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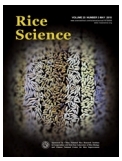

Levels of Crotonaldehyde and 4-hydroxy-(E)-2-nonenal and Expression of Genes Encoding Carbonyl-Scavenging Enzyme at Critical Node During Rice Seed Aging

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Abstract: The critical node (CN) is an important stage during seed aging, which is related to effective genebank conservation. Previous studies have demonstrated that proteins undergo carbonylated modification at the CN in rice, indicating oxidative damage. However, the levels of reactive carbonyl species (RCS) and the associated scavenging system at the CN are largely unknown. In this study, we optimized methods for the extraction and analysis of RCS from dry rice embryos. In order to acquire seeds at the CN, rice seeds were subjected to natural conditions for 7, 9, 11 and 13 months, and the seed germination rates were reduced to 90%, 82%, 71% and 57%, respectively. We chose the stage with seed germination rate of 82% as the CN according to the rice seed vigor loss curve. The levels of crotonaldehyde and 4-hydroxy-(E)-2-nonenal (HNE) were significantly increased at the CN. In addition, genes encoding carbonyl-scavenging enzyme, including *OsALDHs* and *OsAKRs*, were significantly down-regulated at the CN, and reductions in the expression of *OsALDH2-2*, *OsALDH2-5*, *OsALDH3-4*, *OsALDH7*, *OsAKR1* and *OsAKR2* in particular could be responsible for RCS accumulation. Thus, the accumulations of crotonaldehyde and HNE and down-regulation of genes encoding carbonyl-scavenging enzyme might be related to an accelerating loss of seed viability at the CN.

Key words: carbonyl-scavenging system; reactive carbonyl species; seed aging; crotonaldehyde; critical node; rice storage

The important roles of germplasm resources in ensuring food and ecological security have been widely recognized. According to Food and Agriculture Organization (FAO) statistics, more than 7 400 000 accessions have been collected and preserved in genebanks, 10% of which are rice accessions (FAO, 2010). There are 84 284 rice accessions in long-term storage at -18 ºC in the National Crop Genebank of China, Beijing, China, and 78 568 rice accessions in medium-term storage at 4 ºC in the China National Rice Research Institute, Hangzhou, China. However, seeds continue to slowly age even when stored in genebanks under conditions of low temperature and low moisture levels (Xin et al, 2011; Hay et al, 2013; Gao et al, 2016; Sun et al, 2017). At the T.T. Chang Genetic Resources Centre of the International Rice Research Institute, Manila, the Philippines, rice seed germination is reduced from 93% to 85% or less after 33 years (Hay et al, 2015). For effective seed conservation in genebanks, it is important to understand the mechanism of seed viability loss. Seed viability loss during storage exhibits a reverse S-shaped curve, including a plateau phase (Phase I), followed by a rapid decreasing phase (Phase II), and then a slow decreasing phase (Phase III). The transformation from Phase I to Phase II is the critical node (CN), which is

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extremely important for seed conservation. At the CN of the rice seeds, germination is approximately 84% or less (Lu et al, 2005; Yin et al, 2016, 2017).

In our previous studies, we found that rice seeds at the CN have undergone oxidative damage, which might accelerate the loss of seed viability (Yin et al, 2016). The proteins related to energy and defense metabolism are reduced and we observed carbonylated modification at the CN (Yin et al, 2017). Proteins undergo carbonylated modification by reactive carbonyl species (RCS) in response to oxidative damage induced by environmental stress (Mano et al, 2014). In this study, we further investigated the levels of RCS and the carbonyl-scavenging system at the CN of rice seed aging.

RCS are downstream products of reactive oxygen species (ROS). A high content of ROS is toxic to plants, and can lead to tissue injury via oxidative damage to membranes (Mano et al, 2014). Lipids in the membranes can be oxidized by ROS to form lipid peroxide molecules (Mène-Saffrané et al, 2007), which can easily be decomposed to form various aldehydes and ketones in the presence of redox catalysts such as transition metal ions or free radicals, because their molecules are unstable (Farmer and Mueller, 2013). α, β-unsaturated carbonyls, which mainly include crotonaldehyde and 4-hydroxy-(E)-2-nonenal (HNE) are important component of aldehydes and ketones (Farmer and Mueller, 2013; Mano et al, 2014). The content of RCS increases not only when cultured plants are treated with high concentrations of salt (NaCl and $AlCl_3$), but also when the leaves are wounded or treated with abscisic acid (ABA) and H_2O_2 (Yin et al, 2010; Matsui et al, 2012; Mano et al, 2014; Islam et al, 2016). In mitochondria (Taylor et al, 2002) and chloroplasts (Mano et al, 2009), enzymes are inactivated by RCS. Aldehyde dehydrogenases (ALDH) and aldo-keto reductases (AKR) play key roles in scavenging RCS in plants (Stiti et al, 2011; Yamauchi et al, 2011). ALDH superfamily represents a group of enzymes that catalyze the oxidation of endogenous and exogenous aldehydes to the corresponding carboxylic acids by oxidizing α, β-unsaturated aldehydes (Stiti et al, 2011). Eleven *ALDH* genes have been identified in the rice genome (Gao and Han, 2009), whereas three *AKR* genes have been identified in rice, based on the chromosomal localization and homology with other stress-induced AKRs, and these are all stress-inducible (Turóczy et al, 2011).

We previously found that a decrease in antioxidant capacity is accompanied by carbonylation of proteins at the CN of rice seeds (Yin et al, 2017). The proteins are modified by RCS, which is considered to be representative of the extent of oxidative damage. However, no direct studies have been carried out to investigate the content of RCS and the associated scavenging system at the CN of rice seeds. In this study, in order to gain a better understanding of the mechanisms underlying oxidative damage at the CN, we determined the changes in RCS (crotonaldehyde and HNE) contents and relevant scavenging enzyme (ALDH and AKR) gene expression levels at the CN of seed aging in rice. The findings of this research will provide an important experimental basis for identifying the early warning indicators of seed aging at the CN.

MATERIALS AND METHODS

Rice materials and treatments

Nipponbare (*Oryza sativa* L. *japonica*) seeds were obtained from the Jiangxi Agricultural Academy of Sciences, Nanchang, China. The germination rate and moisture content were 99% and 12%, respectively. In order to determine seed vigor loss up to the CN, the rice seeds were sealed in aluminum foil bags at room temperature for 7, 9, 11 and 13 months. For vigor analysis, the seeds were incubated for 7 d in an artificial climate incubator at 28 ºC in dark, as described by the International Seed Testing Association (ISTA, 2014). The number of germinated grains was recorded daily. Germination potential (Gp) was calculated as follows: Gp (%) = Number of germinated seeds in 4 d / Total number of testing seeds × 100. The embryos were extracted from the dry seeds immediately prior to use.

Assay of crotonaldehyde and HNE contents

Crotonaldehyde and HNE were extracted from dry rice embryos, derivatized with 2,4-dinitrophenylhydrazine (DNPH), and subsequently identified and quantified by high performance liquid chromatography (HPLC)-electrospray ionization (ESI)-selective ion monitoring (SIM). As we were unable to obtain crotonaldehyde and HNE from dry rice when using previously established protocols (Matsui et al, 2009; Wang et al, 2012; Csallany et al, 2015), a new protocol was designed with substantial modifications. Dry rice embryos were ground in 1 mL of grinding buffer (sodium acetate, pH 2), followed by the addition of 10