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ABA-Mediated Inhibition of Germination Is Related to the Inhibition of Genes Encoding Cell-Wall Biosynthetic and Architecture: Modifying Enzymes and Structural Proteins in *Medicago truncatula* Embryo Axis

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ABSTRACT Radicle emergence and reserves mobilization are two distinct programmes that are thought to control germination. Both programs are influenced by abscissic acid (ABA) but how this hormone controls seed germination is still poorly known. Phenotypic and microscopic observations of the embryo axis of *Medicago truncatula* during germination in mitotic inhibition condition triggered by 10 μM oryzalin showed that cell division was not required to allow radicle emergence. A suppressive subtractive hybridization showed that more than 10% of up-regulated genes in the embryo axis encoded proteins related to cell-wall biosynthesis. The expression of α-expansins, pectin-esterase, xylogucan-endotransglycosidase, cellulose synthase, and extensins was monitored in the embryo axis of seeds germinated on water, constant and transitory ABA. These genes were overexpressed before completion of germination in the control and strongly inhibited by ABA. The expression was re-established in the ABA transitory-treatment after the seeds were transferred back on water and proceeded to germination. This proves these genes as contributors to the completion of germination and strengthen the idea that cell-wall loosening and remodeling in relation to cell expansion in the embryo axis is a determinant feature in germination. Our results also showed that ABA controls germination through the control of radicle emergence, namely by inhibiting cell-wall loosening and expansion.

Key words: ABA; cell-wall expansion; germination; *Medicago truncatula*; radicle emergence; XET.

INTRODUCTION

In flowering plants, seed germination is the transition state between quiescent embryo and a new photo-synthetically active plant. This transition state itself can be divided into three phases. The first phase is short and is characterized by a sharp and passive increase in water content of the dry and mature seed, while the second phase, during which the water content remains constant, consists of the synthesis of new mRNAs and proteins. During the third and the last phase of germination, the embryo axis elongates and radicle emerges through surrounding tissue. A further increase in water uptake occurs afterwards during post-germination growth and seedling establishment (Bewley, 1997).

Seed germination has been proposed to be under the control of two distinct programs that can progress independently, one driving radicle emergence and the other driving mobilization of reserves (Pritchard et al., 2002). Carbon and nitrogen

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reserves' mobilization during germination has been studied in various cereal, legume, and non-legume species (Fath et al., 2001; Garciarrubio et al., 1997; Glevarec et al., 2004; Gomez-Cadenas et al., 2001; Limami et al., 2002). It has been hypothesized that abscissic acid (ABA) inhibition of germination occurs by preventing reserve mobilization pointing out the dependence of germination upon reserves mobilization (Finkelstein and Lynch, 2000; Garciarrubio et al., 1997). This hypothesis that has been named, 'the nutritional control of germination', was supported by the fact that the inhibitory effects of ABA were strongly repressed in the presence of glucose, sucrose, or fructose; however, this repression was limited to the process of radicle emergence (Finkelstein and Lynch, 2000). This hypothesis has been, however, weakened by the experiments of Pritchard et al. (2002), who showed that ABA inhibition of *Arabidopsis thaliana* was not accompanied by an inhibition of lipid (triacylglycerol) mobilization; moreover, sucrose derived from lipids degradation accumulated in ABA-treated seeds (Pritchard et al., 2002).

Embryo axis elongation and radicle emergence that are turgor-driven processes require loosening of cell walls in the cells of embryo root axes that lie between the root cap and the base of the hypocotyls. Many cellular and metabolic changes that occur in non-dormant seeds before the completion of germination also occur in dormant seeds; but embryo axes of only non-dormant seeds elongate. Several studies suggest that the real inhibition of germination in dormant seeds occurs at the very last stage and that is the inability of cell walls of radicle cells to elongate (Bewley, 1997). Analysis of impact of ABA, a potent inhibitor of germination on *Brassica napus* seeds, showed that neither osmotic potential of embryo axis cells nor their ability to uptake water was affected by the presence of ABA, but rather cell-wall loosening was prevented, resulting in the inhibition of germination. In general, prevention of embryo radicle extension can be achieved by incubating mature seeds in solutions of ABA even when ABA is introduced late during germination. This strengthens the idea that ABA acts to prevent the third phase of germination, such as radicle cell-wall loosening. In sunflower, temporary application of ABA to embryos only prevents radicle extension while its removal leads to the completion of germination (Bewley, 1997). Altogether, these findings highlight the importance of cell-wall loosening in embryo axis cells that allows for cell elongation, probably a determinant event in embryo axis elongation during germination. However, the occurrence and control of this phenomenon, cell-wall loosening or radicle elongation, are poorly known.

Our strategy for improving knowledge of important and vital events of germination was based on a suppressive and subtractive hybridization (SSH) transcriptomics analysis of embryo axes for the identification of genes involved in germination completion. In the present study, the subtraction was carried out between two mRNA populations extracted at two imbibition stages, 6 hours (i.e. the beginning of the second phase of germination) and 23 h (i.e. 2 h after the end of the second phase of germination). It is important to note that new mRNAs are synthesized during the second phase of germination. The fact that this study revealed that more than 10% of up-regulated genes encode proteins related to cell-wall loosening and expansion spurred us to put forward the hypothesis that a late event during germination such as radicle cell-wall loosening, a prerequisite for cell expansion, might be a determinant in germination completion and that event might be controlled by ABA.

RESULTS

Effect of Constant and Transitory Abscisic Acid Treatment on Germination and Post-Germination Growth of *Medicago truncatula*

To determine the effects of a constant and transitory ABA treatment versus water (control) on germination and post-germination growth, germination rate, and radicle length were examined under the three conditions. In water condition, 50% germination (T50) was reached after 22 h of imbibition and 88% after 25 h (Figure 1A). This seed lot has

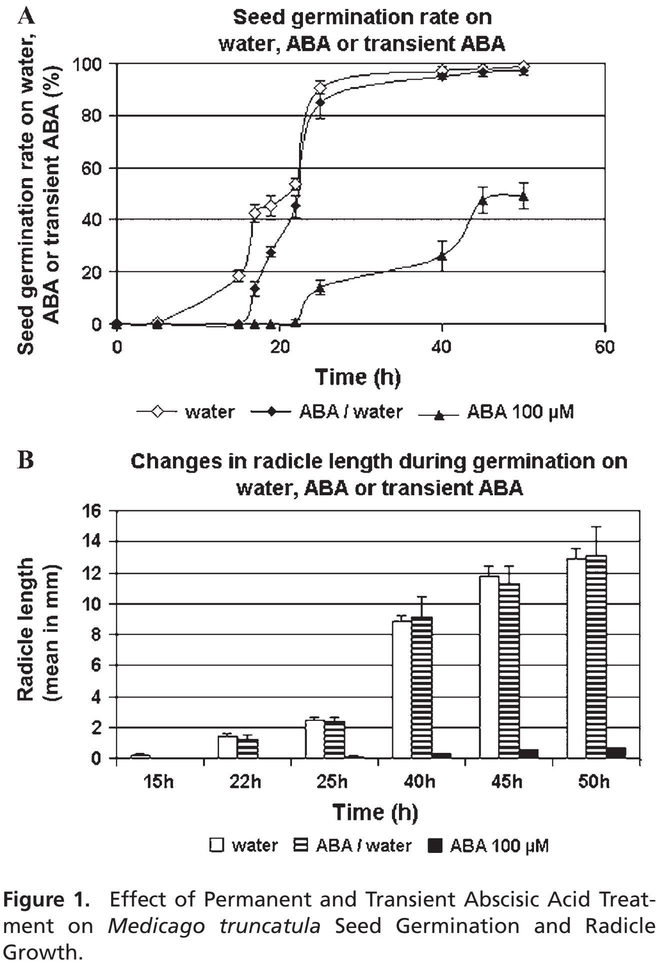

Figure 1. Effect of Permanent and Transient Abscisic Acid Treatment on *Medicago truncatula* Seed Germination and Radicle Growth.

Germination rates **(A)** and radicle length **(B)** are the mean of 150 measurements taken on 150 seeds germinated on water, 100 µM ABA or 100 µM ABA during 15 h before transfer of the seeds on water.