

Transcriptome and Degradome Sequencing Reveals Dormancy Mechanisms of *Cunninghamia lanceolata* Seeds¹

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Seeds with physiological dormancy usually experience primary and secondary dormancy in the nature; however, little is known about the differential regulation of primary and secondary dormancy. We combined multiple approaches to investigate cytological changes, hormonal levels, and gene expression dynamics in *Cunninghamia lanceolata* seeds during primary dormancy release and secondary dormancy induction. Light microscopy and transmission electron microscopy revealed that protein bodies in the embryo cells coalesced during primary dormancy release and then separated during secondary dormancy induction. Transcriptomic profiling demonstrated that expression of genes negatively regulating gibberellic acid (GA) sensitivity reduced specifically during primary dormancy release, whereas the expression of genes positively regulating abscisic acid (ABA) biosynthesis increased during secondary dormancy induction. Parallel analysis of RNA ends revealed uncapped transcripts for ~55% of all unigenes. A negative correlation between fold changes in expression levels of uncapped versus capped mRNAs was observed during primary dormancy release. However, this correlation was loose during secondary dormancy induction. Our analyses suggest that the reversible changes in cytology and gene expression during dormancy release and induction are related to ABA/GA balance. Moreover, mRNA degradation functions as a critical posttranscriptional regulator during primary dormancy release. These findings provide a mechanistic framework for understanding physiological dormancy in seeds.

Seed dormancy mediates the crucial interaction between the environment and the plant life cycle transition from durable seeds to vulnerable seedlings (Willis et al., 2014). Different plant species have adopted

various classes of dormancy to regulate the timing of seed germination, help seedlings emerge under favorable conditions, and maximize the growing season (Baskin and Baskin, 2004; Donohue et al., 2010). Physiological dormancy, the most widely occurring type, is caused by inadequate growth potential of the embryo, which prevents it from overcoming the resistance of its surrounding tissues (Baskin and Baskin, 2014). Seeds with physiological dormancy can change their degree of dormancy, a process known as dormancy cycling: Primary dormant seeds enter nondormancy during seasons favorable for germination, and nondormant seeds may reenter dormancy (secondary dormancy) under unfavorable conditions if they have not germinated (Footitt et al., 2011; Cao et al., 2014). Due to the importance of seed dormancy in the plant life cycle, particularly in agriculture and vegetative restoration, many studies have focused on the regulatory mechanisms underlying seed dormancy.

Genetic studies have revealed some genes that regulate seed dormancy and germination, especially genes involved in abscisic acid (ABA) and gibberellic acid (GA) pathways (Shu et al., 2013; Vaistij et al., 2013; Wang et al., 2013). Also exogenous application of these phytohormones modifies seed germination (Ogawa

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D.C. and J.L. conceived the project idea and designed the experiments; D.C. and H.X. performed the experiments; D.C. performed the data analysis and prepared the figures and tables; W.J.J.S., X.D., Y.L., and Y.Z. advised on the analysis and interpretation of the results; D.C. and H.X. did most of the writing of the manuscript in consultation with all others; all authors approved the final manuscript.

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