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Hormonal control of seed development in GA- and ABA-deficient tomato (*Lycopersicon esculentum* Mill. cv. Moneymaker) mutants

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Abstract

Developing seeds of tomato gibberellin (GA)-deficient *gib1* and abscisic acid (ABA)-deficient *sit*^{w} mutants enabled us to analyze the role of GA in the regulation of embryo histo-differentiation, and the role of ABA in the regulation of maturation and quiescence. Our data show that DNA synthesis and mitotic microtubule arrays are markers for cell division activity and histo-differentiation during early embryogenesis. Cortical microtubular cytoskeleton alone is a marker for expansion growth during maturation, as seed and embryo gain dry weight and attain their final size. During this phase germinability, desiccation tolerance and dormancy are acquired, and a transient increase in ABA occurs, preceding the achievement of physiological maturity and subsequent quiescence. In the *gib1* mutant embryo development was retarded in all parameters studied, except for a transient rise in ABA content. In the *sit*^{w} mutant embryonic DNA synthesis activity was resumed upon completion of histo-differentiation, the microtubular cytoskeleton network was re-established during maturation and followed by viviparous germination. This suggests that ABA controls the suppression of these events during maturation and quiescence. Induction of full seed germinability, desiccation tolerance and dormancy was related to the completion of embryo histo-differentiation but was independent of the state of the microtubular cytoskeleton during maturation.

Keywords: Tomato; Seed development; Hormonal regulation; Gibberellin; ABA; Cell cycle

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1. Introduction

Seed development can be divided in a number of distinct phases that are marked by morphological, genetic and physiological parameters. The first phase is one of histo-differentiation during which the embryo's body plan is established through intensive cell divisions and the embryonic organs and tissues are formed [1–3]. A following phase is a period of seed maturation. It includes the arrest of tissue and organ formation, the accumulation of nutrient reserves, changes in embryo size and in fresh and dry weights, and the suppression of precocious germination [4]. During development and maturation the embryo typically acquires the characteristics that are necessary for survival and growth, such as desiccation tolerance and quiescence and, in many species, dormancy [5]. Concomitantly, programmed transitions occur from a stage of cell proliferation to that of quiescence in the meristematic tissues of the mature embryonic axis [6], followed by germination and post-germination growth during which metabolic and morphogenetic reactivation of the quiescent seed occurs [7–9].

Mutants have proved to be very useful tools in dissecting the genetic regulation of seed developmental processes, e.g. the embryo mutants in *Arabidopsis thaliana* [2] and maize (*Zea mays*) [10]. Abscisic acid (ABA)-deficient mutants of tomato can be considered developmental mutants because there is no arrest of growth during the maturation phase. In studies with the ABA-deficient *sit*^{w} mutant, ABA was shown to be essential for the induction of dormancy and inhibition of precocious germination [11,12]. In contrast with wild type tomato (cv. Moneymaker), dormancy is not expressed in the *sit*^{w} mutant, and the embryo does not enter a quiescent state during seed maturation. Therefore, seeds of this mutant, as well as of ABA-deficient mutants of *A. thaliana* [13,14] and maize [15] may germinate viviparously during development.

Seeds from the gibberellin (GA)-deficient *gib1* tomato mutant appear to develop normally, in that acquisition of dry

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and fresh weights and morphology did not differ from the wild type, but the seeds are not able to germinate in the absence of exogenous GAs [16]. However, Swain et al. [17] reported that GA-deficient *lh-1* and *lh-2* pea (*Pisum sativum*) seeds grew slower than wild type seeds during part of their development but attained the same dry weight at physiological maturity after a similar developmental time as the control seeds. Although in some species GAs are present during early embryogenesis very little is known about the role of GAs in seed development [18,19]. In maize seed development it has been suggested that a GA/ABA balance determines the engagement of either germination or maturation pathways [19,20].

The relationship between the developmental transitions, the action of hormones, and the physiology of seed development is not well understood. The objective of the present study is to identify links between the developmental stages and the acquisition of such typical seed characteristics as germinability, desiccation tolerance and dormancy, and to assess the roles of GA and ABA. Embryonic nuclear DNA synthesis activity and β-tubulin accumulation are good markers for meristematic activity and cell expansion, respectively, and were analyzed to mark the developmental phases and link them with the physiological and morphological markers [12,21].

2. Materials and methods

2.1. Plant materials

Plants from wild type tomato (*Lycopersicon esculentum* Mill. cv. Moneymaker) and its GA- and ABA-deficient mutants, *gib1* and sit^{w}, were grown simultaneously in a greenhouse under natural daylight at 25 °C/20 °C day/night average temperatures. Plants of the *gib1* mutant were sprayed once a week with a solution of 10 μM GA_{4+7} (Plant Protection LTD, Surrey, UK) on the top and flower bud regions to stimulate shoot growth and development of petals and anthers [16]. Plants of the sit^{w} mutant were sprayed once a week with a 10 μM ABA (ACROS, Geel, Belgium) solution to reduce wilting [16,22]. Sufficient flowers of the three genotypes were self-pollinated and tagged, allowing a minimum of five wild type and 15 *gib1* or sit^{w} fruits to be harvested at the same time at different days-after-pollination (DAP). Seeds were isolated from freshly harvested fruits, followed by excision of embryos which were either immediately used for analysis or frozen in liquid nitrogen and stored at −70 °C. The moisture content of three replicates of 20 embryos was determined by measuring the weights of embryos before and after drying at 130 °C for 1 h.

2.2. Germination

Germination analysis was conducted on three replicates of 25 seeds placed on top of two layers of filter paper (Whatman No. 1) in 9 cm Petri dishes soaked with 6 ml distilled water or 10 μM GA_{4+7} in the dark. Germination was monitored daily for 7 days. Seedlings were evaluated at day 7 of imbibition. The presence of dormancy in 42–56 DAP non-germinated wild type seeds was tested by applying a chilling treatment of 10 °C for 24 h and then transferring the seeds back to 25 °C for an additional 7 days. Acquisition of desiccation tolerance was tested by drying the seeds as previously described [23] and testing them for germination at 25 °C.

2.3. ABA extraction and quantification

The ABA extraction from whole seeds was based on the procedure of Raikhel et al. [24], as modified by Berry and Bewley [25]. ABA was quantified in two replicates of 25 fresh seeds, using a Phytodetek ABA immunoassay kit, based on a monoclonal antibody ELISA method (Sigma/Idetek, St. Louis, MO), using (+/−) ABA as a standard.

2.4. DNA replication

Depending on the stage of development, two replicates of 10 (21–28 DAP) or 5 embryos (35 DAP onwards) were used for flow cytometry. With all samples, a minimum of 10,000 nuclei was analyzed. Extraction of nuclei, flow cytometry and measurement of DNA contents were performed according to Sacandé et al. [21].

2.5. Detection of β-tubulin

Total protein extraction from embryos, electrophoresis, western blotting and immuno-chemiluminescent detection of β-tubulin were conducted as described by de Castro et al. [23,26].

2.6. Immuno-histochemical detection of β-tubulin

The sampled material was fixed in paraformaldehyde, dehydrated in ethanol series and embedded in butylmethylmetacrylate, sectioned, and affixed on slides according to Baskin et al. [27]. β-Tubulin immuno-labeling and microscopy were according to Xu et al. [28]. Anti-β-tubulin (1:200, v/v) was used as the first antibody (Amersham, Buckinhamshire, UK) and FITC conjugated goat anti-mouse (1:200, v/v) as second antibody (Amersham, Buckinhamshire, UK). Omission of the first antibody and application of pre-immune serum served as controls and showed no fluorescence. From each of the studied stages at least five embryos were randomly selected, except when a distinction was made between germinated (radicle protruded) and ungerminated seeds. Of each embryo 10–20 sections on the same slide were observed. One of the median sections was selected as representative for the whole population. Independent repetitions, following this protocol, yielded essentially similar results.

3. Results

3.1. Dry weight, germinability, desiccation tolerance, dormancy and ABA content

An increase in dry weight was observed in wild type and sit^{w} embryos between 21 and 49 DAP (Fig. 1A). However, the dry