SHORT COMMUNICATION

Regulative role of calcium signaling on methylglyoxal-improved heat tolerance in maize (*Zea mays* L) seedlings

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ABSTRACT

Nowadays, calcium (Ca^{2+}) and methylglyoxal (MG) are all deemed to be second messengers in plants, which participate in various physiological processes, such as seed germination, seedling establishment, plant growth and development, as well as response to environmental stress. However, the Ca^{2+} -MG interaction in the development of thermotolerance in maize seedlings remains unclear. Here, using maize seedlings as materials, the crosstalk between Ca^{2+} and MG signaling in the acquisition of thermotolerance was explored. The results showed that root-irrigation with Ca^{2+} and MG alone or in combination increased the survival rate of maize seedlings under heat stress, mitigated the decrease in the tissue vitality, and reduced the membrane lipid peroxidation (in term of the content of malondialdehyde), indicating that Ca^{2+} and MG could improve the thermotolerance in maize seedlings. In addition, MG-improved thermotolerance was impaired by ethylene glycol-bis(b-aminoethylether)-N,N,N',N'-tetraacetic acid (a Ca^{2+} chelator), La^{3+} (plasma membrane Ca^{2+} channel blocker), ruthenium red (a mitochondrial Ca^{2+} channel blocker), neomycin (vacuole Ca^{2+} channel blocker), caffeine (an endoplasmic reticulum Ca^{2+} channel blocker), and calmodulin antagonists (chlorpromazine and trifluoperazine), respectively. Also, MG scavengers (N-acetylcysteine, aminoguanidine, and vitamin B6) had no significant effect on Ca^{2+} -triggered thermotolerance (in terms of survival rate, malondialdehyde, and tissue vitality) of maize seedlings. The data illustrated that calcium signaling regulated MG-improved thermotolerance in maize seedlings by mobilizing intracellular and extracellular Ca^{2+} pools.

ARTICLE HISTORY

Received 13 June 2020
Revised 20 June 2020
Accepted 22 June 2020

KEYWORDS

Calcium signaling; heat stress; thermotolerance; maize seedlings; methylglyoxal signaling

Methylglyoxal (MG), also known as pyruvic aldehyde, is a byproduct of sugar and amino acid metabolism, especially in respiration and photosynthesis, which is toxic to plant cells at a high level.¹⁻³ The MG toxicity usually leads to the formation of advanced glycation end products (AGEs) by rapidly reacting with membrane lipids, amino acids (mainly arginine, lysine, and cysteine), and bases (such as guanine), which is collectively known as carboxyl stress or MG stress. MG stress (namely glycation) commonly causes the change in conformation and the loss of function of macromolecules (proteins, nucleic acids, and lipids).¹⁻³ Therefore, MG must be closely controlled and maintained at a low physiological level (or fluctuating in a given range, namely homeostasis) in plant cells under normal conditions.

Except for its toxicity, nowadays, MG, akin to calcium (Ca^{2+}), nitric oxide (NO), reactive oxygen species (ROS), and hydrogen sulfide (H_2S), is deemed to be a novel signaling molecule in plants, which takes part in seed germination, seedling establishment, plant growth and development, and response and adaptation to abiotic and biotic stress.¹⁻⁴ As a signaling molecule, MG can be quickly triggered and then recovered to normal physiological level by synthesizing and degrading in a controllable manner. Generally, MG homeostasis in plant cells is maintained by a combined action of generation (rate-limited enzymes including isomerase,

monoamine oxidase, MG synthase, and cytochrome P450 oxidase) and scavenging (key enzymes involving glyoxalase I, glyoxalase II, and glyoxalase III).¹⁻³

With the development and aggravation of global warming, the high temperature has become a crucial stress factor for many plant species including crop plants. The high temperature commonly leads to variously hierarchical damages at different levels, such as oxidative stress (protein oxidation, nucleic acid damage, and membrane lipid peroxidation), decrease in tissue vitality, protein denaturation, osmotic stress, and so forth.⁵⁻⁷ In view of this, food demand, along with an exponentially growing population, has also become a serious challenge in the future. Exploring the effective approaches and methods for improving the thermotolerance of crop plants and understanding its underlying mechanism are important basis for ensuring sustainable food demand. Among approaches, chemical priming, such as Ca^{2+} , NO, ROS, and H_2S priming, is an effective and economic way,⁸⁻¹⁰ but the Ca^{2+} -MG interaction in the formation of thermotolerance in plants remains unknown.

For a long period, the fact that Ca^{2+} /calmodulin (CaM)-centered calcium signaling system participates in plant growth, development, and response and adaptation to environmental stress has been largely expounded.¹¹⁻¹³ Because of the complexity of signaling network composed of Ca^{2+} /CaM-centered

calcium signaling system, it, till now, is a research hotspot in plant biology, which is in the juvenile stage.^{11–13} The signaling crosstalk of Ca^{2+} and other signaling molecules including MG in the response and adaptation of plants to environmental stress needs to be uncovered in the coming days.

Maize (*Zea mays* L), not only is an important food, energy, and feed crop, but also a novel model plant.^{14,15} As mentioned above, Ca^{2+} and MG signaling alone can induce the plant stress tolerance including thermotolerance, but their interaction in the formation of thermotolerance is not completely clear. Therefore, in this paper, using maize seedlings as materials, the Ca^{2+} -MG interaction in the acquisition of thermotolerance was studied. The objective of this paper was to identify the signaling crosstalk of Ca^{2+} and MG in the development of thermotolerance in plants. In this study, maize (Xuanheyu No 2) seeds were purchased from Chenggong Seed Company, China. The healthy and same size seeds were immersed in 5% (w/v) sodium hypochlorite for 15 min to carry out surface sterilization, and then washed completely with sterile water. The sterilized seeds were imbibed in distilled water for 12 h at 26°C, followed by being sown on the six-layer paper wetted with distilled water in a tray (250 seeds per tray) with cover. The imbibed seeds were germinated at 26°C in dark for 60 h (namely 2.5 d) in a climate chamber and then root-irrigation with the different chemicals as follows.

The 2.5-day-old maize seedlings (at least 200 seedlings per tray) with the same growth were classified into 11 groups (two paralleled replications in each) and irrigated with 100 mL of the following chemicals, respectively [the optimal concentration of chemicals was rooted in our previous studies^{16–18} and preliminary experiments]. (1) distilled water (control); (2) 50 μM MG (expressed as MG in figures, the same as below); (3) 20 mM CaCl_2 (Ca^{2+}); (4) 20 mM CaCl_2 + 50 μM MG (Ca^{2+} + MG); (5) 5 mM ethylene glycol-bis(b-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA, the pH was adjusted to 7.0 with sodium hydroxide) + 50 μM MG (EGTA + MG); (6) 5 mM LaCl_3 + 50 μM MG (La^{3+} + MG); (7) 0.1 mM ruthenium red (RR) + 50 μM MG (RR + MG); (8) 1 mM neomycin (NEC) + 50 μM MG (NEC + MG); (9) 0.5 mM caffeine + 50 μM MG (CAF + MG); (10) 0.5 mM chlorpromazine (CPZ) + 50 μM MG (CPZ + MG); (11) 0.5 mM trifluoperazine (TFP) + 50 μM MG (TFP + MG). After 6 h of treatment, chemicals were drained off, and the seedling roots were washed six times with distilled water to remove the residue chemicals on the surface of the roots. The treated seedlings were then transferred into another climate chamber with 46°C in dark for 16 h to perform heat stress [under this heat stress, the survival rate of the control group was approximately 50%, reaching the half-lethal strength (T_{50})]. After heat stress, the heated seedlings were recovered in a climate chamber with 26°C, 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and 14/10 h (day/night cycle) for a week. The survival rate was calculated as per the formula: survived seedlings/total seedlings $\times$ 100%.

In addition, to further explore the effect of MG on calcium-induced thermotolerance, the 2.5-day-old maize seedlings (at least 200 seedlings per tray) with the same growth were divided into five groups (two paralleled replications in each) and irrigated with 100 mL of the following chemicals, respectively (the optimal concentration of chemicals was come from our

previous studies^{16,18,19} and preliminary experiments) (1) distilled water (control); (2) 20 mM CaCl_2 (Ca^{2+}); (3) 50 μM N-acetyl-cysteine (NAC) + 20 mM CaCl_2 (NAC + Ca^{2+}); (4) 50 μM aminoguanidine (AG) + 20 mM CaCl_2 (AG + Ca^{2+}); (5) 50 μM vitamin B6 (VB_6) + 20 mM CaCl_2 (VB_6 + Ca^{2+}). After 6 h of treatment, the treated seedlings were performed heat stress and counted survival rate based on the above procedure.

After heat stress, membrane lipid peroxidation [in term of malondialdehyde (MDA) content] and tissue vitality [based on the reduction of triphenyl tetrazolium chloride (TTC)] of maize seedlings were spectrophotometrically determined as the methods of Wang et al.¹⁹ The absorbance of methylidene from MDA and methyl hydrazone from TTC was recorded at 532 and 485 nm, respectively, and MDA content and tissue vitality were expressed in nmol g^{-1} fresh weight (FW) and A_{485} . All experiments were performed at least three biological repeats and two paralleled replications in each.

In general, survival rate, membrane lipid peroxidation (MDA content), and tissue vitality (the reducing capacity of TTC by NADH generating from respiration) usually were used as solid stress tolerance indexes including thermotolerance.^{19–21} In this study, the survival rate and tissue vitality of maize seedlings were enhanced by exogenous application of MG and Ca^{2+} alone or in combination, while MDA was reduced by MG, Ca^{2+} or their combination (Figure 1), indicating that MG and Ca^{2+} were able to improve the thermotolerance in maize seedlings. In addition, the MG-enhanced survival rate was reinforced by exogenous Ca^{2+} (CaCl_2 treatment), while weakened by RR (mitochondrion Ca^{2+} channel blocker), NEC (vacuole Ca^{2+} channel blocker), CAF (endoplasmic reticulum Ca^{2+} channel blocker), CPZ (CaM antagonist), and TFP (CaM antagonist), but eliminated by EGTA (Ca^{2+} chelator), LaCl_3 (membrane Ca^{2+} channel blocker) (Figure 1). The results indicated that regulation of Ca^{2+} signaling on MG-induced thermotolerance in maize seedlings, MG might be activated the extracellular and intracellular Ca^{2+} channels.

Also, in order to further explore the effect of MG scavengers on Ca^{2+} -improved heat tolerance in maize seedlings, the seedlings were irrigated with MG or its scavengers NAC, AG, and VB_6 in combination with CaCl_2 . After heat stress, the results indicated that the survival rate could be increased by pretreatment with Ca^{2+} and MG (Figure 1a), and the increase in heat tolerance induced by Ca^{2+} was enhanced by MG (Figure 1a). In addition, Ca^{2+} -induced thermotolerance in maize seedlings was not weakened by MG scavengers NAC, AG, and VB_6 . In the same way, heat stress increased MDA content and reduced tissue vitality of maize seedlings, these effects were mitigated by exogenous application of CaCl_2 , MG, or their combination (Figure 1b, c), similar to the change in survival rate (Figure 1a). Also, pretreatment with MG scavengers NAC, AG, and VB_6 in combination with CaCl_2 had no significant effect on reduced MDA content and increased tissue vitality by Ca^{2+} in maize seedlings (Figure 1b, c), akin to the change in the survival rate of maize seedlings (Figure 1a). These results suggested that Ca^{2+} was able to enhance the thermotolerance in maize seedlings, and this enhancement was strengthened by MG, but the combination of MG scavengers and Ca^{2+} had no significant difference on thermotolerance in maize seedlings

Figure 1. Effect of pretreatment with methylglyoxal (MG), CaCl_2 (Ca^{2+}), CaCl_2 + MG (Ca^{2+} + MG), ethylene glycol-bis(b-aminoethylether)-N,N,N',N'-tetraacetic acid + MG (EGTA + MG), LaCl_3 + MG (La^{3+} + MG), ruthenium red + MG (RR + MG), neomycin + MG (NEC + MG), caffeine + MG (CAF + MG), chlorpromazine + MG (CPZ + MG), trifluoperazine + MG (TFP + MG), N-acetyl-cysteine + CaCl_2 (NAC + Ca^{2+}), aminoguanidine + CaCl_2 (AG + Ca^{2+}), and vitamin B₆ + CaCl_2 (VB_6 + Ca^{2+}) on survival percentage (a), malondialdehyde (MDA) content (b), and tissue vitality (c) of maize seedlings under heat stress. The 2.5-day-old maize seedlings were treated with distilled water (control), 50 μM MG (MG), 20 mM CaCl_2 (Ca^{2+}), and 20 mM CaCl_2 + 50 μM MG (Ca^{2+} + MG), 5 mM EGTA + 50 μM MG (EGTA + MG), 5 mM La^{3+} + 50 μM MG (La^{3+} + MG), 0.1 mM RR + 50 μM MG (RR + MG), 1 mM NEC + 50 μM MG (NEC + MG), 0.5 mM CAF + 50 μM MG (CAF + MG), 0.5 mM CPZ + 50 μM MG (CPZ + MG), 0.5 mM TFP + 50 μM MG (TFP + MG), 0.1 mM NAC + 20 mM CaCl_2 (NAC + Ca^{2+}), 0.1 mM AG + 20 mM CaCl_2 (AG + Ca^{2+}), and 0.1 mM VB_6 + 20 mM CaCl_2 (VB_6 + Ca^{2+}) for 6 h, and then exposed to heat stress at 46°C for 16 h. After heat stress, the survival percentage, MDA content, and tissue vitality of maize seedlings were determined. The data are the mean $\pm$ SE of at least three experiments, significance was analyzed using Duncan's multiple range test, different letters indicate significant difference and the same letters represent non-significant difference.

compared with the Ca^{2+} treatment alone, implied that Ca^{2+} played a role in the downstream of MG signaling.

In conclusion, the current results showed that Ca^{2+} and MG alone could improve the thermotolerance in maize seedlings, and this improvement was enhanced by their combination. Also, MG-improve thermotolerance in maize seedlings was weakened by Ca^{2+} channel blockers (RR, NEC, and CAF) and CaM antagonists (CPZ and TFP), while eliminated by Ca^{2+} chelator (EGTA) and plasma membrane Ca^{2+} blocker (La^{3+}); while MG scavengers (NAC, AG, and VB_6) had no significant effect on the thermotolerance induced by Ca^{2+} . These results illustrated that calcium signaling regulated the MG-induced thermotolerance in maize seedlings. However, the detailed physio-biochemical and molecular mechanism that calcium signaling regulated MG-improved thermotolerance in maize seedlings should be further expounded.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This research is funded by National Natural Science Foundation of China [31760069, 31360057].

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