TRANSCRIPTOME AND PROTEOMICS INTERACTION REVEALS LOW SEED GERMINATION OF CYClobalnopsis gilva TO SAVE FOREST ECOLOGY


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Abstract. Tree species display wide range of heterogeneity in their size, type, dormancy and growth of seed. Data about variation in germination under natural conditions and tree growth in connection with mentioned attributes is of immense value to understand tree distribution and forest stand management. Studies dealing with tree seed germination can enable successful nursery operations and healthy seedling production. This may also increase the seedlings’ establishment in the forest restoration activities on destructed sites through native plant species. Successful germination is not only critical for plantlet establishment but also necessary for crop yield. As seed detaches from mother plant it desiccates continuously and selects suitable environment for germination activity. To understand the low germination rate in Cyclobalnopsis gilva molecular aspects of germination has been elucidated well with the integrated studies of proteomic, transcriptomic molecular biology. In this review, common and different aspects of seed germination including metabolic activation, transcription and translation regulation, have been discussed. This review will help to understand the transcriptomic and proteomic interaction of C. gilva which will be helpful to save the forest ecology and solve the problem of woody plants with low germination rate all over the world.

Keywords: environment, woody plants, forest, metabolic activation, transcription, translation

Introduction

Seed germination comprises of three interlinked processes, such as (I) rapid water uptake, (II) plateau phase and (III) post-germination. Phase (I) starts with the water absorption which helps in the breakdown of starch, proteins, and lipids along with the continuity of glycolytic and oxidative pentose phosphate pathways (Howell et al., 2007; Macovei et al., 2011). Phase II involves mitochondrial synthesis (Howell et al., 2007) and translation of stored mRNA (Dinkova et al., 2011; Fig. 1). Phase III follows the elongation of embryonic axes leading to radicle growth. Previous research studies have explained seed germination with considerable progress from more than two decades. However, there are many small circles of seed germination, which demands more
exploration for the proper understanding of mutual linkages between various pathways (He and Yang, 2013).

In Arabidopsis, barley and rice more than ten thousand stored mRNA and transcripts have been identified and several studies confirmed that mRNA are stored during seed maturation (Rajjou et al., 2004; Nakabayashi et al., 2005; Kimura and Nambara, 2010; Okamoto et al., 2010; Radchuk et al., 2007). Until now, the confirmed transcriptomes from whole dry seeds have presented the diverse type of transcripts according to their genetic and physiological parts like the embryo and endosperm (Le et al., 2010). Furthermore, the transcriptional theory was genuinely authenticated by Kimura and Nambara (2010). Seed germination depends upon the variation in physio-chemistry of different parts of seeds i.e. embryo, endosperm seed coat and interaction among them (Nonogaki et al., 2000). Embryo is a vital part of the seed, as it contains genetic information required for triggering the seed germination process (Sheoran et al., 2005). There are several enzymes involved in metabolic pathways during seed germination and that stored during seed maturation (www.seed-proteome.com) (Rajjou et al., 2004; Fujino et al., 2008; Gallardo et al., 2001). Okamtao (2010) reported that during
imbibition transcriptome profile in germinating seed changes and even these changes are tissue specific. During early germination, transcripts are noticed to share similar or common but highly abundant cis-acting elements in their respective gene promoter followed by high expression pattern (Marcus and Feeley, 1964; Dure and Waters, 1965; Comai and Harada, 1990). Degradation of stored mRNA is associated with a change in expression of a gene (Comai and Harada, 1990; Nakabayashi et al., 2005; Howell et al., 2009; Preston and Hileman, 2009). The mechanisms involved in seed germination, numerous broad-spectrum omics studies have been conducted across the globe, involving transcriptomic approaches (Holdsworth et al., 2008; Sghaier-Hammami et al., 2009a, b). Productivity and survival of woody plants is continuously affected by multiple stresses (Pinheiro et al., 2014). Plants growth is negatively affected by surrounding biotic and abiotic stress agents that cause physical and biological dysfunctions and plants differ in their ability to overcome these stresses. Aside from the huge proteomics data available for woody plants, limited information is available on seed germination. Until now, *Fagus sylvatica* is among the limited plants for which attempts has been made to know the proteomic changes associated with seed germination (Pawlowski, 2007), similarly *P. dactylifera* (Sghaier-Hammami et al., 2009a, b), *J. curcas* (Yang et al., 2009) and *P. campanulata* (Lee et al., 2006) are some other examples. Depending upon seed dormancy woody plants have four categories (Gendreau and Corbineau, 2009) that are physical, mechanical, morphological and embryo dormancy. Fukumoto and Kajimura (2001) reported the poor regeneration of *Cyclobalnopsis* sp is due to insect damage of seeds and deer grazing of regenerating seedlings which are threatening the successful regeneration of *Cyclobalnopsis* sp. Transcriptome and proteomic interaction of woody plants during the seed germination studies has been ignored till now. *Cyclobalnopsis gilva* a woody plant has low germination rate as compared to other plants (Meiqing et al., 2012). It belongs to *Fagaceae* family and because of its hardness it is used in the furniture industry. (Zaynab et al., 2017). It is native in the southeastern part of China (*Fig. 2*). Among evergreen broad leaves, Cyclobalanopsis is one of an abundantly found woody tree which is a good biological filter that reduces environmental pollution (Deng et al., 2014). Being efficient natural cleaners, trees can help to overcome environmental pollution (Seth et al., 2003). Leaves with hairy, spiny and waxy surface can easily absorb gaseous form of pollutants while particle forms of pollutants can be trapped by twigs, branches and tree stems. Trees reduce carbon oxides, disintegrate waste materials and fix nitrogen in atmosphere. Trees act as pollution sinks and metabolize heavy metals and absorb toxic gases from the surrounding environment (Yang et al., 2017). There are no studies reported transcriptome and proteome changes analysis during seed germination of *C. gilva*. The basic purpose of this study/review is to describe transcript and protein change during the complex mechanisms of seed germination of *C. gilva*.

**Transcription and translation activation**

Gene expression changes dramatically at proteomic as well as transcriptomics level as seed imbibes water (*Fig. 3*) (Holdsworth, 2008). Weitbrech et al. (2011) reported that these alterations in gene expression are related to germination. It is considered that transcription is not going on in fully ripened seed, but mRNAs which are essential for germination have already been stored (Howell, 2009). Large number of mRNA species are present in fully ripen seed. Over 12000 mRNA have been found in mature seeds of
Arabidopsis. He et al. (2011) in rice embryo, about 17000 mRNA have been found (Howell, 2009). In rice and Arabidopsis α-amanitin, a transcription inhibitor has found to delay germination but not totally block it (Rajjou, 2004). It looks that transcription inhibitors have greater role to play in seedling establishment than in germination (He et al., 2011). These results suggest that factors assuring the germination speed are synthesized in de novo transcription. High increase in transcription occur in rice embryo after 3 to 12 h of water absorption which results in increased secondary metabolites production that are involved in cell wall, amino acids, carbohydrates metabolism (Howell, 2009). Rice and Arabidopsis germination was diminished in the presence of translation inhibitors which showed that instead of transcription, translation is prerequisite of germination (Rajjou, 2004). New protein synthesis in Arabidopsis starts 8 h after imbibition. That is why it is proposed that stored mRNAs are utilized for this protein synthesis. For metabolites activation and mobilization of reserves translation of stored mRNAs was important in both rice and Arabidopsis seeds (Rajjou, 2012, 2006). In maize seeds in vitro incubation experiments demonstrated that eIF(iso)4E is required for translation of stored mRNAs (Dinkova et al., 2011). Arabidopsis double mutant i4g1/i4g2 knocked out for eIFiso4G1 and eIFiso4G2 (responsible for stored mRNA translation) showed diminished germination rate and long seed viability (Lellis, 2010). From this experiment it was clear that cereal seeds and Arabidopsis possess similar mode of translation for stored mRNA.
Figure 3. Molecular processes occurring in embryo and endosperm during C. gilva seed germination

Role of translation in seed germination

Previous observation inferred that upon imbibition radicle protrude out of seed coat in Arabidopsis seed in the presence a specific, potential inhibitor of DNA-dependent RNA polymerase “α-amanitin”, this showed that seed germination is not limited to de novo transcription (Rajjou, 2004). To speed up the germination vigor, de novo synthesis of transcripts encoding proteins and enzymes involved in GAs biosynthesis and involved in germination sensitivity to this hormone is important because germination uniformity and speed is strongly affected under absence of these transcripts. In contrast, under the presence of translation inhibitor “cycloheximide” Arabidopsis germination was totally blocked (Rajjou, 2001). These results highlight the importance of protein synthesis especially from the stored RNA templates in seed for the completion of germination. As seed store RNA and proteins during maturation on source plant, role of these stored elements is clear from these results and for Arabidopsis seed germination potential is planned during seed maturation. For germination, other studies have also identified the core role of translation. During germination of mature seeds high contribution of stored mRNA has been confirmed by transcriptome analysis (Nakabayashi et al., 2005). The study conducted by Nakabayashi et al. (2005) elaborated the importance of protein synthesis during early germination stages, as 2% to 3% genes showed upregulation while evidence was also provided by ribosomal proteins coding genes and translation initiation and elongation factors.

Recent analysis of sugarbeat (Beta vulgaris L.) seed proteome identified 758 proteins reconstructing and inferring their detailed metabolic status (Catusse et al., 2008). Seeds are prepared for germination mainly during maturation on plan as indicated by sugar beet seeds, which are prepared to mobilize their reserves including starch, proteins, phytate and triglycerides during germination as occur in Arabidopsis and they also
identified proteins not mentioned in seeds. Promisingly, data highlight that sugar beet seeds have opportunity to initiate translation either by using cap-independent mechanism or conventional way of cap-dependent mechanism. It is clear that during some events of cell life like during stress conditions or viral infection and mitosis, cell compromises its standard way of translation i.e. 5-end cap-recognition of mRNA while at the same time some mRNA use cap-independent mechanism of initiation as congregation initiation site for initiation factor (Baird et al., 2006). A model is suggested by these findings. In this model germinating seed has two pools of mRNA comprising of de novo synthesized mRNA and stored mRNA and during germination these pools allow their recruitment by either of two initiation pathways (Catusse et al., 2008). In maize seed, during embryonic axis elongation, stored mRNAs were likely to use cap-independent mechanism for translation initiation (Dinkova et al., 2005).

Comparison of the seed transcriptome and proteome

Comparison of several proteome and transcriptome studies carried out in the same environmental conditions and sampling points, showed little correlation between functional gene classes at proteomics and transcriptomics level (Gallardo et al., 2002; Ogawa et al., 2003; Chibani et al., 2006; Carrera et al., 2008). Germination vigor can possibly change the abundance of RNA present in seed for germination completion, some functions associated with establishment of seedling (translation after germination completion), for observation of changes in protein profiles, proteomic approaches are not sensitive enough. Up till now it is also vague that changes in seed dormancy status are controlled by translational or post-translational regulations (Holdsworth et al., 2008). Gene expression study is critically important to understand the molecular network involved in germination regulation as the list of genes regulated in imbibed seeds obtained through either proteomic (Chibani et al., 2006) or transcriptomic (Cadman et al., 2006) approaches showed similarities. Integration of information from proteomic and transcriptomic studies will be the key objective for further post-genomic research. Favorably, some proteomic and transcriptomic based studies under similar time essays and growth conditions are helping to integrate the information from different studies (Cadman et al., 2006; Chibani et al., 2006; Carrera et al., 2008).

Due to less germination rate of woody plants proteomic studies of seed germination are getting more importance (Zaynab et al., 2017; Canovas et al., 2004). It is difficult to examine woody plants due to laborious sample preparation and high concentrations of resins, tannins and phenolic compounds (Sebastiana et al., 2013). In a study, P. dactylifera L. (Date palm) proteomic studies for seed development and germination were done by Sghaier-Hammami et al. (2009) and they reported that carbohydrates, the major storage reserves are stored in early seed development which are used during initial stages of seed germination.

Comprehensive information regarding access amount of some functional protein types related to seed ripening, dormancy and germination have been provided by the proteomic analysis of germinating seeds. Many other studies have also shown the proteome changes related to dormancy and germination status (Gallardo et al., 2001, 2002; Job et al., 2005; Chibani et al., 2006; Oracz et al., 2007), regarding this information seed biology is a prominent host of plant proteomics. Proteomics is a strategy for global analysis that provides information on a multitude of processes in complex events, such as germination (Gallardo et al., 2001). Despite the increasing
interest in plant proteomics, studies on woody plant seed germination are still very limited. To date, the only attempts to understand proteome changes during the germination of woody plants were carried out in *Prunus campanulata* (Lee et al., 2006), *Fagus sylvatica* (Pawlowski, 2007), *Acer platanoides* (Pawlowski, 2009), *Jatropha curcas* (Yang et al., 2009), *Phoenix dactylifera* (Sghaier-Hammami et al., 2010, 2009b) and *Araucaria angustifolia* (Balbuena et al., 2011). Only a limited number of reports about proteomics in woody plant seed germination have been published. Lu et al. (2016) conducted a study to determine the mechanisms involved in seed dormancy breaking of *M. sieboldii* to better understand the anatomical and translation bases of seed germination in this species and they reported that a small subunit of legumin precursor, ADP-glucose pyrophosphorylase and aspartic proteinase dominate the seed proteome of *M. sieboldii*. Such studies not only provide information regarding the protein abundance in dry stored seeds and imbibed seeds but also elaborate the post-translational modifications for proteome expression. Gallardo et al. (2001) reported the proteome changes related to imbibition germination while Chilbani et al. (2006) studied the imbibed ripened seeds. Rajjou et al. (2004) noticed that germination in alpha-amanitin treated transparent testa (tt) of mutant seeds was not blocked concluded that transcription was not important for germination. On the other hand, cycloheximide was a potential inhibitor of radicle emergence. If transcription was important for expression of genes involved in synchronization and speed of germination was answered by the α-amanitin treatment. Seeds treated with α-amanitin showed great germination potential, but germination rate was slow and less sensitive to GA application and the level of several key proteins for germination (malate synthase) (Eastmond et al., 2000) were very low in treated seeds. Chibani et al. (2006) reported great difference in protein profiles of after-ripened and imbibed seeds of accession CVI. In dormant seeds lack of germination was not associated with less de novo protein production because 35S methionine incorporation quantification showed the same incorporation level in after-ripened and dormant seeds (Carrera et al., 2008). Exogenous ABA application greatly lowered the germination potential in after-ripened imbibed seeds, but it did not alter dormancy at proteomic level (Chibani et al., 2006). 35S methionine incorporation in ABA treated after-ripened imbibed seeds were greater that non-treated and dormant seeds, this indicated that ABA treatment does not hinder RNA translation protein abundance was also high in treated seeds. Both proteomic and transcriptomic studies showed that instead of seed dormancy ABA treatment use another way of inhibiting germination. This is not surprising because physiologically ABA does not reduce germination potential but only delay it (Muller et al., 2006). It is very likely that ABA application to un-germinated seeds just like drought and salinity causes increased ABA production, these responses have already been observed in germinating seedlings (Lopez-Molina et al., 2002).

**Post-translational control of seed germination**

A growing number of evidence indicates that large changes in redox state of protein occur as seed germinates. In dry cereal seeds proteins are present in oxidized (S–S) form in embryo and starchy endosperm, upon imbibition these are converted into sulfhydryl (–SH) or reduced form (Buchanan and Balmer (2005)). Thioredoxin (Trx), a regulatory protein seems to play core role in redox conversion. Trx cause reduction enzymatically in the presence of NADPH and facilitates reserves mobilization during germination in
three ways i.e. enhancing protein susceptibility and sensitivity to proteolysis by reducing them, enhancing breakdown of stored proteins and starch as it inactivates disulfide proteins inhibiting specific proteases and amylases and it activates enzymes functioning in germination (Fig. 3; Buchanan and Balmer, 2005). This issue has recently been investigated in barrel clover (Medicago truncatula) (Alkhalfoui, 2007). Role of Trx in monocotyledons and dicotyledons seed germination have been proved. It can be summarized that in dry and fully mature seeds oxidized proteome keep seed metabolically dormant, which is activated. It can be summarized that oxidized proteome in metabolically dormant seed is by redox reaction in protein bisulfide bridges in the presence of specific Trx during seed germination. Given the large number of proteins affected (more than 100) Alkhalfiou (2007), post-translational mechanism offers an efficient and simple way of controlling complex pathways critical in seed germination. It follows, that investigation of targets like post-translational modifications becomes an emerging question. As post-translational changes may become key regulators of cell activity upon imbibition, so this question is importance increasingly. Carbonylation of proteins by reactive oxygen species may be another oxidative change. In several model systems carbonylated proteins accumulation is referred to aging, but it is not for Arabidopsis where germination rate is high, and seedlings are vigorous. Results indicates that changes in protein carbonylation patterns may occur to nullify the ROS produced in germinating seeds by recovery of metabolic processes. Job et al. (2005) conducted a study in which it is suggested that carbonylation of proteins stored in seed is to facilitate the protein mobilization at time of seed germination. Jatropha and other woody plants store the metabolic reserves as starch, proteins and lipids in embryo and endosperm (Yang et al., 2009). Triacylglycerol (TAG) the major form of lipids is the storage reserve of oilseed plants. TAG is broken down by lipases to release fatty acids which are further catabolized in glyoxilate cycle and β-oxidation. Seed germination and development mechanism of woody plant A. angustifolia was studied by Balbuena et al. (2011). Their study showed the differential expression of proteins in germinated and mature embryos which suggests the activation of robust metabolic pathways, translation initiation and stress regulation, overall indicates active degradation metabolism.

Conclusion and perspectives

Like any other field of plant science seed biology is of importance and attracting attention of plant biologists. Previous studies have suggested complicated signalling network and gene expression regulation are involved in seed germination. Plants may share same molecular mechanisms as transcription and translational activation, phytohormone behavior, radicle protrusion etc. however different plant species possess distinct mechanisms, especially for metabolism activation and reserves mobilization. Furthermore, cross-talk among environmental factor signalling pathways and phytohormones during germination is spurious up till now. With the advent of omics era, molecular pathways underlying the germination are becoming more elaborated. For example, during germination, role of functional proteins and specific signalling components in rice and Arabidopsis have been identified by proteomic and phosphoproteomic studies. However, still much information is required to understand the germination process. Nevertheless, in future integration of newly emerging techniques including ChIP-sequencing, proteomic and transcriptomic techniques and imaging will contribute to understand the process how different signals conciliate seed.
germination. To better understand the underlying mechanisms of desiccation tolerance and intolerance, the physiological responses described require research at the metabolomic and transcriptomic levels in detail.

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