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Proteome changes associated with dormancy release of Dongxiang wild rice seeds

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ABSTRACT

Seed dormancy provides optimum timing for seed germination and subsequent seedling growth, but the mechanism of seed dormancy is still poorly understood. Here, we used Dongxiang wild rice (DXWR) seeds to investigate the dormancy behavior and the differentially changed proteome in embryo and endosperm during dormancy release. DXWR seed dormancy was caused by interaction of embryo and its surrounding structure, and was an intermediate physiological dormancy. During seed dormancy release, a total of 109 and 97 protein spots showed significant change in abundance and were successfully identified in embryo and endosperm, respectively. As a result of dormancy release, the abundance of nine proteins involved in storage protein, cell defense and rescue and energy changed in the same way in both embryo and endosperm, while 67 and 49 protein spots changed differentially in embryo and endosperm, respectively. Dormancy release of DXWR seeds was closely associated with degradation of storage proteins in both embryo and endosperm. At the same time, the abundance of proteins involved in metabolism, glycolysis and TCA cycle, cell growth and division, protein synthesis and destination and signal transduction increased in embryos while staying constant or decreasing in endosperms.

1. Introduction

The crucial function of seed dormancy is to prevent germination when conditions are suitable for germination, but the probability for survival and growth of the seedling is low (Black et al., 2006). Seed dormancy provides an optimum timing for seed germination and subsequent seedling growth (Graeber et al., 2012). To select this time, the depth of seed dormancy continually changes in response to a range of environmental signals that inform the seed about the seasons, its depth in the soil and the presence of competing plants (Footitt et al., 2013). It has been proposed that seed dormancy is an important component of plant fitness (Huang et al., 2010). Low levels of seed dormancy can cause premature germination and high seedling mortality, while high levels of seed dormancy delay germination and decrease the length of the growth season (Donohue et al., 2010). Most crop plant seeds have very low dormancy levels due to selection by breeders for rapid and uniform germination and seedling establishment. As a result, they are susceptible to preharvest sprouting, which causes substantial loss in grain yield and quality (Gubler et al., 2005), especially in years when wet conditions occur along with harvest maturity.

Seed dormancy is a complex trait, because it is influenced by both endogenous and environmental factors, and the dormancy level is determined by the contributions of the different tissues that comprise a seed (Bewley et al., 2013; Black et al., 2006). Seed dormancy is induced during seed maturation and reaches a high level in freshly harvested seeds. During subsequent dry storage

Abbreviations: 2-D, two-dimensional; 2-DE, 2-D gel electrophoresis; ABA, abscisic acid; APX, ascorbate peroxidase; ASA, β-aspartyl-semialdehyde; ASADH, ASA dehydrogenase; CBB, coomassie brilliant blue R-250; CDC, cell division cycle protein; CysPI, cysteine proteinase inhibitor; DOG, delay of germination; DXWR, dongxiang wild rice; DTT, dithiothreitol; eIF, eukaryotic translation initiation factor; GA, gibberellin; GBSS, granule-bound starch synthase; Glc-1-P, glucose-1-phosphate; Glc-6-P, glucose-6-phosphate; Gly-3-P, glyceraldehyde-3-phosphate; GST, glutathione *S*-transferases; HSP, heat shock protein; IEF, isoelectrofocusing; IPMS, isopropylmalate synthase; KARI, ketol-acid reductoisomerase; LAP, leucine aminopeptidase; MDHA, monodehydroascorbate; MDHAR, MDHA reductase; MFT, mother of FT and TFL1; MS, mass spectrometry; NDPK, nucleoside diphosphate kinase; PEBP, phosphatidylethanolamine-binding protein; Prx, peroxiredoxin; TCA, tricarboxylic acid; TCP, T-complex protein; TFA, trifluoroactic acid; UDPG, UDP-glucose.

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(after-ripening), dormancy is slowly reduced. Seeds can also rapidly release dormancy during imbibition under specific conditions, for example, during cold (0–10 °C) or warm (≥15 °C) stratification, or in the presence of phytohormones (Finch-Savage and Leubner-Metzger, 2006; Graeber et al., 2012; Holdsworth et al., 2008). Nonogaki (2014) proposed that hormonal regulation may be a highly conserved mechanism of seed dormancy among seed plants. In many species, seed dormancy is induced and maintained by abscisic acid (ABA), and is released by gibberellin (GA) and ethylene (Gao and Ayele, 2014; Graeber et al., 2012; Nonogaki, 2014). ABA plays a leading role in the regulation of seed dormancy for many species and this role of ABA can be antagonized by GA (Finkelstein et al., 2008; Gao and Ayele, 2014; Graeber et al., 2012; Holdsworth et al., 2008; Nonogaki, 2014) and by ethylene (Corbineau et al., 2014; Nonogaki, 2014; Wang et al., 2013), but the molecular mechanism of these antagonistic effects is less understood (Nonogaki, 2014). Recently, Liu et al. (2015a) de novo assembled and characterized a germinating lettuce seed transcriptome using Illumina paired-end sequencing and obtained many unigenes encoding proteins involved in ABA signaling, including 11 ABA receptors, 94 protein phosphatase 2Cs and 16 sucrose non-fermenting 1-related protein kinases. Interestingly, seed response to light, temperature, nitrate and after-ripening, which varies depending on species, is also controlled through hormone metabolism and signal transduction (Finkelstein et al., 2008; Holdsworth et al., 2008; Nonogaki, 2014; Seo et al., 2009).

Mature seeds, especially their endosperm cells, have cell walls rich in mannan-based polymers that confer a strong mechanical resistance to the radicle protrusion upon seed germination (Iglesias-Fernández et al., 2011). Radicle emergence can be facilitated by reducing the resistance from the surrounding tissues (e.g., the micropylar endosperm) through enzymatic or physical weakening of their cell walls (Morris et al., 2011; Nonogaki et al., 2010; Pinto et al., 2007). Moreover, some new hypotheses, which can be hormone-independent or specific to the seed dormancy pathway, are also emerging from genetic analysis of "seed dormancy mutants". Quantitative trait locus analysis using natural variation in Arabidopsis identified *DELAY OF GERMINATION* 1 (*DOG*1) genes as a "seed dormancy-specific" locus. *DOG*1 is expressed in seeds during the maturation stage, and loss of function of *DOG*1 results in no dormancy (Bentsink et al., 2006). However, *DOG*1 encodes an unknown protein and its biochemical and molecular function is not understood (Bentsink et al., 2006, 2010). Nonogaki (2014) suggested that the chromatin remodeling through histone ubiquitination, methylation and acetylation, which could lead to transcription elongation or gene silencing, may play a significant role in seed dormancy regulation. Small interfering RNA and/or long non-coding RNA might be a trigger of epigenetic changes in the seed dormancy (Nonogaki, 2014). However, these hypotheses remain to be tested.

Proteomic analysis is a very important tool that can be used to examine simultaneous changes in, and to classify temporal patterns of, protein accumulation occurring in seed development and germination (Chibani et al., 2006; Huang et al., 2012; Wang et al., 2015). Proteomic analysis has been performed on seed dormancy in a few of species, including *Arabidopsis thaliana* (Arc et al., 2012; Chibani et al., 2006), *Prunus campanulata* (Lee et al., 2006), *Fagus sylvatica* (Pawłowski, 2007), Norway maple (Pawłowski, 2009; Staszak and Pawłowski, 2014) and wheat (Gao et al., 2013). Proteins involved in genetic information processing, energy, carbon metabolism and cellular antioxidant processes were proposed to be related to seed dormancy induction (maturation) (Staszak and Pawłowski, 2014), and those in metabolism, energy, protein destination, and related to dormancy release (breaking) (Arc et al., 2012; Chibani et al., 2006; Gao et al., 2013; Pawłowski, 2007, 2009). However, some key events (proteins) leading to seed dormancy/dormancy release are still not well understood as only a few of species have been investigated and relative few proteins have been identified. Moreover, in the studies mentioned above, whole seeds or excised embryos (axes) were used as experimental material making it impossible to discern the roles of the embryo and the endosperm in seed dormancy/dormancy release.

Wild rice is generally considered as the progenitor of the cultivated one. It has many excellent agronomic traits, which are absent in cultivated rice, such as the resistance to biotic and abiotic stresses and seed harvest dormancy, and it is, therefore, a valuable genetic resource for improving rice cultivars (Xie et al., 2010). Veasey et al. (2004) observed that wild rice (*Oryza rufipogon* and *O. glumaepatula*) seeds exhibited a pronounced dormancy and that this dormancy could be released by after-ripening. However, the dormancy type and mechanism of wild rice seeds are poorly understood. In the present study, dormant Dongxiang wild rice (DXWR) seeds were used as experimental material. We investigated the water uptake of seeds, changes in germination of excised embryos and seeds with and without hull during dormancy release (stratification), and the proteome changes in embryo and endosperm of seeds stratified for 0, 30, 60, and 90 d, respectively, by two-dimensional (2-D) gel electrophoresis (2-DE) and mass spectrometry (MS) to understand further the key events (proteins) involved in seed dormancy/dormancy release and in that way provide new knowledge for promoting the germination of dormant seeds or preventing pre-harvest sprouting.

2. Materials and methods

2.1. Plant material

Dongxiang wild rice (*Oryza rufipogon* Griff.) seeds were planted in Liantang (28°54′N, 115°93′E; altitude, 28 m), Nanchang, Jiangxi, China on March 26, 2013. During the growth season, March 26–October 10, the daily mean temperature is 22.8 °C, the total rainfall is 1300–1400 mm. The mature seeds, whose hull (palea and inferior palea) had become black-brown, were manually collected at 30 d after flowering. After drying at 15 °C and 42% relative humidity for 72 h, the seeds were used as experimental materials when the water content of seeds was 8.2 ± 0.2% (wet weight basis).

2.2. Determination for water content, length and width of seeds

The water content of seeds was gravimetrically determined (80 °C for 48 h). Three replicates of 20 seeds each were used for determination of water content, and the water content of seeds is expressed in percent of fresh weight.

Length and width of seeds were measured by vernier caliper (Shangliang, Shanghai, China) and expressed in mm. Three replicates of 10 seeds each were used for this determination.

2.3. Water uptake by dry seeds

Four replicates of 50 seeds each were imbibed on two layers of filter paper moistened in 5 ml of distilled water in 90-mm diameter Petri dishes at 28 °C for 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, and 48 h, respectively, and then water content of seeds was determined and expressed in a percentage of their fresh weight.

2.4. Stratification treatment (dormancy release) of seeds

After imbibition in sterile water at 4 °C for 24 h, the seeds were mixed with three volumes of moist perlite (1/5 (w/w) perlite/water) in closed plastic bags, and placed at 4 °C in darkness for 0, 30, 45, 60, 75, and 90 d, respectively.