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Can heavy metal pollution defend seed germination against heat stress? Effect of heavy metals (Cu^{2+}, Cd^{2+} and Hg^{2+}) on maize seed germination under high temperature☆

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ABSTRACT

Heavy metal pollution, as well as greenhouse effect, has become a serious threat today. Both heavy metal and heat stresses can arrest seed germination. What response can be expected for seed germination under both stress conditions? Here, the effects of heavy metals (Cu^{2+}, Cd^{2+} and Hg^{2+}) on maize seed germination were investigated at 20 °C and 40 °C. Compared with 20 °C, heat stress induced thermodormancy. However, this thermodormancy could be significantly alleviated by the addition of a low concentration of heavy metals. Heavy metals, as well as heat stress induced H_2O_2 accumulation in germinating seeds. Interestingly, this low concentration of heavy metal that promoted seed germination could be partly blocked by DMTU (a specific ROS scavenger), irrespective of temperature. Accordingly, H_2O_2 addition reinforced this promoting effect on seed germination, which was induced by a low concentration of heavy metal. Furthermore, we found that the NADPH oxidase derived ROS was required for seed germination promoted by the heavy metals. Subsequently, treatment of seeds with fluridone (a specific inhibitor of ABA) or ABA significantly alleviated or aggravated thermodormancy, respectively. However, this alleviation or aggravation could be partly attenuated by a low concentration of heavy metals. In addition, germination that was inhibited by high concentrations of heavy metals was also partly reversed by fluridone. The obtained results support the idea that heavy metal-mediated ROS and hormone interaction can finally affect the thermodormancy release or not.

1. Introduction

With the arrival of O_2-evolving photosynthetic organisms, reactive oxygen species (ROS) have been the uninvited companions of aerobic life (Gill and Tuteja, 2010). ROS, such as O_2^- and H_2O_2, play a key role in mediating the release of seed dormancy and should be considered as messengers of environmental cues during seed germination (Leymarie et al., 2012; Bailly et al., 2008; Ogawa and Iwabuchi, 2001). The success of germination tightly depends on external factors. It implies that seeds must be endowed with internal sensors able to deliver the environmental cues into the cellular mechanisms leading to germination (Leymarie et al., 2012; Bailly et al., 2008). The stressful situation, associated with excess ROS generation, would prevent radicle emergence. Thus, ROS play a dual role alternating between having a signaling role and being deleterious during seed dormancy release (Bailly et al., 2008).

Global warming is expected to enhance the frequency of the exposure of crops to extreme temperatures and thus damage crop fertility (Porter, 2005; Atkinson and Porter, 1996). High temperature injury can result in serious damage to crops, affecting seed germination and fruit ripening. One mechanism of injury involves the over-production of ROS (Gill and Tuteja, 2010; Pukacka and Ratajczak, 2005). In addition, dormancy release and seed germination are also tightly associated with two important phytohormones: abscisic acid and gibberellins (White et al., 2000). Interestingly, ROS can affect their biosynthesis and catabolism during seed germination (Bahin et al., 2011; Liu et al., 2010).

It is known that the widespread accumulation of heavy metals in the environment is increasingly becoming a problem for all

Abbreviations: ABA, Abscisic acid; DMTU, Dimethyl thiourea; DPI, Diphenyleneiodonium chloride; GR, Germination rate; HM, Heavy metal; H_2O_2, Hydrogen peroxide; NOX, NADPH oxidase; ROS, Reactive oxygen species.

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organisms (Clemens, 2006; Lombi et al., 2001). Heavy metals can be divided into two categories: essential and non-essential elements. All of them are potentially toxic (Nagajyoti et al., 2010). Similarly, one mechanism of toxicity can also be attributed to ROS generation (Sharma and Dietz, 2009; Michalak, 2006).

Abiotic stress (such as high temperature and heavy metals) shows dramatic effects on seed germination behavior (Kranner and Colville, 2011; Li et al., 2005; Hills et al., 2003; Munzuroğlu and Geckil, 2002; Corbineau et al., 1993). Previous reports showed that heat stress can significantly arrest seed germination (Hills et al., 2003; Corbineau et al., 1993). Numerous results have shown that heavy metal treatment can suppress seed germination (Li et al., 2005; Munzuroğlu and Geckil, 2002). Interestingly, low concentrations of heavy metals (e.g. Cu, Cd and As) promoting seed dormancy release were also reported under room temperature conditions (Kranner and Colville, 2011).

Breeders and farmers have long known that often it is the simultaneous appearance of several environmental stresses, rather than a particular stress condition, that is most dangerous to crops (Atkinson and Urwin, 2012; Mittler, 2006; Niinemets and Valladares, 2006). Previous studies have revealed that the response of plants to a combination of two contrasting abiotic stresses is unique and cannot be simply extrapolated from the response of plants to each of the various stresses applied individually (Atkinson and Urwin, 2012; Mittler, 2006).

In general, fewer plants can cope with more than two different environmental stresses simultaneously (Niinemets and Valladares, 2006). Interestingly, treatment with heavy metals can confer plants with greater pathogen and herbivore resistance (Poschenrieder et al., 2006; Mithöfer et al., 2004). However, no data are available for us to learn about seed germination behavior under multiple stress conditions.

Accordingly, the aim of the present study was to investigate the effect of heavy metals on maize seed germination under high temperature conditions and to unveil the possible mechanisms underlying it. In this experiment, one essential (Cu^{2+}) and two non-essential elements (Cd^{2+} and Hg^{2+}) were used.

2. Materials and methods

2.1. Seed germination and treatment

The maize seeds *Jinkenuo* (*Zea mays* L.) were obtained from a seed distributor in Daqing city of China and used for these studies. These seeds were sown in plastic boxes and placed in a seed germinator (LRH-250-A, made in Guangdong Medical Instruments Factory, China) at 20 ± 1 °C (normal temperature) or 40 ± 1 °C (heat stress) for the entire germination period. Germination trials were conducted in plastic boxes equipped with 2-cm deep sand (diameter about 0.5 mm) and moistened with distilled water to ensure adequate moisture for the seeds. The seeds were placed on the sand. Before the heavy metal treatment, the seeds were moistened with distilled water for 120 min. Three kinds of heavy metal ions (Cu^{2+}, Cd^{2+} and Hg^{2+}) were used in this experiment. These metal ions were obtained from their chlorine salt ($CuCl_2$, $CdCl_2$ and $HgCl_2$) and all were dissolved in distilled water before seed treatment. Here, the heavy metal treatment followed such a method: the fully moistened seeds were immersed in heavy metal solution for about 20 min and then washed with distilled water 5 times. After treatment, these seeds were transferred to plastic boxes. However, all pharmacological treatments (these reagents were purchased from a biochemical reagent Co., Ltd. in Harbin city of China) were performed inside the plastic boxes.

The germination experiment was divided into six groups: heavy metal treatment (group 1), DMTU treatment (group 2), DPI/imidazole treatment (group 3), H_2O_2 treatment (group 4), fluridone treatment (group 5) and ABA treatment (group 6).

For group 1, four concentrations of $Cu^{2+}/Cd^{2+}/Hg^{2+}$ (0, 0.1, 1.0 and 10 mM; water as control) were applied on these seeds at 20 °C or 40 °C.

For group 2, three concentrations of DMTU (0, 20 and 100 mM; water as control) were sprayed (sprayed 3 times during the first 24 h after sowing for 4 h) on the seeds after pre-treatment with $Cu^{2+}/Cd^{2+}/Hg^{2+}$ (0.1 mM) at 20 °C or 40 °C.

For group 3, two kinds of inhibitors (DPI, 100 μM; imidazole, 10 mM; water as control) of NADPH oxidase (NOX) were sprayed (sprayed 3 times during the first 24 h after sowing for 4 h) on the seeds after pre-treatment with $Cu^{2+}/Cd^{2+}/Hg^{2+}$ (0.1 mM) at 20 °C or 40 °C.

For group 4, three concentrations of H_2O_2 (0, 10 and 100 mM; water as control) were sprayed (sprayed 3 times during the first 24 h after sowing for 4 h) on the seeds after pre-treatment with $Cu^{2+}/Cd^{2+}/Hg^{2+}$ (0.1 mM) at 20 °C or 40 °C.

For group 5, three concentrations of fluridone (0, 50 and 200 μM; water as control) were sprayed (sprayed 3 times during the first 24 h after sowing for 4 h) on the seeds after pre-treatment with $Cu^{2+}/Cd^{2+}/Hg^{2+}$ (0.1 and 10 mM) at 20 °C or 40 °C.

For group 6, three concentrations of ABA (0, 50 and 200 μM; water as control) were sprayed (sprayed 3 times during the first 24 h after sowing for 4 h) on the seeds after pre-treatment with $Cu^{2+}/Cd^{2+}/Hg^{2+}$ (0.1 and 10 mM) at 20 °C or 40 °C.

In addition, about 1 mL solution was used during each spraying treatment in this experiment. All assays were replicated at least five times to minimize experimental errors; each replicate was carried out on 50 seeds for germination. All concentrations used in this study were based on our preliminary experiments.

2.2. Germination rate assay

When the radicle emergence of seeds exceeded 1 mm, they were considered to have germinated. The number of germinated seeds was counted three times per day, for the time necessary to achieve the final percentage of germinated seeds. Germination rates (GR) were calculated as the percentage of germinated seeds after sowing for 24 and 48 h.

2.3. H_2O_2 extraction and assay

H_2O_2 content in germinating seeds was measured using the xylenol orange oxidation assay (Gay et al., 1999). All germinating seeds were collected and immersed in acetone for termination of germination. Then, these collected seeds were weighed and immediately quenched in liquid nitrogen. Samples (~1 g DW) were ground to a powder in liquid nitrogen using a mortar and pestle. Next, they were homogenized with 10 mL of 5% trichloroacetic acid containing 10 mM EDTA for H_2O_2 extraction. After centrifugation (10,000*g*) at room temperature for 20 min, the supernatants were collected for H_2O_2 assays. One milliliter of assay reagent (25 mM $FeSO_4$ and 25 mM $(NH_4)_2SO_4$, dissolved in 2.5 M H_2SO_4) was added to 100 mL of 125 μM xylenol orange and 100 mM sorbitol. The supernatant (100 μL) was added to 1 mL of xylenol orange reagent. After 30 min of incubation, absorbance by the Fe^{3+}–xylenol orange complex was determined at 560 nm.

2.4. Heavy metal assay

Seeds with different concentration HM treatment were collected and washed. Then, they were air dried and grounded into powder with mortar and pestle. For metal extraction, the digestion tubes were properly acid washed and dried. Dry powdered sample