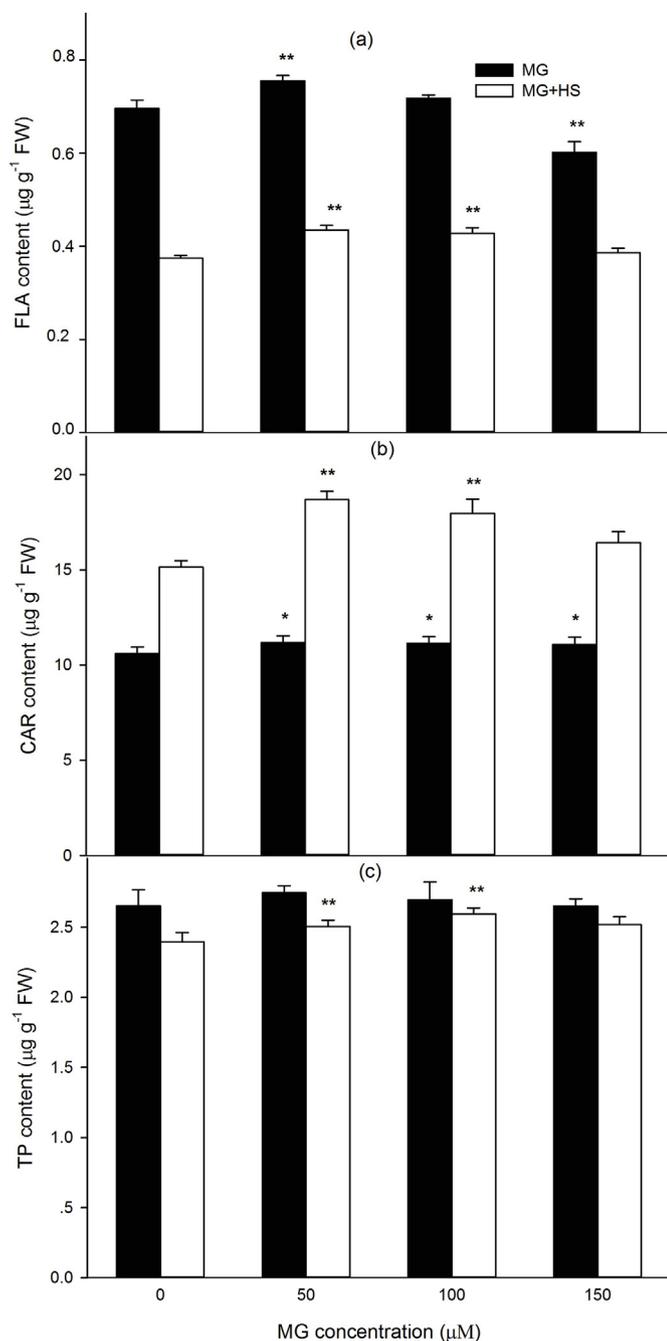


Fig. 5. Effect of irrigation of maize seedlings with different concentrations of methylglyoxal (MG) on flavonoid (FLA, A), carotenoid (CAR, B), and total phenol (TP, C) contents under non-HS and HS conditions. The 2.5-day-old maize seedlings were irrigated with different concentrations of MG for 6 h, and then exposed to HS at 46 °C for 16 h. After MG pretreatment and HS, FLA, CAR, and TP were detected. In figures, the data represented means \pm standard error (SE), and asterisk (*) and double asterisks (**) indicated significant differences ($P < 0.05$) and very significant differences ($P < 0.01$) compared with the control, respectively.

MDHAR, DHAR, AsA, and DHA all dropped (Fig. 3A, B, C, D), but the AsA/AsA + DHA ratio rose (Fig. 3E); however, compared with the control, the maize seedlings pretreated with MG had a higher MDHAR and DHAR activities (Fig. 3A and B), which in turn increased AsA content (Fig. 3C) and AsA/AsA + DHA ratio (Fig. 3E), as well as declined DHA level (Fig. 3D), thus driving the cycle of AsA-GSH. Similarly, in Kentucky bluegrass (*Poa pratensis*), under non-HS conditions, heat-tolerance “Midnight” had a higher gene expression and enzyme

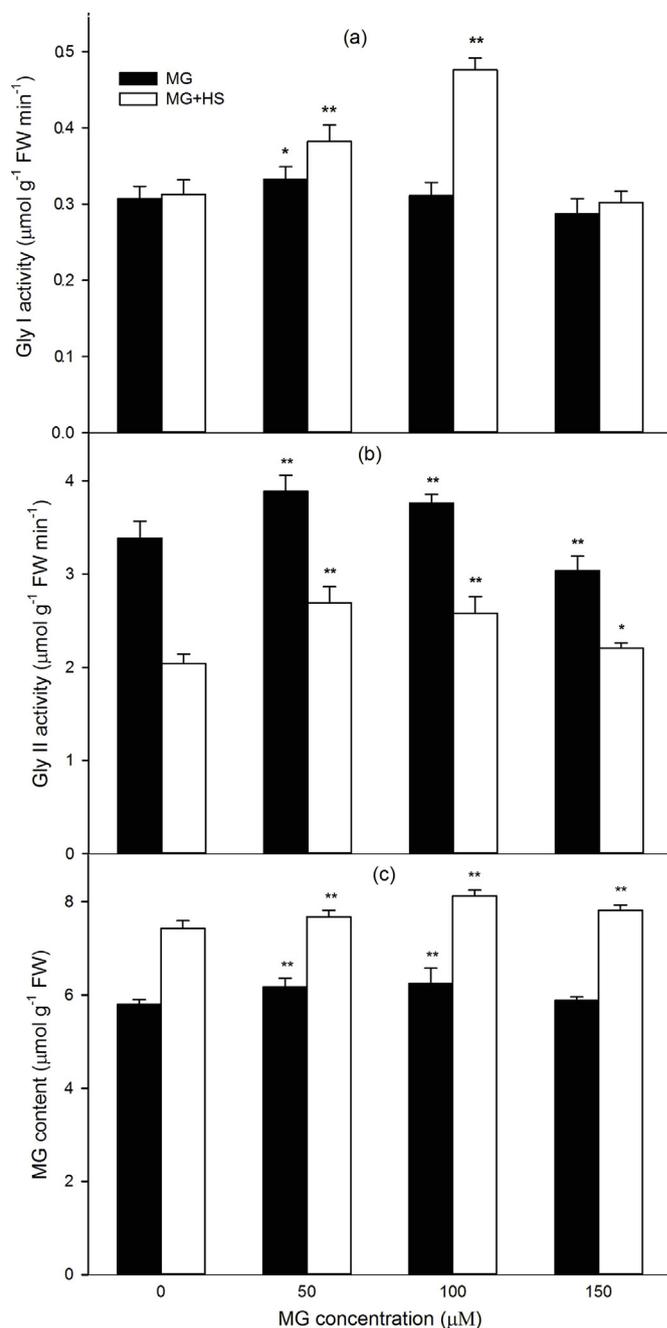


Fig. 6. Effect of irrigation of maize seedlings with different concentrations of exogenous methylglyoxal (MG) on glyoxalase I (Gly I, A) and glyoxalase II (Gly II, B) activities and endogenous MG content (C) under non-HS and HS conditions. The 2.5-day-old maize seedlings were irrigated with different concentrations of exogenous MG for 6 h, and then exposed to HS at 46 °C for 16 h. After MG pretreatment and HS, Gly I, Gly II, and endogenous MG were tested. In figures, the data represented means \pm standard error (SE), and asterisk (*) and double asterisks (**) indicated significant differences ($P < 0.05$) and very significant differences ($P < 0.01$) compared with the control, respectively.

activity related to AsA-GSH cycle (GR, APX, MDHAR, and DHAR) than that of heat-sensitive “Brilliant”; under HS conditions, gene expression and enzyme activity in both varieties were up-regulated, but “Midnight” was more stronger than that of “Brilliant” (Du et al., 2013). In wheat seedlings, our previous data showed that MG pretreatment activated GR, which in turn increase GSH level (Li et al., 2017, 2018b). These results further supported our viewpoint that MG could drive the AsA-GSH cycle by the concerted action of GR, MDHAR, DHAR, GSH,

and AsA to maintain redox balance in plant cells under HS conditions, which might be a physiological basis for MG-induced heat tolerance of maize seedlings.

The cellular redox balance plays a key role in the development of abiotic stress tolerance including heat tolerance in plants (Sewelam et al., 2016; Foyer, 2018; Waszczak et al., 2018; Singh et al., 2019). This balance is not only regulated by AsA-GSH cycle, but also other antioxidant enzymes (CAT, APX, GPX, GST, PAL, and SOD) and non-enzymatic antioxidants (FLA, CAR, and TP) (Sewelam et al., 2016; Foyer, 2018; Waszczak et al., 2018). SOD is the first line of defense against ROS, which converts superoxide radical into H_2O_2 ; CAT, APX, GPX, and GST, with different affinities to H_2O_2 , can scavenge excessive H_2O_2 in the different cellular compartmentations to maintain its homeostasis, which acts as signaling molecule in plant cells; in the meanwhile, antioxidants GSH and AsA were oxidized to GSSG and MDHA by ROS, and then enter into AsA-GSH cycle to remain cellular redox homeostasis (Sewelam et al., 2016; Foyer, 2018; Waszczak et al., 2018). In addition, PAL is a key enzyme in the biosynthesis of flavonoids (FLA) and total phenols (TP) in plants, both of which, along with the CAR, can directly scavenge ROS such as singlet oxygen, superoxide radical, and hydroxyl radical, further keeping cellular redox balance (Mustafa et al., 2010; Agati et al., 2012; Falcone et al., 2012; Amri and Hossain, 2018). In the present study, under non-HS conditions, compared with the control, MG activated CAT activity and enhanced FLA and CAR level in maize seedlings (Figs. 4A and 5A, B), followed by maintaining a higher antioxidant capacity (Figs. 2–5), laying the foundation of the subsequent development of heat tolerance (Fig. 1A and B). Under HS conditions, in comparison to the control, the MG-pretreated maize seedlings showed a higher activity of APX, GPX, GST, and PAL (Fig. 5B, C, D, E), which in turn raised the level of FLA, CAR, and TP (Fig. 6A, B, C), thus enhancing the antioxidant capacity of maize seedlings under HS conditions. The enhancement of antioxidant capacity induced by MG reduced the oxidative damage to cells, namely a lower ROS (data not shown) and MDA accumulation (Fig. 1C). As discussed above, in Kentucky bluegrass, addition to genes and enzymes related to AsA-GSH cycle, other antioxidant enzyme (GPX, CAT, and SOD) gene and activity in both “Midnight” and “Brilliant” also were up-regulated under both non-HS and HS conditions, and the “Midnight” was higher than that of “Brilliant” (Du et al., 2013). In addition, MG pretreatment could activate antioxidant enzymes SOD, CAT, and APX in wheat seedlings, which in turn increased the resistance to salt stress (Li et al., 2017). Similar effect of MG on antioxidant enzymes were also reported by Bless et al. (2017) in *Brassica rapa* under zirconium stress. Therefore, besides AsA-GSH cycle, antioxidant enzymes (CAT, APX, GPX, GST, and PAL) and non-enzymatic antioxidants (FLA, CAR, and TP) also took part in the development of heat tolerance induced by MG in maize seedlings.

Accumulating evidences indicated that glyoxalase system (Gly I and Gly II) plays more and more important role in the cellular metabolism, plant growth, development, and response and tolerance to environmental stress by maintaining MG homeostasis (Kaur et al., 2016; Hoque et al., 2016; Li, 2016; Hossain et al., 2018; Mostofa et al., 2018; Cai et al., 2019; Mahlanza et al., 2019; You et al., 2019). Besides signaling molecule, as mentioned above, excessive MG can lead to carbonyl stress, damage to proteins, nucleic acids, and membrane lipids. Therefore, the maintenance of MG homeostasis is a very important physiological basis for adapting changing environments. Gly I, along with GSH, scavenges excessive MG and generates intermediate hemithioacetal (HTA), which is then changed into S-D-lactoylglutathione (SLG) by Gly I. Afterwards, Gly II catalyzes the conversion of SLG to non-toxic lactate and regenerates GSH, the former is converted into pyruvate (by lactate dehydrogenase: LDH), which enters subsequently into tricarboxylic acid cycle (TCA) to generate more reducing power NADH and energy ATP; the latter (GSH) enters into AsA-GSH cycle to maintain redox balance (Kaur et al., 2016; Hoque et al., 2016; Li, 2016; Welchen et al., 2016; Hossain et al., 2018; Mostofa et al., 2018). In

Brassica napus L. seedlings, NO pretreatment could protect from the toxicity of herbicide paraquat by modulating MG-detoxification and ROS-scavenging systems (Hasanuzzaman et al., 2018). In this study, under non-HS conditions, compared with the control, exogenous MG priming increased the level of endogenous MG (Fig. 6C), which might act as a signaling molecule, followed by up-regulating the activities of Gly I and Gly II (Fig. 6A and B), and accumulated more GSH (Fig. 2B), thus enhancing the MG-scavenging capacity of maize seedlings. Under HS conditions, endogenous MG level and Gly I activity were parallelly up-regulated compared with the control seedlings (Fig. 6A, C), while the MG-pretreated maize seedlings maintained a higher Gly II activity (but lower than that of non-HS maize seedlings) (Fig. 6B) to scavenge excessive MG under HS conditions. Similarly, MG pretreatment stimulated Gly I and Gly II activities, which in turn reduced the accumulation of endogenous MG in wheat seedlings under salt or cadmium stress, followed by improving stress tolerance (Li et al., 2017, 2018a). Similar stimulative effect of MG on glyoxalase system were also found in *Brassica rapa* under zirconium stress (Bless et al., 2017). These results further illustrated that the activation of MG-driven glyoxalase system might be a novel physiological mechanism of MG-triggered heat tolerance in maize seedlings.

5. Conclusion

In sum, exogenous MG pretreatment was capable to trigger the heat tolerance of maize seedlings by alleviating a decrease in tissue vitality and biomembrane integrity and an increase in membrane lipid peroxidation. In addition, MG pretreatment was able to drive the AsA-GSH cycle by stimulating GR, MDHAR, DHAR, GSH, and AsA as well as ROS/MG-scavenging system (CAT, APX, GPX, GST, PAL, Gly I, Gly II, FLA, CAR, and TP), which might be a novel physiological basis for MG-triggered heat tolerance in maize seedlings (Fig. 7). However, the development of heat tolerance in plants is involved in the crosstalk among

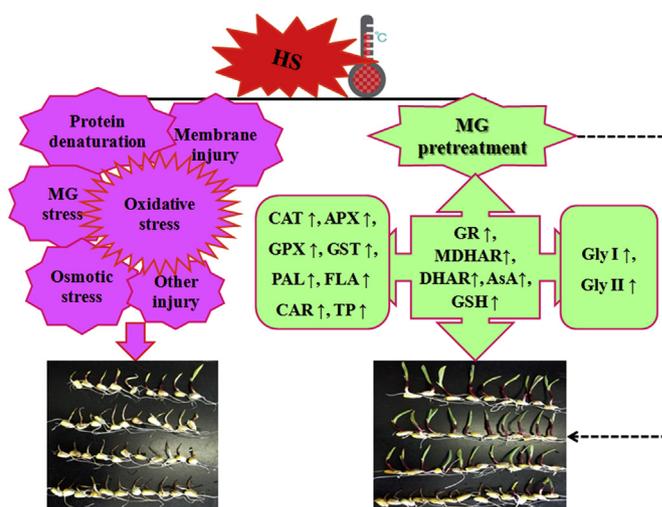


Fig. 7. The mechanisms underlying methylglyoxal (MG)-triggered heat tolerance in maize seedlings. Heat stress (HS) leads to intertwined protein denaturation, membrane injury, oxidative stress, MG stress, osmotic stress, and other injury, thus resulting in plant death. Exogenous MG pretreatment drove the ascorbate (AsA)-glutathione (GSH) cycle by stimulating glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), AsA, and GSH; and enhanced reactive oxygen species (ROS)-scavenging system (catalase: CAT, ascorbate peroxidase: APX, guaiacol peroxidase: GPX, glutathione-S-transferase: GST, phenylalanine ammonia-lyase: PAL, flavonoids: FLA, carotenoids: CAR, and total phenols: TP) and MG-scavenging system (glyoxalase I: Gly I, glyoxalase II: Gly II) under non-HS and HS conditions. Thus, the heat tolerance of maize seedlings was enhanced by the joint action of AsA-GSH cycle, ROS-scavenging system, and MG-scavenging system. The solid and dashed lines indicate the known and unknown pathways.

signaling molecules (Ca²⁺, ROS, NO, H₂S, MG, and phytohormones), which needs to be further uncovered in the future.

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Authors contribution statement

ZGL conceived and designed experiments and wrote manuscript, YW and XYX performed experiments and wrote manuscript, XMJ assisted the whole experiment process. All authors have approved the final version of the manuscript.

Conflicts of interest

The authors declare that they have no conflict of interest.

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