

Precise Expression of *DREB1A* Gene Is Required for Proper Seed Germination, Vegetative and Reproductive Development, and Seed Grain Yield in *Arabidopsis thaliana*

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ABSTRACT

Life-history traits and biometrics of plant development are greatly influenced by their changing environment. To survive under various stressful environments, plants develop a multifaceted regulatory network that is mainly governed by transcription factors, including the dehydration responsive element binding (DREB) family. The roles of *DREB1A* have been investigated in responses of plants to various abiotic stresses, however, its effects on plants growth and development over the whole life cycle has not yet been fully described. Here, we studied detailed developmental characterizations of *drebl1a* T-DNA insertional mutant alongside a previously reported *DREB1A* over-expressing plants (OX28) in *Arabidopsis thaliana*. Seed germination, vegetative and reproductive growth stages and plant yield were also investigated. Under normal growth conditions, both *drebl1a* and OX28 plants exhibited reduced seed germination and delayed early development. In addition, both *drebl1a* and OX28 plants showed prolonged vegetative growth and delayed transition from vegetative to reproductive development. At the reproductive phase, the time between the emergence of flower stem bolting and opening of the first flower in *drebl1a* was 15% shorter in comparison to wild type (WT-Col0). In contrast, the OX28 plant had a prolonged reproductive development with a remarkably increased number of flowers per plant. Interestingly, lateral branches on the main inflorescence stem showed a lower number in *drebl1a*, as opposed to the OX28 plant. Despite these observations, in both *drebl1a* and OX28 plants the total seed weight was decreased significantly. Our findings proposed that there was a relationship between a high expression level of the *DREB1A* gene with the development and seed yield of *A. thaliana*.

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Introduction

Life-history traits of plants (from seed to seed) could be affected by various internal and/or

external factors. Abiotic stresses, as environmental stimuli, are constantly surrounding plants and trigger different aspects



of their growth and development (Shao *et al.*, 2007). The integration of plant response to stress and regulation of growth coordinated through a comprehensive network of molecular and physiological pathways. Two major players of this network that have central roles are transcription factors (*i.e.*, gene regulatory factors) and hormones (*i.e.*, growth regulatory factors) (Nakashima *et al.*, 2009; Peleg and Blumwald, 2011). Several families of transcription factors (TFs) including AREB/ABF, DREB1/CBF, DREB2, NAC, WRKY, and some members of MYC/MYB were identified as regulators of abiotic stress-responsive genes (Nakashima *et al.*, 2009; Park *et al.*, 2015; Yamaguchi-Shinozaki and Shinozaki, 2006; Zhu *et al.*, 2019).

The DREB1/CBF regulon (*i.e.*, regulated genes as a unit) have between 100 (Park *et al.*, 2015) to 400 (Zhao *et al.*, 2016) downstream genes which contain a cis-regulatory conserved CCGAC core DNA sequence in their promoters. All three members of the DREB1/CBF family, including DREB1B/CBF1, DREB1C/CBF2, and DREB1A/CBF3 (Yamaguchi-Shinozaki and Shinozaki, 1994), were differently expressed at low levels in most plant organs and most developmental stages (Schmid *et al.*, 2005). The expression of DREB1/CBF TFs in increased levels, especially the DREB1A gene, which encodes a protein with 213-amino acids, confer tolerance to environmental stresses, including cold, salinity, and drought (Agarwal *et al.*, 2006). The tolerance mechanism raises from downstream genes which modulate diverse stress-inducible metabolic pathways, such as sugar, lipid, and osmoprotectant biosynthesis (Seki *et al.*, 2002; Shi *et al.*, 2015). Furthermore, this mechanism has crosstalk with most plant hormones, including ethylene (Kazan, 2015), abscisic acid (ABA) (Knight *et al.*, 2004), gibberellic acid (GA) (Suo *et al.*, 2012; Tonkinson *et al.*, 1997), salicylic acid (SA) (Chinnusamy *et al.*, 2003), and jasmonic acid (JA) (Hu *et al.*, 2013) to organize plant development with environmental signals.

Elevated levels of DREB1A expression in plants caused abiotic stress tolerance, and also led to changes in the development and growth, such as low length with small dark green leaves, delayed emergence of flower, fewer axillary shoots, and

low seed yield (Gilmour *et al.*, 2000; Kasuga *et al.*, 1999; Liu *et al.*, 1998b; Suo *et al.*, 2012). On the other hand, DREB1A mutant lines have been applied to understand DREB1s roles in abiotic stresses tolerance and to establish functional redundancy among DREB transcription factors (Novillo *et al.*, 2004; Novillo *et al.*, 2007; Zhao *et al.*, 2016). However, the plants lacking basal levels of DREB1A expression have not yet been fully described to investigate the whole life cycle (seed to seed) and DREB1A effects on plant growth and development. Therefore, here we detailed developmental characterization of a *dreb1a* T-DNA insertional mutant and a DREB1A over-expression line in *A. thaliana*. The life-history traits and biometrics of plant development affected by DREB1A gene expression were also analyzed and discussed.

Material and Methods

Plant seeds

Five different types of *A. thaliana* seeds were used. Two of the five were Columbia (Col-0) accession seeds including the wild type (WT-Col0 as background control of DREB1A mutant line (*dreb1a*)) and the T-DNA transformed line (ID: N413033) in the DREB1A gene purchased from Nottingham Arabidopsis Seed Center (Kohan-Baghkheirati *et al.*, 2018). The other three types were Wassilewskija (WS-2) accession seeds including the wild type WS-2 (WT-WS2), the DREB1A-overexpressing (OX28), and the empty vector containing plant (Vector), which was previously reported by Professor M. F. Thomashow (Michigan State University, USA) (Gilmour *et al.*, 2000). The WT-WS2 and Vector lines were used as background controls of the DREB1A overexpressing line, OX28. Two wild-type lines (*i.e.*, WT-Col0 and WT-WS2) were applied to normalize the background effects of two different accessions in the same treatments.

Seeds were surface sterilized with 15 % (v/v) Clorox commercial bleach for 15 min, washed with ddH₂O five times, and sown either on plates containing Murashige and Skoog (MS) (Murashige and Skoog, 1962) medium supplemented with 3 % sucrose and 0.7 % agar or on 100 mm x 100 mm x 125 mm pots with Fafard (Fafard professional peat moss and peat-

based mixes, Conrad Fafard Inc. manufacturer #8063028, USA) soil. Plates and pots were incubated for four days at 4 °C and transferred to a growth chamber with a 12 hours light cycle, 23°C, and light intensity of 272.0 $\mu\text{mol s}^{-1} \text{m}^{-2}$.

Biometrics of germination and post-germination development

To assess the role of the *DREB1A* gene in germination and post-germination growth, phenotypes of seed germination from Stage 0.1

to 0.7, (Fig. 1, based on Boyes *et al.* (Boyes *et al.*, 2001)) to the appearance of the second pair of true leaves (Stage 1.04) were observed on seeds sowing and growing on agar plates. Seedlings images were captured from an Olympus DP71 camera connected to an Olympus BX51 microscope. The images were processed and analyzed in the ImageJ Java version (Rueden *et al.*, 2017).

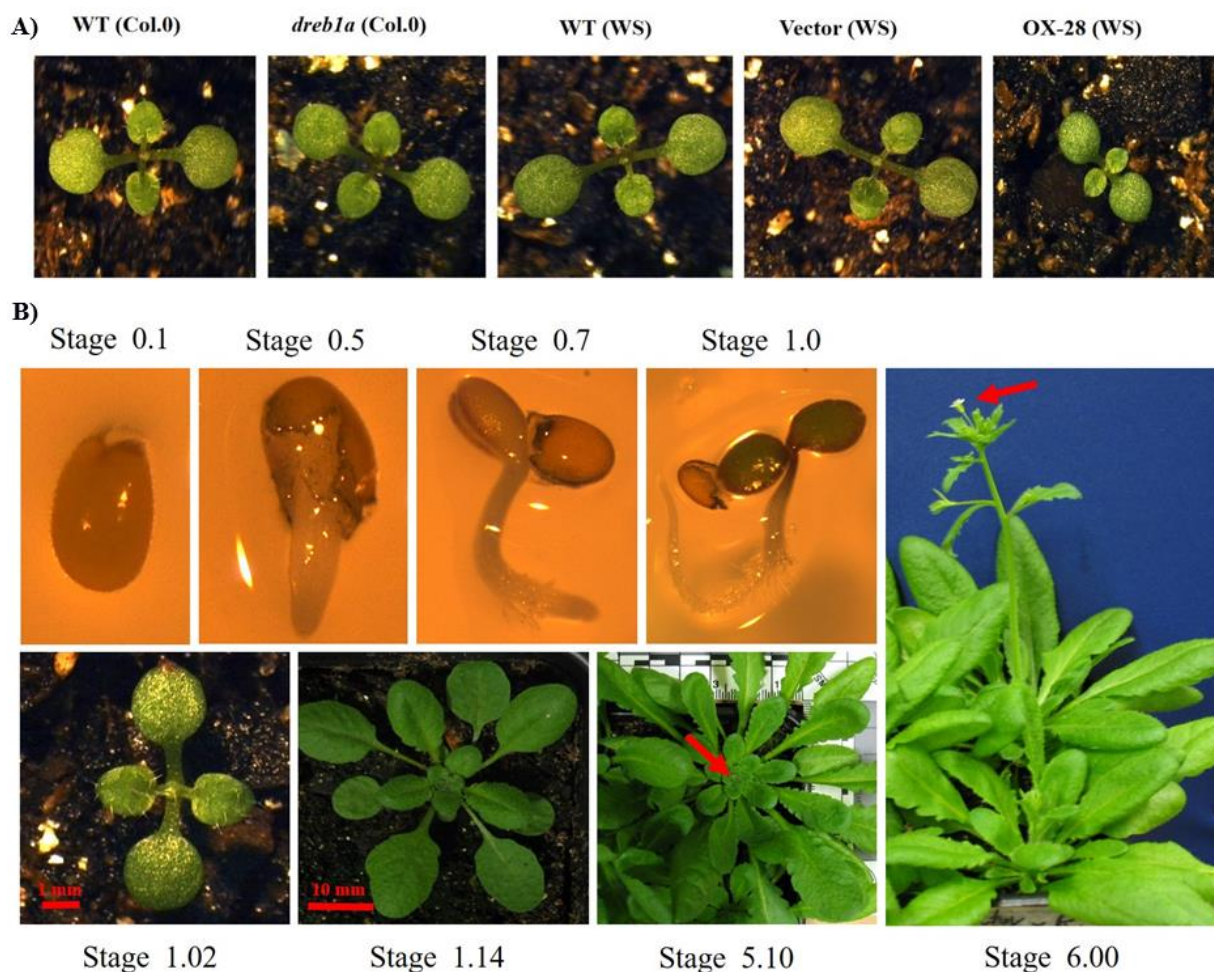


Fig. 1. Principal growth stage analysis: A) Representative *dreb1a* and OX28 plants alongside their control wild types 17 days after planting at stage 1.02; B) The definition of standard growth stages from germination to flowering. The seed imbibition - stage 0.1, the emergence of radicle - stage 0.5, emergence of hypocotyl - stage 0.7, fully opened of cotyledons - stage 1.0, two rosette leaves - stage 1.02, fourteen rosette leaves - stage 1.14, first flower buds - stage 5.10, and first flower open - stage 6.00 were defined.

Life-history traits and biometrics of plant development

The life-history traits of the five different lines were studied to investigate the probable role(s) of the *DREB1A* gene in plant development.

Vegetative and reproductive growths (*i.e.*, stages 1.02 to 6.90 according to Boyes *et al.* (Boyes *et al.*, 2001)), of soil-grown plants were recorded (Fig. 1). Two technical replicates with four biological replicates were applied for each

group. Their phenotypes were assessed every day unless otherwise indicated. From stage 1.02 to the emergence of the first flower bud (Stage 5.10), the number of rosette leaves was recorded, and every two days, digital images were taken and analyzed using the ImageJ (Schneider *et al.*, 2012).

Statistical analyses

The experiment was performed in four biological replicates and two technical replicates. The student's t-test by SPSS V22 software was applied for comparing the means, and the significance of the variations.

Results

Seed germination and DREB1A expression

Two vital stages in plant development were seed germination and post-germination investigated in this study (Fig. 2A-B). When in the normal

condition, the germination rate of the *dreb1a* seeds was significantly less (92 ± 2.2) than that of the WT-Col0 seeds (98 ± 0.13 ; $p\leq 0.01$) (Fig. 3A). These tests demonstrate that the loss of the DREB1A gene decreases seed germination of *A. thaliana* even in normal conditions. A similar reduction also occurred in the OX28 seeds (Fig. 3A). These two lines of evidence suggest that fine-tuning of DREB1A is necessary for proper seed germination in *A. thaliana*. There were no visible differences in the *dreb1a* seedlings in comparison to WT-Col0, while the OX28 seedlings were much smaller than their corresponding wild-type counterparts (Fig. 2A). Furthermore, the OX28 seedlings lasted a much longer time from S0.5 (i.e., radicle emergence) to S0.7 (i.e., complete emergence of hypocotyl) while the *dreb1a* seedlings possessed a longer duration from S0.7 to S1.0 (i.e., cotyledons fully opened) (Fig. 2B).

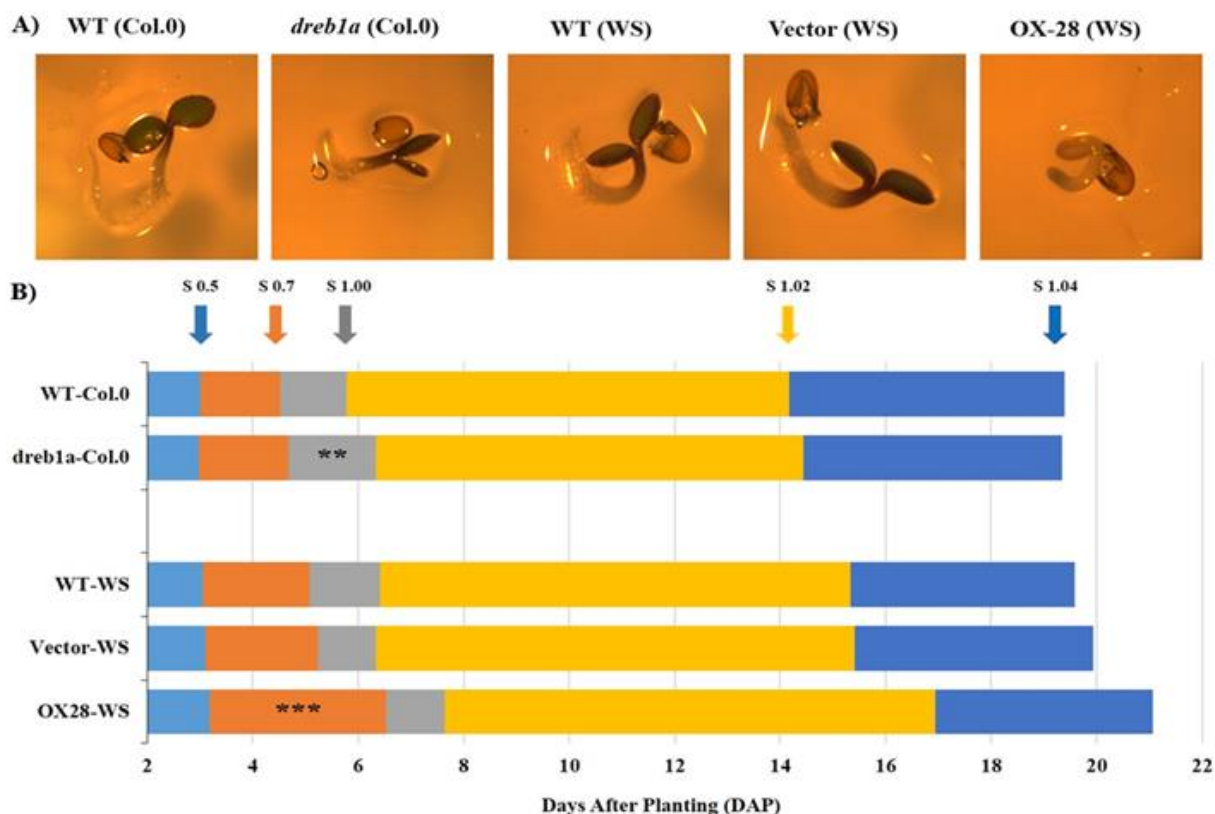


Fig. 2. Plate-based early analysis of growth stage progression: A) Representative *dreb1a* and OX28 plants alongside their control wild types 10 days after planting; B) Growth stage progression of the plants grown on MS medium under normal growth conditions. The arrows show the days after planting (DAP) that the WT-Col0 line reached the indicated growth stage. The boxes show the length of time for passes between two stages. Stars indicate the confidence levels that were confirmed by t-test at $p<0.05$ (*), $p<0.01$ (**), or $p<0.001$ (***)

According to Fig. 3B and C, the leaf area (*i.e.*, rosette diameter) per plant at S1.14 and the number of rosette leaves per plant at the time of bolting (S5.10) in the *drebl1a* plants were similar to those in the WT-Col0 seedlings. However, the OX28 plant (*i.e.*, 0.93 cm²) appeared much different from the WT-WS2 and Vector plants (*i.e.*, 8.75 cm²). As previously reported (Gilmour *et al.*, 2000), the OX28 plant exhibited a dwarf

phenotype with prolonged and much extended vegetative development growth (Fig. 4). The OX28 plant not only had a near to 10-fold less rosette diameter per plant at the S1.14 (Fig. 3B) but also possessed 1.5-fold (*i.e.*, 34 in comparison to 22) more rosette leaves per plant at S5.10 than those of the WT-WS2 and Vector plants (Fig. 3C and Supplement 1).

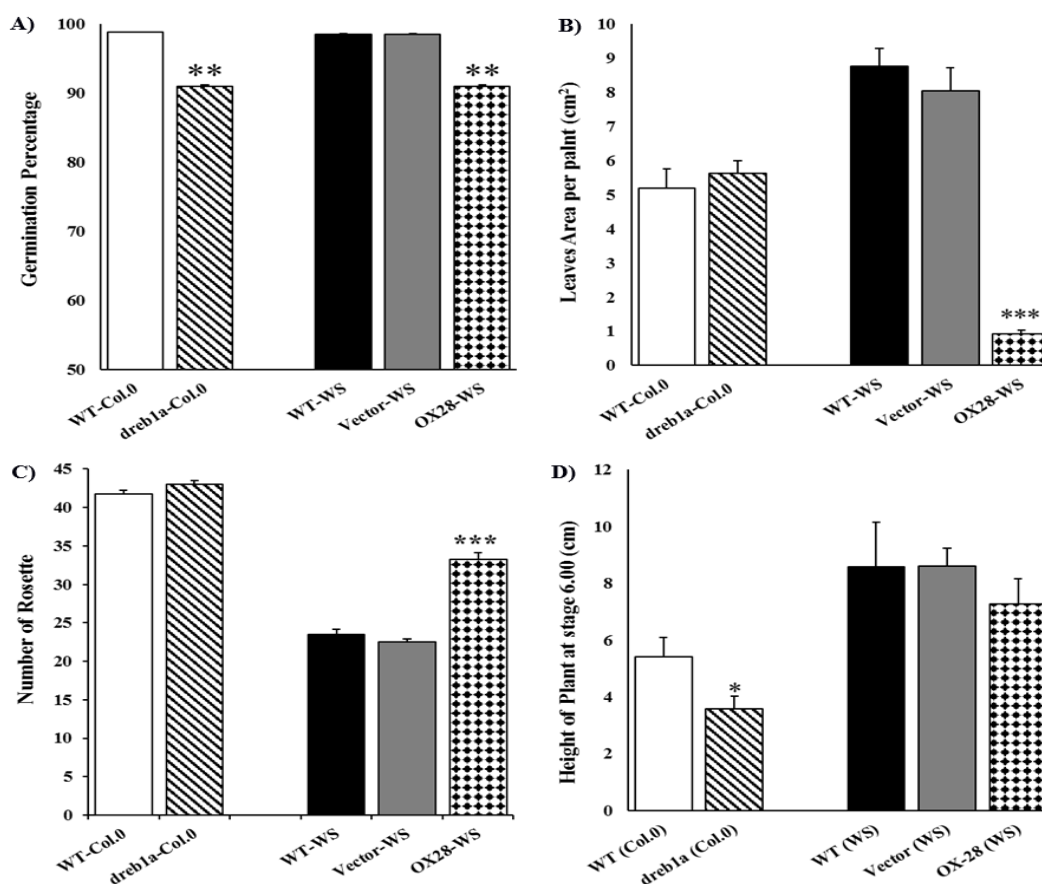


Fig. 3. Detection of germination percentage and vegetative traits of *drebl1a* and OX28 plants alongside their control wild types: A) Comparison of germination rate in five lines. The germination of seeds was recorded every day to 14 after planting (DAP); B) Comparison of total leaves area per plant at stage 1.14 in five lines using computerized analysis of rosettes' digital images; C) Comparison of the rosette number per plant at stage 5.10 in five lines. D) Comparison of the height of the plant at stage 6.00 in five lines. Bars indicate standard deviations of replicates that were confirmed by t-test at the confidence level $p < 0.05$ (designated as *), $p < 0.01$ (**), or $p < 0.001$ (***)

Reproductive development-delayed in the *drebl1a* and prolonged in the DREB1A overexpressing plants

The measurement of related parameters to vegetative-reproductive phase transition in Fig. 3B-D and Fig. 4B indicated a significant phase transition delayed in the *drebl1a* plants in comparison to the WT-Col0 plants.

Accompanying phenotypes to this delay at S6.00 (*i.e.*, first flower opening) had a shorter height of the main inflorescence stem and shorter bolting (elongation of the first internode of an inflorescence) time of the *drebl1a* plants than those in the WT-Col0 plants (Fig. 3D, Supplement 2, and Fig. 4B). In addition, the number of lateral branches on each main inflorescence stem of the *drebl1a* plant and the

total weight of their harvested seeds per plant was significantly less than those in the WT-Col0 plant (Fig. 5B and D). This is while no differences were observed between the *drebl1a*

and the WT-Col0 plants in the duration of flower production (S6.00- S6.90), and the number of flowers and inflorescence stems per plant (Fig. 4, Fig. 5A, and Supplement 3).

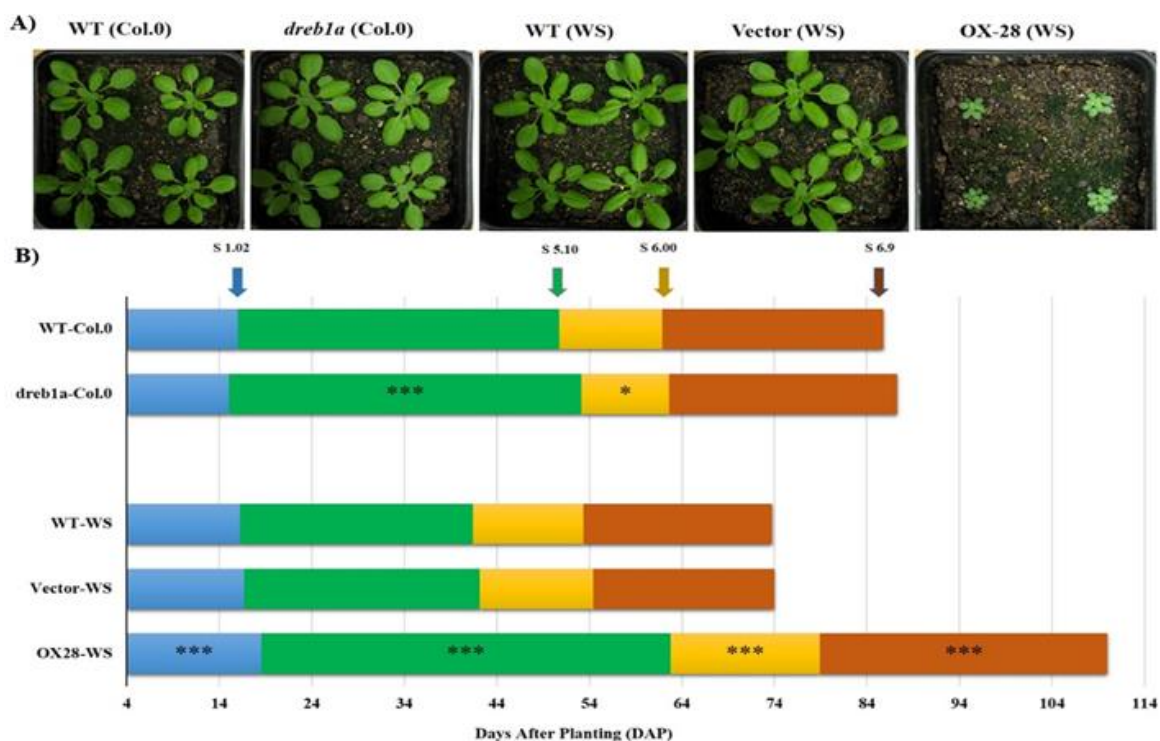


Fig. 4. Soil-based analysis of growth stage progression in *drebl1a* and OX28 plants: A) Representative of the five plant lines at stage 1.14; B) Growth stage progression of five different lines grown on soil under normal growth conditions. The arrows show the days after planting (DAP) that the WT-Col0 line reached the indicated growth stage. The boxes show the length of time for passes between two stages. Stars indicate the confidence levels that were confirmed by *t*-test at $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***)

Despite the delayed and shortened reproductive phase in the *drebl1a* plant, the OX28 plant had a prolonged reproductive development - significantly longer emergence of inflorescence (S5.10) and significantly longer flower production (S6.00–S6.90) than the WT-WS2 and Vector plants (Fig. 4B, Fig. 5A, and Supplement 3). The number of lateral branches on the main inflorescence stem of the OX28 plant showed a significant increase in comparison to WT-WS2 (Fig. 5D). The total weight of the harvested seeds per plant from the OX28 plants was half of that of the WT-WS2 plants (Fig. 5B).

Discussion

Possible roles of DREB1A in seed germination

Previous studies (Novillo *et al.*, 2007; Sliwinska *et al.*, 2009) have had reported that DREB1s

transcription factors may affect seed germination in *A. thaliana*; e.g., *cbf* triple mutants have reduced germination at a normal growth condition (Zhao *et al.*, 2016). In this study, the germination of the *drebl1a* seeds was reduced. This suggests that DREB1A homologs (i.e., DREB1B and DREB1C) cannot compensate for the absence of DREB1A in its latter role in seed germination. In addition, the OX28 seeds exhibited a similar trend of reduced germination although this phenomenon has not been reported previously.

Seed germination depends on the seed's properties (i.e., quality, development, and maturation) (Demir and Ellis, 1992; Zanakis *et al.*, 1994), and is a GA-dependent process. Possible roles of DREB1A may be in the seeds and GA-dependent processes. They include cell

elongation in the lower hypocotyl and hypocotyl-radicle transition zone through increasing the nuclear DNA content and accumulation of carbohydrate-containing bodies (Ogawa *et al.*, 2003; Sliwinska *et al.*, 2009). The vital role of DREB1A could be supported by its high-level expression throughout seed maturation and seed germination (Novillo *et al.*, 2007). The high levels also occurred in the early seedling developmental stages of wild type *A. thaliana* seedlings (Novillo *et al.*, 2007), and in the final flower development stage as well as seed filling siliques (refer to Supplement 4). The low germination rate of the *drebl1a* seeds could result from the low quality of premature seeds. As seed quality is the potential performance of a

seed, premature seeds do not complete seed maturation/desiccation and filling. It is incurred that *drebl1a* seeds may have defective seed maturation and unripen seed. Moreover, in the DREB1A over-expressing line, the level of GA was decreased (Suo *et al.*, 2012) suggesting that the reduced rate of seed germination in this line might be caused by low levels of GA, which is necessary for proper seed germination (Koornneef *et al.*, 2002). Altogether, it seems that proper seed germination depends on accurate expression levels of the *DREB1A* gene. The observed reduced seed germination in the absence of DREB1A and also in the *DREB1A* over-expressing lines are probably caused by two different mechanisms.

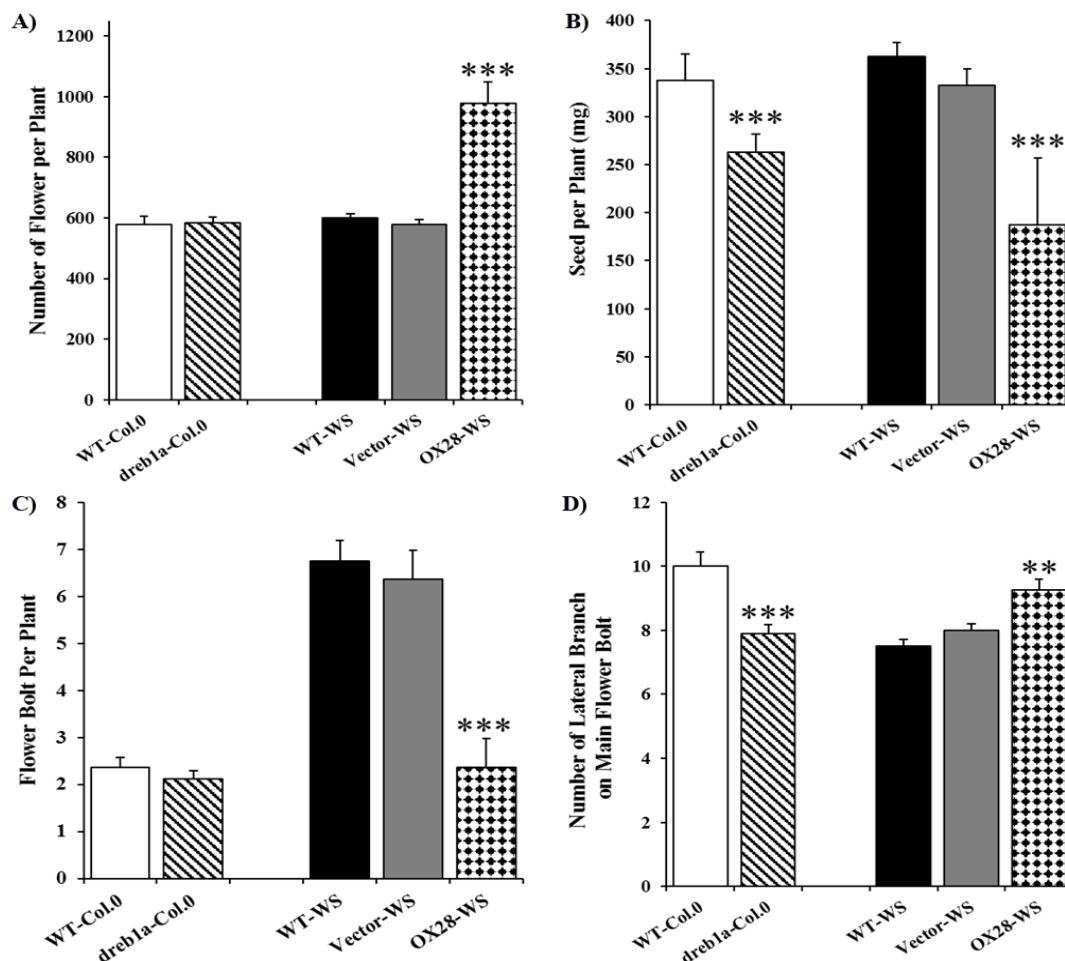


Fig. 5. Detection of reproductive traits of *drebl1a* and OX28plants: A) Comparison of the number of flowers per plant in the five lines; B) Comparison of seed weight per plant based on randomly pooled 1000 seeds from each line; C) Comparison of flower bolt per plant in the five lines; D) Comparison of the number of lateral branches on the main flower bolt in the five lines. Bars indicate standard deviations of replicates that were confirmed by t-test at $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***) confidence level.

Early *dreb1a* seedling development was delayed in the stage of fully opened cotyledons (S1.0). S1.0 is critical for the *A. thaliana* plant to transition from heterotrophic to autotrophic growth. This growth delay at S1.0 was recently reported in glucose 6-phosphate/phosphate translocator mutant lines (*gpt2*), of which sensing sugar was defective (Dyson *et al.*, 2014). Increased levels of specific soluble sugars were reported in *A. thaliana* DREB1A overexpressing lines (Gilmour *et al.*, 2000). Both lines of evidence suggest a connection between the DREB1A regulon and sugar metabolism. Upon the increase of DREB1A expression (e.g., in DREB1A overexpressing lines), levels of gene expression and metabolites increased in key sugar metabolism enzymes (Maruyama *et al.*, 2009). Interestingly, the sugar metabolism profile of DREB1A overexpressing plants was similar to those in plants exposed to cold stress (Gilmour *et al.*, 2000; Maruyama *et al.*, 2009). Cold acclimation was triggered by soluble sugars (Tarkowski and Van den Ende, 2015). A portion of sugar metabolism in cold acclimation is regulated by DREB1A downstream genes and galactinol synthase (*Gals*) included (Gilmour *et al.*, 2000; Maruyama *et al.*, 2009). When plants are exposed to abiotic stresses, the expression of *Gals* can lead to the accumulation of raffinose that acts as an osmoprotectant (Liu *et al.*, 1998a; Zuther *et al.*, 2004). Sucrose and soluble sugars (including glucose as the most ancient and conserved regulator), in cooperation with hormones (especially GA, ABA, and ethylene), can integrate a wide variety of signals between environmental changes with metabolic fluxes that control seedling development (Gibson, 2005; Koch, 2004; Rognoni *et al.*, 2007; Sheen, 2014).

The OX28 line exhibited a significant delay in the emergence of cotyledons - before S1.0 (i.e., the fully opened cotyledon stage) - in comparison to the WT-WS2 plant. This delay might result from the decrease of GAs in OX28 plants, due to the decreased levels of GA20 oxidase and increased levels of GA2 oxidase (Cong *et al.*, 2008; Suo *et al.*, 2012). GA20 oxidase induces activation of GAs while GA2 oxidase induces inactivation of GAs; up-regulation of GA2 oxidase and down-regulation of GA20 oxidase results in decreased levels of

activated GAs. In *A. thaliana*, transcripts of GA20 oxidase accumulated during germination to regulate downstream genes involved in cell elongation. This caused the acceleration of cotyledon and hypocotyl emergence (Ogawa *et al.*, 2003). Moreover, elevated amounts of sucrose in the OX28 line stabilized DELLA proteins; thus, stabilized DELLA proteins had more time to suppress GA signaling (Li *et al.*, 2014). These stabilized DELLA proteins decrease GA signal in the early developmental stages of OX28 plants and consequently result in the delay of cotyledon emergence. A high level of soluble sugars in the OX28 line could cause germination delay and early growth arrest (Koch, 2004; Rognoni *et al.*, 2007).

Role of DREB1A in reproductive growth

The number of rosette leaves and transition to reproductive development (regulated by internal and external environments) determine when the *A. thaliana* plant flowers (Huijser and Schmid, 2011; Koornneef *et al.*, 1991; Simpson *et al.*, 1999). Both phenomena apply to the OX28 plants to describe a delay of developmental phase transition by this study and by Gilmour and her associates (Gilmour *et al.*, 2000). The delay could result from a multifaceted network of connections between the ICE1-DREB1A regulon and developmental switches (including flowering time loci) (Lee *et al.*, 2015; Seo *et al.*, 2009; Suo *et al.*, 2012). DREB1A positively regulated the expression of flowering locus C (*FLC*) gene, while *FLC* suppressed a key floral activator *Suppressor of overexpression of constans 1* (*SOC1*) which caused delayed flowering (Lee *et al.*, 2015; Seo *et al.*, 2009). As a loop, *SOC1* negatively regulated the expression of the DREB1A gene. Then, a feedback loop of *FLC-SOC1-DREB1A* regulated the expression level of ICE1-DREB1A regulon and the time of flowering. Overexpression of DREB1A may shift the feedback loop to increase the *FLC* level, which consequently results in delayed flowering (Lee *et al.*, 2015; Seo *et al.*, 2009). Indigenous GAs decreased the levels of *DREB1A* over-expressing plants (Suo *et al.*, 2012) related to delay flowering. Without a significant increase in the number of rosette leaves (according to (Huijser and Schmid, 2011; Koornneef *et al.*, 1991;

Simpson *et al.*, 1999)), *drebl1a* lines showed slightly later (i.e., delayed) the emergence of flowering and earlier opening of the first flower than that of the WT-Col0 plant. Late flowering in the *drebl1a* plants probably is not a delay of developmental phase transition like OX28 plants (Gilmour *et al.*, 2000). One reason is that the delayed flowering phenotype was not established in *cbf* triple mutants and their flowering time was comparable to wild type (Zhao *et al.*, 2016). Second, the *DREB1A* gene acts as a key regulator of early response to drought in *A. thaliana* flowers (Su *et al.*, 2013). It suggests that *DREB1A* has an important role, through unknown mechanisms, in plant response to environmental stresses during flowering. Thus, the absence of *DREB1A* in the *drebl1a* plant may result in slightly later flowering. However, once *A. thaliana* plants enter the reproductive phase, high-level expression of *SOC1* can induce acceleration of flower opening and differentiation, even at the basal level of *DREB1A* (Lee *et al.*, 2015; Seo *et al.*, 2009).

DREB1A affects life-history traits and seed yields

Changes in the life-history traits in *A. thaliana* altered its productivity. It was reported that *DREB1A* overexpressing plants with a prolonged life cycle significantly reduced seed yield (Gilmour *et al.*, 2000); however, ectopic expression of cotton *DREB1* gene in *A. thaliana* resulted in a higher seed yield (Huang *et al.*, 2009; Liu *et al.*, 1998a). There was no report of *DREB1A* null mutation on seed yield, even *cbf* triple mutants (Zhao *et al.*, 2016). In this study, regardless of the duration of the flowering period and the number of flowers, the total seed yield was significantly reduced in the *drebl1a* plants. Reduced seed yield can be measured by at least four parameters, seed biomass, seed number, inflorescence height, and flower number (Van Daele *et al.*, 2012). Our results showed that the *drebl1a* plants were comparable to the WT-Col0 in the number of flowers, the number of inflorescence stems, and the 1000 seeds weight. This result suggests that the seed yield reduction in the *drebl1a* plants results from a reduction of seed numbers. This also suggests that some of the flowers were not fertile or seed maturation did not complete in the *drebl1a* plants. Although

there is no report of the *DREB1A* gene directly involved in flower fertility and/or seed maturation, there are two lines of indirect evidence. First, the expression of *DREB1A* is high in the pistil development, exposing pollen, in *A. thaliana* flowers to early-drought treatment (Su *et al.*, 2013). Second, some of the *DREB1A* downstream genes such as late embryogenesis-abundant (LEAs), *RD29*, and *RD28* have high expression levels during pollen production and seed maturation (Wise and Tunnacliffe, 2004; Yamaguchi-Shinozaki and Shinozaki, 1993). Our current understanding proposes a multilateral role for *DREB1A* in flower fertility and seed maturation/desiccation.

In contrast to the *drebl1a* plant, the OX28 plant exhibited a prolonged life history. This resulted from the repressing effects of the *DREB1A* gene on *SOC1* (Lee *et al.*, 2015) and reduced levels of activated GA (Suo *et al.*, 2012). This results in late flowering initiation and a long flowering period. The aforementioned connections between *ICE1-DREB1A* regulon and *FLC-SOC1* loop (Lee *et al.*, 2015; Seo *et al.*, 2009) and between *DREB1A* level and gibberellin activated level (Cong *et al.*, 2008; Suo *et al.*, 2012) could affect flowering time and life-history traits in the OX28 plants. Although the OX28 lines produced flowers twice as many WT-WS2 flowers, the seed yield of the former plant was significantly reduced in contrast to the latter. Gilmour and associates determined fewer inflorescence stems as the causes of the reduced yield of the OX28 plants (Gilmour *et al.*, 2000). A similar result - fewer inflorescence stems - was observed in this study; however, we also observed an increased number of flowers in the OX28 plants (Fig. 5). Thus, we propose that the cause(s) would be the aforementioned flower fertility and seed maturation/desiccation.

Reduction of seed yields in both *drebl1a* and OX28 plants suggests a precise role for the *DREB1A* gene in flower fertility and/or seed maturation. Moreover, the high expression level of *DREB1A* in siliques (Supplement 2) suggests that this gene is probably required for proper seed desiccation. These data indicate that fine-tune expression of *DREB1A* may be important for entering flowering time, completing seed maturation, and the modulation of reproductive

traits as an evolutionary adaptation strategy against diverse stress conditions in *A. thaliana*.

Conflicts of interest

The authors declared they have no conflicts of interest.

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بیان دقیق ژن DREB1A برای جوانه زنی مناسب بذر، رشد رویشی و زایشی، و عملکرد بذر در گیاه آرابیدوپسیس تالیانا (*Arabidopsis thaliana*) ضروری است

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چکیده

ویژگی‌ها و شاخص‌های رشد و نمو گیاهان تا حد زیادی تحت تأثیر محیط در حال تغییر آنها است. برای زنده ماندن در محیط‌های مختلف استرس‌زا، گیاهان یک شبکه تنظیمی چند وجهی ایجاد کردند که عمدتاً توسط فاکتورهای رونویسی از جمله خانواده DREB (dehydration responsive element binding) کنترل می‌شود. نقش *DREB1A* در پاسخ گیاه به تنش‌های غیرزیستی مختلف بررسی شده است، با این حال، اثرات آن بر رشد و نمو گیاه در کل چرخه زندگی هنوز به طور کامل شرح داده نشده است. در این مطالعه، خصوصیات نمو جهش‌یافته *drebla* T-DNA همراه با لاین بیش بیان *DREB1A* (OX28) در گیاه آرابیدوپسیس تالیانا مطالعه شد. جوانه زنی بذر، مراحل رشد رویشی و زایشی، و عملکرد گیاه نیز مورد بررسی قرار گرفت. تحت شرایط رشد نرمال، هر دو گیاه *drebla* و OX28 کاهش در جوانه زنی بذر و تاخیر در استقرار گیاهچه نشان دادند. علاوه بر این، هر دو گیاه *drebla* و OX28 رشد رویشی طولانی‌مدت و تاخیر در انتقال از فاز رویشی به زایشی را نشان دادند. در فاز زایشی، مدت زمان بین ظهور جوانه محور گل و باز شدن اولین گل در *drebla* در مقایسه با نوع وحشی (WT-Col0) پانزده درصد کوتاه‌تر بود. در مقابل، گیاه OX28 فاز زایشی طولانی‌مدت با افزایش قابل توجه تعداد گل در هر محور داشت. جالب این که تعداد شاخه‌های جانبی روی محور گل دهی در *drebla* در مقایسه با گیاه OX28 کمتر بود. با وجود این مشاهدات، در هر دو گیاه *drebla* و OX28 وزن کل دانه به طور معنی‌داری کاهش یافت. یافته‌های ما نشان داد که بین سطح بیان دقیق ژن *DREB1A* با رشد و عملکرد دانه در گیاه آرابیدوپسیس تالیانا ارتباط وجود دارد.

واژگان کلیدی: آرابیدوپسیس تالیانا؛ *DREB1A*؛ استقرار گیاهچه؛ انتقال فاز نمو؛ جوانه زنی بذر

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