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Research article

Mitochondrial structural and antioxidant system responses to aging in oat (*Avena sativa* L.) seeds with different moisture contents

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ABSTRACT

We observed the relationship between lifespan and mitochondria, including antioxidant systems, ultrastructure, and the hydrogen peroxide and malondialdehyde contents in 4 h imbibed oat (*Avena sativa* L.) seeds that were aged with different moisture contents (4%, 10% and 16%) for 0 (the control), 8, 16, 24, 32 and 40 d at 45 °C. The results showed that the decline in the oat seed vigor and in the integrity of the mitochondrial ultrastructure occurred during the aging process, and that these changes were enhanced by higher moisture contents. Mitochondrial antioxidants in imbibed oat seeds aged with a 4% moisture content were maintained at higher levels than imbibed oat seeds aged with a 10% and 16% moisture content. These results indicated that the levels of mitochondrial antioxidants and malondialdehyde after imbibition were related to the integrity of the mitochondrial membrane in aged oat seeds. The scavenging role of mitochondrial superoxide dismutase was inhibited in imbibed oat seeds aged at the early stage. Monodehydroascorbate reductase and dehydroascorbate reductase played more important roles than glutathione reductase in ascorbate regeneration in aged oat seeds during imbibition.

1. Introduction

Seed aging, which decreases seed viability during storage, is a major problem for successful agricultural production and productivity. The seed aging process is dependent primarily upon their moisture content, the oxygen level and the temperature at which seeds are stored (Bewley et al., 2013; Groot et al., 2012). Seed aging may, in part, be due to the accumulation of reactive oxygen species (ROS) damage, which causes lipid peroxidation, impairment of RNA and protein synthesis, and the degradation of DNA during storage or treatments (Chen et al., 2013; McDonald, 1999). The scavenging of ROS largely depends on the availability of molecular antioxidants, such as tocopherols and ascorbic acid in dry seeds (Pukacka and Ratajczak, 2007). However, the detailed mechanisms of seed aging caused by moisture content, which leads to the loss of seed viability, have not been determined, and the current understanding of seed quality and storage conditions do not allow for reliable means to predict or prevent this critical problem (Walters et al., 2010).

Mitochondria are a principal source of ROS in plant cells, particularly in root tissues, and are the early targets of oxidative injuries, which can be accelerated to a greater degree than in other organelles during deterioration (Gupta, 2011; Jacoby et al., 2012). Mitochondria also play an important role in the production and scavenging of ROS during seed germination, and provide energy compounds and intermediates for cellular biosynthesis (Xin et al., 2014). Hydrogen peroxide (H_2O_2) is crucial for the production and scavenging of ROS, and the enhanced H_2O_2 accumulation indirectly induces an increase in lipid peroxidation. Lipid peroxidation in the mitochondrial context of meristematic cells refers to free radical autoxidation of the polyunsaturated fatty acids of membrane lipids (Jacoby et al., 2012). In addition, malondialdehyde (MDA), a symbolic product of lipid peroxidation, can interact with multiple cells to reduce or even eliminate their functions. Biotic or abiotic stress increases mitochondrial MDA and H_2O_2 contents in moist tissues, which seriously damages the mitochondrial membrane structure and function (Zhan et al., 2014).

Mitochondrial H_2O_2 production is tightly controlled by enzymatic and non-enzymatic antioxidant systems, such as the

Abbreviations: APX, ascorbate peroxidase; AsA, ascorbate; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; GR, glutathione reductase; GSH, glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; MDHAR, monodehydroascorbate reductase; ROS, reactive oxygen species; SOD, superoxide dismutase.

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manganese-superoxide dismutase (Mn-SOD) and the ascorbate-glutathione (AsA-GSH) cycles, during the germination process (Martía et al., 2013; Minibayeva et al., 2012). These systems not only limit H_2O_2 production and scavenge H_2O_2, they may also serve as redox sensors and repair damage to macromolecules (Hamanaka and Chandel, 2010; Navrot et al., 2007). In mitochondria, Mn-SOD presumably acts in anticipation of the forthcoming ROS burst that occurs upon re-oxygenation (Millar et al., 2004). Ascorbate peroxidase (APX) is the most important peroxidase in H_2O_2 detoxification using AsA, which can be regenerated in the matrix by NADH-dependent monodehydroascorbate reductase (MDHAR) or by GSH-dependent dehydroascorbate reductase (DHAR), and oxidized glutathione (GSSG) can be regenerated by glutathione reductase (GR) using NADPH as an electron donor (Wu et al., 2009). However, the reduction in the antioxidant system of mitochondria results in enhanced H_2O_2 production and membrane damage (Gupta, 2011). Although advances have been made in understanding mitochondrial H_2O_2 processing, the mechanisms of mitochondrial H_2O_2 elimination remain poorly understood (Bewley et al., 2013; Macherel et al., 2007). This may be due to a lack of studies focusing on the relationship between mitochondrial antioxidant systems and their ultrastructures in aged seeds during imbibition.

Oat (*Avena sativa* L.), an annual herb plant, is a low-carbon and eco-friendly crop that is planted in regions facing a variety of environmental stresses, such as infertility, salinity, drought, and cold. The higher lipid content limits the extensive use of oat as food and seed because of the potential for lipid-derived rancidity and the associated deterioration (Claudine et al., 2012; Heini et al., 2001; Leonova et al., 2008). This study was designed to research mitochondrial damage, ultrastructure, antioxidant system failure and lipid peroxidation, in aged oat seed embryo cells during imbibition, and to determine the relationship between mitochondrial antioxidant systems and their ultrastructures in aged seeds during imbibition.

2. Methods

2.1. Seed samples

Oat seeds (variety: Triple Crown) were collected by the Forage Seed Laboratory of the China Agricultural University in 2009, and were then sealed in plastic bags and stored at −20 °C. The experiment was launched in September 2013. The seed moisture content was 9.8% (fresh weight basis), and the oil content was 5.0%.

2.2. Determination of seed moisture contents

The seed moisture contents were determined in accordance with ISTA Rules, chapter IX (ISTA, 2013). Approximately 4.5 g of seeds were placed in a sample container and weighed, and then they were oven-dried at ~130–133 °C for 1 h (two replicates). After cooling for 30 min in a desiccator, seeds were weighed again and the moisture content was calculated.

2.3. Adjusting the seed moisture content

The seed moisture content was regulated to 4%, 10% and 16% (fresh weight basis). Approximately 25 g of seeds were placed into aluminum foil bags for the methods described below:

i) When the required moisture content was above the natural moisture content, the required amount of distilled water to reach the desired moisture content was calculated. The oat seeds were placed on filter paper wetted with distilled water and weighed continually. Seeds reaching the required weight were immediately sealed in an aluminum foil bag (12×17 cm^2) at ~5–10 °C.

ii) When the required moisture content was below the natural moisture content, the seeds were weighed, and the seed weight required to reach the desired moisture content under natural conditions was calculated. Seeds were placed into a desiccator containing allochroic silica gel and weighed frequently. Seeds reaching the required weight were immediately sealed in an aluminum foil bag.

2.4. Seed aging treatments

After regulating the seed moisture contents, seed samples with 4%, 10% and 16% moisture contents (10%, 53% and 80% equilibrium relative humidity, respectively) in aluminum foil bags were aged for 0 (the control), 8, 16, 24, 32 and 40 d at 45 °C in a constant temperature water-bath, and were prepared as experimental samples. Thermohygrometers, which measure temperature and humidity, were sealed inside the aluminum foil bags used to age the seeds with different moisture contents to determine the bags' relative humidity. The level of seed vigor was indicated by the seeds' germination percentage after the aging treatment. The germination percentage of oat seeds with a 4% moisture content reduced from 88% to 80% after 40 d, those with 10% moisture content reduced from 89% to 0% after 40 d, while those with 16% moisture content reduced from 82% to 0% after 8 d. Detailed analyses were performed as previously described (Xia et al., 2015).

2.5. Ultrastructural observations of embryo cells

Seed samples with 4%, 10% and 16% moisture contents were selected randomly after being aged for 0 (control), 16 and 40 d. The embryonic roots, after imbibition for 4 h and without radicle protrusions, were removed and fixed in a 4% glutaraldehyde solution for 24 h and then placed at 4 °C. Embryo samples were processed by rinsing with buffer, fixing with osmium tetroxide, and rinsing with buffer again. They were then dehydrated using an alcohol gradient, embedded in epoxy resin and sliced ultrathin using an LKB8800 III ultramicrotome. Observations were then performed using a transmission electron microscope (Hitachi H-7500).

2.6. Isolation of mitochondria

Mitochondria were extracted according to Yin et al. (2009). Briefly, 200 embryonic axes were collected from aged oat seeds without radicle protrusions after imbibition for 4 h. For the preparation of mitochondria, the axes were ground with a mortar and pestle in 20 mL of precooled grinding medium. The grinding medium was composed of 50 mM potassium phosphate buffer (pH 8.0), 0.3 M mannitol, 0.5% (w/v) bovine serum albumin (BSA), 0.5% (w/v) polyvinylpyrrolidone-40, 2 mM EGTA and 20 mM cysteine. The homogenate was squeezed through a 40×40 μm mesh nylon cloth and centrifuged at $2000 \times g$ for 15 min. The supernatant was centrifuged at $12\,000 \times g$ for 15 min. The precipitate was suspended in a wash medium buffer containing 0.3 M mannitol, 0.1% (w/v) BSA and 10 mM TES (pH 7.5), and centrifuged again at $12\,000 \times g$ for 15 min. The final precipitate was washed once with wash medium and suspended in a small volume of the washing medium (mitochondrial fraction). The suspension was then loaded onto a Percoll step gradient consisting of a 1:2 ratio, bottom to top, of 40% Percoll:23% Percoll in a mannitol wash buffer. The mixture was centrifuged for 1 h at $40\,000 \times g$, and the mitochondria presented as an opaque band at the 23%/40% interface. The mitochondrial band was collected and washed three times by centrifugation at