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The mechanism underlying fast germination of tomato cultivar LA2711

Rongchao Yang[a,c], Zhuannan Chu[b], Haijun Zhang[a], Ying Li[b], Jinfang Wang[a], Dianbo Li[a], Sarah Weeda[d], Shuxin Ren[d], Bo Ouyang[b,**], Yang-Dong Guo[a,*]

[a] College of Agriculture and Biotechnology, China Agricultural University, Beijing 100193, China
[b] College of Horticulture and Forestry Science, Huazhong Agricultural University, Wuhan 430070, China
[c] Institute of Facilities Agriculture, Chinese Academy of Agricultural Engineering, Beijing 100026, China
[d] School of Agriculture, Virginia State University, Petersburg, USA

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ABSTRACT

Seed germination is important for early plant morphogenesis as well as abiotic stress tolerance, and is mainly controlled by the phytohormones abscisic acid (ABA) and gibberellic acid (GA). Our previous studies identified a salt-tolerant tomato cultivar, LA2711, which is also a fast-germinating genotype, compared to its salt-sensitive counterpart, ZS-5. In an effort to further clarify the mechanism underlying this phenomenon, we compared the dynamic levels of ABA and GA_4, the transcript abundance of genes involved in their biosynthesis and catabolism as well as signal transduction between the two cultivars. In addition, we tested seed germination sensitivity to ABA and GAs. Our results revealed that insensitivity of seed germination to exogenous ABA and low ABA content in seeds are the physiological mechanisms conferring faster germination rates of LA2711 seeds. *SlCYP707A2*, which encodes an ABA catabolic enzyme, may play a decisive role in the fast germination rate of LA2711, as it showed a significantly higher level of expression in LA2711 than ZS-5 at most time points tested during germination. The current results will enable us to gain insight into the mechanism(s) regarding seed germination of tomato and the role of fast germination in stress tolerance.

1. Introduction

Seed germination is accomplished by a well-orchestrated series of events which includes complex interactions with the environment mediated by phytohormones and other small molecules which signal a suitable environment for germination to ensure plant survival. The physiological mechanism of germination includes abscisic acid (ABA), gibberellins (GAs), brassinosteroids, ethylene, reactive oxygen species, and nitrogen-containing compounds such as nitrate and nitric oxide (NO) [1]. Specifically, ABA and GAs are considered to be absolutely required, and their dynamic equilibrium is central to seed dormancy and the control of germination [2,3].

ABA is a major plant hormone that functions in the initiation and maintenance of seed dormancy, and it induces transcription factors and other regulatory proteins involved in suppressing germination [4–6]. The level of ABA in seeds is determined by its biosynthesis, catabolism and transport [7]. ABA biosynthesis in seeds is mainly regulated by the rate-limiting enzyme 9-cis-epoxycarotenoid dioxygenase (NCED) [8,9], while its catabolism is mediated by ABA 8′-hydroxylase encoded by CYP707A gene family members [10–12]. In tomato, ABA levels are mainly determined by *SlNCED1* during fruit set and under drought or salt stress [13,14], however, *SlNCED2* has not been characterized. In addition, four predicted CYP707A genes have been identified; among them, the expression patterns of *SlCYP707A1*, *SlCYP707A2* and *SlCYP707A4* correlate with ABA levels. Furthermore, transgenic plants over-expressing *SlCYP707A1* have reduced ABA levels and exhibit ABA deficient phenotypes [15]. The level of *SlCYP707A2* is the highest

Abbreviations: ABA, abscisic acid; GA, gibberellic acid; RT-qPCR, reverse transcription-quantitative real-time PCR; NO, nitric oxide; CTK, cytokinin; IAA, auxin; ETH, ethylene; BR, brassinosteriods; NCED, nine-cis-epoxycarotenoid dioxygenase; PYL, PYR1-like; PP2Cs, type 2C protein phosphatases; SnRK2s, SNF1-related kinases; ABI 5, ABA insensitive 5; GPS, geranyl diphosphate synthase; GPS, geranyl diphosphate synthase; CPS, copalyldiphosphate synthase; GID1, gibberellin insensitive dwarf 1; EXP, expansin; GulB, b-1,3-glucanase; MAN, endo-b-mannanase; XTH, xyloglucan endotransglucosylase/hydrolase; B9, daminozide; PBZ, paclobutrazol; EF 1α, elongation factor 1α.

* Corresponding author. Tel.: +86 10 62734845; fax: +86 10 6273 3404.
** Corresponding author. Tel.: +86 27 8728 1679; fax: +86 27 8728 0016.
E-mail addresses: bouy@mail.hzau.edu.cn (B. Ouyang), yaguo@cau.edu.cn (Y.-D. Guo).

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during tomato seed germination, and *SlCYP707A4* is induced by salt stress [13,14]. To date, *SlCYP707A3* has not been examined. The studies of ABA receptors [16–18] and ABA signaling [19,20] have demonstrated that the ABA signal transduction pathway includes three core components: ABA receptors (PYR/PYL/RCARs), type 2C protein phosphatases (PP2Cs) acting as negative regulators, and SNF1-related kinases (SnRK2s) acting as positive regulators. Transcriptional regulation of the three core components has been investigated during tomato fruit development and under drought stress [21].

Gibberellins (GAs), a large family of tetracyclic diterpenes, are essential to stimulate seed germination in a wide range of plant species. This has been demonstrated by the inability of GA-deficient tomato (*gib-1*) and *Arabidopsis* (*ga1-3*) mutants to germinate without exogenous GA [22,23]. The predominantly active GA is dependent on plant species and physiological processes. GAs are produced through two parallel pathways including the non-13-hydroxylation pathway and the early 13-hydroxylation pathway, which lead to the production of GA_4 and GA_1, respectively. In tomato, the GA biosynthetic genes copalyldiphosphate synthase (CPS), GA 20-oxidase (GA20ox), GA 3-oxidase (GA3ox) and GA 2-oxidase (GA2ox) have been identified [24,25]. During fruit set in tomato, the early 13-hydroxylation pathway is the primary metabolic pathway, and GA_1 is the predominant bioactive GA molecule [26,27]. Conversely, during tomato seed germination, the non-13-hydroxylation pathway is the primary metabolic pathway, and GA_4 is the predominant bioactive GA molecule. GAs stimulate germination by inducing hydrolytic enzymes that weaken the endosperm, allowing for the expansion of the embryo [22]. The transcriptional expression of several genes encoding expansin (EXP), b-1,3-glucanase (GulB), endo-b-mannanase (MAN) and xyloglucan endotransglucosylase/hydrolase (XTH), are induced by GA. It has been shown that the weakening of tomato endosperm cap is a biphasic process: the first step is ABA-independent weakening of the endosperm cap associated with several enzymes as mentioned above, the second step is radical emergence inhibited by ABA through enzymes such as GulB [28–31]. Although other plant hormones (ETH, IAA, CTK, BR) also play key roles during seed germination, overall, researchers pay far more attention to the ABA and GA pathways. However, the detailed mechanism of ABA and GA in regulating tomato seed germination remains elusive.

In this study, we investigated the dynamic levels of ABA and GA_4 during seed germination and the mRNA transcripts involved in their biosynthesis and catabolism as well as signal transduction in two tomato cultivars, LA2711 (a fast-germinating genotype) and ZS-5 (a relatively slow-germinating genotype). Furthermore, we investigated ABA and GA sensitivity of the two genotypes during seed germination. The results will enable us to gain further insight into the mechanisms responsible for the difference in seed germination rates in the two cultivars.

2. Materials and methods

2.1. Plant materials

Tomato (*Solanum lycopersicum*) seeds of Edkawi (Accession no. LA2711) and ZS-5 were kindly provided by the Tomato Genetic Resource Center (TGRC, Davis, CA, USA) and the Chinese Academy of Agricultural Science (CAAS, Beijing, China), respectively. LA2711 is salt-tolerant [14,32,33], while ZS-5 is a salt sensitive accession [32,34]. Both accessions belong to cultivated tomato with large fruits, and their genetic backgrounds are relatively close. Tomato seeds were soaked for 20 mins with 10% Na_3PO_4, and then rinsed several times with sterile distilled water. Seeds were allowed to germinate at approximately 24 °C in the dark for 72 h. Seedlings with 5–6 true leaves were transplanted and grown in a glasshouse under optimal conditions. Only the first three flower clusters were kept for each plant and 2–3 fruits were kept for each flower cluster. Each fruit from every flower cluster was marked individually. The fruits were harvested when fully mature, and seeds from fresh fruits were treated with 4% HCl for 2–3 h at room temperature, and then rinsed with water. Seeds were air-dried and preserved at 4 °C.

2.2. Seed germination under different conditions

The seeds used in the germination test were harvested from the fruit of the same flower cluster for the same treatment. For each cultivar, 200 hand-selected seeds of uniform size were used in the same treatment. Seeds for the first treatment were placed on five layers of wet filter paper in 9 cm petri dishes. Seeds for the second treatment were surface-sterilized in 70% (v/v) ethanol for 15 s, followed by 15 min in 4% (w/v) sodium hypochlorite, then rinsed several times with sterile distilled water. Seeds for the third treatment were sterilized as the second one, but the seeds were sown on MS medium containing 30 g L^{-1} sucrose and 7 g L^{-1} agar. Four replicates were used for each treatment. Seeds were allowed to germinate at approximately 24 °C in the dark for 48 h then transferred to an incubator with a 16 h photoperiod and an optimal temperature regime of 24 °C/21 °C (light/dark). The germination response was scored visually as radicle protrusion at 12-h intervals.

2.3. Measurement of endogenous GA and ABA

Similar to the seed germination test, seeds were germinated in petri dishes with wet filter paper. The germinating seeds were harvested separately at 0, 12, 18, 24, 36 and 48 h after imbibition, frozen in liquid nitrogen and kept at −70 °C.

Endogenous GA_4 and ABA were extracted as described by the previous study [3]. The GA_4 and ABA measurements were performed using an LC–MS/MS system consisting of a quadruple/time-of-flight tandem mass spectrometer (Q-Tof Premier; Waters MS Technologies, Manchester, UK) and an Acquity Ultra Performance liquid chromatography system (Waters) equipped with a reverse-phase column (Acquity UPLC BEH-C18, Waters) as described by previous studies [35,36]. MassLynx software (ver. 4.1, Waters) was used to calculate the GA and ABA concentrations from the LC–MS/MS data.

2.4. Quantitative real-time PCR analysis

Total RNA was extracted from tomato seed samples using a phenol extraction method [30]. The quantity and quality of all RNA samples were assessed by agarose gel analysis. Genomic DNA was removed using an RNase-Free DNase I kit (Takara, Dalian, China). The cDNA was synthesized from the total RNA using the PrimeScript™ RT reagent kit (Takara) according to the manufacturer's instructions. PCR was used to further determine whether contaminating genomic DNA was present in the synthesized cDNA, with a single pair of primers spanning an intron of EF1α gene (accession no: SGN-U578831).

Real-time PCR was performed in an optical 96-well plate with a Light Cycler 480 instrument (Roche Diagnostics, Basel, Switzerland). Each 20 μL reaction contained 0.8 μL of 10 μM gene-specific primers (see Tables S1 and S2 for details), 1.0 μL cDNA samples, 10 μL SYBR Premix Ex Taq (Takara, Kyoto, Japan) and 8.2 μL water. The thermalcycle was as follows: 95 °C for 30 s; 40 cycles of 95 °C for 5 s and 60 °C for 25 s. Tomato *PP2Acs* gene was used as an internal control [37]. Real-time PCR was performed in triplicate and data was analyzed using the $2^{-\Delta\Delta CT}$ method [38].