J Genet Resour2022;8(1): 99-110 *Homepage: http://sc.journals.umz.ac.ir/*

RESEARCH ARTICLE *DOI: 10.22080/jgr.2022.22267.1279*

J|G|R

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

Eisa Kohan-Baghkheirati[1, 2, 3], Mohammad Bagherieh-Najjar[1*], Ahmad Abdolzadeh[1], Jane Geisler-Lee[3]

[1]Department of Biology, Golestan University, Gorgan, Iran
[2]Department of Biology, Hakim Sabzevari University, Sabzevar, Iran
[3]Department of Plant Biology, Southern Illinois University Carbondale, Carbondale, IL 62901, USA

ARTICLE INFO

Article history:
Received 27 July 2021
Accepted 06 December 2021
Available online 09 February 2022

Keywords:
Arabidopsis thaliana
DREB1A
Early Development
Growth Phase Transition
Seed Germination

Supplementary information:
Supplementary information for this article is available at http://sc.journals.umz.ac.ir/

Corresponding authors:
✉ M. Bagherieh-Najjar
mb.bagherieh@gu.ac.ir

p-ISSN 2423-4257
e-ISSN 2588-2589

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 *dreb1a* 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 *dreb1a* and OX28 plants exhibited reduced seed germination and delayed early development. In addition, both *dreb1a* 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 *dreb1a* 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 *dreb1a*, as opposed to *the* OX28 plant. Despite these observations, in both *dreb1a* 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*.

Please cite this paper as: Kohan-Baghkheirati E, Bagherieh-Najjar MB, Abdolzadeh A, Geisler-Lee J. 2022. A precise expression level of *dreb1a* gene is required for proper seed germination, vegetative and reproductive development, and seed yield in *Arabidopsis thaliana*. *J Genet Resour* 8(1): 99-110. doi: 10.22080/jgr.2022.22267.1279.

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 ddH_2O 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 µmol s^{-1} 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).

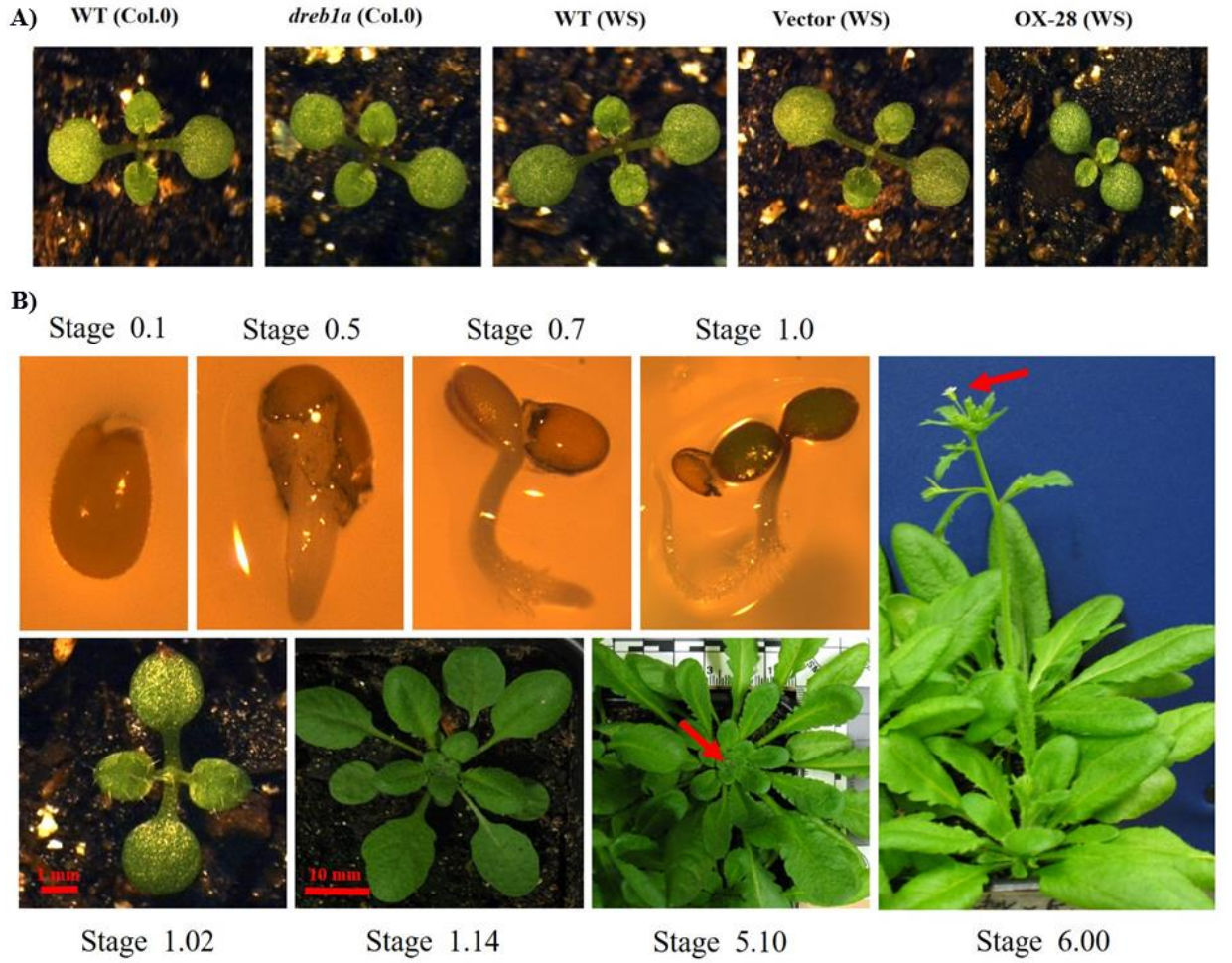

Fig. 1. Principal growth stage analysis: A) Representative *dreb1a* and OX28 plants alongside their control wild types 17 days after planting at stage 1.02; B) The definition of standard growth stages from germination to flowering. The seed imbibition - stage 0.1, the emergence of radicle - stage 0.5, 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).

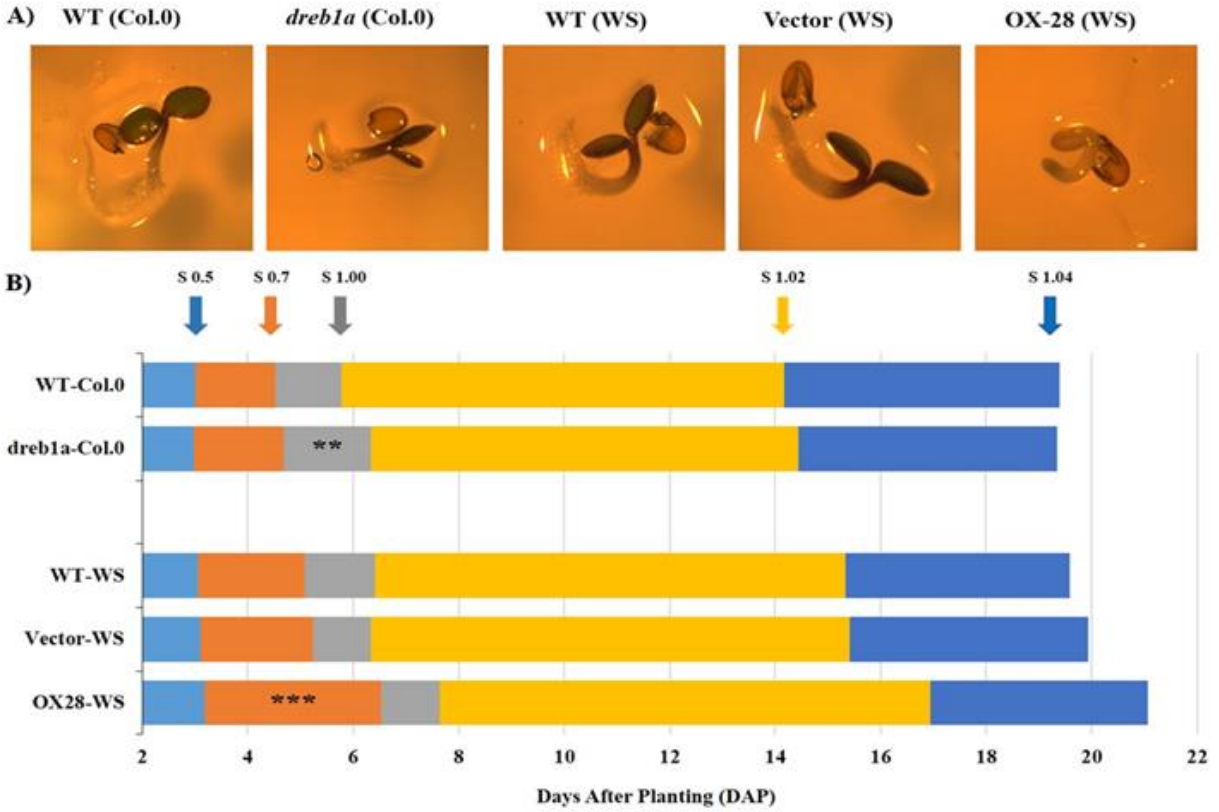

Fig. 2. Plate-based early analysis of growth stage progression: A) Representative dreb1a and OX28 plants alongside their control wild types 10 days after planting; B) Growth stage progression of the plants grown on MS medium under normal growth conditions. The arrows show the days after planting (DAP) 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 dreb1a plants were similar to those in the WT-Col0 seedlings. However, the OX28 plant (*i.e.*, 0.93 cm^2) appeared much different from the WT-WS2 and Vector plants (*i.e.*, 8.75 cm^2). 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).

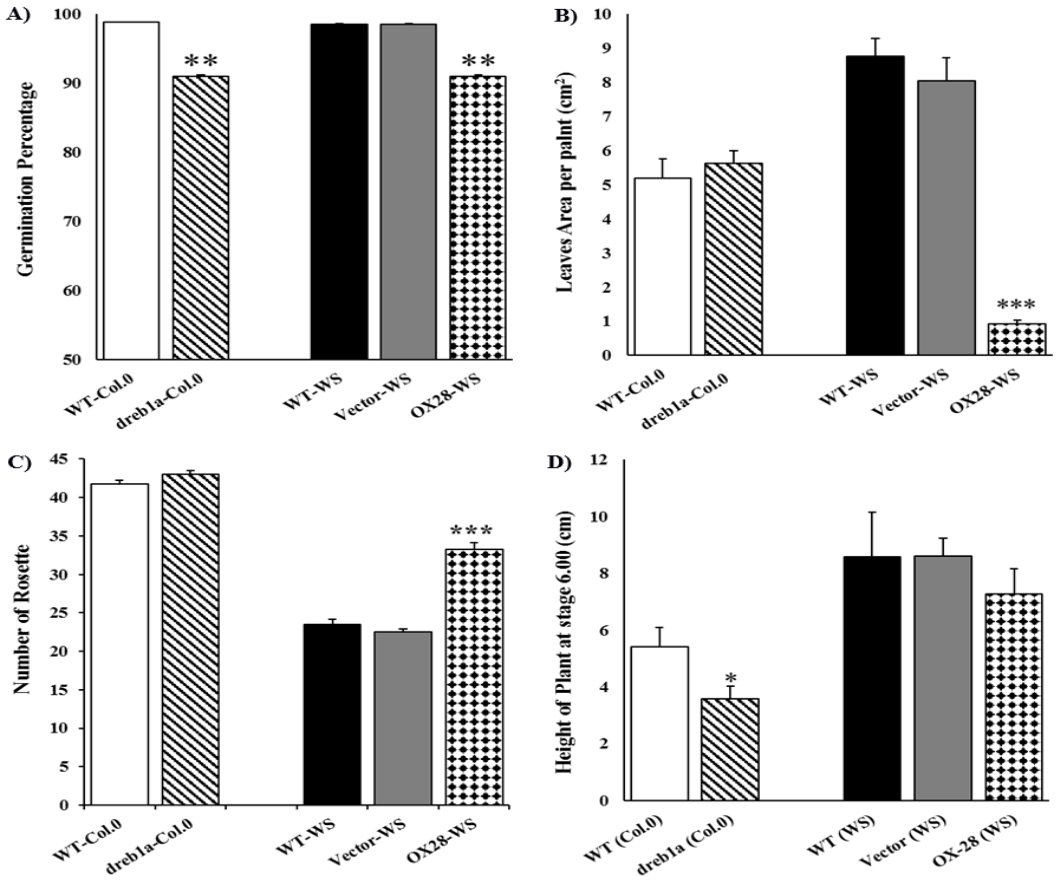

Fig. 3. Detection of germination percentage and vegetative traits of dreb1a and OX28 plants alongside their control wild types: A) Comparison of germination rate in five lines. The germination of seeds was recorded every day to 14 after planting (DAP); B) Comparison of total leaves area per plant at 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 dreb1a 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 *dreb1a* 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 dreb1a 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 *dreb1a* 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 *dreb1a* 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).

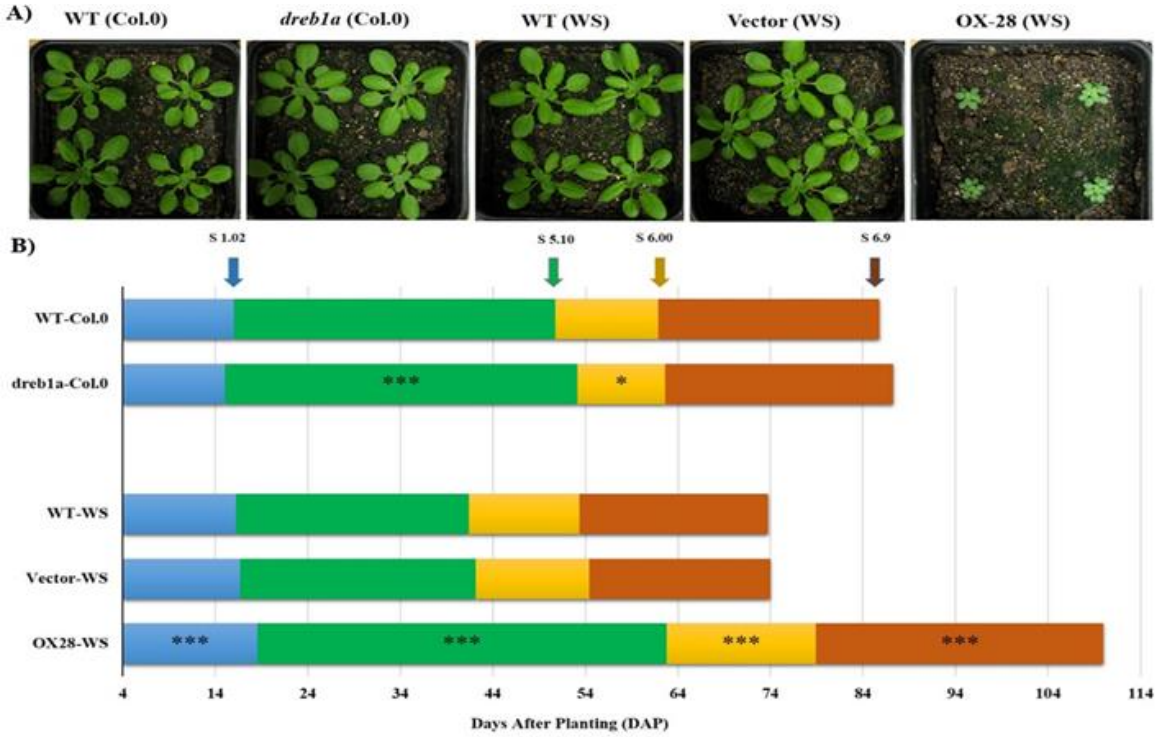

Fig. 4. Soil-based analysis of growth stage progression in *dreb1a* and OX28 plants: A) Representative of the five plant lines at stage 1.14; B) Growth stage progression of five different lines grown on soil under normal growth conditions. The arrows show the days after planting (DAP) that the WT-Col0 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 *dreb1a* 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 dreb1a 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 dreb1a 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 dreb1a 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.

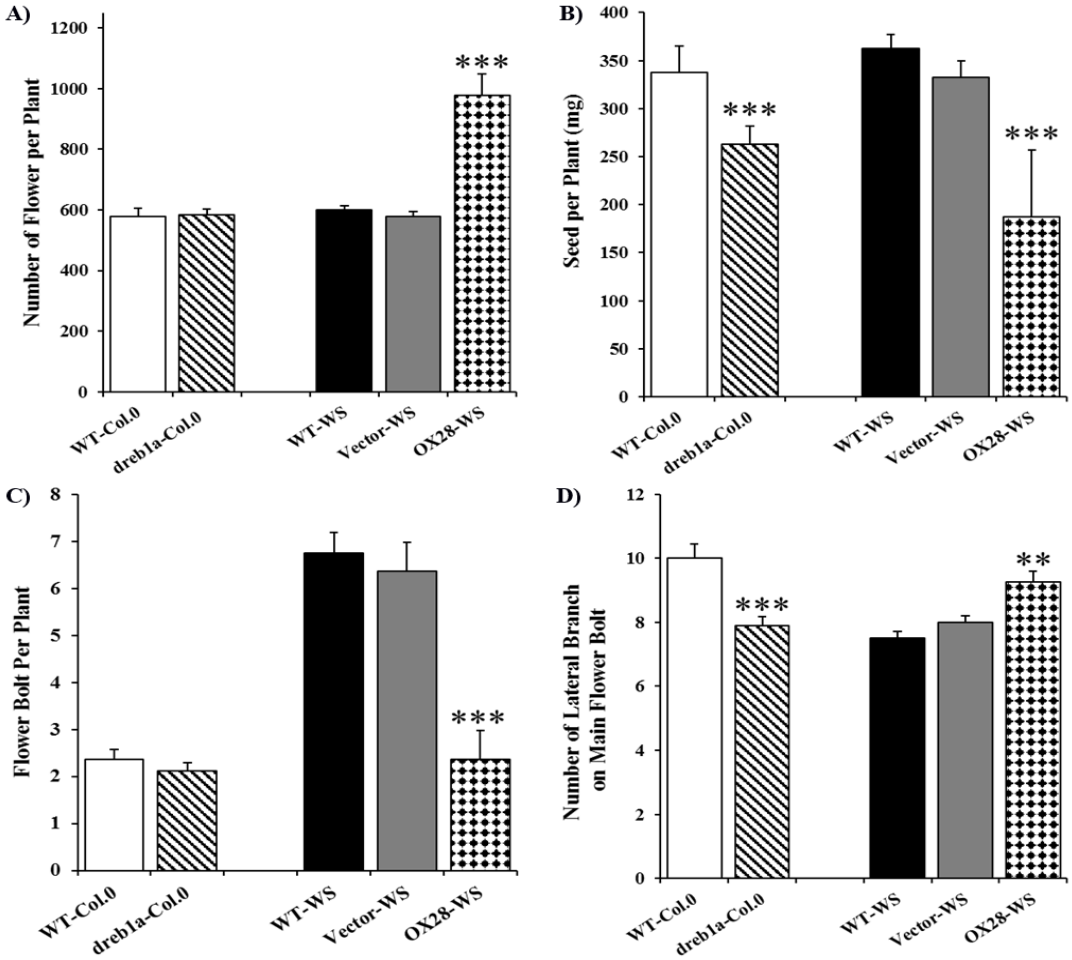

Fig. 5. Detection of reproductive traits of dreb1a and OX28plants: A) Comparison of the number of flowers per plant in the five lines; B) Comparison of seed weight per plant based on randomly pooled 1000 seeds from each line; C) Comparison of flower bolt per plant in the five lines; D) Comparison of 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 over-expressing 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)), dreb1a 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 dreb1a 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 dreb1a 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 dreb1a 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 dreb1a 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 dreb1a 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 dreb1a 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 dreb1a 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 dreb1a 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.

References

Agarwal PK, Agarwal P, Reddy M, Sopory SK. 2006. Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. *Plant cell rep* 25(12): 1263-1274.

Boyes DC, Zayed AM, Ascenzi R, McCaskill AJ, Hoffman NE, Davis KR, Görlach J. 2001. Growth stage–based phenotypic analysis of Arabidopsis a model for high throughput functional genomics in plants. *Plant Cell* 13(7): 1499-1510.

Chinnusamy V, Ohta M, Kanrar S, Lee BH, Hong X, Agarwal M, Zhu JK. 2003. ICE1: a regulator of cold-induced transcriptome and freezing tolerance in Arabidopsis. *Genes Dev* 17(8): 1043-1054.

Cong L, Zheng H-C, Zhang Y-X, Chai T-Y. 2008. Arabidopsis DREB1A confers high salinity tolerance and regulates the expression of GA dioxygenases in Tobacco. *Plant sci* 174(2): 156-164.

Demir I, Ellis RH. 1992. Changes in seed quality during seed development and maturation in tomato. *Seed Sci Res* 2(2): 81-87.

Dyson BC, Webster RE, Johnson GN. 2014. GPT2: a glucose 6-phosphate/phosphate translocator with a novel role in the regulation of sugar signalling during seedling development. *Ann bot* 113(4): 643-652

Gibson SI. 2005. Control of plant development and gene expression by sugar signaling. *Curr Opin Plant Biol* 8(1): 93-102.

Gilmour SJ, Sebolt AM, Salazar MP, Everard JD, Thomashow MF. 2000. Overexpression of the Arabidopsis CBF3Transcriptional Activator Mimics Multiple Biochemical Changes Associated with Cold Acclimation. *Plant Physiol* 124(4): 1854-1865.

Hu Y, Jiang L, Wang F, Yu D. 2013. Jasmonate regulates the inducer of CBF expression–c-repeat binding factor/DRE binding factor1 cascade and freezing tolerance in Arabidopsis. *Plant Cell* 25(8): 2907-2924.

Huang JG, Yang M, Liu P, YANG GD, WU CA, ZHENG CC. 2009. GhDREB1 enhances abiotic stress tolerance, delays GA-mediated development and represses cytokinin signalling in transgenic Arabidopsis. *Plant Cell Environ* 32(8): 1132-1145.

Huijser P, Schmid M. 2011. The control of developmental phase transitions in plants. *Develop* 138(19): 4117-4129.

Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, Shinozaki K. 1999. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nat Biotechnol* 17(3): 287-291.

Kazan K. 2015. Diverse roles of jasmonates and ethylene in abiotic stress tolerance. *Trends plant sci* 20(4): 219-229.

Knight H, Zarka DG, Okamoto H, Thomashow MF, Knight MR. 2004. Abscisic acid induces CBF gene transcription and subsequent induction of cold-regulated genes via the CRT promoter element. *Plant physiol* 135(3): 1710-1717.

Koch K. 2004. Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Curr Opin Plant Biol* 7(3): 235-246.

Kohan-Baghkheirati E, Bagherieh-Najjar M, Abdolzadeh A, Geisler-Lee J. 2018. Altered DREB1A gene expression in Arabidopsis thaliana leads to change in root growth, antioxidant enzymes activity, and response to salinity but not to cold. *Conserv Genet Resour* 4(2): 90-104.

Koornneef M, Bentsink L, Hilhorst H. 2002. Seed dormancy and germination. *Curr Opin Plant Biol* 5(1): 33-36.

Koornneef M, Hanhart C, Veen J. 1991. A genetic and physiological analysis of late flowering mutants in Arabidopsis thaliana. *Mol Genet Genom* 229(1): 57-66.

Lee JH, Jung JH, Park CM. 2015. Inducer of cbf expression 1 integrates cold signals into flowering locus C-mediated flowering pathways in Arabidopsis. *Plant J* 84(1): 29-40.

Li Y, Van den Ende W, Rolland F. 2014. Sucrose induction of anthocyanin biosynthesis is mediated by DELLA. *Mol plant* 7(3): 570-572.

Liu J-JJ, Krenz DC, Galvez AF, de Lumen BO. 1998a. Galactinol synthase (GS): increased enzyme activity and levels of mRNA due to cold and desiccation. *Plant Sci J* 134(1): 11-20.

Liu Q, Kasuga M, Sakuma Y, Abe H, Miura H, Yamaguchi-Shinozaki K, Shinozaki K. 1998b. Two Transcription Factors, DREB1 and DREB2, with an EREBP/AP2 DNA Binding Domain Separate Two Cellular Signal Transduction Pathways in Drought- and Low- Temperature-Responsive Gene Expression, Respectively, in Arabidopsis. *Plant Cell* 10(8): 1391–1406.

Maruyama K, Takeda M, Kidokoro S, Yamada K, Sakuma Y, Urano K, Fujita M, Yoshiwara K, Matsukura S, Morishita Y. 2009. Metabolic pathways involved in cold acclimation identified by integrated analysis of metabolites and transcripts regulated by DREB1A and DREB2A. *Plant physiol* 150(4): 1972-1980.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol Plant* 15(3): 473-497.

Nakashima K, Ito Y, Yamaguchi-Shinozaki K. 2009. Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. *Plant physiol* 149(1): 88-95.

Novillo F, Alonso JM, Ecker JR, Salinas J. 2004. CBF2/DREB1C is a negative regulator of CBF1/DREB1B and CBF3/DREB1A expression and plays a central role in stress tolerance in Arabidopsis. *Proc Natl Acad Sci USA* 101(11): 3985-3990.

Novillo F, Medina J, Salinas J. 2007. Arabidopsis CBF1 and CBF3 have a different function than CBF2 in cold acclimation and define different gene classes in the CBF regulon. *Proc Natl Acad Sci USA* 104(52): 21002-21007.

Ogawa M, Hanada A, Yamauchi Y, Kuwahara A, Kamiya Y, Yamaguchi S. 2003. Gibberellin biosynthesis and response during Arabidopsis seed germination. *Plant Cell* 15(7): 1591-1604.

Park S, Lee CM, Doherty CJ, Gilmour SJ, Kim Y, Thomashow MF. 2015. Regulation of the Arabidopsis CBF regulon by a complex low-temperature regulatory network. *Plant J* 82(2): 193-207.

Peleg Z, Blumwald E. 2011. Hormone balance and abiotic stress tolerance in crop plants. *Curr Opin Plant Biol* 14(3): 290-295.

Rognoni S, Teng S, Arru L, Smeekens SC, Perata P. 2007. Sugar effects on early seedling development in Arabidopsis. *Plant Growth Regul* 52(3): 217-228.

Rueden CT, Schindelin J, Hiner MC, DeZonia BE, Walter AE, Arena ET, Eliceiri KW. 2017. ImageJ: Image analysis interoperability for the next generation of biological image data. *BMC Bioinformatics* 18(1):529. doi: 10.1186/s12859-017-1934-z.

Schmid M, Davison TS, Henz SR, Pape UJ, Demar M, Vingron M, Schölkopf B, Weigel D, Lohmann JU. 2005. A gene expression map of Arabidopsis thaliana development. *Nat genet* 37(5): 501-506.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9(7): 671-675.

Seki M, Narusaka M, Ishida J, Nanjo T, Fujita M, Oono Y, Kamiya A, Nakajima M, Enju A, Sakurai T et al. 2002. Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. *Plant J* 31(3): 279-292.

Seo E, Lee H, Jeon J, Park H, Kim J, Noh Y-S, Lee I. 2009. Crosstalk between cold response and flowering in Arabidopsis is mediated through the flowering-time gene SOC1 and its upstream negative regulator FLC. *Plant Cell* 21(10): 3185-3197.

Shao HB, Guo QJ, Chu LY, Zhao XN, Su ZL, Hu YC, Cheng JF. 2007. Understanding molecular mechanism of higher plant plasticity under abiotic stress. *Colloids Surf B Biointerfaces* 54(1): 37-45.

Sheen J. 2014. Master regulators in plant glucose signaling networks. *J Plant Biol* 57(2): 67-79.

Shi Y, Ding Y, Yang S. 2015. Cold signal transduction and its interplay with phytohormones during cold acclimation. *Plant Cell Physiol* 56(1): 7-15.

Simpson GG, Gendall AR, Dean C. 1999. When to switch to flowering. *Annu Rev Cell Dev Biol* 15(1): 519-550.

Sliwinska E, Bassel GW, Bewley JD. 2009. Germination of Arabidopsis thaliana seeds is not completed as a result of elongation of the

radicle but of the adjacent transition zone and lower hypocotyl. *J Exp Bot* 60(12): 3587-3594.

Su Z, Ma X, Guo H, Sukiran NL, Guo B, Assmann SM, Ma H. 2013. Flower development under drought stress: morphological and transcriptomic analyses reveal acute responses and long-term acclimation in Arabidopsis. *Plant Cell* 25(10): 3785-3807.

Suo H, Ma Q, Ye K, Yang C, Tang Y, Hao J, Zhang ZJ, Chen M, Feng Y, Nian H. 2012. Overexpression of AtDREB1A causes a severe dwarf phenotype by decreasing endogenous gibberellin levels in soybean [Glycine max (L.) Merr.]. PloS One 7: e45568.

Tarkowski ŁP, Van den Ende W. 2015. Cold tolerance triggered by soluble sugars: a multifaceted countermeasure. *Front Plant Sci* 6: 203.

Tonkinson C, Lyndon R, Arnold G, Lenton J. 1997. The effects of temperature and the Rht3 dwarfing gene on growth, cell extension, and gibberellin content and responsiveness in the wheat leaf. *J Exp Bot* 48(4): 963-970.

Van Daele I, Gonzalez N, Vercauteren I, de Smet L, Inzé D, Roldán-Ruiz I, Vuylsteke M. 2012. A comparative study of seed yield parameters in Arabidopsis thaliana mutants and transgenics. *Plant Biotechnol J* 10(4): 488-500.

Wise MJ, Tunnacliffe A. 2004. POPP the question: what do LEA proteins do? *Trends Plant Sci* 9(1): 13-17.

Yamaguchi-Shinozaki K, Shinozaki K. 1993. Characterization of the expression of a desiccation-responsive rd29 gene of Arabidopsis thaliana and analysis of its promoter in transgenic plants. *Mol Gen Genet* 236(2): 331-340.

Yamaguchi-shinozaki K, Shinozaki k. 1994. A novel cis-acting element in an Arabidopsis gene is involved in responsiveness to drought, low-temperature, or high-salt stress. *Plant Cell* 6(2): 251-264.

Yamaguchi-Shinozaki K, Shinozaki K. 2006. Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu Rev Plant Biol* 57: 781-803.

Zanakis G, Ellis R, Summerfield R. 1994. Seed quality in relation to seed development and maturation in three genotypes of soyabean (Glycine max). *Exp Agric* 30(2): 139-156.

Zhao C, Zhang Z, Xie S, Si T, Li Y, Zhu JK. 2016. Mutational evidence for the critical role of CBF transcription factors in cold acclimation in Arabidopsis. *Plant Physiol* 171(4), 2744-2759.

Zhu D, Hou L, Xiao P, Guo Y, Deyholos MK, Liu X. 2019. VvWRKY30, a grape WRKY transcription factor, plays a positive regulatory role under salinity stress. *Plant Sci* 280: 132-142.

Zuther E, Büchel K, Hundertmark M, Stitt M, Hincha DK, Heyer AG. 2004. The role of raffinose in the cold acclimation response of Arabidopsis thaliana. *FEBS Lett* 576(1-2): 169-173.