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Research article

Change in desiccation tolerance of maize embryos during development and germination at different water potential PEG-6000 in relation to oxidative process

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ABSTRACT

Desiccation tolerance is one of the most important traits determining seed survival during storage and under stress conditions. However, the mechanism of seed desiccation tolerance is still unclear in detail. In the present study, we used a combined model system, desiccation-tolerant and -sensitive maize embryos with identical genetic background, to investigate the changes in desiccation tolerance, malonyldialdehyde (MDA) level, hydrogen peroxide (H_2O_2) content and antioxidant enzyme activity during seed development and germination in 0, −0.6 and −1.2 MPa polyethylene glycol (PEG)-6000 solutions. Our results indicated that maize embryos gradually acquired and lost desiccation tolerance during development and germination, respectively. The acquirement and loss of desiccation tolerance of embryos during development and germination were related to the ability of antioxidant enzymes including superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.11), catalase (CAT, EC 1.11.1.6), glutathione reductase (GR, EC 1.6.4.2) and dehydroascorbate reductase (DHAR, EC 1.8.5.1) to scavenge reactive oxygen species (ROS) and to control MDA content. Compared with treatment in water, PEG-6000 treatment could markedly delay the loss of desiccation tolerance of germinating embryos by delaying water uptake and time course of germination, increasing GR activity and decreasing MDA content. Our data showed the combination of antioxidant enzyme activity and MDA content is a good parameter for assessing the desiccation tolerance of maize embryos. In addition, H_2O_2 accumulated in mature embryos and PEG-treated embryos after drying, which was at least partially related to a longer embryo/seedling length in rehydration and the physiological mechanisms of priming.

1. Introduction

Desiccation tolerance is one of the most important traits determining seed survival during storage and under stress conditions. Orthodox seeds acquire gradually desiccation tolerance during development. After undergoing a maturation drying phase, the seeds pass into a metabolically inactive or quiescent state and could be stored for an extended period of time. Once these seeds germinate, desiccation tolerance is rapidly lost after only a few hours of germination [1]. During desiccation sensitive phase, re-drying may seriously impair subsequent germination and seedling establishment [2]. Conversely, recalcitrant seeds originated from tropical and subtropical plant species are characterized by the absence of maturation drying, and most of them have a high water content and active metabolism when they are shed from the mother plant. They are sensitive to drying and low temperatures, and quickly lose viability during storage [1,3]. Seeds of many important economical plants are recalcitrant, including many important tropical plantation crop species such as rubber and cocoa, tropical fruit crops such as mango, lychee and longan, and tropical timber species which belong to the families Dipterocarpaceae and Araucariaceae, etc. It is now difficult to find a suitable strategy to preserve these species' seeds. Thus, it is essential to understand the mechanisms of seed desiccation tolerance in more detail.

Abbreviations: APX, ascorbate peroxidase; AsA, ascorbic acid; CAT, catalase; DAP, days after pollination; DHAR, dehydroascorbate reductase; DW, dry weight; EDTA, ethylenediamine tetraacetic acid; EFGS embryo, embryo excised from germinating seeds; g g^{-1}, g H_2O g^{-1} DW; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; MGT, mean germination time; MDA, malonyldialdehyde; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NBT, nitroblue tetrazolium; O_2^-, superoxide radical; PEG, polyethylene glycol; PVPP, polyvinylpolypyrrolidone; ROS, reactive oxygen species; SOD, superoxide dismutase.

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Oxidative processes and free radicals are induced by a wide range of stresses, including dehydration [4]. Reactive oxygen species (ROS) cause lipid peroxidation, protein oxidation and DNA damage, all of which contribute to cell death [5,6]. It has been proposed that desiccation tolerance is associated with a capacity to effectively scavenge ROS because it involves increased antioxidant enzyme activities [7,8], such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and dehydroascorbate reductase (DHAR). Antioxidant defense systems accumulate in orthodox seed when desiccation tolerance is acquired, and degrade when desiccation tolerance is lost [9,10]. The relationship between changes in ROS content and the acquisition and loss of seed desiccation tolerance has been reported in maize [6], silver maple [11], *Trichilia dregeana* [12], and wheat [13]. However, these studies were only involved in individual development or germination process, or in diverse species with different genetic background such as recalcitrant or orthodox seeds, which make understanding the mechanism of desiccation tolerance difficult.

The operation and controlling of hydration conditions might be an effective pathway to decrease the rate of desiccation tolerance loss of seeds during germination. Hydration under controlled conditions, achieved by incubation in solution containing an osmotic agent, causes the transient activation of antioxidant response and DNA repair systems that preserve genome integrity and seed quality. Primed seeds are subsequently re-dried for storage, distribution, and planting. This technique, known as osmopriming, is routinely used by seed companies and germplasm banks to improve seed vigor [14]. Polyethylene glycol (PEG) molecule with a $M_r \geq 6000$ (PEG-6000) is inert, non-ionic, and virtually impermeable chains that have frequently been used to induce water stress and maintain uniform water potential throughout the experimental period [15]. PEG-6000 is small enough to influence the osmotic potential, but large enough not penetrate membranes and stays in apoplast fluid [15]. Chen et al. [16] reported that PEG osmopriming enhanced desiccation tolerance of spinach seeds.

Germinating orthodox seed is sometime studied instead of recalcitrant seed because both are desiccation sensitive and have a high degree of subcellular development and metabolic activity [17]. In the present study, we used a combined model system, desiccation-tolerant and -sensitive maize embryos with identical genetic background, to investigate the changes in desiccation tolerance, malonyldialdehyde (MDA) level, hydrogen peroxide (H_2O_2) content and antioxidant enzyme activity during seed development and germination in PEG solutions with different water potentials. Our results indicated that the combination of antioxidant enzyme activity and MDA content might be a good parameter for assessing seed desiccation tolerance under drying stress. PEG treatment can delay the loss of desiccation tolerance of germinating maize embryos by delaying water uptake and time course of germination.

2. Results

2.1. Acquisition of desiccation tolerance during development

During seed development, the water content of embryos decreased from 3.28 g H_2O g^{-1} DW (g g^{-1}) at 24 days after pollination (DAP) to 1.32 g g^{-1} at 52 DAP; and dry weight of embryos gradually increased, for example, the mean dry weight was 2.0 mg $embryo^{-1}$ at 28 DAP and 15.8 mg $embryo^{-1}$ at 44 DAP (Fig. 1a). The maize embryos began to acquire desiccation tolerance (% survival) during 32–36 DAP (Fig. 1b), and their survival increased from zero at 28 DAP to 100% at 52 DAP. We have observed that embryo/seedling length produced by dried embryos significantly increased with increasing development periods of time. For example, seedling length produced by dried embryos at 44 and 52 DAP was 17.8 and 57.1 mm $embryo^{-1}$, respectively (Fig. 1b).

2.2. MDA and H_2O_2 content during development

Malonyldialdehyde is a product of lipid peroxidation, which has considerable potential to damage membranes and may be a principal cause of deterioration in orthodox seeds [5]. To test the relationship between lipid peroxidation and drying injury, we comparatively assayed the changes in MDA contents of fresh and dried maize embryos at different developmental stage. MDA content of fresh embryos gradually decreased from 24 (0.32 μmol g^{-1} DW) to 40 DAP (0.08 μmol g^{-1} DW), and then clearly increased from 40 to 52 DAP (0.24 μmol g^{-1} DW) (Fig. 2a). Compared with fresh embryos at different developmental stage, the MDA content of dried embryos decreased with seed development, and was lower than that of fresh ones at 24, 28, 48 and 52 DAP, respectively (Fig. 2a).

H_2O_2 is a ROS, their contents of fresh embryos markedly decreased with seed development (Fig. 2b). Drying decreased H_2O_2 content of embryos at 24 DAP and increased after 36 DAP as compared with fresh embryos at different developmental stage. We have noted that H_2O_2 contents of dried embryos have a little changes from 24 (1.06 μmol g^{-1} DW) to 52 (0.96 μmol g^{-1} DW) DAP (Fig. 2b).

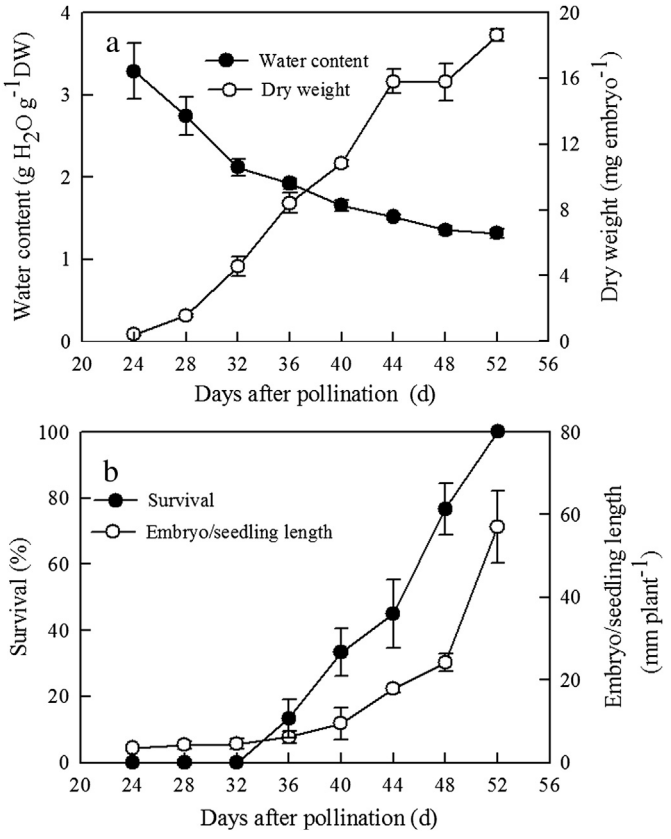

Fig. 1. Changes in water content and dry weight of embryos (a), and in survival and length of embryo/seedling of dried embryos (b) during maize seed development. a, After maize seeds at different days after pollination (DAP) were collected, and the embryos were immediately excised, and the water content and dry weight of fresh embryos were measured. b, The excised embryos at different DAP were dried to a water content of 0.07 ± 0.01 g g^{-1}, and then the survival of dried embryos and length of embryo/seedling produced by dried embryos were assayed. All values are means ± SD of three replicates of 20 embryos each.