J. Plant Biol. (2019) 62:410-418
DOI 10.1007/s12374-019-0348-z

RESEARCH ARTICLE

# Shoot Meristem Activity is Involved in Salt Tolerance on Arabidopsis Shoot Growth

**Ye Sol Jun, Ok-Kyoung Cha, Jeong Hyun Kim and Horim Lee***

*Department of Biotechnology, Duksung Women's University, Seoul 01369, Korea*

Received: August 19, 2019 / Accepted: September 27, 2019


**Abstract** All aerial tissues and organs of higher plants are generated from the shoot apical meristem (SAM) containing a pluripotent stem cell population. The maintenance of shoot meristem activity, such as cell proliferation and differentiation, is important for appropriate normal growth as well as adaptive plastic development under abiotic stress conditions. Here we report that the effects of salt tolerance on Arabidopsis shoot growth may occur via shoot meristem activity. Interestingly, the *clv3-2* and *clv1 bam1* mutants showed the salt tolerant phenotype on shoot growth and development through the increased number of actively dividing cells in the SAM but not the promoted expression levels of the typical stress-responsive genes. The reduction of 5-ethynyl-2'-deoxyuridine (EdU)-labeled dividing cells in the SAM by the treatment of abiotic stresses occurred most likely through abscisic acid (ABA) and reactive oxygen species (ROS). In addition, shoot meristem activity via cell proliferation was regulated dynamically depending on the presence or absence of abiotic stimuli. Therefore, these results suggest that stem cell maintenance and differentiation in the SAM may play an important role in stress tolerance for developmental plasticity on plant growth against unfavorable environmental conditions.

**Key words:** Abiotic stress, Cell proliferation, Salt tolerance, Shoot meristem activity

## Introduction

The shoot apical meristem (SAM) in plants determines all above-ground architectures, including the leaves, flowers, and stems, via the replenishment of undifferentiated stem cells during the entire lifespan (Wang et al. 2018a). Continuously dividing pluripotent stem cells are located in the central zone (CZ) and support new cells toward differentiation regions, such as the peripheral zone (PZ) and rib zone (RZ) to form lateral organs (Wang et al. 2018a; Kitagawa and Jackson 2019). The organizing center (OC) faced with the CZ, PZ and RZ helps maintain shoot meristem activity in the stem cell niche (Dinney and Benfey 2008; Wang et al. 2018a). Therefore, the accurate maintenance of stem cell division and differentiation in the SAM is essential to develop the proper plant body structure on shoot growth and development after embryogenesis.

The activity of shoot meristems, including proliferation and differentiation, is tightly maintained by key regulatory mechanisms composed of *SHOOT MERISTEMLESS* (*STM*) and *CLAVATA* (*CLV*)-*WUSCHEL* (*WUS*) pathways. The *STM* gene encodes a KNOTTED1-related homeodomain transcription factor (TF) and involves the initial establishment of shoot meristems (Long et al. 1996; Kitagawa and Jackson 2019). Moreover, *STM* partially acts as an upstream regulator of the cytokinin (CK) biosynthetic gene, *ISOPENTENYL TRANSFERASE 7* (*IPT7*), in the SAM (Yanai et al. 2005). CK activates *WUS* expression via the *ARABIDOPSIS HISTIDINE KINASE 4* (*AHK4*) CK sensor (Gordon et al. 2009), contributing to the position and proliferation of stem cell population in the SAM (Cheng et al. 2017).

The constant stem cell number in the SAM is also controlled by complex interactions via a negative feedback loop, which consists of CLAVATA3 peptide (CLV3p)-mediated CLV receptor signaling pathway repressing the expression of *WUSCHEL* (*WUS*) homeodomain TF (Wang et al. 2018a; Kitagawa and Jackson 2019). CLV3p secreted from stem cells is perceived by CLV receptor complexes, including CLV1, CLV2, CORYNE (CRN), RECEPTOR LIKE PROTEIN KINASE 2 (RPK2), BARELY ANY MERISTEM1/2/3 (BAM1/2/3), and CLAVATA3 INSENSITIVE RECEPTOR KINASES (CIKs) (Kitagawa and Jackson 2018). Recently, the loss-of-function *clv1 bam1* double mutant exhibited the phenocopy with the *clv3* mutant (Shinohara and Matsubayashi

*Corresponding author; Horim Lee
Tel : +82-10-3762-6331
E-mail : hrlee1375@duksung.ac.kr

2015), showing that CLV1 and BAM1 are direct receptors for CLV3p. In addition, another report showed recently that the CLV3p-mediated phosphorylation of downstream mitogen-activated protein kinases MPK3 and MPK6 was removed almost completely in *clv1 bam1* double mutant SAM tissues (Lee et al. 2019). CLV3p-triggered CLV1/BAM1 signaling represses the expression of *WUS*, which promotes stem cell proliferation via cell-to-cell movement of the WUS protein from the OC to the stem cell zone (Kitagawa and Jackson 2019). The WUS proteins activate *CLV3* expression directly in stem cells through direct binding to the *CLV3* promoter in the absence of *HAIRY MERISTEM* (*HAM*) (Zhou et al. 2018).

As a sessile organism, plants have an intrinsic property of developmental plasticity to adapt to their growth and organ formation under surrounding environmental/abiotic stress conditions, such as salt, drought, cold/heat, and osmotic stresses (Zhou 2016; Wang et al. 2018a). Because plants develop post-embryonically, this plasticity is presumably derived from shoot meristems that produce new cells, tissues, and organs. Recent studies have supported this hypothesis that there is a correlation between abiotic stresses and shoot meristem development. Accordingly, different types of reactive oxygen species (ROS), which are induced by a number of diverse abiotic stresses (Apel and Hirt 2004), are distributed antagonistically for meristem maintenance in shoot and root stem cell niches (Zeng et al. 2017; Yang et al. 2018). Moreover, another recent report showed that the shoot meristem endogenously has an oxygen-gradient along apical-basal or radial axis and this hypoxic condition is required for the differentiation process to sustain leaf production (Weits et al. 2019), suggesting the involvement of ROS in shoot meristem development. On the other hand, it is unclear how unfavorable abiotic stresses regulate shoot meristem activity via cell proliferation and differentiation.

In this study, we discovered that both *clv3* and *clv1 bam1* mutant alleles containing increased stem cell population in the SAM show salt tolerance on shoot growth and development but not roots. Although the typical stress-responsive genes were strongly induced by salt stresses, their expression patterns were not correlated with the salt tolerant phenotype. Instead, EdU-labeled actively dividing cells in the SAM reflect the salt-resistant shoot growth of *clv3-2* compared to wild-type (WT) Landsberg *erecta* (L*er*). Moreover, the stress-mediated changes in cell division in the SAM occurred via abscisic acid (ABA) and ROS, which are downstream regulators of abiotic stresses, and cell proliferation in shoot meristems responded dynamically with or without stress signals. Finally, these results show that shoot meristem activity may be involved in regulating the stress tolerance to adapt to ever-changing environmental conditions.

## Results

### Increased Stem Cell Population Promotes Salt Tolerance

To determine the correlation between abiotic stresses and shoot growth and development via stem cell homeostasis, we examined the effects of salt tolerance of WT L*er* and the *clv3-2* mutant, which has a lesion of an unbalanced stem cell population in the SAM. Although shoot growth and development were slightly delayed under the condition of 100 mM NaCl, the growth phenotype of WT L*er* and *clv3-2* mutant plants was rarely affected (Fig. 1A). On the other hand, *clv3-2* mutant plants strongly increased the survival rate compared to the WT L*er* plants under the condition of 200 mM NaCl (WT L*er* 8.9% and *clv3-2* 88.9%) (Fig. 1A, B). The CLV3p signal is perceived by downstream CLV receptors in the SAM (Kitagawa and Jackson 2019) and recent reports have shown that CLV1 and BAM1 act as the main receptor of CLV3p to activate MAPK signaling (Shinohara and Matsubayashi 2015; Lee et al. 2019). For this reason, salt tolerance between WT Columbia-0 (Col-0) and the *clv1 bam1* double mutant were also compared. Consistently, WT Col-0 plants displayed a more sensitive response to the treatment of 200 mM NaCl, but not 100 mM NaCl, compared to *clv1 bam1* mutant plants (WT Col-0 54.4% and *clv1 bam1* 91.1%) (Fig. 1A, B). These results suggest that the unbalanced increased stem cell population shown in the *clv3-2* or *clv1 bam1* SAMs enhances salt tolerance on shoot growth and development.

### Salt Tolerance of clv3-2 or clv1 bam1 Mutants is a Shoot-specific Phenotype

CLV3p-CLV1/BAM1 signaling functions in the regulation of stem cell homeostasis organizing shoot meristem development (Wang et al. 2018a; Kitagawa and Jackson 2019). Therefore, to test whether this salt tolerance phenotype shown in *clv3-2* or *clv1 bam1* is restricted or universal on plant growth, we examined the tolerance effect on root growth and development after transferring seedlings to salt stress conditions. Seven-day-old seedlings were grown more on new MS plates without (Control) or with (200 mM NaCl) stress conditions for two days (Fig. S1A). Under the control conditions, the root growth rate of *clv3-2* or *clv1 bam1* mutant seedlings was slightly slower than that of each WT (Fig. S1A, B). However, the root growth of L*er*, *clv3-2*, Col-0 and *clv1 bam1* was almost arrested from the 200 mM NaCl treatment (Fig. S1A, B), which is in contrast to the tolerant phenotypes on shoot growth (Fig. 1). Consistent with the response of root growth, decreased cell-dividing areas labeled with 5-ethynyl-2'-deoxyuridine (EdU) in *clv3-2* or *clv1 bam1* by the treatment of salt stresses showed no difference compared to that of

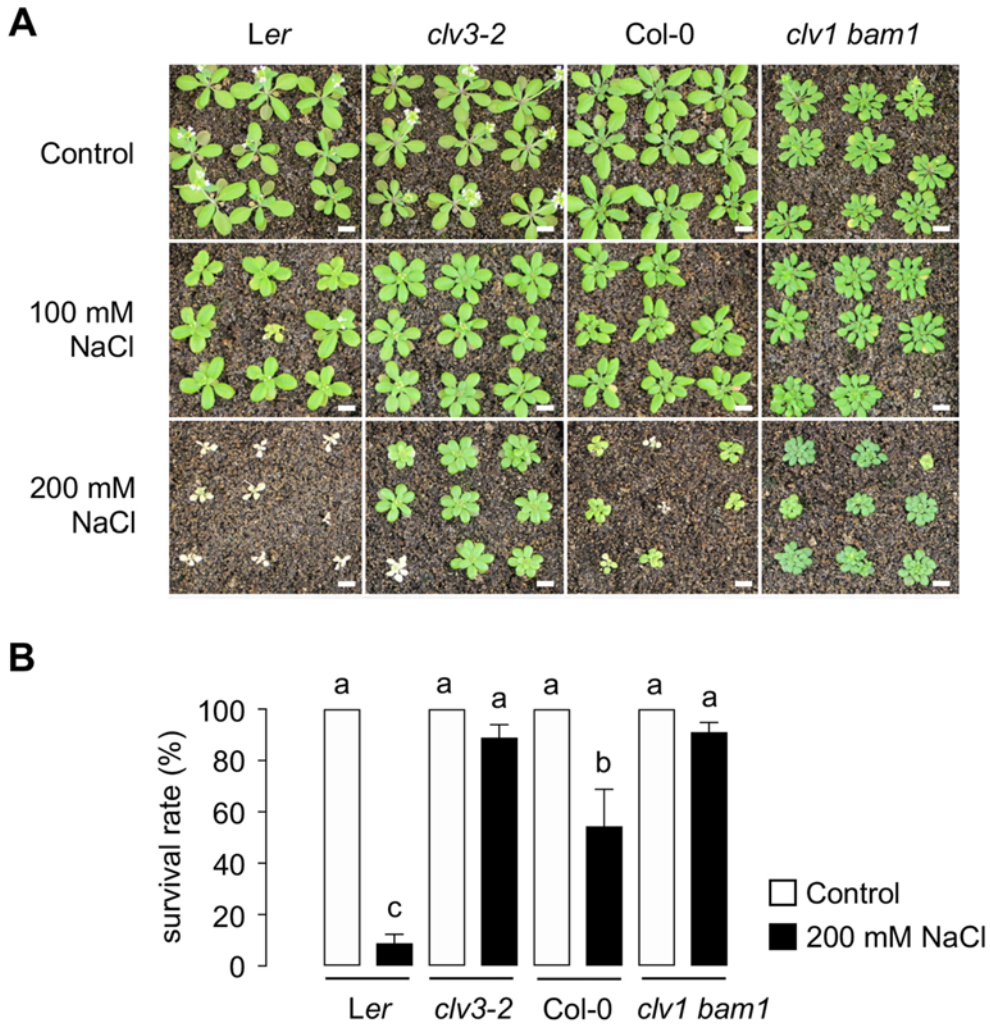


**Fig. 1.** Increased stem cell population promotes salt tolerance. (A) Salt tolerance phenotypes of WT L*er*, *clv3-2*, WT Col-0 and *clv1 bam1* plants. The plants were grown for two weeks and then 100 or 200 mM NaCl were treated twice with a week interval. The control was just applied with water without salt stress. Colorless or pale green plants were found mainly in L*er* or Col-0 plants. Scale bars, 1 cm. (B) Survival rates of L*er*, *clv3-2*, Col-0 and *clv1 bam1* plants on shoot growth resistance to 200 mM NaCl conditions. The data are presented as the mean ± SE ($n$=4). The different letters indicate a significant difference ($p$<0.05) according to the Duncan test.

each WT L*er* or Col-0, respectively (Fig. S1C). As a result, because the effect of salt tolerance was not found in root growth and development of *clv3-2* or *clv1 bam1* mutant seedlings compared to each WT seedling, it suggests that salt tolerance shown in *clv3-2* or *clv1 bam1* mutants is a shoot-specific phenotype.

## Expression Patterns of the Typical Abiotic Stress-responsive Genes are not Involved in Salt Tolerance of clv3-2 or clv1 bam1 on Shoot Growth

To elucidate the molecular mechanism underlying the different shoot growth phenotypes caused by the treatment of salt stresses, the shoot apex tissues of *clv3-2*, *clv1 bam1*, and each WT were isolated to perform the gene expression assays. The expression levels of the abiotic stress-responsive genes, such as *KIN1*, *RD29A*, *RAB18*, and *DREB2A* (Jakab et al. 2005; Sakuma et al. 2006; Msanne et al. 2011; Yu et al. 2017), were analyzed without or with the 200 mM NaCl treatment. Under salt stresses, the expression levels of four stress-responsive genes were obviously promoted in WT L*er* or Col-0 compared to the control treatment (Fig. 2A-D). In addition, these responses promoted by salt stresses were also observed in *clv3-2* or *clv1 bam1* with similar or slightly lower levels compared to those of each WT (Fig. 2A-D). Although the salt tolerant phenotype of WT Col-0 plants on shoot growth was less sensitive than WT L*er* plants (Fig. 1), the expression patterns of stress-responsive genes between both ecotypes, such as Col-0 and L*er*, were similar or slightly higher in L*er* than Col-0 (Fig. 2E). Overall, these results suggest that the typical role of the abiotic stress-responsive genes does not reflect directly the physiological

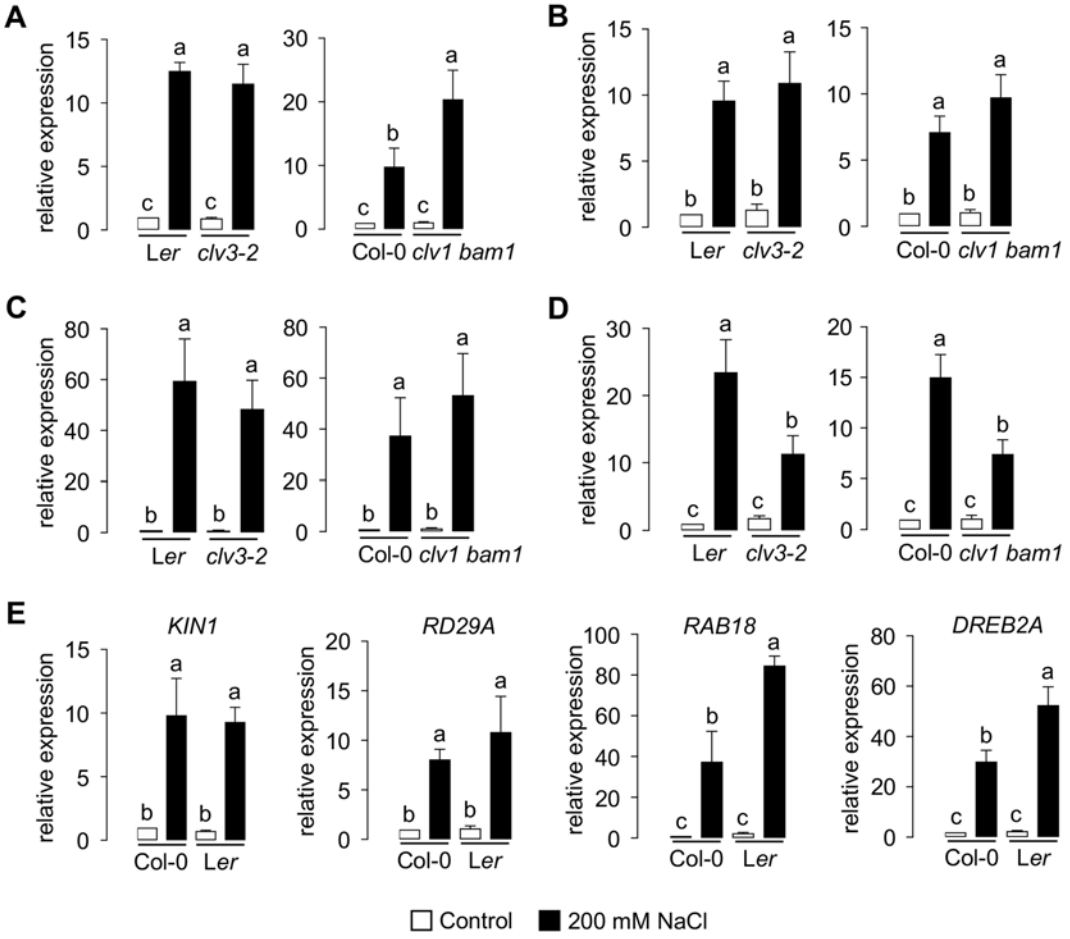


**Fig. 2.** Expression patterns of the typical abiotic stress-responsive genes are not involved in salt tolerance of *clv3-2* or *clv1 bam1* on shoot growth. The expression of typical stress-responsive genes including *KIN1* (A), *RD29A* (B), *RAB18* (C) and *DREB2A* (D) in WT L*er* and *clv3-2* or WT Col-0 and *clv1 bam1*. The total RNAs were isolated from the shoot apex tissues. Each expression was normalized by *ACT2* and the relative expression was determined by a comparison with each WT applied by the control condition. (E) Comparison of stress-responsive gene expression between different WT ecotypes, such as Col-0 and L*er*. Relative expression was determined by a comparison with WT Col-0 applied by the control condition. The data are presented as the mean ± SE ($n$=4). The different letters indicate a significant difference ($p$<0.05) according to the Duncan test.

salt resistant phenotypes shown in *clv3-2* or *clv1 bam1*.

## Shoot Meristem Activity Affects Salt Tolerance on Shoot Growth

Postembryonic development and organ formation of the aerial parts of mature plants occur via meristem activities, including homeostatic cell proliferation and differentiation in the SAM region. To determine how cell proliferation in the SAM of WT L*er* and the *clv3-2* mutant is affected by salt stresses, we carried out EdU assays monitoring actively dividing cells. In WT L*er*, EdU-labeled dividing cells were observed throughout multiple layers of the SAM regions (inside white dots) and decreased dramatically one day after the 200 mM NaCl treatment (Fig. 3A). Similarly, the number of actively dividing cells were also decreased in the *clv3-2* mutant SAM under 200 mM NaCl (Fig. 3A). Despite the reduced tendency of cell proliferation in both WT L*er* and *clv3-2* SAMs by salt stresses, the *clv3-2* SAM contains more actively dividing cells than the WT L*er* SAM under salt stress conditions. The remaining rates of EdU-labeled cells at 200 mM NaCl in WT L*er* and *clv3-2* were 23.8% and 37.6%, respectively, compared to each control condition (Fig. 3A, B). Indeed, the number of EdU-labeled cells in the *clv3-2* SAM at 200 mM NaCl was statistically undistinguishable from the conditions of the control, 50, 100, and 150 mM NaCl conditions in WT L*er* SAMs (Fig. 3B), and WT L*er* plants exposed to 100 mM NaCl showed the resistant phenotype (Fig. 1A). Intriguingly, *WUS* expression was induced under the stress condition of 200 mM NaCl even in WT L*er* and the highest level was detected in *clv3-2* treated with 200 mM NaCl (Fig. 3C), suggesting the possibility of WUS-mediated cell proliferation under salt stresses for adaptation. Taken together, a more active state of shoot meristems leads to the

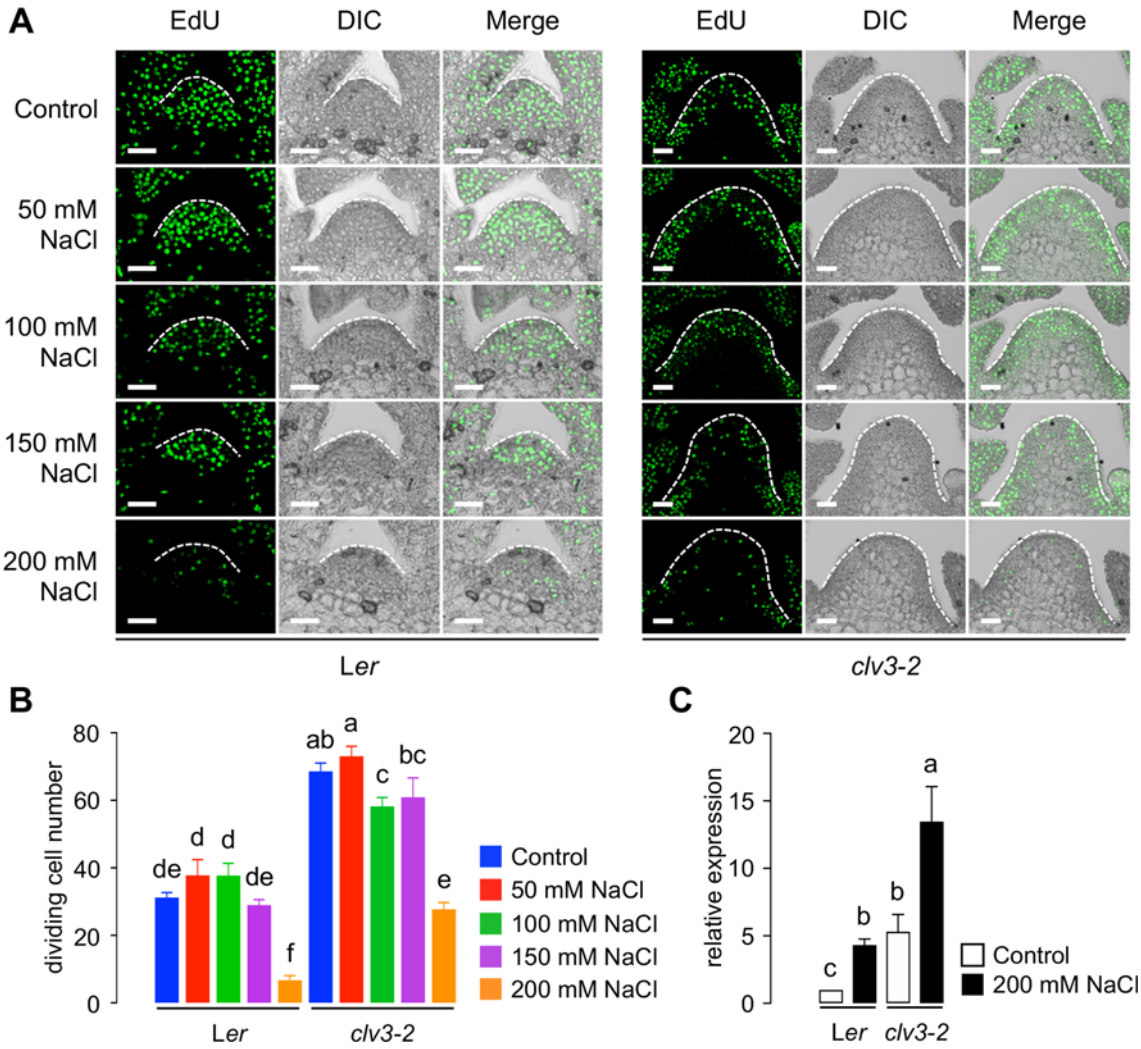


**Fig. 3.** Shoot meristem activity affects salt tolerance on shoot growth. (A) Shoot meristem phenotypes with proliferating cells labeled by EdU in WT L*er* and *clv3-2* under without (Control) or with 50, 100, 150 or 200 mM NaCl conditions. The dotted lines show the outline of the SAM regions. DIC, differential interference contrast. Merge, merged images of EdU-signal and DIC. Scale bars, 20 μm. (B) Quantified number of EdU labeled cells in the SAM of WT L*er* and *clv3-2* without or with various concentrations of NaCl. The data are presented as the mean ± SE ($n$=10). (C) Comparison of *WUS* expression in WT L*er* and *clv3-2* without (Control) or with 200 mM NaCl conditions. Each expression was normalized by *ACT2* and relative expression was determined by a comparison with WT L*er* applied by the control condition. The data are presented as the mean ± SE ($n$=4). The different letters indicate a significant difference ($p$<0.05) according to the Duncan test.

salt tolerant phenotype in *clv3-2* compared to that of WT L*er*.

## Shoot Meristem Activity is Dynamically Controlled by Abiotic Stresses

We next asked whether the fluctuations of cell proliferation by salt stresses can also be applied to other abiotic stresses. Interestingly, a significant decrease in the number of EdU-labeled dividing cells was also detected along increased concentrations of osmolyte mannitol in shoot meristems of WT L*er* and *clv3-2* as well as WT Col-0 and *clv1 bam1* (Fig. 4A; Fig. S2), indicating that shoot meristem activity is also affected by osmotic stress as well as salt stress. The ABA pathway is an intracellular signaling herb to relay various abiotic stimuli, including salt, drought, and osmotic stresses through mainly the SnRK protein kinase families (Zhou 2002; Zouh 2016). As expected, there was a decrease in the number of actively dividing cells in *clv3-2* SAMs at the 10 μM ABA treatment compared to the ABA mock treatment (Fig. 4B). In the case of WT L*er* SAMs, EdU-labeled cell division activities were already influenced by the mock treatment without ABA (Fig. 4B). Therefore, we could not determine the responsive behavior under the higher concentrations of ABA. Moreover, because these decreased patterns of cell proliferation in the SAM was also observed in the treatment of reactive oxygen species (ROS), e.g., hydrogen peroxide ($H_2O_2$) (Fig. 4C), which is known to be induced by ABA in controlling the stomata aperture (Watkins et

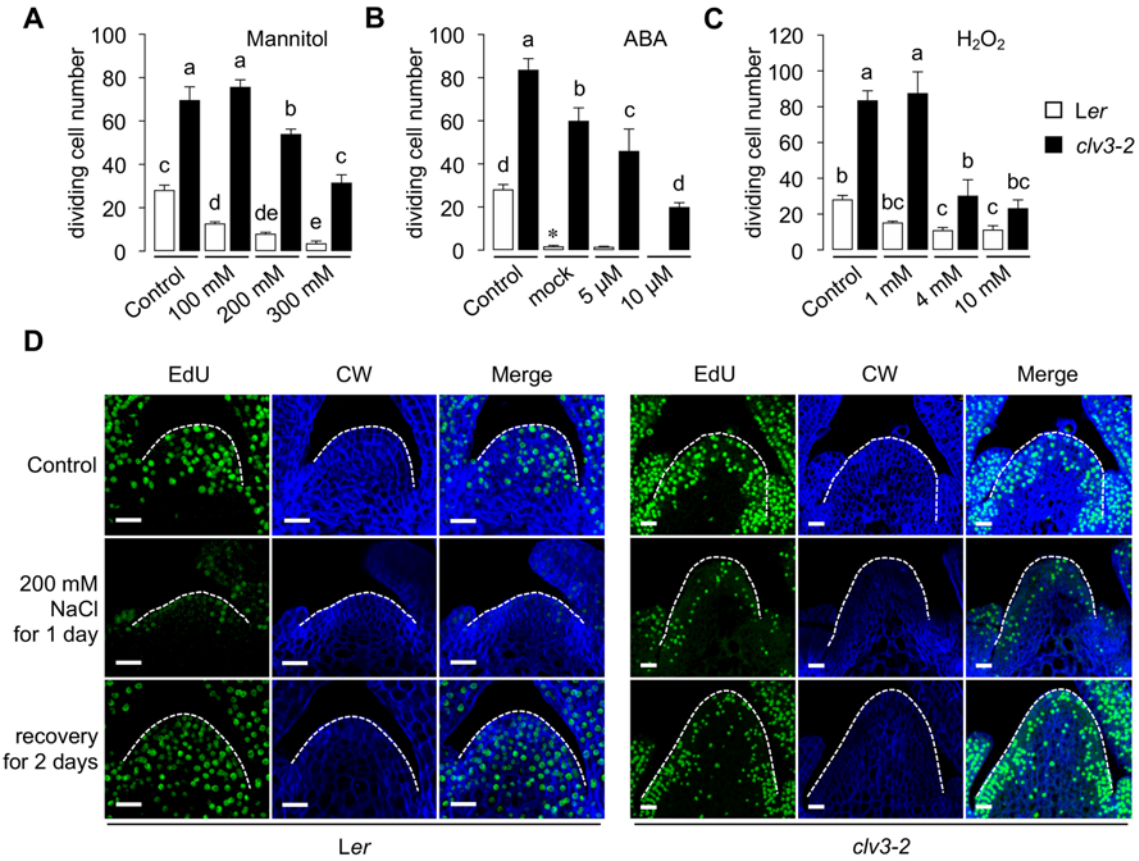


**Fig. 4.** Shoot meristem activity is dynamically controlled by abiotic stresses. The number of EdU labeled cells in the SAMs of WT L*er* and *clv3-2* without (Control) or with various concentrations of different abiotic stimuli, such as mannitol (A), ABA (B) and $H_2O_2$ (C) was quantified. The control indicates the condition without treatment. The mock condition was treated with 0.01M NaOH, in which the ABA solution was prepared (B). Asterisk indicates that the number of EdU labeled cells was already affected by the mock condition in WT L*er* SAMs (B). The data are presented as the mean ± SE ($n$=4-15). The different letters indicate a significant difference ($p$<0.05) according to the Duncan test. (D) Recovered cell proliferation in the absence of salt stresses. The shoot meristem phenotypes with proliferating cells labeled by EdU in WT L*er* and *clv3-2* were observed under the without (Control) or with 200 mM NaCl condition for 1 day. The EdU-labeled cells were also observed 2 days after removing the salt stresses by transferring seedlings to a new medium. The dotted lines showed the outline of the SAM regions. CW, Calcofluor White staining plant cell walls. Merge, merged images of EdU-signal and CW. Scale bars, 20 μm.

al. 2017), the regulation of shoot meristem activity triggered by abiotic stresses may occur via ABA and $H_2O_2$ as downstream signaling regulators. Furthermore, when an input stimulus of salt stresses was removed from a culture medium, actively dividing cells in shoot meristems were recovered completely in both WT L*er* and *clv3-2* (Fig. 4D), suggesting that shoot meristem activity is capable of dynamically responding to unfavorable abiotic stresses for plastic development on shoot growth.

## Discussion

### SAM Activity Via Meristem Maintenance Genes is Involved in Stress Tolerance to Abiotic Stimuli

In this study, we found that shoot meristems containing actively dividing cells are more resistant to salt stresses (Fig. 1; Fig. 3). Although the typical stress-responsive genes were strongly induced by salt stresses, the expression patterns were similar among genotypes, such as WT L*er* and *clv3-2* or WT Col-0 and *clv1 bam1* (Fig. 2), indicating that another mechanism is required for displaying salt tolerance on shoot growth and development. Usually, the biological role of shoot meristem activity via cell proliferation and differentiation is considered mainly for maintaining undifferentiated cells in the SAM and controlling postembryonic organogenesis. Therefore, the salt tolerant phenotype shown in *clv3-2* or *clv1 bam1* mutants may be evidence that the homeostatic regulation controlling stem cell population in the SAM is involved in stress tolerance to abiotic stresses. CLV3 secreted from stem cells is a signal peptide to trigger the downstream CLV1/BAM1 receptor signaling pathway to maintain the homeostatic stem cell pool in the shoot stem cell niche (Shinohara and Matsubayashi 2015; Wang et al. 2018a; Kitagawa and Jackson 2019; Lee et al. 2019). In addition, the salt stress condition did not

influence root growth and cell proliferation in root meristems (Fig. S1), suggesting that the salt tolerant phenotype of *clv3-2* or *clv1 bam1* mutants is the shoot-specific effect related to the CLV3p-mediated signaling pathway.

In addition, highly induced *WUS* promoting stem cell proliferation was observed in the *clv3-2* mutant under the salt stress condition (Fig. 3C). On the other hand, because salt stresses also displayed the decreased number of actively dividing cells in shoot meristems via ABA and $H_2O_2$ (Fig. 3A; Fig. 4A-C), WUS-mediated stem cell proliferation by salt stresses may act as a parallel pathway to regulate the appropriate cell proliferation in the SAM for developmental plasticity under abiotic stresses. Interestingly, a recent report showed that transgenic plants overexpressing *WUS* have the resistant phenotype for drought stresses (Lee et al. 2016). Furthermore, *WUS* expression is induced in the stressful, dedifferentiated, embryonic callus via the auxin gradient for shoot regeneration (Su et al. 2009; Grafi et al. 2011) and some *WUSCHEL-related homeobox* (*WOX*) genes functioning developmental processes have recently been reported to be responsive to abiotic stimuli of drought, salt, and cold in other plant species, such as *Oryza sativa* (rice), *Brassica napus*, *Gossypium hirsutum* (cotton), and *Camellia sinensis* (tea plant) (Cheng et al. 2014; Yang et al. 2017; Wang et al. 2018b; Wang et al. 2019), supporting the correlation with abiotic stresses.

A recent study reported that the overexpression line of another meristem maintenance gene *STM* showed the drought tolerant phenotype (Lee et al. 2016). The ABA-induced R2R3-type MYB96 TF bound directly to the *STM* promoter and the expression of *STM* was induced by the ABA treatment (Lee et al. 2016). Because the loss-of-function *stm* mutant has a complete defect in the shoot meristem structure after the formation of cotyledons, the function of *STM* is to initiate the shoot meristem structure during embryogenesis (Long et al. 1996). Moreover, *STM* is partly involved in maintaining shoot meristem activity with *WUS* (Lenhard et al. 2002), indicating the synergistic regulation on stem cell proliferation and differentiation in the SAM, even though their function is not completely overlapped. Because the expression of *WUS* was not induced by R2R3-type MYB96 TF in contrast to *STM* (Lee et al. 2016), these results suggest that at least two different pathways, such as ABA-dependent and ABA-independent manners via *STM* and *WUS*, may be involved in the abiotic stress tolerance on shoot growth and development through complicated molecular mechanisms based on shoot meristem activities.

### ROS is Involved in Abiotic Stress Tolerance in the SAM

Recent studies support the biological roles of abiotic stresses (e.g., hypoxia and ROS homeostasis) for shoot meristem maintenance (Zeng et al. 2017; Yang et al. 2018; Weits et al. 2019). In this study, our results showed that abiotic stresses, such as salt and osmotic stresses reduced actively dividing cells in the SAM via ABA and $H_2O_2$ (Fig. 3; Fig. 4). Accordingly, it has been well known that $H_2O_2$ is involved in the differentiation process of the animal stem cell population (Hamanaka et al. 2013; Ludin et al. 2014). Similarly, a recent report showed that $H_2O_2$ is also accumulated at the PZ, in which plant stem cells undergo the differentiation process to form new developing lateral organs (Zeng et al. 2017). In addition, $H_2O_2$ negatively regulates stem cell proliferation through the inhibition of NADH or NADPH, which produce the superoxide anion ($O_2^-$) that accumulates in the shoot stem cell zone (Zeng et al. 2017). Therefore, we cannot rule out the possibility that salt tolerance shown in *clv3-2* or *clv1 bam1* SAMs may be caused by $H_2O_2$-meidated differentiation processes of previously increased stem cells to a new cell fate harboring the property of stress adaptation (Shoshani and Zipori 2011).

Developmental plasticity on plant growth and development under abiotic stresses must be an important factor in the intrinsic nature of plants that live in ever-changing environments. Overall, these results provide an insight that stress tolerance mediated by the CLV-WUS pathway controlling shoot meristem activity is probably a key mechanism allowing plants to develop properly under external environmental stress conditions. Therefore, future studies will examine how different types of ROS involving shoot meristem activity are re-distributed under abiotic stresses and how the CLV-WUS pathway interacts with abiotic stresses to understand the molecular basis of stress tolerance mediated by shoot meristem activity.

## Materials and Methods

### Plant Materials and Growth Conditions

Columbia-0 (Col-0) and Landsberg *erecta* (L*er*) ecotypes were used as the wild-type (WT) Arabidopsis plants in this study. The *clv3-2* mutant has a L*er* background and the *clv1 bam1* mutant has a Col-0 background. The *clv1 bam1* double mutant was prepared by crossing *clv1* (WiscDsLox489-492B1) and *bam1* (Salk_015302) single mutants. For the plant growth and development study under salt stresses, L*er*, *clv3-2*, Col-0 and *clv1 bam1* seeds were germinated and grown on soil at 22–23°C with a 100 μmol $m^{-2}$ $s^{-1}$ light intensity under 16-h light/8-h dark photoperiod conditions for 4 weeks. For liquid culture of seedlings to perform the EdU assay, 10 seeds of L*er* and *clv3-2* were germinated and grown in 6-well plates containing 1 ml of liquid medium [0.5 × Murashige and Skoog (MS) and 0.5% sucrose, pH 5.8 adjusted with KOH] for 10 days. For the half MS plate culture of seedlings to perform the gene expression assay, L*er*, *clv3-2*, Col-0 and *clv1 bam1* seedlings were grown in half MS plates containing 0.5 × MS, 0.5% sucrose and 0.8% phytoagar for seven days. For the root growth and EdU assay, seedlings were grown in half MS plates for 9 days.

### Salt Tolerance Assay and Survival Test

For the observation of plant growth and development under salt stresses, the plants were grown for two weeks on soil and then applied

with water containing 100 or 200 mM NaCl twice with a one-week interval. The salt tolerant phenotype and survival ratio of the plants were measured using 4-week-old plants grown on soil.

EdU-based Cell Proliferation Assay using Paraffin Sections

Seedlings were grown in 6-well plates with 1 mL of liquid medium for 9 days and treated with stress conditions for 1 day. A 100X (1 mM) working solution of 5-ethynyl-2'-deoxyuridine (EdU) (Invitrogen, Carlsbad, CA, USA) was prepared from a 10 mM stock solution. For EdU staining, 9-day-old seedlings were incubated with 1X EdU (10 μM) for 16 h in their own culture medium at room temperature. After EdU incubation, SAM tissues were harvested using forceps and a blade. The SAM tissue was fixed for 20 min in chilled 90% acetone and transferred to a FAA (formaldehyde-acetic acid-alcohol) fixation solution for 4 h at room temperature. Paraffin embedding and sections were prepared using a Lieca RM2165, as described previously (Lee et al. 2019). After removing the paraffin through histoclear (National Diagnostic, Sommerville, NJ, USA) and rehydration steps, sections were then incubated in EdU reaction cocktails (Invitrogen) with plastic coverslips (Chemicon; Fisher Scientific, Waltham, MA, USA) at room temperature for 2 h. This incubation was protected from light. The reaction cocktails were mixed with the following components (1.6 μL EdU buffer additive, 14 μL EdU reaction buffer, 6.7 μL $CuSO_4$, 0.07 μL Alexa Fluor azide and 144 μL distilled water. A total volume of approximately 166 μL was used for one slide reaction. After the EdU reaction, the slides were washed with distilled water and stained immediately with 0.1mg/ml of Calcofluor White solution (Sigma, St. Louis, MO, USA) for 5 min and rinsed briefly with water. The EdU (488 nm laser line) and Calcofluor White (433 nm laser line) images were taken using a confocal microscope (ZEISS LSM-700). The number of EdU-labeled stem cell were counted using an Image J program in the SAM region.

For EdU staining using roots, the seedlings were treated with 10 μM EdU in 6-well plates containing a liquid medium for 2 h. The seedlings were then fixed with 3.7% formaldehyde for 15 min and treated with EdU reaction cocktails for 30 min. The seedlings were washed with distilled water and treated with Calcofluor White for 5 min. The fluorescent signals of EdU and Calcofluor White were detected using a ZOE fluorescent cell imager (Bio-Rad, Hercules, CA, USA).

Gene Expression Assay

For expression assays, the abiotic stress-responsive genes were performed using the shoot apex tissues from seedlings that were grown on MS plates for 6 days and then transferred to new MS plates containing 200 mM NaCl for 1 day. The total RNAs were extracted from the shoot apex tissues of 7-day-old seedlings with TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Residual genomic DNA from the total RNAs were removed using 1 unit of DNase I (Roche), and the first-stranded cDNAs from 1 μg of total RNA were synthesized using the M-MLV reverse transcriptase (Promega, Madison, USA) according to the manufacturer's instructions. Reverse transcription-quantitative polymerization chain reaction (RT-qPCR) assays were performed with the stress-responsive gene primers [*KIN1-F*: 5'-GGC AGC GGG AGG TGT TAAC-3' and *KIN1-R*: 5'-TGA CCC GAA TCG CTA CTT GTT-3' for *KIN1* (At5g15960) gene expression; *RD29A-F*: 5'-GTG CCG ACG GGA TTT GAC-3' and *RD29A-R*: 5'-CTG ATG CCT CAC CGT ATC CA-3' for *RD29A* (At5g52310) gene expression; *RAB18-F*: 5'-TTC GGT CGT TGT ATT GTG CTT T-3' and *RAB18-R*: 5'-CCA GAT GCT CAT TAC ACA CTC ATG-3' for *RAB18* (At5g66400) gene expression; *DREB2A-F*: 5'-CAG TGT TGC CAA CGG TTC AT-3' and *DREB2A-R*: 5'-AAA CGG AGG TAT TCC GTA GTT GAG-3' for *DREB2A* (At5g05410) gene expression] using a Step One Plus real time PCR detection system with SYBR Green Real time PCR Master Mix (TOYOBO). *ACT2* (At3g18780) was used as a control gene to normalize each gene expression (*ACT2-F*: 5'-TCC CTC AGC ACA TTC CAG CAG AT-3' and *ACT2-R*: 5'-AAC GAT TCC TGG ACC TGC CTC ATC-3' for *ACT2* gene expression).

Statistical Analyses

All quantitative results were analyzed by one-way analysis of variation (ANOVA) using Prism (ver. 7.0b) and SPSS (ver. 25) statistical software. Significant differences and different letters were determined by one-way ANOVA analysis with a Duncan multiple comparison test.

## Acknowledgements

This study was supported by the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2018R1D1A1A02048850).

## Author's Contributions

HL designed the experiments and wrote the manuscript; YSJ performed the phenotype analyses and EdU assays against salt stress treatment; OKC and JHK performed gene expression assays and additional experiments for revised manuscript. All authors agreed on the contents of the final manuscript and post no conflicting interest.

## Supporting Information

**Fig. S1.** Salt stress tolerance of *clv3-2* or *clv1 bam1* mutants was not found on root growth and development.
**Fig. S2.** Shoot meristem activity is also affected by osmotic stresses.

## References

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–399

Cheng S, Huang Y, Zhu N, Zhao Y (2014) The rice WUSCHEL-related homeobox genes are involved in reproductive organ development, hormone signaling and abiotic stress response. Gene 549:266–274

Cheng ZJ, Shang B, Zhang XS, Hu Y (2017) Plant hormones and stem cells, In JY Li, CY Li, SM Smith, Hormone Metabolism and Signaling in Plants, ed, Elsevier, London, pp 405–430

Gordon SP, Chickarmance VS, Ohno C, Meyerowitz EM (2009) Multiple feedback loops through cytokinin signaling control stem cell number within the Arabidopsis shoot meristem. Proc Natl Acd Sci USA 106:16529–16534

Grafi G, Chalifa-Caspi V, Nagar T, Plaschkes I, Barak S, Ransbotyn V (2011) Plant response to stress meets dedifferentiation. Planta 233:433–438

Hamanaka RB, Glasauer A, Hoover P, Yang SN, Blatt H, Mullen AR, Getsios S, Gottardi CJ, DeBerardinis RJ, Lavker RM, Chandel NS (2013) Mitochondrial reactive oxygen species promote epidermal epidermal differentiation and hair follicle development. Sci Signal 6:ra8

Jakab G, Ton J, Flors V, Zimmerli L, Métraux J-P, Mauch-Mani B (2005) Enhancing Arabidopsis salt and drought stress tolerance

by chemical priming for its abscisic acid responses. Plant Physiol 139:267–274
Kitagawa M, Jackson D (2019) Control of meristem size. Annu Rev Plant Biol 70:269–291
Lee H, Jun YS, Cha O-K, Sheen J (2019) Mitogen-activated protein kinases MPK3 and MPK6 are required for stem cell maintenance in the Arabidopsis shoot apical meristem. Plant Cell Rep 38:311–319
Lee HG, Choi Y-R, Seo PJ (2016) Increased *STM* expression is associated with drought tolerance in Arabidopsis. J Plant Physiol 201:79–84
Lenhard M, Jürgens G, Laux T (2002) The *WUSHCEL* and *SHOOTMERISTEMLESS* genes fulfil complementary roles in Arabidopsis shoot meristem regulation. Development 129:3195–3206
Long JA, Moan EI, Medford JI, Barton MK (1996) A member of the KNOTTED class of homeodomain proteins encoded by the *STM* gene of Arabidopsis. Nature 379:66–69
Ludin A, Gur-Cohen S, Golan K, Kaufmann KB, Itkin T, Medaglia C, Lu XJ, Ledergor G, Kollet O, Lapidot T (2014) Reactive oxygen species regulate hematopoietic stem cell self-renewal, migration and development, as well as their bone marrow microenvironment. Antioxid Redox Signal 21:1605–1619
Msanne J, Lin J, Stone JM, Awada T (2011) Characterization of abiotic stress-responsive *Arabidopsis thaliana RD29A* and *RD29B* genes and evaluation of transgenes. Planta 234:97–107
Sakuma Y, Maruyama K, Osakabe Y, Qin F, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2006) Functional analysis of an Arabidopsis transcription factor, *DREB2A*, involved in drought-responsive gene expression. Plant Cell 18:1292–1309
Shinohara H, Matsubayashi Y (2015) Reevaluation of the CLV3-receptor interaction in the shoot apical meristem: dissection of the CLV3 signaling pathway from a direct ligand-binding point of view. Plant J 82:328–336
Shoshani O, Zipori D (2011) Mammalian cell differentiation as a possible outcome of stress. Stem Cell Rev and Rep 7:488–493
Su YH, Zhao XY, Liu YB, Zhang CL, O'Neil SD, Zhang XS (2009) Auxin-induced *WUS* expression is essential for embryonic stem cell renewal during somatic embryogenesis in Arabidopsis. Plant J 59:448–460
Wang B, Smith SM, Li J (2018a) Genetic regulation of shoot architecture. Annu Rev Plant Biol 69:437–468
Wang M-M, Liu M-M, Ran F, Guo P-C, Ke Y-Z, Wu Y-W, Wen J, Li P-F, Li J-N, Du H (2018b) Global analysis of *WOX* transcription factor gene family in *Brassica napus* reveals their stress- and hormone-responsive patterns. Int J Mol Sci 19:3470
Wang P, Guo Y, Chen X, Zheng Y, Sun Y, Yang J, Ye N (2019) Genome-wide identification of *WOX* genes and their expression patterns under different hormone and abiotic stress treatments in tea plant (*Camellia sinensis*). Trees 33:1129–1142
Watkins JM, Chapman JM, Muday GK (2017) Abscisic acid-induced reactive oxygen species are modulated by flavonols to control stomata aperture. Plant Physiol 175:1807–1825
Yanai O, Shani E, Dolezal K, Tarkowsk P, Sablowski R, Sandberg G, Samach A, Ori N (2005) Arabidopsis KNOXI proteins activate cytokinin biosynthesis. Curr Biol 15:1566–1571
Yang S, Yu Q, Zhang Y, Jia Y, Wan S, Kong X, Ding Z (2018) ROS: The fine-tuner of plant stem cell fate. Trends Plant Sci 23:850–853
Yang Z, Gong Q, Qin W, Yang Z, Cheng Y, Lu L, Ge X, Zhang C, Wu Z, Li F (2017) Genome-wide analysis of *WOX* genes in upland cotton and their expression pattern under different stresses. BMC Plant Biol 17:113
Yu Y, Li Y, Jia F, Zhao M, Li W, Sun Q, Li N, Li W, Meng Z (2017) *ZmFKBP20-1* improves the drought and salt tolerance of transformed Arabidopsis. J Plant Biol 60:558–570
Weits DA, Kunkowska AB, Kamps NCW, Portz KMS, Packbier NK, Venza ZN, Gaillochet C, Lohmann JU, Pedersen O, van Dongen JT, Licausi F (2019) An apical hypoxic niche sets the pace of shoot meristem activity. Nature 569:714–717
Zeng J, Dong Z, Wu H, Tian Z, Zhao Z (2017) Redox regulation of plant stem cell fate. EMBO J 36:2844–2855
Zhuo Y, Yan A, Han H, Li T, Geng Y, Liu X, Meyerowitz EM (2018) HAIRY MERISTEM with WUSCHEL confines CLAVATA3 expression to the outer apical meristem layers. Science 361:502–506
Zhu J-K (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 53:247–273
Zhu J-K (2016) Abiotic stress signaling and responses in plants. Cell 167:313–324