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Proteome analysis reveals an energy-dependent central process for *Populus* × *canadensis* seed germination

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ABSTRACT

Poplar (*Populus* × *canadensis*) seeds rapidly germinated in darkness at 10, 15, and 20 °C and reached 50% seed germination after about 22, 4.5, and 3.5 h, respectively. Germination of poplar seeds was markedly inhibited by abscisic acid (ABA) at 50 μM and cycloheximide (CHX) at 100 μM, and these inhibitive roles were temperature-dependent. In the present study, mature poplar seeds were used to investigate the differentially changed proteome of seeds germinating in water, ABA, and CHX. A total of 130 protein spots showed a significant change (1.5-fold increase/decrease, $P < 0.05$) in abundance, and 101 protein spots were successfully identified. Most of the proteins were associated with cell defense and rescue (21%), storage proteins (21%), protein synthesis and destination (20%), metabolism (16%), and energy (14%). The germination of poplar seeds is closely related with the increase in those proteins involved in amino acid and lipid metabolism, the tricarboxylic acid cycle and pentose phosphate pathway, protein synthesis and destination, cell defense and rescue, and degradation of storage proteins. ABA and CHX inhibit the germination of poplar seeds by decreasing the protein abundance associated with protein proteolysis, protein folding, and storage proteins. We conclude that poplar seed germination is an energy-dependent active process, and is accompanied by increasing amino acid activation, protein synthesis and destination, as well as cell defense and rescue, and degradation of storage proteins.

1. Introduction

The *Populus* genus includes a number of common species, which play an important role in dominating the riparian woodland ecosystem in the Northern hemisphere (Gonzalez et al., 2010). Poplar is also a fast-growing tree and it is therefore widely used for biofuel production (Aylott et al., 2008). A single mature female *Populus* can produce thousands or even millions of cottony seeds in most years (Karrenberg and Suter, 2003), but the recruitment of new individuals is rare in the natural environment (Braatne et al., 2007).

The success of seed germination and the establishment of normal seedlings are a prerequisite for the propagation of plant species and are the basis for crop production (Rajjou et al., 2012). Seed germination is a complex trait that is influenced by many genetic, endogenous, and environmental factors, and includes a number of cellular processes, such as translation, cell elongation, cell cycle activation, repair mechanisms, and organellar reassembly (Han and Yang, 2015; Rajjou et al., 2012; Rosental et al., 2015). Although the study on seed germination has achieved much progress, the molecular mechanisms underlying seed germination still remain elusive (Han and Yang, 2015; Shu et al., 2016).

Gibberellic acid (GA) promoted, and abscisic acid (ABA) inhibited, seed germination, and they have antagonistic roles in seed germination (Dong et al., 2012; Finkelstein et al., 2008; Nonogaki, 2014; Shu et al., 2016; Weitbrecht et al., 2011). Holdsworth et al. (2008) proposed that environmental factors influence seed germination by regulating the biosynthesis and catabolism of GA and ABA. Except for phytohormone regulation of seed germination, histone methylation (Cho et al., 2012), reactive oxygen species (ROS), and reactive nitrogen species (Arc et al., 2011; Liu et al., 2010;

Abbreviations: 2-D, two-dimensional; 26SP, 26S proteasome; ABA, abscisic acid; AKR, aldo-keto reductase; AlaAT, alanine aminotransferase; AspAT, aspartate aminotransferase; CBB, Coomassie brilliant blue R-250; CHX, cycloheximide; CysPI, cysteine proteinase inhibitor; DXP, 1-deoxy-D-xylulose 5-phosphate; DXPR, DXP reductoisomerase; EF, elongation factor; eIF5A, eukaryotic initiation factor 5A; ER, endoplasmic reticulum; GA, gibberellic acid; GST, glutathione S-transferases; HSP, heat shock protein; KARI, ketol-acid reductoisomerase; KAS, β-ketoacyl-[acyl carrier protein (ACP)] synthase; LEA, late embryogenesis abundant; MEP, 2-C-methyl-D-erythritol 4-phosphate; MS, mass spectrometry; 6-PGDH, 6-phosphogluconate dehydrogenase; PPIase, peptidyl prolyl cis-trans isomerases; Prx, peroxiredoxin; RBP, RNA binding protein; ROS, reactive oxygen species; SOD, superoxide dismutase; TCA, tricarboxylic acid; Tcp, T-complex protein.

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Lounifi et al., 2013) all participate in the regulation of seed germination. Furthermore, the germination of *Arabidopsis thaliana* (Rajjou et al., 2004) and rice (Sano et al., 2012) seeds is inhibited by treatment with the translational inhibitor cycloheximide (CHX), but not by transcriptional inhibitor α-amanitin or actinomycin D.

Proteomic analyses involved in seed germination have only been reported in several plant species—*A. thaliana* (Gallardo et al., 2001, 2002), rice (Han et al., 2014a, 2014b; He et al., 2011a, 2011b; Kim et al., 2009; Yang et al., 2007), maize (Fu et al., 2011; Lu et al., 2008), tomato (Sheoran et al., 2005), mungbean (Ghosh and Pal, 2012), pea (Wang et al., 2012a), date palm (Sghaier-Hammami et al., 2009), and *Jatropha curcas* (Yang et al., 2009). Moreover, during seed germination, the patterns of protein oxidation (Job et al., 2005), the proteome changes modulated by GA and ABA (Kim et al., 2008) and affected by high temperature and ABA (Liu et al., 2015), as well as the proteomic analysis of the effect of salicylic acid on seed germination and early defense mechanism establishment (Rajjou et al., 2006) have also been investigated. In this way, the proteins involved in seed germination included those associated with metabolism, energy, protein synthesis and destination, cell growth and division, cell defense and rescue, and storage proteins (Job et al., 2005; Kim et al., 2008; Liu et al., 2015; Rajjou et al., 2006). However, because of the spectacular progresses in mass spectrometry (MS), deeper proteome analyses of seed germination are still valuable and needed.

In the present study, mature poplar (*Populus* × *canadensis*) seeds were used to investigate the behavior of seed germination, the response of seed germination to ABA and CHX, and the differential proteome of seeds germinating in water, ABA, and CHX with the aim of understanding further the mechanism of seed germination in tree plants.

2. Materials and methods

2.1. Seed collection and drying

The mature poplar (*Populus* × *canadensis* Moench) seeds with cotton were collected in the Beijing Botanical Garden (N 39°59′, E 116°139′; altitude, 73 m), Xiangshan, Beijing, China, on 9 May, 2013. After collection, the cotton on the seed surface was manually removed, and then the seeds were dried at 28 ± 2 °C and 75 ± 5% relative humidity (RH) for 4 d. When water content of seeds was 9.9 ± 0.1% (on a fresh weight basis), the seeds were used as experimental material.

2.2. Water content determination

Water content of seeds was determined according to the international rules for seed testing (International Seed Testing Association, 2010) and is expressed in% of fresh weight.

2.3. Seed germination

Two layers of filter paper were put into 60-mm-diameter Petri dishes, and then three ml of distilled water was added. Three replicates of 100 seeds each were germinated on two layers of filter paper in darkness at 10, 15, and 20 °C, respectively. To maintain constant moisture, distilled water was added to the filter paper each day during seed germination. Radicle protrusion of 2 mm was used as the criterion for completion of germination. When no further germination of seeds was observed within 5 d, the germination test was stopped.

2.4. Effect of ABA and CHX treatment on seed germination

Two layers of filter paper were put into 60-mm-diameter Petri dishes, and then three ml of 0, 1, 5, 10, 25, and 50 μM ABA or 0, 10, 50, 100, and 200 μM CHX solution was added, respectively. Three replicates of 100 seeds each were germinated on the filter paper in darkness at 10, 15, and 20 °C, respectively. Radicle protrusion of 2 mm was used as the criterion for completion of germination. When no further germination of seeds was observed within 5 d, the germination test was stopped.

2.5. Preparation of protein samples

Poplar seeds were germinated at 15 °C in water for 0, 12, 24, and 36 h, or in 50 μM ABA or 100 μM CHX for 24 h, respectively. The protein preparation of different seed samples was performed based on the methods of Zhang et al. (2015) and Xu et al. (2016).

2.6. Two-dimensional (2-D) gel electrophoresis

The protein sample was dissolved in 300 μl sample buffer composed of 7 M urea, 2 M thiourea, 2% (w/v) CHAPS, 20 mM dithiothreitol, and 0.5% (v/v) immobilized pH gradient buffer (pH 5–8). The protein concentration was assayed according to the method of Bradford (1976) using bovine serum albumin as the standard. 2-D gel electrophoresis was performed as described by Zhang et al. (2015) and Xu et al. (2016).

2.7. Image analysis, in-gel digestion with trypsin, and protein identification by MALDI-TOF-TOF mass spectrometry

The 2-D gels were scanned at a 300 dpi resolution with a UMAX Power Look 2100XL scanner (Maxium Tech., Taipei, China). Spot detection and gel comparison were made according to the methods of Zhang et al. (2015) and Xu et al. (2016). Well-resolved gels of three independent biological replicates were used for proteomic comparison. Spots were considered reproducible when they were well resolved in three biological replicates. The normalized volume of each spot was assumed to represent its protein abundance. When comparing spot size between groups, a difference was considered significant when the change was ≥ 1.5-fold and $P < 0.05$ (t-test).

The protein spots showing a significant change were excised from the stained gels. In-gel digestion with trypsin and peptide extraction were performed as described by Zhang et al. (2015) and Xu et al. (2016).

The peptide mixtures were desalted and identified as described by Zhang et al. (2015). The peptide mass fingerprints obtained were used to search protein databases using Mascot (Matrix Science, Boston, MA, USA). The following search parameters were used: Database—NCBInr and/or SwissProt, Taxonomy—Green plants, maximum 1 missed cleavage, cysteine carbamidomethylation as a fixed modification, methionine oxidation and N-terminal acetylation as variable modifications, mass tolerance of 70 ppm in MS mode, and 0.6 Da for MS/MS. The MOWSE score of identified proteins had to exceed 100 ($P < 0.05$) in SwissProt and NCBI database.

2.8. Statistical analysis

All data were analyzed using a one-way ANOVA model from the SPSS 19.0 package for Windows (SPSS, 2010) and were expressed as mean ± SD for at least three independent biological replicates.