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Research article

Comparative metabolome analysis of wheat embryo and endosperm reveals the dynamic changes of metabolites during seed germination

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ABSTRACT

In this study, we performed the first comparative metabolomic analysis of the wheat embryo and endosperm during seed germination using GC-MS/MS. In total, 82 metabolites were identified in the embryo and endosperm. Principal component analysis (PCA), metabolite-metabolite correlation and hierarchical cluster analysis (HCA) revealed distinct dynamic changes in metabolites between the embryo and endosperm during seed germination. Generally, the metabolite changes in the embryo were much greater than those in the endosperm, suggesting that the embryo is more active than the endosperm during seed germination. Most amino acids were upregulated in both embryo and endosperm, while polysaccharides and organic acids associated with sugars were mainly downregulated in the embryo. Most of the sugars showed an upregulated trend in the endosperm, but significant changes in lipids occurred only in the embryo. Our results suggest that the embryo mobilises mainly protein and lipid metabolism, while the endosperm mobilises storage starch and minor protein metabolism during seed germination. The primary energy was generated mainly in the embryo by glycolysis during seed imbibition. The embryo containing most of the genetic information showed increased nucleotides during seed germination process, indicating more active transcription and translation metabolisms.

1. Introduction

Seed germination is the start and crucial stage in the life cycle of cropss, which is strongly related to seedling survival rate and grain yield. In general, seed germination commences with the uptake of water by the quiescent dry seed and reaches completion with visible radicle protrusion through the seed coat (Bewley and Black, 1994). Seed germination is controlled by a large number of environmental factors (e.g. temperature or light) that directly impact the entire crop growth cycle (Gul and Weber, 1999). The phytohormones gibberellin acid (GA) and abscisic acid (ABA) play crucial roles in the regulation of seed germination by stimulating or suppressing, respectively, seed germination (Finch-Savage and Leubner-Metzger, 2006; Penfield et al., 2006). Abundant storage mRNA, which accumulates during the late maturation process in the dry seed, can be used for *de novo* and instant protein biosynthesis upon imbibition (He and Yang, 2013).

The transition from a dry seed to a dynamic seed has been shown to be associated with a major metabolic switch, resulting in changes in storage proteins, carbohydrates, and lipids (Bewley and Black, 1994). Metabolomics, which serve as a tool to analyze substrates and products in various metabolic pathways, has recently become an area of major research interest. Historically, gas chromatography-mass spectrometry (GC-MS) was one of the first techniques used for high-throughput metabolite profiling in plants and is generally performed using electron impact (EI) quadrupole or time-of-flight (TOF) MS (Lisec et al., 2006). Using GC-MS, it is possible to profile several hundred compounds belonging to diverse chemical classes including sugars, organic acids, amino acids, and fatty acids (Shulaev, 2006).

Metabolite analysis has been performed on different kinds of tissues/organs, mainly focused on root, leaf, shoot, and fruit (Guo et al., 2015; Nicolas and Alisdair, 2006). A few studies regarding metabolism during seed germination were conducted in *Arabidopsis* (Sreenivasulu et al., 2008), barley (Fait et al., 2006), and rice (Hu et al., 2016). In wheat, limited reports have focused on the

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metabolite profiling of wheat grains derived from organic and conventional agriculture (Zörb et al., 2006; Frank et al., 2011), chemical analysis of semolina and the volatile composition of semolina and pasta samples from durum wheat cultivars (Beleggia et al., 2009), and metabolite profiling of a diverse collection of wheat lines to assess the effects of environmental factors on their characteristics (Matthews et al., 2012). More recently, our group has reported metabolomic profiling during wheat grain development and in response to high-nitrogen fertiliser (Zhen et al., 2016). However, metabolomic profiling during wheat seed germination, especially for isolated embryo and endosperm, remains unknown. In this study, we performed the first integrated metabolomics analysis of the wheat embryo and endosperm during seed germination using GC-MS. Our results provide novel insights into the biochemical mechanisms of cereal seed germination at the metabolome level.

2. Materials and methods

2.1. Plant material and seed germination

The Chinese elite bread wheat cultivar Zhengmai 366 (*Triticum aestivum* L., 2n = 6 × = 42, AABBDD) was used as the experimental material. Seeds of similar size were selected for germination treatment. Six biological replicates were performed and each replicate included 500 seeds. Surface-sterilised seeds were placed on moistened paper in Petri dishes, after which 8 mL of ddH_2O was added. All Petri plates were covered by lids and placed in an incubator at 22 °C under dark conditions and 80% humidity. The seeds were collected at five different germination stages (0, 6, 12, 18, and 24 h) after imbibition with six biological replicates. The embryos were separated from the endosperms and stored at −80 °C prior to metabolite extraction. The changes of 1000 seeds' weight were tested. The seed morphology changes from five germination phases were monitored by stereo microscopy.

2.2. Metabolite extraction

The collected samples (100 mg) were ground in a mortar using liquid nitrogen, and then transferred into 10-mL centrifuge tubes. After adding 1.4 mL of 100% methanol (pre-cooled at −20 °C) and vortexing for 30 s, 60 μL of polar internal standard (20 mL of 0.2 mg mL^{-1} ribitol in water) was added to the tube. The samples were vortexed again for 30 s and placed into an ultrasound machine for 15 min. Next, 750 μL of chloroform and 1.4 mL of ddH_2O were added and vortexed for 30 s, followed by centrifugation for 15 min at 4000 rpm. The supernatant was transferred into a new tube and blow-dried with nitrogen. Subsequently, 60 μL of methoxypyridine (15 mg mL^{-1}) was added and vortexed for 30 s. After reacting for 16 h, 60 mL of N, O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) reagent (containing 1% trimethylchlorosilane) was added to the tube. After reacting for 60 min, the samples were used for subsequent GC-MS analysis.

2.3. Metabolite analysis by GC-MS

Metabolites from embryo and endosperm were analysed by GC-MS using a modified method of Lisec et al. (2006). The GC column was an HP-5MS Capillary column (5% phenyl methyl silox: 30 mm × 250 μm i.d., 0.25 μm film thickness). A 1-μL sample was injected into the column in the split flow and the distribution ratio of flow was 20:1. The injector temperature was set at 280 °C, the ion source at 250 °C, and the interface at 150 °C. For metabolite analysis, the oven programme was as follows: 40 °C for 5 min, 40–300 °C over 10 min, and 300 °C for 5 min. Helium was used as carrier at a flow rate of 1 mL min^{-1}.

Peak identification, data baseline filtering, and integration were processed using XCMS software. Raw data from the XCMS software were exported to Microsoft Excel software for further processing. Normalised processing of raw data was performed as described previously (Lawton et al., 2008). Missing values were assumed to be below the limits of detection and were imputed with the observed minimum after the normalization step. Based on the retention time, mass-to-charge ratio (*m/z*), and mass spectral signature of all detectable ions, the variables were analysed for their annotation. The samples were identified using the Golm Metabolome Database (GMD) and NIST MS Search Program database. We also use 39 standard chemicals (Suppl. Table S1 with green color) to confirm the qualitative of the identified chemical. Metabolites that had a confirmed identity were used for further date analysis.

2.4. Data analysis

Statistical differences of the metabolites during the five time points were calculated by one-way ANOVA and Bonferroni-type correction for false discovery rate (FDR). Principal component analysis (PCA) models were generated using Simca-P software (version 11.0). Metabolite-metabolite correlation analysis was performed by Pearson correlation coefficients. Correlation matrix was performed using the R language heatmap package together with Cytoscape version 3.1.0 software. Hierarchical cluster analysis (HCA) was performed using the R program (www.r-project.org) called heatmap.2.

3. Results

3.1. Seed morphological changes during the germination process

The absorption of moisture can accelerate seed metabolism and promote nutrient absorption by the embryo upon imbibition. Seed morphology underwent clear changes concurrent with the seed germination process (Fig. 1a). The radicle and bud emerged and the radicle length reached 1–2 cm at 24 h, indicating the completion of seed germination and the beginning of seedling growth.

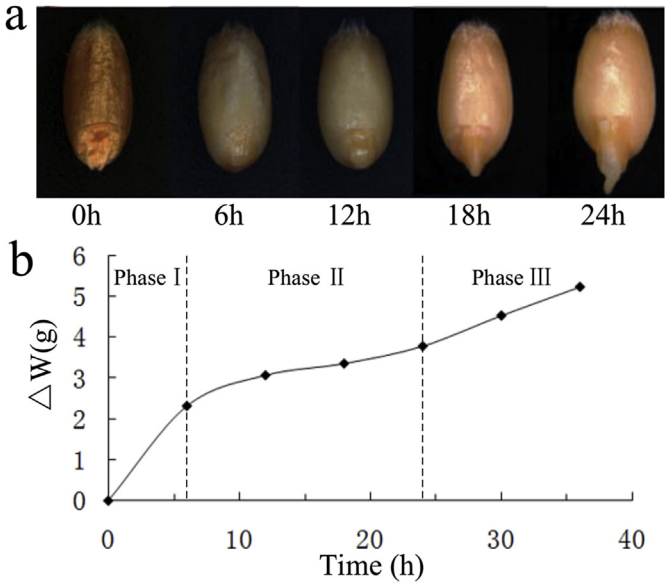

Fig. 1. Grain morphology and water content changes during seed germination. **a.** Morphology changes. **b.** 1000-seed water content changes.