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cDNA-AFLP analysis of seed germination in *Arabidopsis thaliana* identifies transposons and new genomic sequences

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Summary

A cDNA-AFLP experiment was designed to identify and clone nucleotide sequences induced during seed germination in *Arabidopsis thaliana*. Sequences corresponding to known genes involved in processes important for germination, such as mitochondrial biogenesis, protein synthesis and cell cycle progression, were isolated. Other sequences correspond to *Arabidopsis* BAC clones in regions where genes have not been annotated. Notably, a number of the sequences cloned did not correspond to available sequences in the databases from the *Arabidopsis* genome, but instead present significant similarity with DNA from other organisms, for example fish species; among them, some may encode transposons. A number of the sequences isolated showed no significant similarity with any sequences in the public databases. Oligonucleotides derived from these new sequences were used to amplify genomic DNA of *Arabidopsis*. Expression analysis of representative sequences is presented. This work suggests that, during germination, there may be a massive transposon mobilization that may be useful in the annotation of new genome sequences and identification of regulatory mechanisms.

Introduction

Germination is the process by which a seed initiates growth after a period of quiescence. It requires seed imbibition, and in a strict sense, is

Abbreviations: AFLP, amplification fragment length polymorphism; cDNA, complementary DNA; GA, gibberellic acid

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defined as the process leading to emergence of the radicle through the testa, a tissue of maternal origin that surrounds the embryo (Bewley, 1997; Koornneef et al., 2002). Germination is thus finished once the radicle has emerged.

Imbibition, i.e. water uptake by the seeds, is accompanied by cell expansion, cell wall synthesis and activation of metabolism. Accumulating evidence indicates that, in general, cell division occurs following germination (de Castro et al., 2000; Barroco et al., 2005). The increase in cell growth that is required for germination is due to cell elongation.

In a very short time interval, a limited number of cells elongate and go through differentiation processes based in rapid metabolic changes preceding cell division. The process of germination is under the control of environmental and hormonal factors thus making the system appropriate for the study of plant development and the cellular responses to these factors.

In *Arabidopsis*, as in many other plant species, germination is under the control of phytochrome. The seeds require the presence of light to germinate, and after activation by red light, phytochrome regulates the production of Gibberellic acid (GA) biosynthetic enzymes as well as of other genes, including transcription factors (Yamaguchi et al., 1998; Kamiya and García-Martínez, 1999; Tepperman et al., 2001). Nevertheless, the classical view that explains seed germination as a consecutive series of causes and effects ending in the activation of genes that encode proteins necessary for radicle elongation may, in the light of recent results, be modified for a more integrative view that takes into consideration global genome dynamics (Lippman et al., 2004; Nakabayashi et al., 2005)

GA is needed for *Arabidopsis* seed germination: *ga1* mutants, unable to synthesize GA, do not complete germination unless GA is added exogenously or the testa broken or removed artificially (Silverstone et al., 1997). GA is required to overcome ABA induced dormancy and seed coat resistance to germination (Debeaujon and Koornneef, 2000). Processes under the control of GA during germination of *Arabidopsis thaliana* seeds have been recently analyzed by microarray techniques. Upregulated genes included those whose products are involved in cell elongation and division, transcriptional regulation and enzymes responsible for hormone biosynthesis (Ogawa et al., 2003). The variations in gene expression in the microarray study are limited to the genes included in the array. An alternative approach, such as complementary DNA (cDNA)-amplification fragment length polymorphism (AFLP), may be helpful to uncover new transcripts whose products are involved in germination.

Proteins involved in the repression of germination are negatively regulated by GA and include members of the GRAS multiprotein family of transcriptional regulators involved in cell differentiation. In *Arabidopsis*, the DELLA subfamily of GRAS regulatory genes consists of *GAI* (*Gibberellic Acid Insensitive*), *RGA* (*Regulator of gibberellin response*), *RGA*-LIKE1 (*RGL1*), *RGL2*, and *RGL3*. All contain DELLA protein motifs. Among them, *RGL1* and *RGL2* have been demonstrated to be involved in germination (Peng and Harberd, 2002). Mutants in *RGL1* present reduced sensitivity to paclobutrazol, an inhibitor of GA biosynthesis (Wen and Chang, 2002) and mutations in *RGL2*, restore germination to germination-deficient mutants or wild-type seeds treated with inhibitors (Lee et al., 2002). Thus, an important effect of GA in the induction of germination may be the reduction of the levels of these inhibitors. GA action is regulated by the ubiquitin–proteasome pathway. The genes *Sleepy1* (*SLY1*) and *Sneezy* (*SNE*) encode F-box proteins of a Skp1-cullin-F-box (SCF), E3 ubiquitin ligase complex that positively regulates GA signaling trough targeted proteolysis of the DELLA proteins using ubiquitin mediated destruction (McGinnis et al., 2003; Strader et al., 2004).

The mechanism by which DELLA proteins exert their inhibitory effect is still unclear. For RGA, its nuclear localization is reduced by GA in coordination with auxin and ethylene, leading to root elongation (Achard et al., 2003; Fu and Harberd, 2003) but the nuclear targets of RGA have not yet been identified.

It has been shown that many non-coding RNAs may be expressed in a variety of tissues and developmental conditions. Among them, some may be precursors of microRNAs, whose roles in differentiation processes have been recently demonstrated both in plant and in animal systems (Reinhart et al., 2002; Houbaviy et al., 2003). This work reports on the identification of sequences regulated by GA early during imbibition. The cDNA-AFLP approach allows the identification of expressed sequences independent of their coding capacity. The analysis of their regulation in diverse mutant backgrounds and during the course of germination may provide insights into this process.

Materials and methods

Plant material

A. thaliana cv. Wassilewskija, as well as its derived mutant *abc33* (*ga1* allele described by