CRABS CLAW and SUPERMAN coordinate phytohormone-mediated spatio-temporal regulation of floral meristem activities in Arabidopsis

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Graduate School of Biological Science **Abstract of Doctoral Thesis**

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The appropriate timing of the termination of floral meristem activity (FM determinacy) determines the number of floral organs. In *Arabidopsis*, two transcription factors, CRABS CRAW (CRC) and SUPERMAN (SUP), play key roles in FM determinacy. CRC belongs to the YABBY transcription factor family, whose members contain a zinc finger and a helix-loop-helix domain. The *crc* mutation causes the formation of unfused carpels and leads to an increase in carpel number in sensitized backgrounds. The *SUP* gene encodes a C2H2-type zinc-finger protein, and *sup* mutants produce extra carpels and stamens. Even though both of the genes have been reported to regulate floral organs development, floral meristem (FM) determinacy and auxin homeostasis, the genetic interaction between them is not fully understood. The *crc sup* double mutant had significantly more stamens and carpels than the parental lines and an enlarged floral meristem. The transcriptomic data showed that these two transcription factors might regulate multiple common downstream genes which include several cytokinin- and auxin-related genes as well as stress- and metabolic-related genes to function downstream of CRC and SUP during stamen development and possibly FM determinacy. The regulation of common downstream genes by CRC and SUP might contribute to the initiation of an appropriate number of stamens and to subsequent growth and development. Besides that, the confocal live imaging of cytokinin reporter line *TCSn:GFP* on several genotypes which include *crc-1*, *sup-5* and *crc-1 sup-5* mutants implicated that cytokinin might plays important role during spatio-temporal regulation on FM determinacy. This study might open the breakthrough point for further investigation on the transcriptional-hormonal crosstalk network on flower development.

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1. INTRODUCTION

Meristematic activity during plant development

Plant development is dependent on the persistent activity of pluripotent meristematic cells that are responsible for organ formation (Laux et al., 1996). There are two main different groups of pluripotent meristematic cell populations in plants, termed as shoot apical meristem (SAM) and root apical meristem (RAM). SAM is responsible for the post-embryonic growth and organogenesis in aerial environment whereas RAM promotes the growth of roots underground for nutrients intake. Although the cell architecture of SAM and RAM differ, the working mechanism and pattern of their genetic, epigenetic and chemical signaling approaches for stem cell maintenance and differentiation are relatively conserved **(**Miwa et al., 2009**)**. SAM is located at the plant apex and the maintenance of stem cell reservoir in SAM is the core factor to uphold the production lines for the formation of aerial organs, which include flowers, leaves and stems **(**Barton and Poethig, 1993; Bowman and Eshed, 2000; Lee et al., 2019b**)**. To sustain proper continuous growth, the SAM maintains the balance between self-renewal of stem cells and cell differentiation for lateral organ formation (Lee et al., 2019a).

In *Arabidopsis thaliana*, homeodomain transcription factor WUSCHEL (WUS) and CLAVATA (CLV) ligand-receptor system form the central part of the genetic and epigenetic regulatory networks in the maintenance of stem cell pool in SAM (Mayer et al., 1998; Schoof et al., 2000; Sharma and Fletcher 2002; Williams and Fletcher, 2005). In SAM, WUS-mediated signaling from the organization center (OC) located at the lower cell layers manipulates the cell identity of overlying neighbor cells for the replenishment of stem cell pool. Hence, WUS expression has to be restricted spatially in a precise manner by CLV to avoid swaying of the organ primordia initiation at upper cell layers (Carles and Fletcher, 2003; Adibi et al., 2016). As reported, ectopic *WUS* expression is enough to shift the balance between stem cell proliferation and differentiation rates in SAM (Mayer et al., 1998; Williams and Fletcher, 2005). Corresponding to that, *WUS-CLV* negative feedback loop acts as the rheostat for this spatial control of WUS signal transduction and the effective teamwork of this self-regulatory loop with other transcription factors and phytohormones are also mandatory (Schoof et al., 2000; Leibfried et al., 2005). Auxin and cytokinin are two core phytohormones function in the regulatory network of SAM. In fact, both of them play distinct roles in cellular level: auxin promotes stem cell differentiation and subsequently organogenesis while cytokinin triggers cell division for stem cell activity maintenance and cell cycle progression (Zhao et al., 2010).

Floral meristem (FM) and floral patterning

In general, flower development initiates from the floral stem-cell pool that is located in the center of the floral meristems (FMs). The model plant *Arabidopsis thaliana* produces flowers with four whorls of floral organs, from outermost to center part: four sepals, four petals, six stamens and two fused carpels (Smyth et al., 1990; Figure 1A). In 1991, the genetic ABC model which describes the roles of floral homeotic proteins in floral organ identity specification was proposed (Coen and Meyerowitz, 1991; Ito, 2011). Two more classes, D- and E-class were then included into this ABC model later. The D-class genes are required for ovule development, while E-class genes form homo- and hetero-oligomeric complexes to determine the floral organ identity (Guo et al., 2015; Figure 1B). Quadruple mutant of the founding members in E-class genes, *sepallata1/2/3/4* (*sep1/2/3/4*) produces flower with only leaf-like floral organs, indicating the need of *SEP* genes in each of the flower development stages (Honma and Goto, 2001; Jetha et al., 2014). The revised ABC model indicates that sepals identity is under the control of A-class gene *APETALA1* (*AP1*) and *SEP*; A-, B- and E-class proteins complex AP1-SEP-AP3-PISTILLATA (PI) specifies petals; B-, C-and E-class proteins complex (AG-SEP-AP3-PI) is required for stamens specification; C- and E-class (AG-SEP) for carpels and lastly, D- and E-class protein complex SEEDSTICK(STK)-SEP for ovules identity determination (Honma and Goto, 2001; Yanofsky et al., 1990; Jack et al., 1992; Mandel et al., 1992; Goto and Meyerowitz, 1994; Mendes et al., 2013; Figure 1B). Besides the proper spatial functioning of floral homeotic genes, the timing for FM activity termination (FM determinacy) is also crucial for proper regulation of flower development since the meristem size determines the flower size and also floral organ number. As compared to SAM and RAM, FM activity is determinate and the stem cell pool is lost during differentiation (Sun et al., 2014). Delayed FM determinacy or expansion of FM expression domain would result in higher stem cell proliferation rate and consequently flower size enlargement and extranumerary floral organs formation (Sablowski, 2007).

FM is derived from SAM and it is important for the transition of plants from vegetative to reproductive phase. As the stem continues to elongate, secondary SAMs are generated in axils of leaves, and FM formation are induced on the peripheral zones (PZ) of both primary and secondary SAMs to promote flowering (Fletcher, 2002). Balance between the rates of stem cell proliferation and differentiation in FM is pivotal for the proper flower development (Sun et al., 2009). As similar to SAM, FM activity is also regulated by WUS activity and its spatial expression pattern is under the control of *WUS-CLV* pathway during early stage of flower

development. At the beginning of flower development, *W*US is expressed in the OC to specify and maintain the stem cell identity of the overlying cells. The expansion of *WUS* expression is then restricted by the CLV signaling pathway via transcriptionally activation of CLV3 peptide by WUS during stage 2 (Sun and Ito, 2015; Figure 2). On the other hand, *WUS* activates the expression of *AG* during stage 3 under the collaboration with *LEAFY* (*LFY*) and AG subsequently trigger the activity of another downstream gene of *AG*, namely *KNUCKLES* (*KNU*) to form the *WUS-AG-KNU* negative feedback loop to terminate the floral meristem activity during stage 6 of flower development to produce floral organs with defined numbers (Mizukami and Ma, 1992; Sun et al., 2014; Figure 2). Correspondingly, *clv* mutants show broader expression localization of *WUS* and subsequently produce meristems with size enlargement and flowers with extra organs. Whereas, disrupted *ag* or *knu* expression would lead to delayed floral meristem termination, FM over-proliferation and consequently supernumerary organs formation (Yanofsky et al., 1990; Mizukami and Ma, 1997; Prunet et al., 2009; Sun et al., 2014). Even though most of the early reports about the regulation of SAM and FM activities mainly discussed about the roles of distinct transcriptional regulatory networks or cascades, more and more studies revealed that phytohormones homeostasis and transcription factors are inseparable to preserve the stem cell niches and control organs specialization in plants (Aloni et al., 2006; Shani et al., 2006; Achard et al., 2007; Kurakawa et al., 2007; Kyozuka, 2007; Veit, 2009).

Precise spatio-temporal regulation of stem cell activity is crucial in FM for proper flower development. *WUS* expression needs to be shut down during stage 6 of flower development which is after the initiation of carpel primordia to terminate FM activity. This timing mechanism is under the control of *WUS-AG-KNU* negative loop, apart from the *WUS-CLV* spatial regulatory pathway (Sun et al., 2009; Payne et al., 2004; Figure 2). *KNU* encodes a C2H2 zinc finger protein and its expression is induced by the C-class floral homeotic gene *AG*, two days after *AG* induction by WUS during stage 3, showing a precise timing control for FM determinacy (Sun et al., 2009; Yanofsky et al., 1990; Lenhard et al., 2001; Lohmann et al., 2001). Studies from other groups also discovered another *AG*-dependent pathway of *WUS* regulation through cytokinin which work redundantly with *AG-KNU* pathway. ETTIN/ AUXIN RESPONSE FACTOR 3 (ETT/ARF3) integrates *AG* function by down-regulating the expression of cytokinin biosynthesis and signaling genes such as *ISOPENTENYLTRANSFERASE*s (*IPT*s), *LONELY GUY*s (*LOG*s) and *ARABIDOPSIS HISTIDINE KINASE 4* (*AHK4*) to reduce cytokinin activity in flower (Liu et al., 2014; Li et al., 2016; Zhang et al., 2018). Since cytokinin acts as the mitogenic phytohormone and it is

capable of increasing the cell mitotic rate in Arabidopsis (Stals and Inzé, 2001), it is possible that *AG*-mediated FM determinacy is dependent to cell division process driven by phytohormone homeostasis based on the available reports.

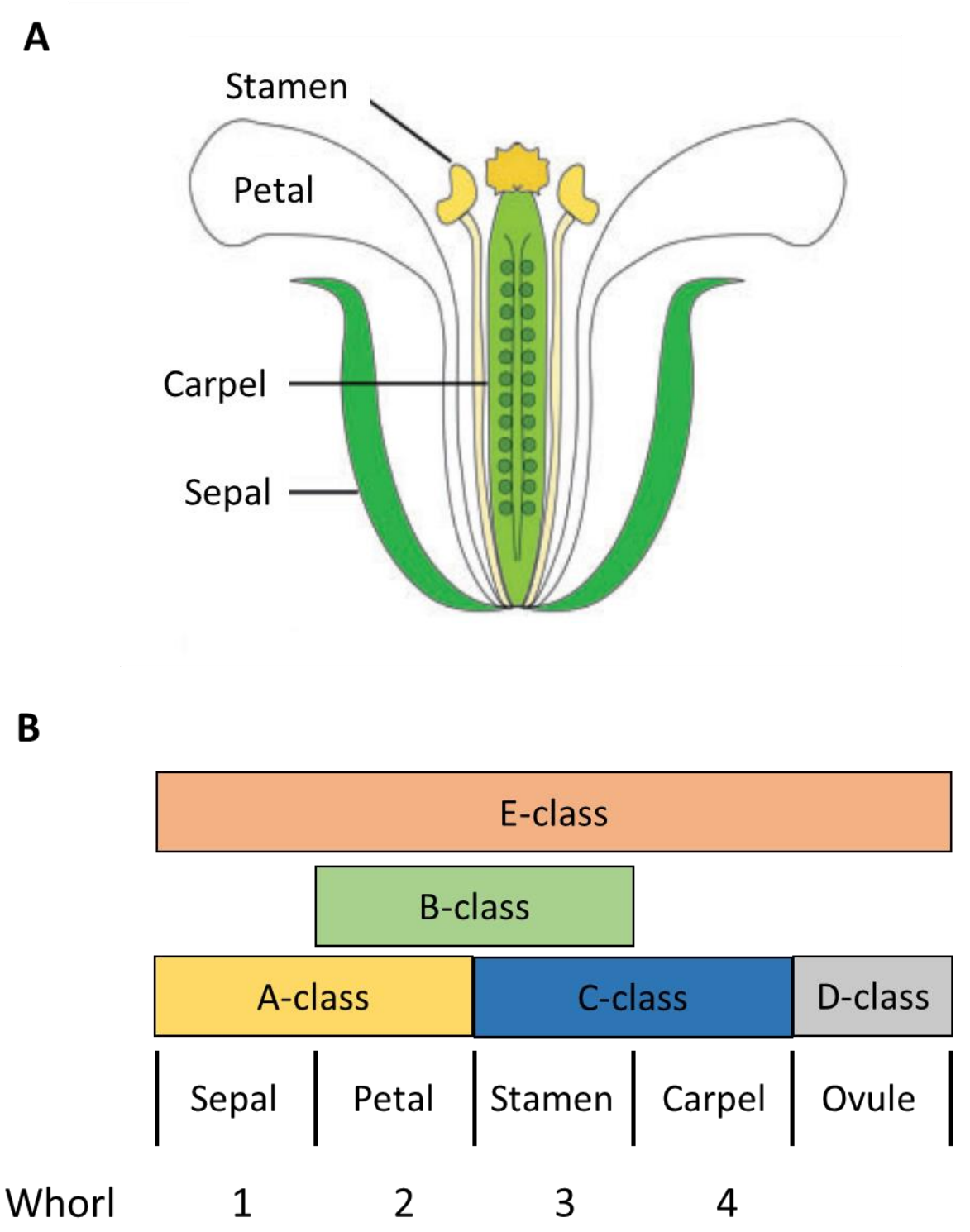


Figure 1 Illustration diagram of *Arabidopsis thaliana* flower and revised ABC model. (A) Wild type Arabidopsis flower consists of four sepals, four petals, six stamens and two fused carpels. Picture is adapted from Guo et al. (2015). **(B)** Revised ABC model which categorizes the genes required for the development of respective floral organs.

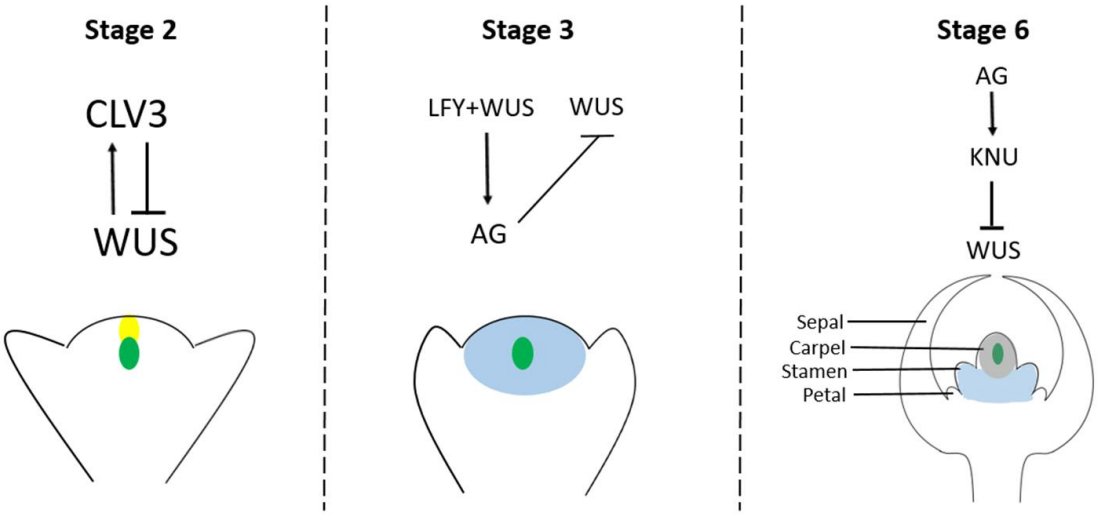


Figure 2 Illustration diagram of spatio-temporal regulation of FM activity termination in Arabidopsis. Green – *WUS* expression domain; Yellow – *CLV3* expression domain; Blue – *AG* expression domain; Grey – *KNU* expression domain

CRABS CLAW (*CRC*) and *SUPERMAN* (*SUP*)

Another direct downstream target of *AG* is *CRABS CLAW* (*CRC*), which contributes to a *KNU*-independent pathway of FM determinacy. CRC belongs to the YABBY family transcription factors, which contain a zinc finger as well as a helix-loop-helix domain. Its expression starts from stage 6 of flower development and localizes to the abaxial region of the developing carpels (Bowman and Smyth, 1999; Figure 3). The *crc* single mutant shows only minor or no FM indeterminacy, however, in sensitized background with strong FM indeterminate phenotype such as *ag* or *knu* mutant background, the FM indeterminacy phenotype could be further enhanced by introducing *crc* mutation (Alvarez & Smyth, 1999; Prunet et al., 2008; Breuil-Broyer et al., 2016). This phenomenon makes the role of *CRC* in the FM determinacy pathway mysterious and puzzling. Recent study from Yamaguchi et al. identified a direct downstream target of *CRC*, which is *TORNADO2* (*TRN2*) (Yamaguchi et al, 2017). *TRN2* encodes a transmembrane protein of the tetraspanin family and loss-of-function *trn2* mutant showed disrupted auxin distribution in axillary meristem and also mis-expression of stem cell marker genes in SAM periphery (Cnops et al., 2006; Chiu et al., 2007; Boavida et al., 2013; Wang et al., 2015). In FM, CRC represses the expression of *TRN2* via transcriptional silencing, thus interfering the polar auxin transport and creating auxin maxima for gynoecium development and FM determinacy (Yamaguchi et al, 2017). Another back-to-back study from the same group further discovered that *CRC* up-regulates biosynthetic (*YUCCA 4*) *YUC4* gene and this mechanism works in parallel with down-regulation of *TRN2* to form a feed forward network for auxin level elevation in medial region of developing carpel (Yamaguchi et al., 2018). These findings may explain why *crc* loss-of-function mutant shows almost no FM indeterminacy. It is plausible that the loss of CRC-mediated auxin homeostasis fine-tuning could be compensated by other auxin regulatory pathway or function of other common upstream regulators of *YUC4* and *TRN2*.

SUPERMAN (*SUP*) encodes a C2H2 zinc-finger protein, which has been proposed to act as a boundary gene that specifies the separation between floral whorls 3 and 4 by regulating the expression of two B-class genes *APETALA3* (*AP3*) and *PISTILLATA* (*PI*) (Bowman et al, 1991; Sakai et al., 1995). *SUP* expression is induced by AG during late stage 3 or early stage 4 at the boundary of whorls 3 and 4 where the stamen primordia give rise (Sakai et al., 1995; 2000; Xu et al., 2019; Figure 3) The loss-of-function *sup* mutation leads to the formation of supernumerary stamens, suggesting that *SUP* acts as the regulator of both floral patterning and FM determinacy (Sakai et al., 1995, 2000; Xu et al., 2018). It was reported that the formation

of extra stamens in *sup* mutant is at the expense of carpel tissues from whorl 4 that switch identity from female to male due to the expansion of *AP3* and *PI* expression closer to the center of the FM as compared with the wild type (Bowman et al., 1991; Prunet et al., 2017). However, the expression domain of *SUP* is at the boundary between whorls 3 and 4 and mostly not overlapped with that of FM regulator *CLV3* or *WUS* throughout the flower development process (Prunet et al., 2017). This indicates that SUP regulates floral meristem activity in a non-cell autonomous manner. Further study confirmed that SUP down-regulates the expression of *YUC1* and *YUC4* genes at the boundary of stamen and carpel by depositing repressive genetic mark H3K27me3 to decrease local auxin biosynthesis, thus generating non-cell autonomous auxin signaling to induce FM determinacy and determine floral organs identity (Xu et al., 2018). Besides auxin, it was also strongly suggested that cytokinin signaling pathway coordinated by SUP activity along the border between whorls 3 and 4 results in the suppression of stamen development and promotion of female organ differentiation in the fourth whorl (Nibau et al., 2010). Taken together, these reports suggest a model of auxin- and cytokinin-regulated hormonal pathway under the effect of SUP on the modulation of cell growth and proliferation as well as floral patterning (Zhao et al., 2001; Cheng et al., 2006, 2007; Nibau et al., 2010; Sassi and Vernoux, 2013; Xu et al., 2018).

Despite the fact that both *SUP* and *CRC* play important roles in floral patterning, floral reproductive organs development and FM determinacy, the relationship between them is not well understood. However, some evidences suggest that there might be relationship between them in FM determinacy as well as floral organ development regulation. *SUP* is responsible to restrict the expression domain of B-class genes *AP3* and *PI* within third whorl to ensure proper stamen and carpel development while *CRC* expression is activated by AG to establish the abaxial-adaxial polarity of carpel in fourth whorl. Several studies had reported the antagonistic roles of B-class and C-class genes during flower development in which C-class gene *AG* activates the expression of *CRC* while B-class genes *AP3* and *PI* repress it for proper floral organ identity determination (Wuest et al., 2012; O'Maoileidigh et al., 2013; Wellmer et al., 2014). This suggest that *SUP* might play the role of restricting *CRC* expression in fourth whorl genetically through the down-regulation of *AP3* and *PI*. Besides that, another study also reported that certain *sup* mutant backgrounds showed different degree of floral organs phenotype and FM indeterminacy in early and late flowers respectively, indicating that functions of *SUP* and *CRC* are overlapped to work on the FM termination–carpel patterning switch especially in late flowers (Breuil-Broyer et al., 2016).

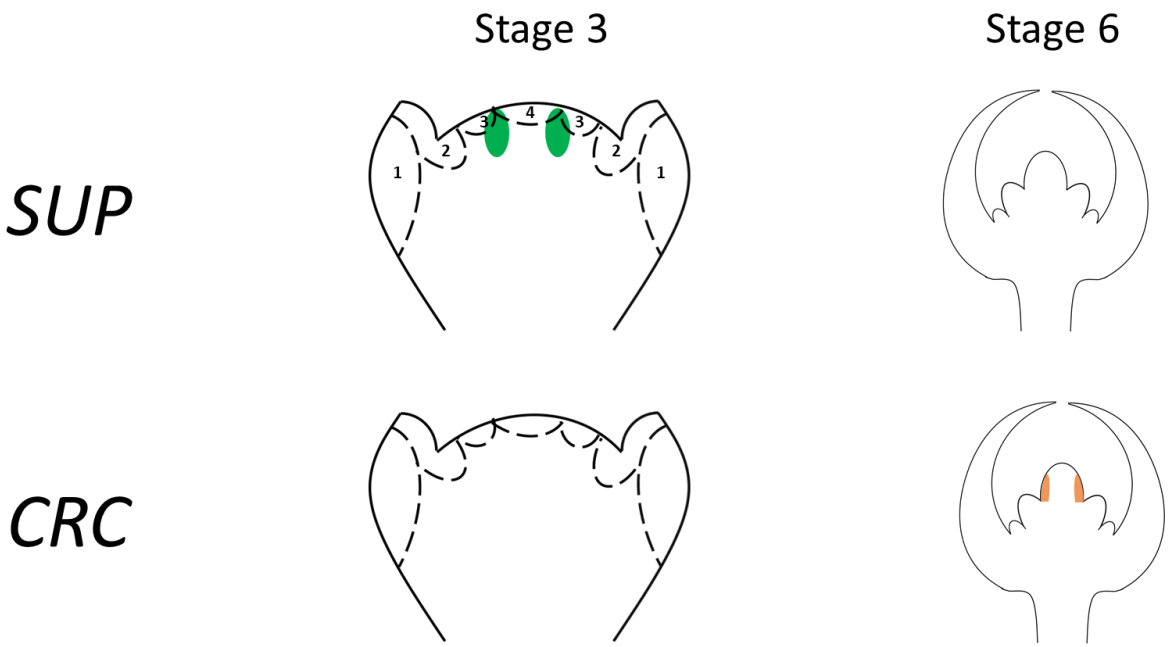


Figure 3 Illustration diagram of spatio-temporal regulation of SUP and CRC expression in Arabidopsis. *SUP* starts to be expressed at the boundary of whorls 3 and 4 during late stage 3 or early stage 4 while *CRC* starts to be expressed at the abaxial region of the developing carpel during late stage 5 or early stage 6. Green – *SUP* expression domain; Orange – *CRC* expression domain

Auxin-cytokinin regulatory network during flower development

Auxin is essential for organogenesis in vegetative SAM to produce organ primordia which includes flowers and leaves primordia. Two loss-of-function mutants of an auxin efflux carrier *PIN-FORMED1* (PIN1) and a positive regulator of cellular auxin efflux *PINOID* (*PID*) showed defect in polar auxin transport which leads to the formation of naked inflorescence without FM and flower (Okada et al., 1991; Bennett et al., 1995). Besides auxin polar transporters, mutation in *AUXIN RESPONSE FACTOR 5*/*MONOPTEROS* (*ARF5*/*MP*), one of the key regulators of auxin signaling response in Arabidopsis, also produces inflorescences with similar phenotypes as the mentioned mutants (Przemeck et al., 1996). This indicates that the polar auxin transport and auxin signaling transduction are tightly linked for FM formation. Both flowers and leaves primordia arise from peripheral zones of SAM; however, leaves primordia are still able to be produced in *mp*, *pin1* and *pid* mutants (Schuetz et al., 2008). This suggests that flower onset could be more dependent to auxin maxima and distribution as contrast to leaf primordia initiation or effects of PIN1 and MP may be more dominant in FM (Wang and Jiao, 2018). Corresponding to these phenomena, it can be deduced that there must be other regulators that act downstream of these auxin polar transporters as well as signaling transducer to initiate the flower primordia and flower onset upon auxin maxima introduction.

During the early phase of inflorescence development, cauline leaves emerge from the organ primordia at the SAM periphery which subtend secondary inflorescence branches (Yamaguchi et al., 2014a). The primordia would give rise to flowers once the plant is ready for meristematic identity transition that is from vegetative to reproductive or floral stage (Poethig, 2003; Yamaguchi et al., 2014a). During this floral transition, auxin maxima activates expression of LFY, a master regulator of reproductive growth in Arabidopsis which encodes a transcription factor that is responsible for floral fate specification of meristems and also a master coordinator of the entire floral network (Moyroud et al., 2010; Yamaguchi et al., 2013; Figure 4). In *pin1* mutant, *LFY* expression is greatly disrupted, indicating that *LFY* expression and the maintenance of flower development network is tightly linked to the cellular auxin level and distribution (Yamaguchi et al., 2013; Vernoux et al., 2010). In fact, auxin response element AuxRE could be found in the promoter region of *LFY* which acts as the binding site of MP (Yamaguchi et al., 2014b; Wakeel et al., 2018). Besides that, LFY mRNA and protein levels were greatly reduced in hypomorphic *mp* mutant and this reduction effect could be rescued by auxin treatment or further enhanced after treated with auxin transport inhibitor 1-N-

naphthylphthalamic acid (NPA), further proving that auxin signaling is essential to regulate the flower development or floral organs formation (Yamaguchi et al., 2013).

After the initial growth stage, the FM acquires meristematic features (Denay et al., 2017) and are capable to establish the stem cell reservoir for flower development. To initiate the stem cell proliferation, MP mediates repression of the cytokinin response negative regulators, *ARABIDOSPIS RESPONSE REGULATOR 7* and *15* (*ARR7* and *15*) (Zhao et al., 2010) accompanied by the repression of *ARR7* by LFY simultaneously (Chahtane et al., 2013), thereby locally increasing the cytokinin response in FMs (Figure 4). The role of MP as a cytokinin response activator can be proven by the compensation of the loss of primordia initiation in *mp* mutants by inhibition of ARR7/15 activity through amiRNA (Zhao et al., 2010). In stage 2 flower, expression of *WUS* and *CLV3* after the activation of the cytokinin signaling established the OC and a stem cell niche respectively to enable the continuous provision of stem cell for flower development and floral organs formation (Yoshida et al., 2011). Hence, it is evident that function of cytokinin during early flower development is to maintain the stem cell pool pluripotency after the meristematic identity transition coordinated by auxin maxima in flower primordia.

Besides the cytokinin positive signaling cascade, another cytokinin negative signaling component, *ARABIDOPISIS HISTIDINE PHOSPHOTRANSFER PROTEIN6* (*AHP6*) is also functionally important in hormonal regulatory pathway of flower development (Mähönen et al., 2006; Moreira et al., 2013; Besnard et al., 2014a, 2014b). During flower development, AHP6 acts as the downstream target of MP upon auxin signaling to inhibit cytokinin signaling in surrounding areas, and consequently control the emergence of new primordia and regulate the positioning of floral primordia spatially (Moreira et al., 2013; Besnard et al., 2014a, 2014b; Denay et al., 2017; Figure 4). *In situ* hybridization data from another report also demonstrated that expression of *AHP6* in the inflorescence meristem is located at the position of organogenesis during flower primordium formation besides early stage 1 primordium, in floral organ primordia of stage 3 flowers, and in the distal part of developing gynoecia in stage 6 flowers (Bartrina et al., 2011). It is generally reported that auxin and cytokinin function antagonistically, recent studies have revealed that they also act synergistically during gynoecium formation (Wolters and Jurgens, 2009; El-Showk et al., 2013; Schaller et al., 2015; Müller et al., 2017; Reyes-Olalde et al., 2017a, 2017b). During early development of gynoecium primordia, SPATULA (SPT) which belongs to basic Helix-Loop-Helix (bHLH) transcription factor family increases the cytokinin level at the basal medial domain of carpel region (Reyes-Olalde et al., 2017a, 2017b]. The increase in cytokinin level would eventually

activates the expression of auxin biosynthesis genes *YUC1* and *YUC4* as well as auxin efflux carriers *PIN7* which in turn activates the expression of *AHP6* at lateral domain. This causes the restriction of cytokinin signaling from the lateral domain. At the same time, auxin signaling is induced at the carpel apex by activating *YUC4* and blocking *PIN3* simultaneously to create auxin maxima at the apical region (Reyes-Olalde et al., 2017a; Müller et al., 2017). *AHP6* mutant also showed prolonged FM activity, indicating that cytokinin inhibitory field also involves in the regulation of FM determinacy (Bartrina et al., 2011). These reports indicate that the role of AHP6 is mainly on the spatial control of cytokinin signaling no matter it is during organogenesis or carpel development. Although these findings have demonstrated that the interplay between auxin and cytokinin via AHP6 is important for floral primordium positioning and gynoecium formation, the role of AHP6 in FM determinacy and floral organ development still remains unclear.

Both SUP and CRC function in the regulation of FM determinacy and floral organs development by fine-tuning auxin signaling pathways at early (stages 4–5) and intermediate (around stage 6) floral stages respectively; however, how they dynamically the auxin signal in distinct spatio-temporal pattern are still remain unknown (Xu et al., 2019). In addition to that, it has been proven that cytokinin inhibition by ETT is necessary to promote FM determinacy, what is the role of cytokinin in this FM termination and floral organs development process modulated by *SUP* and *CRC* is also worthy to be further investigated to elucidate the role of auxin-cytokinin network that might involve other regulatory pathways as well (Liu et al., 2014, Zhang et al., 2018; Xu et al., 2019). Hence, this study aims to investigate the interaction between *SUP* and *CRC* based on the genetic analyses. The genetic analyses and cytokinin reporter assay revealed that *SUP* and *CRC* might cooperatively fine-tune cytokinin signaling to regulate stamen formation during flower development; whereas the transcriptomic analysis identified several possible candidates which might contribute to complete a complex regulatory pathway which involves hormonal and metabolic coordination.

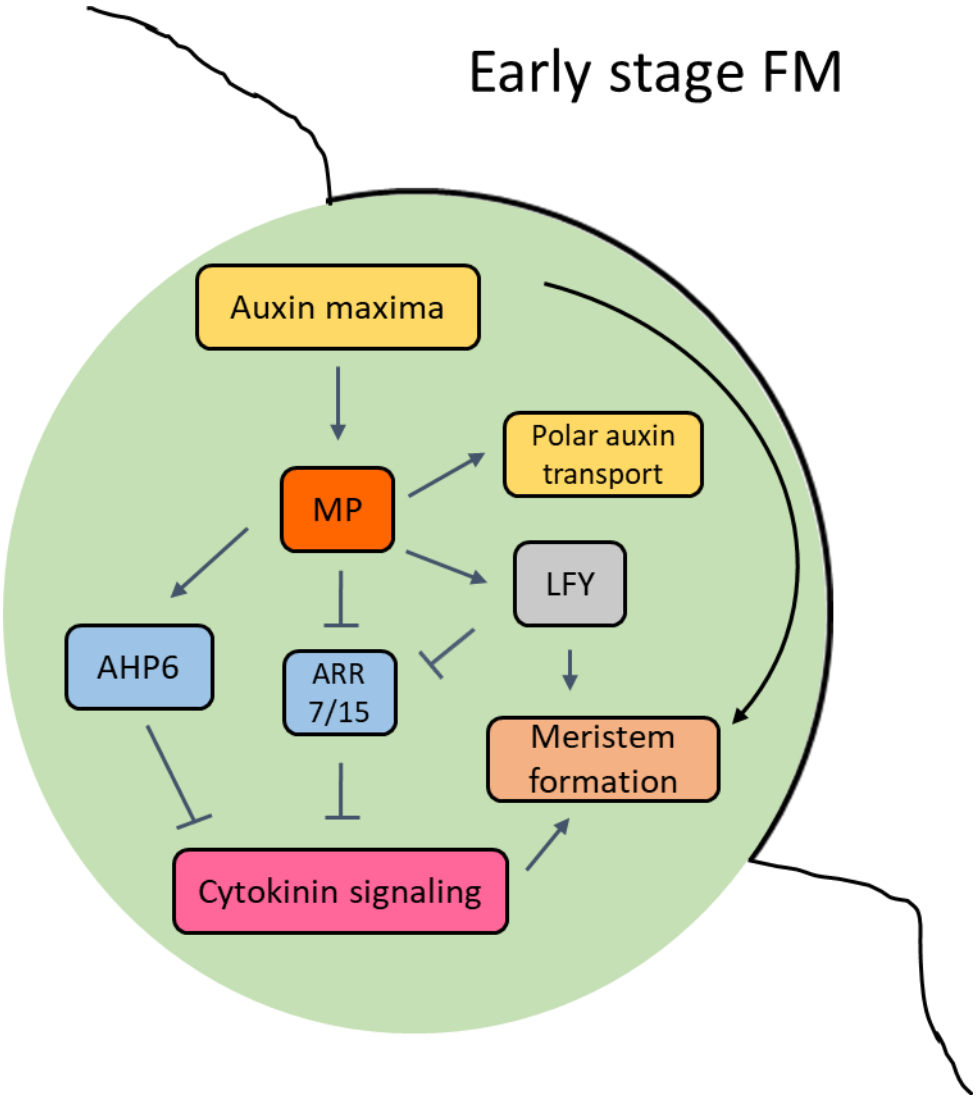


Figure 4 Genetic and hormonal network during FM formation. MP triggers the polar auxin transport after activation by auxin maxima to initiate the meristematic activity in flower primordia. It also activates the expression of *LFY* to complete the floral identity transition as well as mediate the floral development. Both MP and LFY down-regulate the activity of ARRs to promote cytokinin signaling and consequently maintenance of stem cell niche pluripotency. The cytokinin signaling inhibitor AHP6 also acts as the downstream target of MP to spatially restrict the cytokinin activity to coordinate the formation of new organ primordia.

2. MATERIALS AND METHODS

2.1 Plant materials and growth conditions

The *Arabidopsis thaliana* mutants *crc-1* and *sup-5* used in this study were in the Landsberg *erecta* (L*er*) ecotype background. The cytokinin reporter line *TCSn::GFP* was in the Col-0 background. The *crc-1*, *sup-5*, and *TCSn::GFP* lines were described previously (Gaiser et al., 1995; Bowman and Smyth 1999; Jacobsen et al., 2000; Sakai et al., 2000; Zürcher et al., 2013; Yamaguchi et al., 2017, 2018). The double mutants were generated by genetic crossing and genotyped by PCR in subsequent generations. Seeds were sown on soil and stratified at 4°C for 3 to 7 days. Plants were grown at 22°C under 24 h of continuous light. Plants to be directly compared were grown side-by-side to minimize environmental differences within the growth chamber. The genotyping primers used in this study are listed in Table 1.

Table 1 List of genotyping primers

Genotyping primers	Sequence
sup-5-FW	5′-GATAATGCGTCCAAGAATCAGTC-3′
sup-5-RV	5′-CATAAAACGGTAACAAGCGCATAC-3′
	*Wild type -1116 bp; sup-5 (homo) - 501 bp; sup-5 (hetero) - 501 & 1116 bp
crc-1-FW	5′-CCTTTGACATATACTCTTTAGTTCC-3′
crc-1-RV	5′-CTTTTGATGCGTTGGATCTCAAGC -3′
	*After digestion with HindIII, Wild type -133 bp; crc-1 (homo) - 23 bp & 110bp; crc-1 (hetero) - 23 bp, 110bp & 133 bp

2.2 Phenotyping open flowers

The first 5 to 10 flowers at developmental stage 13 (according to Smyth et al., 1990) were harvested for phenotyping open flowers. To measure flower size, flowers of L*er*, *crc-1*, *sup-5*, and *crc-1 sup-5* were removed with forceps and fixed onto agar, and photos were taken from above. Flower size was measured using Image J (http://imagej.nih.gov/ij/) software. Thirty flowers (five flowers each from six individual plants) from each genotype were measured. The number of floral organs (sepals, petals, stamen, and carpels) in the wild type (L*er*), *crc-1*, *sup-5*, and *crc-1 sup-5* of stage 13 flowers was counted under a dissecting microscope. Forty flowers (five each from eight individual plants) were counted for each genotype. To test for statistical significance, one-way ANOVA was followed by the post-hoc Tukey HSD test.

2.3 Measurement of floral meristem size

To measure the size of the FM, inflorescences 1 to 3 cm tall were harvested immediately after bolting. Inflorescences were fixed with FAA overnight. The resulting inflorescences were dehydrated in an ethanol series (50, 60, 70, 80, 90, 95, and 100%; not less than 20 min each). The fixed samples were then removed from 100% ethanol and placed in Technovit 7100 resin (Heraeus) before overnight incubation for polymerization. Eight, 10-μm thick sections were prepared using a RM2255 microtome (Leica Microsystems) for each genotype or floral developmental stage. Significance was tested using the Student's *t*-test and one-way ANOVA followed by the post-hoc Tukey HSD test.

2.4 RNA-seq

For RNA extraction, floral buds up to floral stage 8 from inflorescences 1 to 3 cm tall were harvested. Five biological replicates were harvested from wild-type (L*er*), *crc-1*, *sup-5*, and *crc-1 sup-5* backgrounds. Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen), and genomic DNA was removed using an RNase-Free DNase Set (Qiagen). Library preparation and sequencing were performed as described previously (Uemura et al., 2017; Ichihashi et al., 2018). The created libraries were sequenced by next-generation sequencing (Illumina), and the produced bcl files were then converted into fastq files by bcl2fastq (Illumina). Mapping of sequences to the Arabidopsis TAIR10 genome was performed using Bowtie with the following options ("--all -- best --strata --trim5 8"). The number of reads for each reference was then counted, and the false discovery rate (FDR), log concentration (Conc) and log fold change (FC) were obtained using the edge R package (Robinson et al., 2010). To

determine DEGs, FDR < 0.05 was used. The data were deposited into the DNA Data Bank of Japan (DRA008874).

2.5 Transcriptomic analysis

To identify common differentially expressed genes (DEGs), online software (bioinformatics.psb.ugent.be/webtools/Venn) was used to calculate the overlap between DEGs lists in all mutant backgrounds. Furthermore, Gene Ontology (GO) term analysis was performed using online software agriGO v2.0 (systemsbiology.cau.edu.cn/agriGOvs/) (Tian et al., 2017), followed by REVIGO (Reduced + Visualize Gene Ontology; revigo.irb.hr) (Supek et al., 2011) to reduce the redundant GO terms.

2.6 RT-qPCR

For RNA extraction, floral buds up to floral stage 8 from inflorescences 1 to 3 cm tall were harvested. Plants from mutant backgrounds (*crc-1*, *sup-5*, and *crc-1 sup-5*) and the controls [wild-type (L*er*)] were grown side-by-side. Approximately 100 mg of floral bud tissue was prepared and frozen immediately after trimming, without fixation. Tissues were kept at -80°C until use (less than 5 months). RNA extraction was performed using the RNeasy Plant Mini Kit (Qiagen). Genomic DNA was then removed using an RNase-Free DNase Set (Qiagen) to minimize contamination by genomic DNA. The RNA concentration was determined with an IMPLEN NanoPhotometer P-Class spectrophotometer. Synthesis of cDNA was performed with a PrimeScript first-strand cDNA Synthesis Kit (Takara) using less than 5 µg total RNA, 50 µM oligo dT primer and 200 U PrimeScript RTase with RNase Inhibitor, at 42°C for 30 min. The resulting cDNA was quantified by a LightCycler 480 (Roche) using FastSmart Essential DNA Green Master Mix (Roche) and C_q values were obtained. The expression levels of *AHP6* (*AT1G80100*), *IAA19* (*AT3G15540*), *REM25* (*AT5G09780*) and *TPPI* (*AT5G10100*) were quantified; *EIF4A-1* (*AT3G13920*) was used for the normalization of signals. Five biological replicates were performed and similar results were obtained. The RT-qPCR primers used in this study are listed in Table 2.

Table 2 List of RT-qPCR primers

RT-qPCR primers	Sequence
AHP6-FW	5′-CAGCTGGAGCAGCAGAGAAT-3′
AHP6-RV	5′-TTTCGCTTCGGTAGCTTATAACACA-3′
IAA19-FW	5′-GATCTAGCCTTTGCTCTTGATAAGC-3′
IAA19-RV	5′-ATGACTCTAGAAACATCCCCCAAG-3′
REM25-FW	5′-CTTGGGAGACCACGAGTTTCTTA-3′
REM25-RV	5′-TTTTGACACGACTAGAAGAAGCGAA-3′
TPPI-FW	5′-TACAG GTTCGGTCGGTATTAAAGAA-3′
TPPI-RV	5′-TTGTTAGTGTTCCCAAATCCAAGTG-3′
EIF4-FW	5′-ACCAGGCGTAAGGTTGATTG-3′
EIF4-RV	5′- GGTCCATGTCTCCGTGAGTT-3′

2.7 Confocal microscopy

To observe GFP signals, inflorescence apices from 4-8 cm tall plants were used. Old floral buds were removed by using forceps and needle under dissecting microscope. Tissues were placed on glass slides and covered with cover glasses. Mixed solution of full-strength MS medium (Duchefa Biochemie), 0.4% FM4-64 dye (Sigma-Aldrich), 0.0003% silwet L-77 (BMS) was pipetted into the space between glass slide and cover glass to embed the tissue. Air bubbles were removed by tapping on the cover glass gently using needle without damaging the tissue. GFP signals were observed under confocal microscope LSM710 (ZEISS).

3. RESULTS

3.1 The *sup crc* double mutant has significantly larger flowers and supernumerary stamens and carpels

To analyze the genetic interaction between *SUP* and *CRC*, we generated a *crc-1 sup-5* double mutant and compared the size of flowers between the wild type, *crc-1*, *sup-5*, and *crc-1 sup-5* (Figure 5A–E). A wild-type flower consists of four sepals, four petals, six stamens, and two carpels (Figure 5A). The mean size of wild-type flowers was 7.5 ± 0.1 mm^2 (Figure 5E). Similar to the wild type, the *crc-1* single mutant had a fixed number of four types of floral organs (Figure 5B). The size of *crc-1* flowers was 7.5 ± 0.2 mm^2 (Figure 5E), at which significant difference was not detected as compared to wild-type and *crc-1* flowers ($p > 0.01$) (Figure 5E). As reported previously (Uemura et al., 2017), *sup-5* plants produce significantly larger flowers (9.6 ± 0.2 mm^2) than the wild type ($p < 0.01$) (Figure 5C, E). An increase in the size of *sup-5* mutant flowers was accompanied by the presence of extra whorls of stamens, due to sustained floral stem-cell activity (Xu et al., 2018; Figure 5C). In *crc-1 sup-5* double mutants, a large number of stamens and carpels arose from whorls 3 and 4 (Figure 5C). The mean size of *crc-1 sup-5* flowers was 11.1 ± 0.2 mm^2 (Figure 5D, E) and was thus significantly larger than that of the wild type or either single mutant ($p < 0.01$) (Figure 5E).

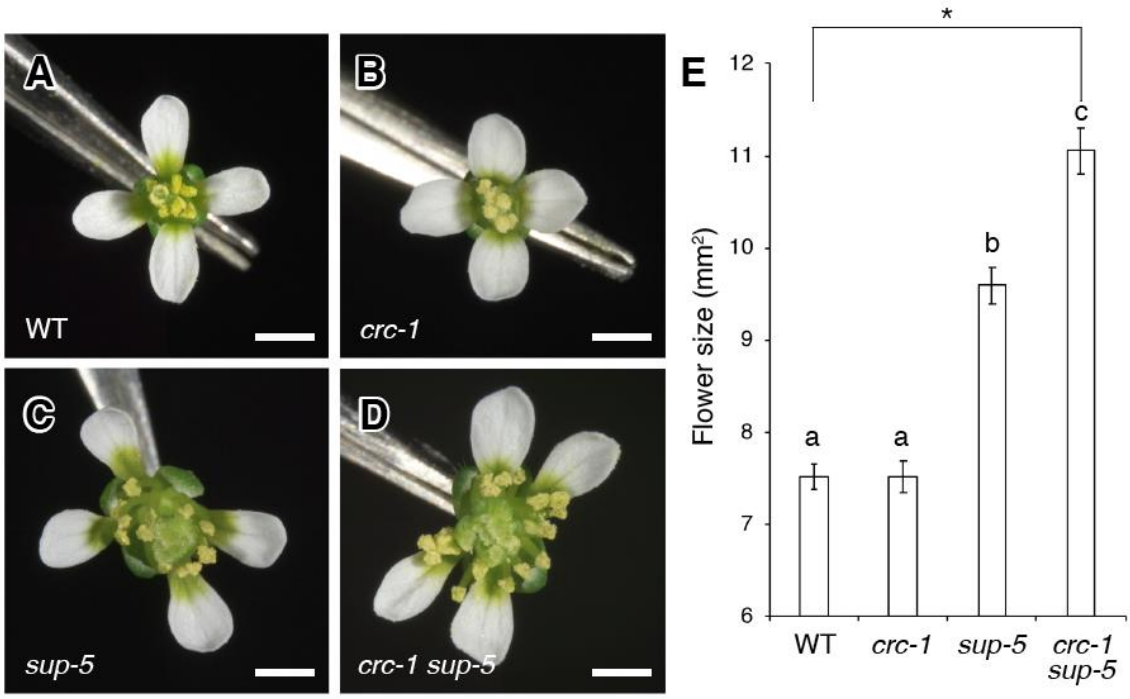


Figure 5 Comparison of flower size among the wild type, *crc-1*, *sup-5*, and *crc-1 sup-5* at floral stage 13. **(A–D)** Top view of flowers. **(A)** The wild type (WT), **(B)** *crc-1*, **(C)** *sup-5*, and **(D)** *crc-1 sup-5*. **(E)** Quantification of flower size. Scale bars represent 500 μm. The asterisk indicates significant difference based on one-way ANOVA. The same letters indicate nonsignificant differences, whereas different letters indicate significant differences based on the post-hoc Tukey HSD test ($p < 0.01$). N=30, error bars represent standard deviation.

3.2 Combination of *crc* with *sup* results in a synergistic increase in the number of stamens and carpels

To investigate the genetic interaction between *CRC* and *SUP* further, the number of organs was counted in wild-type, *crc-1*, *sup-5*, and *crc-1 sup-5* double mutant flowers (Figure 6A–T). Wild-type flowers had four sepals, four petals, six stamens, and two carpels (Figure 6A, E, F, J, K, O, P, T). Similarly, the *crc* mutant produced four sepals, four petals, and six stamens (Figure 6B, E, G, J, L, O, Q, T). Although *crc-1* mutants produced three or even occasionally four carpels in very subtle chance, the mean carpel number for *crc-1* was 2.1 ± 0.0 (Figure 6T), at which significant difference was not detected as compared to the wild type. Indeed, significant difference was not detected in the number of all four floral organs between the wild type and *crc-1* mutants ($p > 0.01$) (Figure 6E, J, O, T). No significant difference was observed in the number of sepals and petals between wild-type and *sup-5* flowers ($p > 0.01$) (Figure 6A, C, E, F, H, J). However, the mean numbers of stamens and carpels in *sup-5* were 10.7 ± 0.3 and 4.3 ± 0.1, respectively (Figure 6K, M, O, P, R, T), significantly higher than those of the wild type ($p < 0.01$) (Figure 6O, T). Similar to the wild type or the parental lines, *crc-1 sup-5* double mutant flowers also produced four sepals and four petals ($p > 0.01$) (Figure 6A–J) but produced significantly more stamens and carpels than the wild type or either single mutant ($p < 0.01$) (Figure 6K–T). Thus, the combination of *crc* with *sup* enhanced the *sup* phenotype.

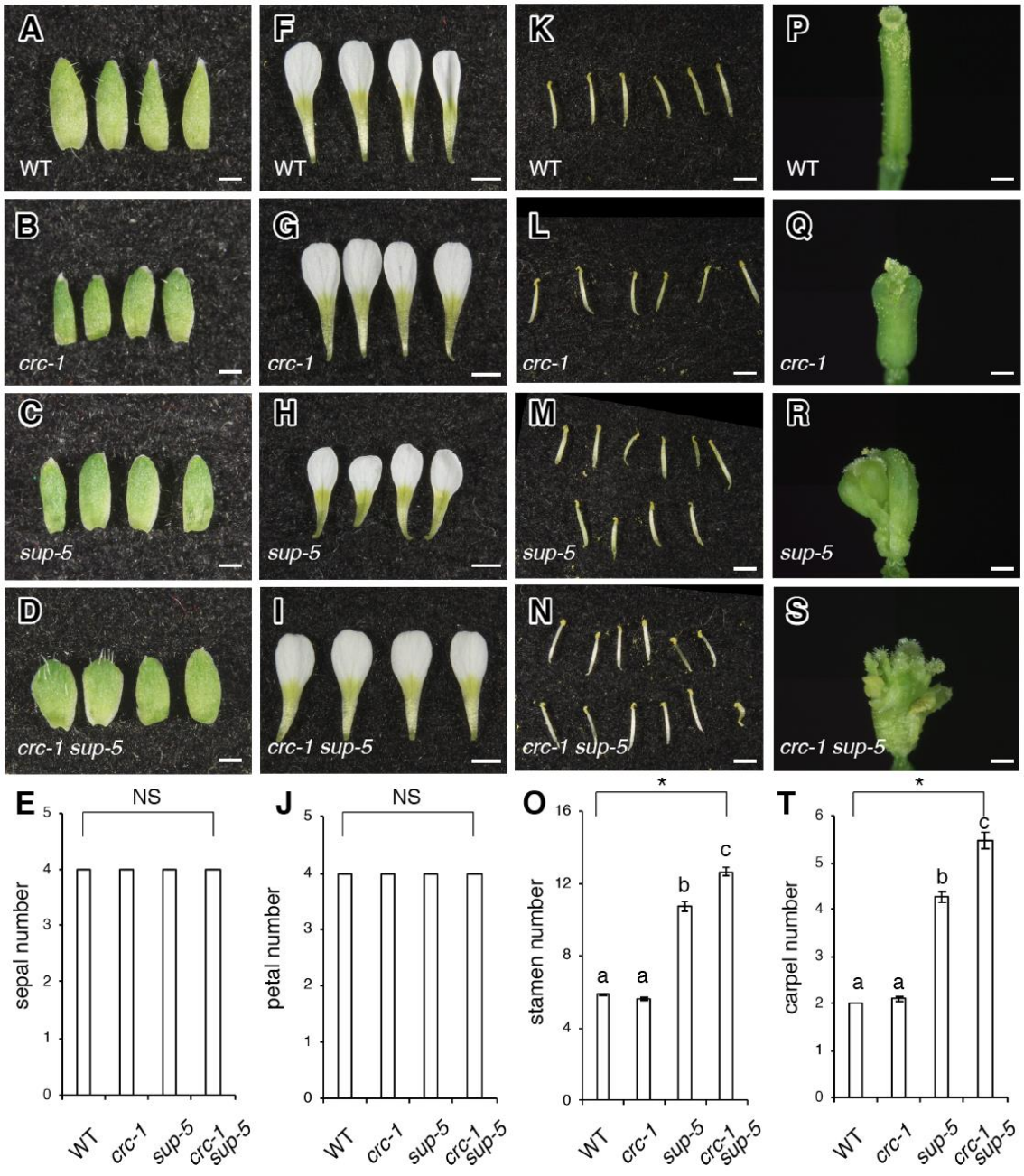


Figure 6 Comparison of floral organ number among the wild type, *crc-1*, *sup-5*, and *sup-5 crc-1* at floral stage 13. **(A–D)** Side view of sepals. **(A)** The wild type (WT), **(B)** *crc-1*, **(C)** *sup-5*, and **(D)** *crc-1 sup-5*. Scale bars represent 500 μm. **(E)** Quantification of sepal number. **(F–I)** Side view of petals. **(F)** The wild type, **(G)** *crc-1*, **(H)** *sup-5*, and **(I)** *crc-1 sup-5*. Scale bars represent 1 mm. **(J)** Quantification of petal number. **(K–N)** Side view of stamens. **(K)** Wild type, **(L)** *crc-1*, **(M)** *sup-5*, and **(N)** *crc-1 sup-5*. Scale bars represent 1 mm. (**O**) Quantification of stamen number. **(P–S)** Side view of carpels. **(P)** The wild type, **(Q)** *crc-1*, **(R)** *sup-5*, and **(S)** *crc-1 sup-5*. Scale bars represent 500 μm. **(T)** Quantification of carpel number. Scale bars represent 500 μm. Asterisks indicate significant differences based on one-way ANOVA. The

same letters indicate nonsignificant differences, whereas different letters indicate significant differences based on the post-hoc Tukey HSD test ($p < 0.01$). N=40, error bars represent standard deviation.

3.3 Combining *crc* and *sup* enhances the FM width phenotype of *sup*

Next, we aimed to determine whether the changes in the size of flowers and/or the number of floral organs correlated with changes in FM height and width. Therefore, the height (from the groove between sepal primordia and the FM to the top of the floral meristem) and the width (between the two grooves along the lateral axis) of the FM were quantified by sectioning (Figure 7A–O). In wild-type plants, mean FM height and width at stage 3 were 12.0 ± 0.5 µm and 58.0 ± 1.1 µm, respectively (Figure 7A, E), and were 29.2 ± 0.9 µm and 75.2 ± 2.0 µm, respectively, at stage 5 (Figure 7A, J). In *crc-1* mutants, no significant difference in FM height and width at stage 3 or stage 5 was observed compared to wild-type plants ($p > 0.01$) (Figure 7A, B, E, F, G, J). Similarly, no significant difference in FM height and width was observed between wild-type and *sup-5* FMs at stage 3 ($p > 0.01$). In *crc-1 sup-5*, FM height and width were similar to in *sup-5* by stage 5 (Figure 7C–E, H–J). A significant difference in FM width was observed between *sup-5* and *crc-1 sup-5* FMs at stage 6, but no significant difference was detected in height (Figure 7O, $p < 0.01$, Student's *t*-test). Therefore, combining the *crc* mutation with *sup* enhanced the *sup* mutant FM width phenotype.

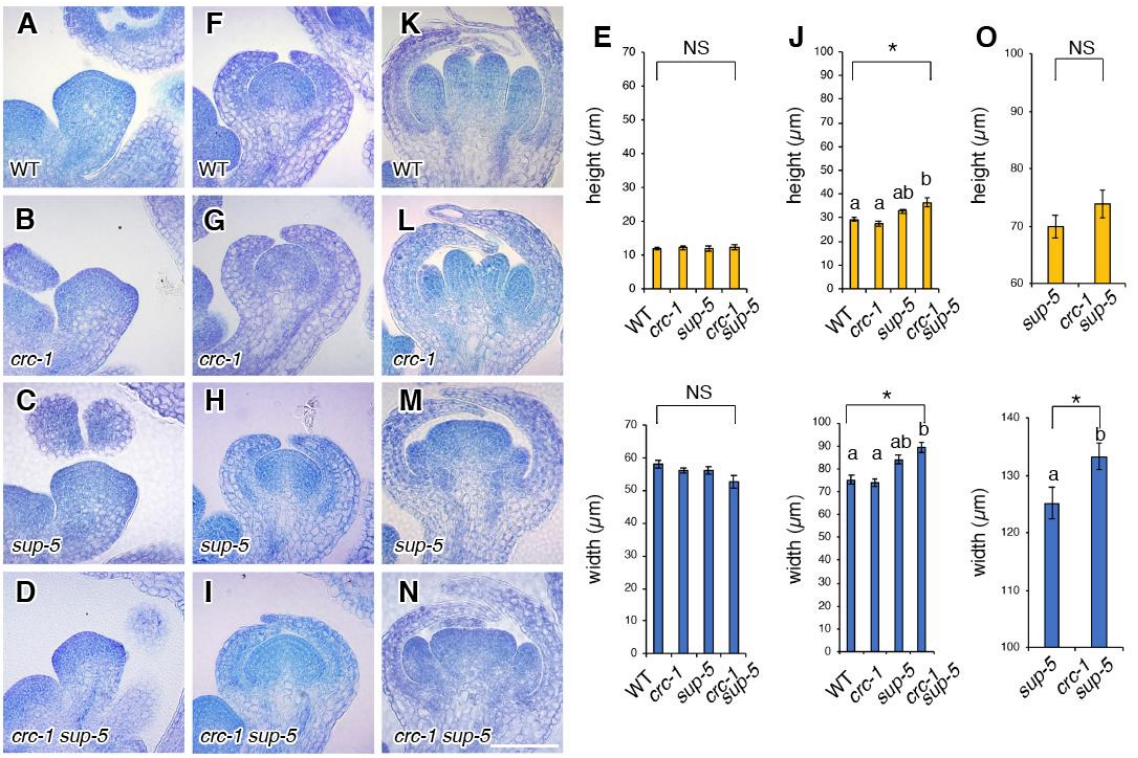


Figure 7 Comparison of floral meristem height and width among the wild type, *crc-1*, *sup-5*, and *sup-5 crc-1* at different floral stages. (A–D) Vertical cross sections of stage 3 floral meristems. **(A)** The wild type, **(B)** *crc-1*, **(C)** *sup-5*, and **(D)** *crc-1 sup-5*. **(E)** Quantification of floral meristem height and width at stage 3. **(F–J)** Vertical cross sections of floral meristems at stage 5. **(F)** The wild type, **(G)** *crc-1*, **(H)** *sup-5*, and **(I)** *crc-1 sup-5*. **(J)** Quantification of floral meristem height and width at stage 5. **(K–O)** Vertical cross sections of floral meristems at stage 6. **(K)** The wild type, **(L)** *crc-1*, **(M)** *sup-5*, and **(N)** *crc-1 sup-5*. **(O)** Quantification of floral meristem height and width at stage 6. Scale bars represent 50 μm. For multiple comparisons, asterisks indicate significant differences based on one-way ANOVA. The same letters indicate non-significant differences, whereas different letters indicate significant differences based on the post-hoc Tukey HSD test ($p < 0.01$). For single comparisons, the *p*-value was determined using the Student's *t*-test (*$p < 0.01$). N=12, error bars represent standard deviation.

3.4 Differentially expressed genes (DEGs) in *crc sup* mutants

RNA-seq was performed to identify DEGs in *crc*, *sup*, and *crc sup* (DRA008874). Approximately 10 M reads were sequenced per sample, which were then mapped onto the *Arabidopsis* TAIR 10 genome. In *crc-1* mutants, 263 genes were differentially expressed compared to the wild type (FDR < 0.05) (Figure 8). Similarly, 65 and 281 genes in total were differentially expressed in *sup* and *crc sup* mutants, respectively (FDR < 0.05) (Figure 9A, B).

To identify genes involved in the enhancement of the *sup* phenotype in the *crc sup* double mutant, we focused on three different categories of DEGs, containing either 20 genes, 11 genes, or 216 genes (Figure 9A–C; 10A–C). The "20 genes" category contained the DEGs common to all *crc-1*, *sup-1*, and *crc-1 sup-5* backgrounds (Figure 8). This category contained genes that were differentially expressed in single mutants and whose expression was further altered in the double mutant. To examine the probable functions of these 20 genes, GO term enrichment analysis was performed using agriGO v2.0 online software (Tian et al, 2017). Stimulus-related GO terms such as "response to hormone stimulus", "response to endogenous stimulus" and "response to stimulus" were identified (Figure 9A; Table 3). A further reduction of redundant GO terms by REVIGO categorized approximately 70% of the GO terms as "response to endogenous stimulus" (Figure 10A; Table 4). The "11 genes" category contained the DEGs shared by *sup-5* and *crc-1 sup-5* mutants. This category consisted of the downstream genes of SUP, whose expression was affected by combination with the *crc* mutation. In addition to stimulus-related terms, this category of GO terms also contained development-related GO terms, such as "developmental process" and "anatomical structure development" (Figure 9B; 10B; Tables 5, 6). The "216 genes" category contained genes that were differentially expressed only in *crc-1 sup-5*. The GO terms in this category included "response to chemical stimulus", "response to stimulus" and "response to hormone stimulus" which were also present in the "20 genes" and "11 genes" categories (Figure 9A–C; Tables 7, 8). In addition, unique GO terms such as "abscission", "programmed cell death", "immune response", and "cell wall organization or biogenesis" were present (Figure 9C; Tables 7, 8).

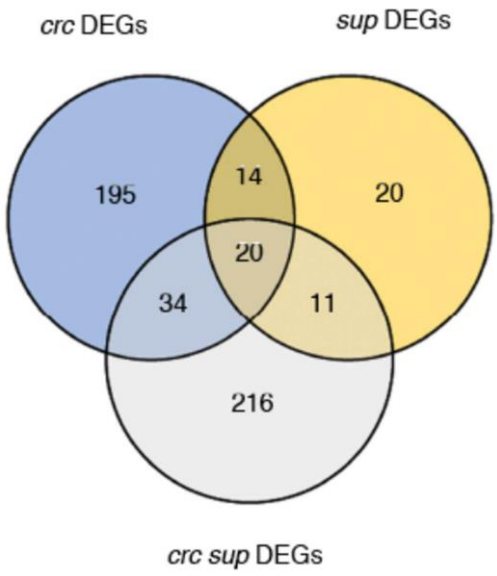
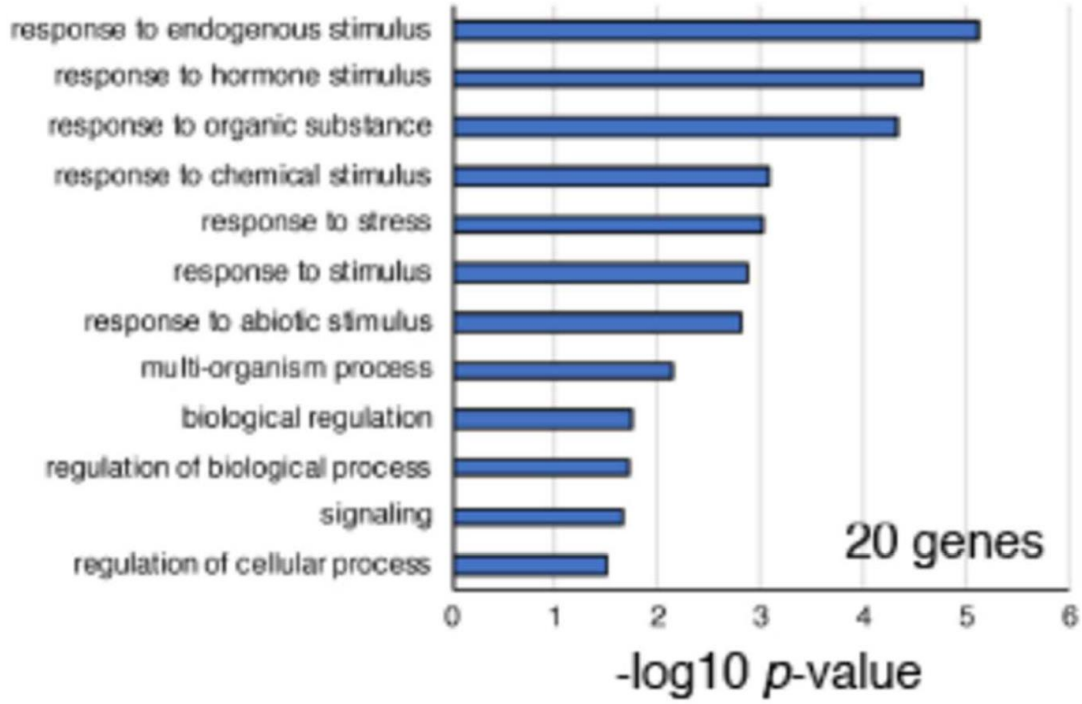
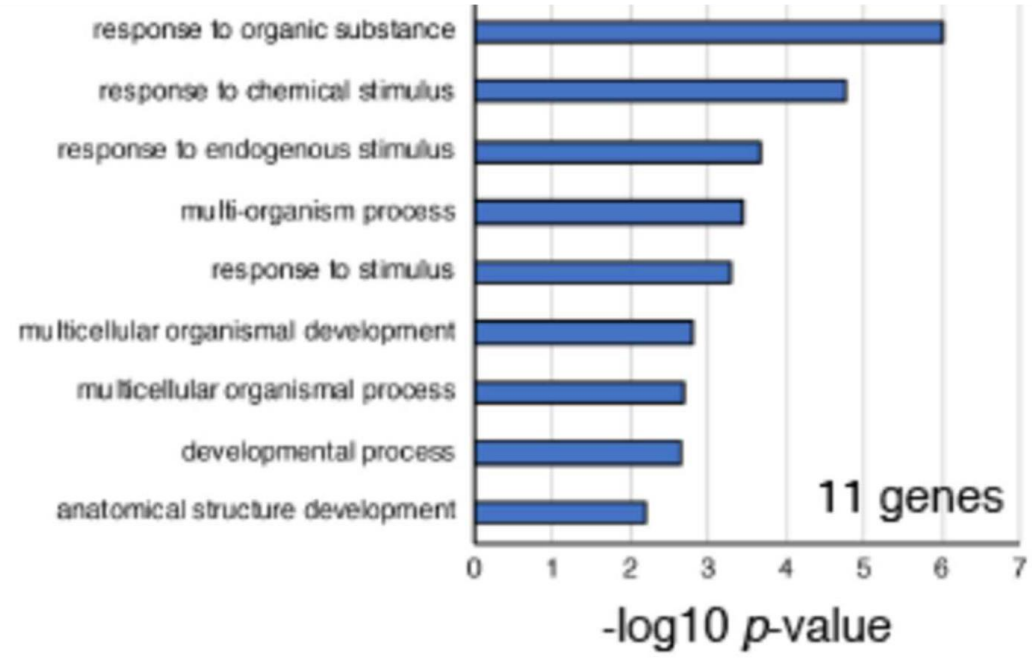


Figure 8 Venn diagram showing the number of DEGs in *crc*, *sup*, and *crc sup* mutants.

A


response to endogenous stimulus
response to hormone stimulus
response to organic substance
response to chemical stimulus
response to stress
response to stimulus
response to abiotic stimulus
multi-organism process
biological regulation
regulation of biological process
signaling
regulation of cellular process
20 genes
0
1
2
3
4
5
6
-log10 p-value

B


response to organic substance
response to chemical stimulus
response to endogenous stimulus
multi-organism process
response to stimulus
multicellular organismal development
multicellular organismal process
developmental process
anatomical structure development
11 genes
0
1
2
3
4
5
6
7
-log10 p-value

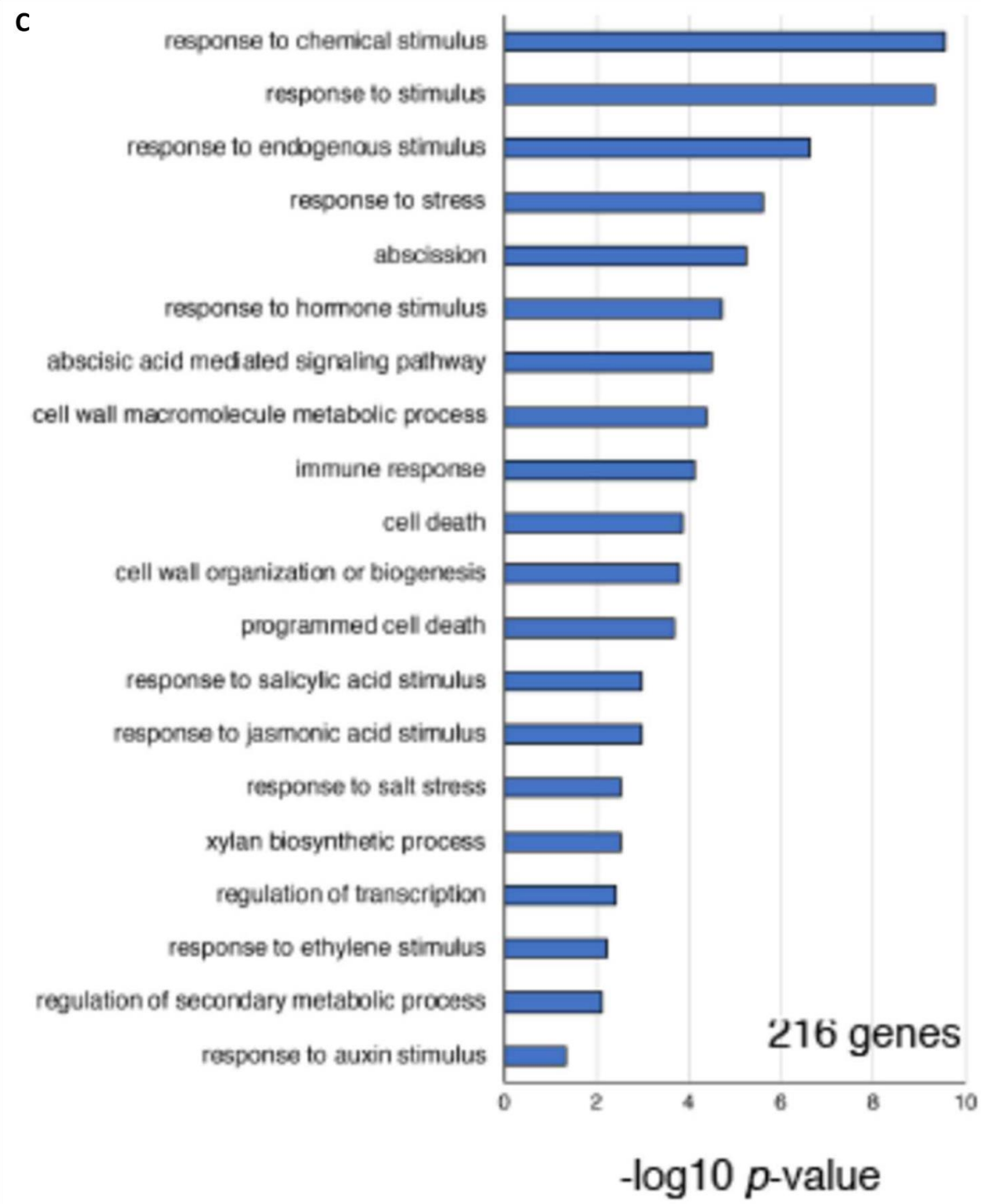


Figure 9 GO term enrichment analysis of each categories. (A) containing 20 genes, 11 genes **(B)**, and 216 genes **(C)**. Selected terms determined by their -$\log_{10}$-adjusted *p*-values are shown.

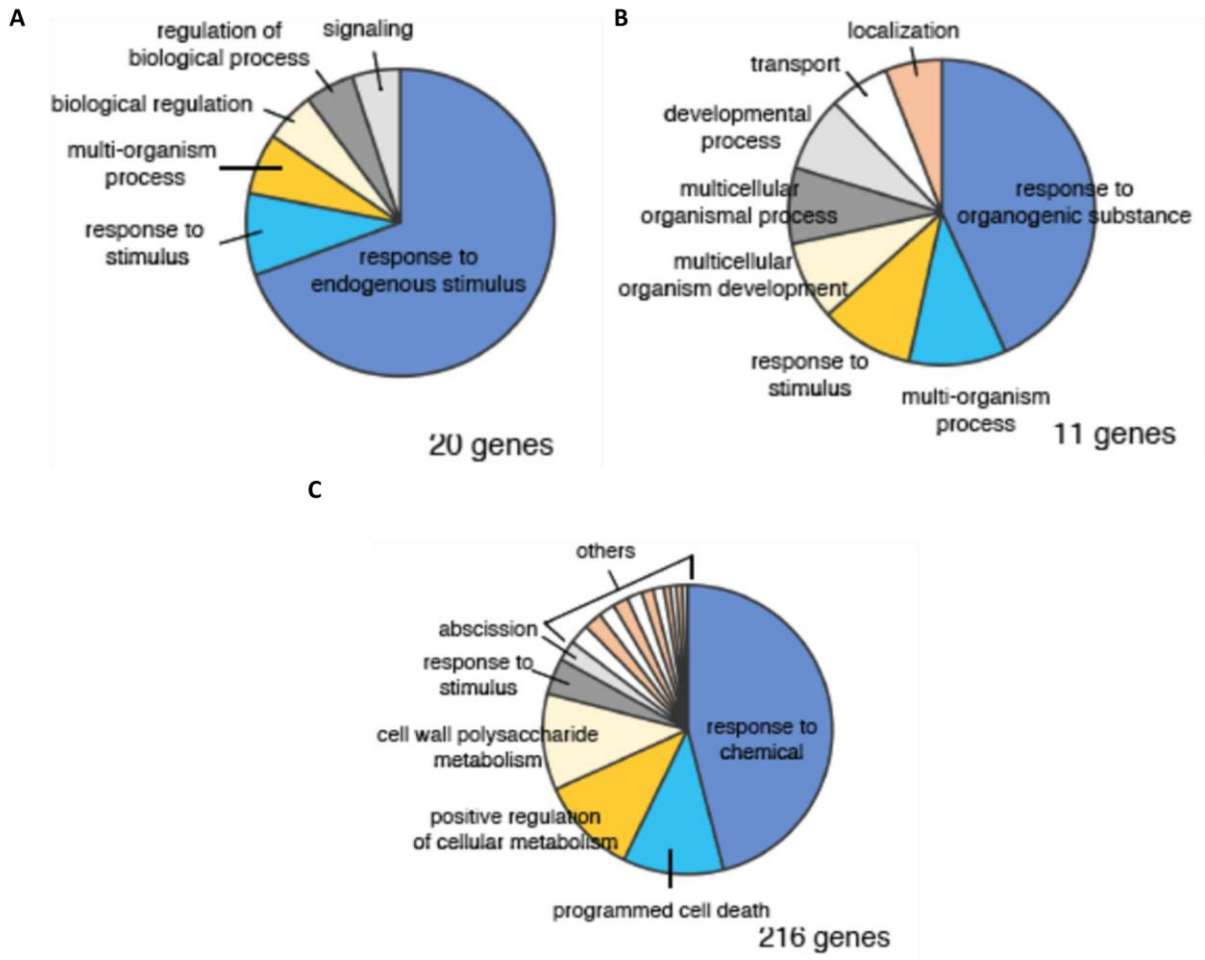


Figure 10 Pie charts showing summarized GO terms by REVIGO in each categories. (E) 20 genes, **(F)**11 genes, and (**G**) 216 genes within the GO term category "biological process."

Table 3 GO term analysis of "20 genes"

GO term	Ontology	Description	Number in input list	Number in BG/Ref	p-value	FDR
GO:0009719	P	response to endogenous stimulus	8	1615	7.50E-06	0.00065
GO:0009725	P	response to hormone stimulus	7	1375	2.80E-05	0.0012
GO:0010033	P	response to organic substance	9	2754	4.70E-05	0.0014
GO:0042221	P	response to chemical stimulus	9	3978	0.00081	0.017
GO:0006950	P	response to stress	9	4089	0.00099	0.017
GO:0009628	P	response to abiotic stimulus	7	2635	0.0016	0.019
GO:0050896	P	response to stimulus	11	6292	0.0014	0.019
GO:0051704	P	multi-organism process	5	1820	0.0075	0.08
GO:0023052	P	signaling	5	2376	0.022	0.17
GO:0050789	P	regulation of biological process	8	5235	0.02	0.17
GO:0065007	P	biological regulation	9	6222	0.018	0.17

Table 4 REVIGO analysis of "20 genes"

Term ID	Description	Frequency in Db	$\log_{10}$ p-value	Uniqueness	Dispensability	Representative
GO:0009719	response to endogenous stimulus	0.53%	-5.1249	0.539	0	response to endogenous stimulus
GO:0009628	response to abiotic stimulus	0.57%	-2.7959	0.537	0.374	response to endogenous stimulus
GO:0009725	response to hormone	0.34%	-4.5528	0.51	0.649	response to endogenous stimulus
GO:0042221	response to chemical	3.07%	-3.0915	0.498	0.451	response to endogenous stimulus
GO:0006950	response to stress	4.58%	-3.0044	0.49	0.562	response to endogenous stimulus
GO:0010033	response to organic substance	0.90%	-4.3279	0.489	0.371	response to endogenous stimulus
GO:0023052	signaling	6.77%	-1.6576	0.838	0	signaling
GO:0050789	regulation of biological process	19.37%	-1.699	0.735	0	regulation of biological process
GO:0050896	response to stimulus	12.21%	-2.8539	0.847	0	response to stimulus
GO:0051704	multi-organism process	0.75%	-2.1249	0.828	0	multi-organism process
GO:0065007	biological regulation	20.50%	-1.7447	0.861	0	biological regulation

Table 5 GO term analysis of "11 genes"

GO term	Ontology	Description	Number in input list	Number in BG/Ref	p-value	FDR
GO:0010033	P	response to organic substance	8	2754	1.00E-06	6.30E-05
GO:0042221	P	response to chemical stimulus	8	3978	1.70E-05	0.00052
GO:0009719	P	response to endogenous stimulus	5	1615	0.00021	0.0043
GO:0051704	P	multi-organism process	5	1820	0.00036	0.0057
GO:0050896	P	response to stimulus	8	6292	0.0005	0.0063
GO:0007275	P	multicellular organismal development	6	3864	0.0016	0.017
GO:0032502	P	developmental process	6	4094	0.0022	0.017
GO:0032501	P	multicellular organismal process	6	4020	0.002	0.017
GO:0048856	P	anatomical structure development	5	3396	0.0061	0.042
GO:0006810	P	transport	5	3577	0.0076	0.048
GO:0051234	P	establishment of localization	5	3652	0.0083	0.048
GO:0051179	P	localization	5	3819	0.01	0.053

Table 6 REVIGO analysis of "11 genes"

Term ID	Description	Frequency in Db	log_{10} p-value	Uniqueness	Dispensability	Representative
GO:0006810	transport	17.62%	-2.1192	0.727	0	transport
GO:0007275	multicellular organism development	1.56%	-2.7959	0.689	0	multicellular organism development
GO:0010033	response to organic substance	0.90%	-6	0.698	0	response to organic substance
GO:0042221	response to chemical	3.07%	-4.7696	0.693	0.451	response to organic substance
GO:0009719	response to endogenous stimulus	0.53%	-3.6778	0.701	0.371	response to organic substance
GO:0032501	multicellular organismal process	2.37%	-2.699	0.83	0	multicellular organismal process
GO:0032502	developmental process	2.81%	-2.6576	0.831	0	developmental process
GO:0050896	response to stimulus	12.21%	-3.301	0.847	0	response to stimulus
GO:0051179	localization	18.50%	-2	0.857	0	localization
GO:0051704	multi-organism process	0.75%	-3.4437	0.828	0	multi-organism process

Table 7 GO term analysis of "216 genes"

GO term	Ontology	Description	Number in input list	Number in BG/Ref	p-value	FDR
GO:0010033	P	response to organic substance	53	2754	1.80E-10	2.50E-07
GO:0042221	P	response to chemical stimulus	66	3978	2.70E-10	2.50E-07
GO:0050896	P	response to stimulus	88	6292	4.80E-10	3.00E-07
GO:0009415	P	response to water	17	424	4.40E-08	2.00E-05
GO:0009414	P	response to water deprivation	16	416	1.90E-07	6.30E-05
GO:0009719	P	response to endogenous stimulus	33	1615	2.40E-07	6.30E-05
GO:0010200	P	response to chitin	16	421	2.20E-07	6.30E-05
GO:0071495	P	cellular response to endogenous stimulus	21	815	1.40E-06	0.0003
GO:0009743	P	response to carbohydrate stimulus	21	812	1.40E-06	0.0003
GO:0006950	P	response to stress	57	4089	2.40E-06	0.00045
GO:0071310	P	cellular response to organic substance	26	1234	3.00E-06	0.00051
GO:0007242	P	intracellular signaling cascade	26	1252	3.90E-06	0.0006
GO:0002679	P	respiratory burst during defense response	8	121	6.00E-06	0.00075
GO:0045730	P	respiratory burst	8	121	6.00E-06	0.00075
GO:0009838	P	abscission	6	54	5.80E-06	0.00075
GO:0009755	P	hormone-mediated signaling pathway	16	600	1.80E-05	0.0021
GO:0009725	P	response to hormone stimulus	26	1375	1.90E-05	0.0021

GO:0006952	P	defense response	29	1653	2.40E-05	0.0025
GO:0070887	P	cellular response to chemical stimulus	26	1417	3.20E-05	0.003
GO:0009738	P	abscisic acid mediated signaling pathway	10	252	3.10E-05	0.003
GO:0050794	P	regulation of cellular process	58	4595	3.90E-05	0.0032
GO:0009628	P	response to abiotic stimulus	39	2635	3.90E-05	0.0032
GO:0032870	P	cellular response to hormone stimulus	16	641	3.90E-05	0.0032
GO:0044036	P	cell wall macromolecule metabolic process	11	319	4.20E-05	0.0033
GO:0071215	P	cellular response to abscisic acid stimulus	10	267	4.90E-05	0.0036
GO:0048583	P	regulation of response to stimulus	16	667	6.20E-05	0.0044
GO:0002376	P	immune system process	20	984	7.20E-05	0.0048
GO:0006955	P	immune response	20	984	7.20E-05	0.0048
GO:0065007	P	biological regulation	71	6222	0.0001	0.0064
GO:0045087	P	innate immune response	19	930	0.0001	0.0064
GO:0050789	P	regulation of biological process	62	5235	0.00012	0.0075
GO:0016265	P	death	13	500	0.00014	0.008
GO:0008219	P	cell death	13	500	0.00014	0.008
GO:0071554	P	cell wall organization or biogenesis	19	963	0.00016	0.0087
GO:0012501	P	programmed cell death	12	451	0.00021	0.011

GO:0031347	P	regulation of defense response	13	529	0.00024	0.013
GO:0002252	P	immune effector process	9	273	0.00029	0.015
GO:0080134	P	regulation of response to stress	13	544	0.00031	0.015
GO:0050832	P	defense response to fungus	10	342	0.00034	0.016
GO:0007165	P	signal transduction	26	1670	0.00042	0.019
GO:0051716	P	cellular response to stimulus	33	2355	0.00044	0.02
GO:0009620	P	response to fungus	12	499	0.0005	0.022
GO:0070882	P	cellular cell wall organization or biogenesis	10	367	0.00058	0.025
GO:0010363	P	regulation of plant-type hypersensitive response	10	371	0.00063	0.027
GO:0009889	P	regulation of biosynthetic process	35	2634	0.00076	0.03
GO:0080135	P	regulation of cellular response to stress	10	379	0.00074	0.03
GO:0031326	P	regulation of cellular biosynthetic process	35	2631	0.00074	0.03
GO:0006612	P	protein targeting to membrane	10	392	0.00095	0.035
GO:0023046	P	signaling process	26	1768	0.00096	0.035
GO:0031325	P	positive regulation of cellular metabolic process	13	615	0.00096	0.035
GO:0023060	P	signal transmission	26	1767	0.00095	0.035
GO:0009751	P	response to salicylic acid stimulus	11	470	0.0011	0.036

GO:0009753	P	response to jasmonic acid stimulus	11	471	0.0011	0.036
GO:0009893	P	positive regulation of metabolic process	13	623	0.0011	0.036
GO:0009737	P	response to abscisic acid stimulus	13	621	0.001	0.036
GO:0043067	P	regulation of programmed cell death	10	397	0.001	0.036
GO:0009626	P	plant-type hypersensitive response	10	401	0.0011	0.037
GO:0034050	P	host programmed cell death induced by symbiont	10	402	0.0011	0.037
GO:0010941	P	regulation of cell death	10	405	0.0012	0.038
GO:0031323	P	regulation of cellular metabolic process	37	2928	0.0013	0.041
GO:0019748	P	secondary metabolic process	20	1247	0.0014	0.043
GO:0045088	P	regulation of innate immune response	10	415	0.0014	0.043
GO:0048585	P	negative regulation of response to stimulus	9	349	0.0016	0.045
GO:0050776	P	regulation of immune response	10	419	0.0015	0.045
GO:0009863	P	salicylic acid mediated signaling pathway	9	349	0.0016	0.045
GO:0002682	P	regulation of immune system process	10	419	0.0015	0.045
GO:0071446	P	cellular response to salicylic acid stimulus	9	351	0.0016	0.046
GO:0080090	P	regulation of primary metabolic process	35	2761	0.0017	0.047
GO:0023052	P	signaling	31	2376	0.0021	0.056

GO:0006970	P	response to osmotic stress	15	842	0.0021	0.056
GO:0010383	P	cell wall polysaccharide metabolic process	7	231	0.0022	0.057
GO:0009891	P	positive regulation of biosynthetic process	12	600	0.0023	0.06
GO:0031328	P	positive regulation of cellular biosynthetic process	12	600	0.0023	0.06
GO:0010413	P	glucuronoxylan metabolic process	6	182	0.003	0.075
GO:0009651	P	response to salt stress	14	788	0.003	0.075
GO:0045492	P	xylan biosynthetic process	6	183	0.0031	0.075
GO:0045491	P	xylan metabolic process	6	186	0.0033	0.079
GO:0070589	P	cellular component macromolecule biosynthetic process	6	187	0.0034	0.079
GO:0070592	P	cell wall polysaccharide biosynthetic process	6	187	0.0034	0.079
GO:0044038	P	cell wall macromolecule biosynthetic process	6	187	0.0034	0.079
GO:0006355	P	regulation of transcription, DNA-dependent	30	2372	0.0038	0.088
GO:0045449	P	regulation of transcription	30	2376	0.0039	0.089
GO:0051252	P	regulation of RNA metabolic process	30	2388	0.0042	0.095

GO:0010410	P	hemicellulose metabolic process	6	198	0.0045	0.099
GO:0009611	P	response to wounding	8	340	0.0049	0.11
GO:0009617	P	response to bacterium	11	577	0.005	0.11
GO:0010382	P	cellular cell wall macromolecule metabolic process	6	203	0.005	0.11
GO:0031348	P	negative regulation of defense response	7	273	0.0053	0.11
GO:0019222	P	regulation of metabolic process	37	3186	0.0054	0.11
GO:0061025	P	membrane fusion	7	275	0.0055	0.11
GO:0006944	P	cellular membrane fusion	7	275	0.0055	0.11
GO:0051707	P	response to other organism	20	1421	0.006	0.12
GO:0009723	P	response to ethylene stimulus	8	353	0.006	0.12
GO:0006886	P	intracellular protein transport	16	1044	0.0063	0.12
GO:0009867	P	jasmonic acid mediated signaling pathway	7	282	0.0063	0.12
GO:0071395	P	cellular response to jasmonic acid stimulus	7	282	0.0063	0.12
GO:0044264	P	cellular polysaccharide metabolic process	11	601	0.0066	0.13
GO:0033692	P	cellular polysaccharide biosynthetic process	10	523	0.0071	0.14
GO:0034613	P	cellular protein localization	16	1059	0.0072	0.14

GO:0045184	P	establishment of protein localization	17	1160	0.0075	0.14
GO:0043455	P	regulation of secondary metabolic process	5	157	0.0076	0.14
GO:0010556	P	regulation of macromolecule biosynthetic process	30	2491	0.0076	0.14
GO:0015031	P	protein transport	17	1160	0.0075	0.14
GO:0019219	P	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	30	2496	0.0078	0.14
GO:0048522	P	positive regulation of cellular process	13	793	0.008	0.14
GO:0032774	P	RNA biosynthetic process	31	2621	0.0085	0.15
GO:0006350	P	transcription	31	2620	0.0085	0.15
GO:0006351	P	transcription, DNA-dependent	31	2618	0.0084	0.15
GO:0051171	P	regulation of nitrogen compound metabolic process	30	2517	0.0087	0.15
GO:0005976	P	polysaccharide metabolic process	12	712	0.0087	0.15
GO:0048518	P	positive regulation of biological process	14	896	0.0089	0.15
GO:0000160	P	two-component signal transduction system (phosphorelay)	5	165	0.0092	0.15
GO:0070727	P	cellular macromolecule localization	16	1093	0.0095	0.16
GO:0008104	P	protein localization	17	1216	0.012	0.19

GO:0006810	P	transport	39	3577	0.012	0.19
GO:0009699	P	phenylpropanoid biosynthetic process	7	327	0.013	0.21
GO:0009862	P	systemic acquired resistance, salicylic acid mediated signaling pathway	6	251	0.013	0.21
GO:0034637	P	cellular carbohydrate biosynthetic process	14	941	0.013	0.21
GO:0048519	P	negative regulation of biological process	17	1243	0.014	0.22
GO:0019438	P	aromatic compound biosynthetic process	11	680	0.015	0.24
GO:0000271	P	polysaccharide biosynthetic process	10	590	0.015	0.24
GO:0051234	P	establishment of localization	39	3652	0.016	0.24
GO:0006984	P	ER-nuclear signaling pathway	5	193	0.017	0.25
GO:0015698	P	inorganic anion transport	6	266	0.017	0.25
GO:0009607	P	response to biotic stimulus	21	1687	0.018	0.26
GO:0016137	P	glycoside metabolic process	6	268	0.017	0.26
GO:0023034	P	intracellular signaling pathway	8	433	0.018	0.27
GO:0023033	P	signaling pathway	9	519	0.018	0.27
GO:0010167	P	response to nitrate	5	199	0.019	0.27
GO:0010468	P	regulation of gene expression	30	2695	0.021	0.3
GO:0009987	P	cellular process	124	14419	0.021	0.3
GO:0006605	P	protein targeting	13	906	0.021	0.3
GO:0015706	P	nitrate transport	5	207	0.022	0.31

GO:0009814	P	defense response, incompatible interaction	9	536	0.022	0.31
GO:0009664	P	plant-type cell wall organization	7	369	0.024	0.33
GO:0042158	P	lipoprotein biosynthetic process	9	551	0.026	0.35
GO:0033036	P	macromolecule localization	19	1541	0.025	0.35
GO:0006497	P	protein amino acid lipidation	9	551	0.026	0.35
GO:0042157	P	lipoprotein metabolic process	9	552	0.026	0.35
GO:0045893	P	positive regulation of transcription, DNA-dependent	8	467	0.027	0.36
GO:0045941	P	positive regulation of transcription	8	467	0.027	0.36
GO:0051254	P	positive regulation of RNA metabolic process	8	471	0.028	0.37
GO:0009827	P	plant-type cell wall modification	5	222	0.028	0.37
GO:0071669	P	plant-type cell wall organization or biogenesis	8	473	0.029	0.37
GO:0042545	P	cell wall modification	7	386	0.029	0.37
GO:0010628	P	positive regulation of gene expression	8	473	0.029	0.37
GO:0034645	P	cellular macromolecule biosynthetic process	51	5243	0.03	0.38
GO:0009813	P	flavonoid biosynthetic process	5	225	0.03	0.38
GO:0051179	P	localization	39	3819	0.03	0.38

GO:0045935	P	positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	8	479	0.031	0.38
GO:0051173	P	positive regulation of nitrogen compound metabolic process	8	480	0.031	0.38
GO:0042742	P	defense response to bacterium	7	394	0.032	0.39
GO:0005982	P	starch metabolic process	5	230	0.032	0.39
GO:0016051	P	carbohydrate biosynthetic process	14	1070	0.034	0.41
GO:0010557	P	positive regulation of macromolecule biosynthetic process	8	491	0.035	0.42
GO:0009698	P	phenylpropanoid metabolic process	7	405	0.036	0.43
GO:0060255	P	regulation of macromolecule metabolic process	30	2829	0.036	0.43
GO:0009059	P	macromolecule biosynthetic process	51	5312	0.037	0.43
GO:0009605	P	response to external stimulus	14	1087	0.038	0.44
GO:0048523	P	negative regulation of cellular process	11	789	0.039	0.45
GO:0010604	P	positive regulation of macromolecule metabolic process	8	508	0.041	0.48
GO:0009411	P	response to UV	5	247	0.041	0.48
GO:0046907	P	intracellular transport	17	1423	0.043	0.49
GO:0009812	P	flavonoid metabolic process	5	251	0.044	0.5

GO:0042546	P	cell wall biogenesis	6	336	0.044	0.5
GO:0071555	P	cell wall organization	9	613	0.045	0.51
GO:0009733	P	response to auxin stimulus	7	431	0.047	0.53
GO:0044042	P	glucan metabolic process	7	432	0.048	0.53
GO:0051649	P	establishment of localization in cell	18	1554	0.048	0.53
GO:0006725	P	cellular aromatic compound metabolic process	13	1022	0.048	0.53

Table 8 REVIGO analysis of "216 genes"

Term ID	Description	Frequency in Db	log_{10} p-value	Uniqueness	Dispensability	Representative
GO:0002376	immune system process	0.60%	-4.1427	0.988	0	immune system process
GO:0009812	flavonoid metabolic process	0.02%	-1.3565	0.969	0	flavonoid metabolism
GO:0009838	abscission	0.00%	-5.2366	0.971	0	abscission
GO:0009987	cellular process	63.78%	-1.6778	0.995	0	cellular process
GO:0016265	(obsolete) death	0.14%	-3.8539	0.988	0	(obsolete) death
GO:0023052	signaling	6.77%	-2.6778	0.988	0	signaling
GO:0023060	(obsolete) signal transmission	0.14%	-3.0223	0.988	0	(obsolete) signal transmission
GO:0042221	response to chemical	3.07%	-9.5686	0.742	0	response to chemical
GO:0010167	response to nitrate	0.00%	-1.7212	0.752	0.694	response to chemical
GO:0051716	cellular response to stimulus	9.56%	-3.3565	0.69	0.68	response to chemical
GO:0009743	response to carbohydrate	0.04%	-5.8539	0.72	0.65	response to chemical
GO:0002679	respiratory burst involved in defense response	0.00%	-5.2218	0.756	0.252	response to chemical

GO:0010200	response to chitin	0.00%	-6.6576	0.743	0.571	response to chemical
GO:0009719	response to endogenous stimulus	0.53%	-6.6198	0.777	0.423	response to chemical
GO:0048583	regulation of response to stimulus	1.12%	-4.2076	0.649	0.463	response to chemical
GO:0050832	defense response to fungus	0.03%	-3.4685	0.741	0.531	response to chemical
GO:0009628	response to abiotic stimulus	0.57%	-4.4089	0.775	0.427	response to chemical
GO:0009651	response to salt stress	0.04%	-2.5229	0.77	0.691	response to chemical
GO:0006984	ER-nucleus signaling pathway	0.01%	-1.7696	0.722	0.297	response to chemical
GO:0009611	response to wounding	0.13%	-2.3098	0.77	0.497	response to chemical
GO:0000160	phosphorelay signal transduction system	2.57%	-2.0362	0.584	0.517	response to chemical
GO:0009607	response to biotic stimulus	0.34%	-1.7447	0.784	0.403	response to chemical
GO:0009605	response to external stimulus	1.37%	-1.4202	0.759	0.475	response to chemical
GO:0006970	response to osmotic stress	0.08%	-2.6778	0.76	0.662	response to chemical

GO:0009414	response to water deprivation	0.02%	-6.7212	0.688	0.514	response to chemical
GO:0006952	defense response	0.57%	-4.6198	0.744	0.427	response to chemical
GO:0006950	response to stress	4.58%	-5.6198	0.732	0.562	response to chemical
GO:0006955	immune response	0.34%	-4.1427	0.727	0.603	response to chemical
GO:0080134	regulation of response to stress	0.34%	-3.5086	0.635	0.542	response to chemical
GO:0010033	response to organic substance	0.90%	-9.7447	0.711	0.451	response to chemical
GO:0071495	cellular response to endogenous stimulus	0.40%	-5.8539	0.717	0.672	response to chemical
GO:0050896	response to stimulus	12.21%	-9.3188	0.989	0	response to stimulus
GO:0051179	localization	18.50%	-1.5229	0.99	0	localization
GO:0065007	biological regulation	20.50%	-4	0.99	0	biological regulation
GO:0071554	cell wall organization or biogenesis	0.95%	-3.2366	0.961	0	cell wall organization or biogenesis
GO:0031325	positive regulation of cellular metabolic process	1.00%	-3.0177	0.692	0.035	positive regulation of cellular metabolism
GO:0048519	negative regulation of biological process	1.98%	-1.8539	0.799	0.332	positive regulation of cellular metabolism

GO:0048518	positive regulation of biological process	1.74%	-2.0506	0.802	0.306	positive regulation of cellular metabolism
GO:0031326	regulation of cellular biosynthetic process	10.82%	-3.1308	0.642	0.68	positive regulation of cellular metabolism
GO:0043455	regulation of secondary metabolic process	0.01%	-2.1192	0.822	0.261	positive regulation of cellular metabolism
GO:0019222	regulation of metabolic process	11.94%	-2.2676	0.741	0.698	positive regulation of cellular metabolism
GO:0050794	regulation of cellular process	18.84%	-4.4089	0.709	0.474	positive regulation of cellular metabolism
GO:0009699	phenylpropanoid biosynthetic process	0.01%	-1.8861	0.879	0.685	positive regulation of cellular metabolism
GO:0006725	cellular aromatic compound metabolic process	29.63%	-1.3188	0.922	0.102	positive regulation of cellular metabolism
GO:0045730	respiratory burst	0.00%	-5.2218	0.958	0.039	respiratory burst
GO:0010383	cell wall polysaccharide metabolic process	0.08%	-2.6576	0.786	0.051	cell wall polysaccharide metabolism

GO:0032774	RNA biosynthetic process	10.93%	-2.0706	0.802	0.31	cell wall polysaccharide metabolism
GO:0042545	cell wall modification	0.05%	-1.5376	0.863	0.622	cell wall polysaccharide metabolism
GO:0016051	carbohydrate biosynthetic process	1.08%	-1.4685	0.822	0.604	cell wall polysaccharide metabolism
GO:0009813	flavonoid biosynthetic process	0.02%	-1.5229	0.94	0.111	cell wall polysaccharide metabolism
GO:0009059	macromolecule biosynthetic process	19.55%	-1.4318	0.865	0.462	cell wall polysaccharide metabolism
GO:0042158	lipoprotein biosynthetic process	0.19%	-1.585	0.878	0.105	cell wall polysaccharide metabolism
GO:0019438	aromatic compound biosynthetic process	16.95%	-1.8239	0.855	0.429	cell wall polysaccharide metabolism
GO:0071669	plant-type cell wall organization or biogenesis	0.04%	-1.5376	0.876	0.615	cell wall polysaccharide metabolism
GO:0005976	polysaccharide metabolic process	0.91%	-2.0605	0.871	0.472	cell wall polysaccharide metabolism
GO:0005982	starch metabolic process	0.01%	-1.4949	0.859	0.572	cell wall polysaccharide metabolism

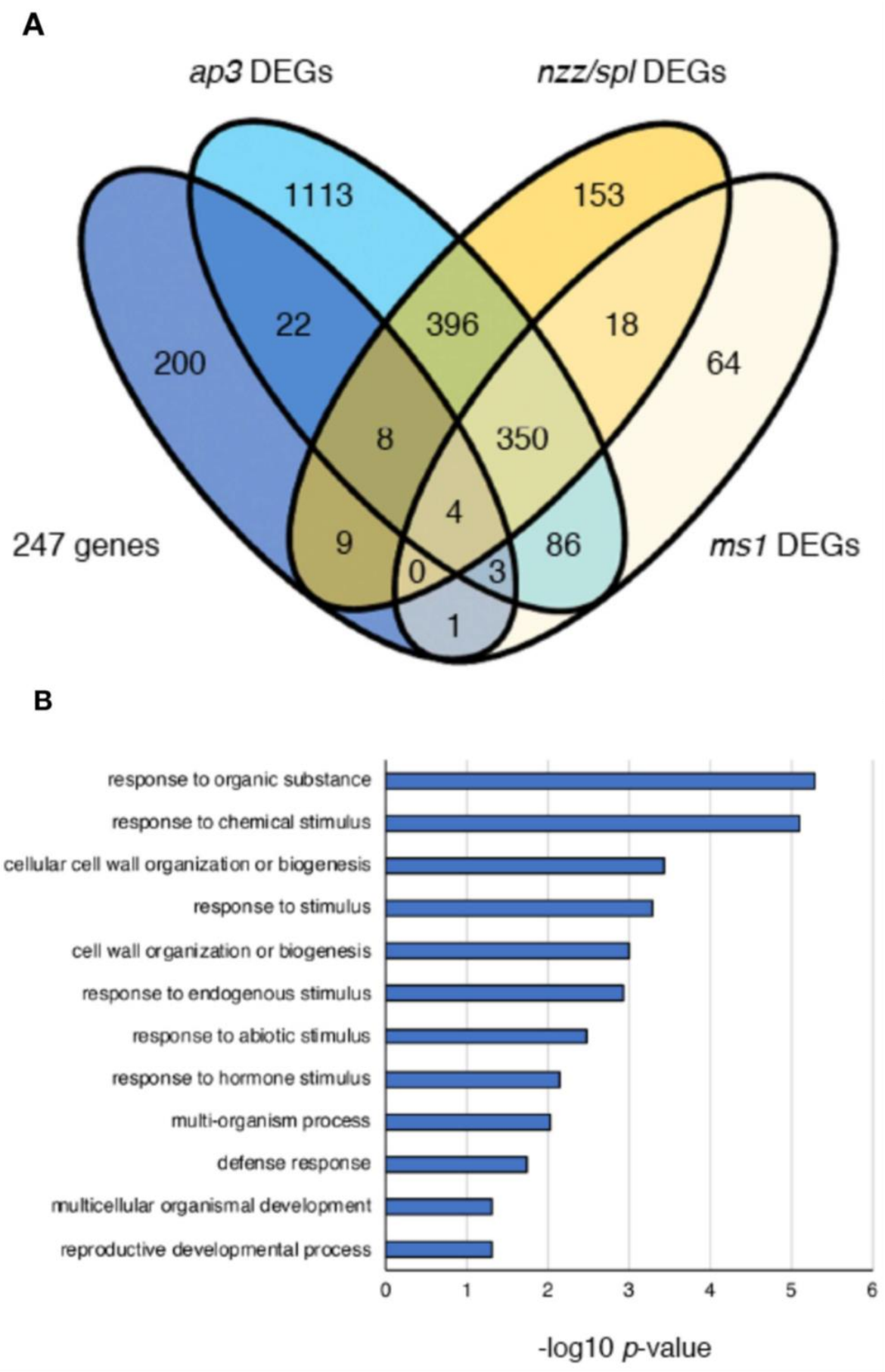
GO:0009664	plant-type cell wall organization	0.03%	-1.6198	0.863	0.604	cell wall polysaccharide metabolism
GO:0045491	xylan metabolic process	0.05%	-2.4815	0.78	0.669	cell wall polysaccharide metabolism
GO:0034645	cellular macromolecule biosynthetic process	19.29%	-1.5229	0.812	0.68	cell wall polysaccharide metabolism
GO:0012501	programmed cell death	0.43%	-3.6778	0.895	0.053	programmed cell death
GO:0006810	transport	17.62%	-1.9208	0.908	0.526	programmed cell death
GO:0033036	macromolecule localization	3.03%	-1.6021	0.925	0.334	programmed cell death
GO:0006612	protein targeting to membrane	0.18%	-3.0223	0.803	0.159	programmed cell death
GO:0034050	host programmed cell death induced by symbiont	0.00%	-2.9586	0.918	0.684	programmed cell death
GO:0008219	cell death	0.46%	-3.8539	0.933	0.173	programmed cell death
GO:0009626	plant-type hypersensitive response	0.00%	-2.9586	0.699	0.684	programmed cell death

GO:0070589	cellular component macromolecule biosynthetic process	0.59%	-2.4685	0.836	0.392	programmed cell death
GO:0015698	inorganic anion transport	0.87%	-1.7696	0.928	0.259	programmed cell death
GO:0015706	nitrate transport	0.02%	-1.6576	0.944	0.583	programmed cell death
GO:0042157	lipoprotein metabolic process	0.21%	-1.585	0.941	0.085	lipoprotein metabolism
GO:0019748	secondary metabolic process	0.14%	-2.8539	0.951	0.094	secondary metabolism
GO:0016137	glycoside metabolic process	0.03%	-1.7696	0.945	0.107	secondary metabolism

3.5 Identification of DEGs related to stamen development

To identify the genes involved in supernumerary stamen initiation in *crc-1 sup-5* double mutants, we computationally identified genes involved in stamen development using a published transcriptome dataset. A list which consists 247 DEGs by combining the categories containing 20 genes**,** 11 genes and 216 genes (Figure 8). These categories were selected based on their common involvement of DEGs with *sup* mutant background. Previous studies have determined the global expression profile of *Arabidopsis* stamen development using *ap3*, *spl/nzz*, and *ms1* mutants. Among 247 DEGs identified in Figures 9 and 10, 47 genes were predicted to be expressed at early, intermediate, and late stages of stamen development each represented by DEGs lists of *ap3*, *spl/nzz*, and *ms1* mutants respectively (Figure 11A; Table 9). Out of 47 identified genes, 37 were differentially expressed in *ap3* (early stage) (Figure 11A). This is consistent with the functions of *CRC* or *SUP*, since both genes are highly expressed from early stages of flower development. Furthermore, an additional 21 and 8 identified genes were differentially expressed in *nzz/spl* (intermediate stage) and *ms1* (late stage), respectively (Figure 11A).

To understand the function of the 47 genes involved in stamen development, GO term analysis was performed (Figure 11B). The enriched terms included 'response to organic substance,' 'response to chemical stimulus,' 'cell wall organization or biogenesis,' 'response to stimulus,' 'response to hormone,' 'multicellular organismal development,' and 'reproductive developmental process' (Figure 11B; Table 10). The interactive graph view of 47 genes generated by REVIGO identified a cluster of GO terms that contain five different terms: 'response to organic substance,' 'response to hormone,' 'response to chitin,' 'response to carbohydrate,' and 'response to water deprivation' (Figure 11C). After removing redundant GO terms, approximately 70% of GO terms were involved in 'response to chemical' (Figure 11D; Table 11).


A
ap3 DEGs
nzz/spl DEGs
1113
153
22
396
18
200
64
8
350
4
247 genes
9
86
ms1 DEGs
0
3
1
B
response to organic substance
response to chemical stimulus
cellular cell wall organization or biogenesis
response to stimulus
cell wall organization or biogenesis
response to endogenous stimulus
response to abiotic stimulus
response to hormone stimulus
multi-organism process
defense response
multicellular organismal development
reproductive developmental process
0
1
2
3
4
5
6
-log10 p-value

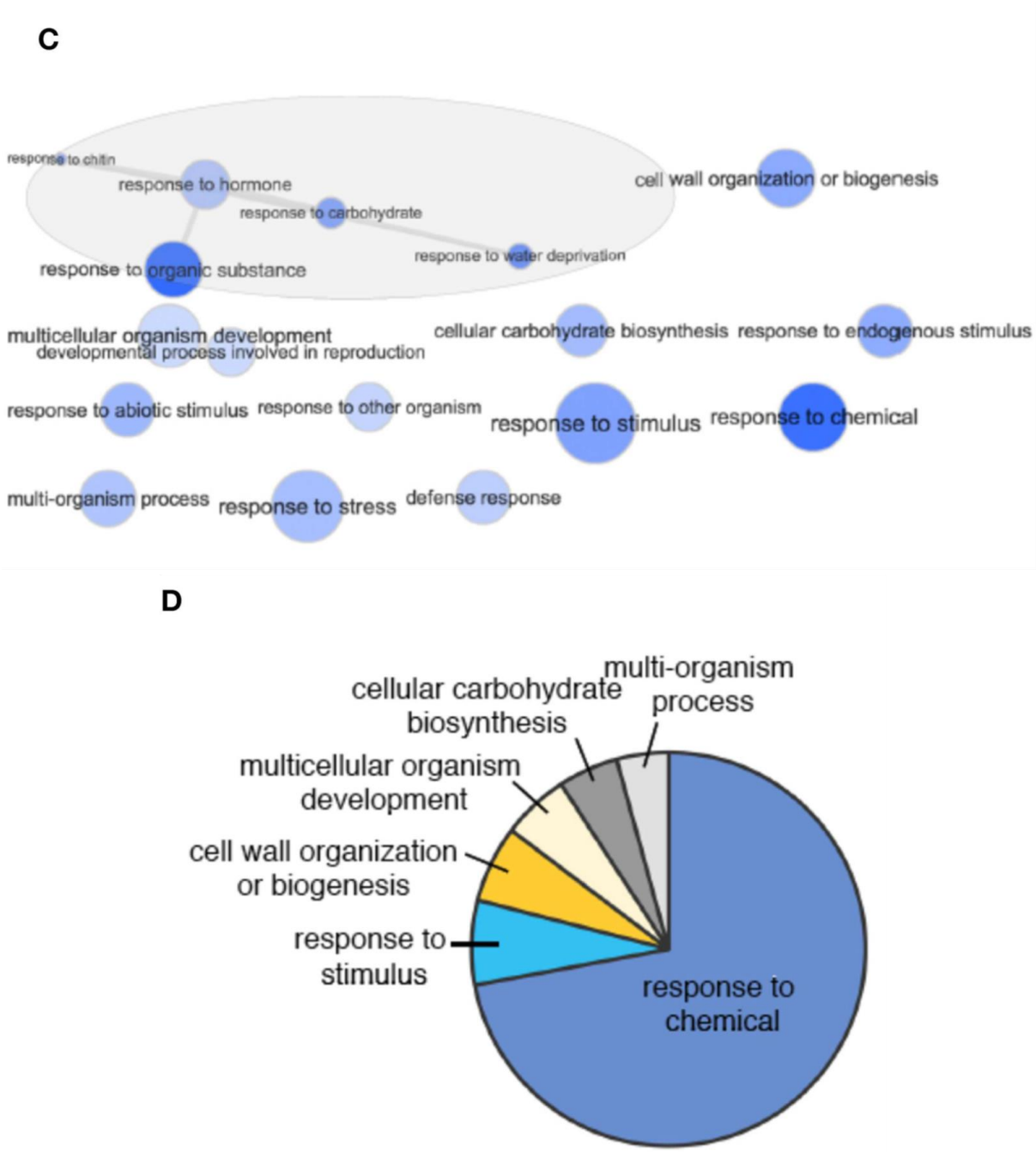


Figure 11 DEGs in *crc sup*, *ap3*, *nzz/spl*, and *ms1* flowers. (A) Venn diagram showing 247 DEGs in *ap3*, *nzz/spl*, and *ms1*. **(B**) GO term enrichment analysis of 47 genes co-regulated by CRC and SUP and related to stamen development. Selected terms determined by their -$\log_{10}$-adjusted *p*-values are shown. **(C)** The interactive graph view of 47 genes generated by REVIGO. **(D)** Pie charts showing summarized GO terms by REVIGO for 47 genes within the GO-term category "biological process."

Table 9 List of "47 genes"

Reference ID		
AT1G02360	AT2G38530	AT4G28500
AT1G10220	AT2G40000	AT4G30250
AT1G12010	AT2G45210	AT4G36700
AT1G12080	AT2G47770	AT5G03210
AT1G14860	AT3G02480	AT5G05410
AT1G21130	AT3G08860	AT5G07530
AT1G65970	AT3G15540	AT5G09780
AT1G72260	AT3G16920	AT5G10100
AT1G73260	AT3G22740	AT5G16570
AT1G76650	AT3G48340	AT5G23160
AT1G80100	AT3G62290	AT5G24860
AT2G25450	AT4G02280	AT5G25280
AT2G26410	AT4G16590	AT5G39670
AT2G26530	AT4G18780	AT5G40690
AT2G30230	AT4G23900	AT5G51800
AT2G35300	AT4G26280	

Table 10 GO term analysis of "47 genes"

GO term	Ontology	Description	Number in input list	Number in BG/Ref	p-value	FDR
GO:0042221	P	response to chemical stimulus	19	3978	8.10E-06	0.0012
GO:0010033	P	response to organic substance	16	2754	5.00E-06	0.0012
GO:0009414	P	response to water deprivation	6	416	6.60E-05	0.0055
GO:0009415	P	response to water	6	424	7.30E-05	0.0055
GO:0009743	P	response to carbohydrate stimulus	7	812	0.00037	0.018
GO:0070882	P	cellular cell wall organization or biogenesis	5	367	0.00036	0.018
GO:0050896	P	response to stimulus	21	6292	0.0005	0.021
GO:0010200	P	response to chitin	5	421	0.00067	0.025
GO:0071554	P	cell wall organization or biogenesis	7	963	0.001	0.033
GO:0009719	P	response to endogenous stimulus	9	1615	0.0012	0.036
GO:0009628	P	response to abiotic stimulus	11	2635	0.0033	0.089
GO:0034637	P	cellular carbohydrate	6	941	0.0045	0.11

		biosynthetic process				
GO:0006950	P	response to stress	14	4089	0.0052	0.12
GO:0009725	P	response to hormone stimulus	7	1375	0.0072	0.15
GO:0016051	P	carbohydrate biosynthetic process	6	1070	0.0083	0.16
GO:0051704	P	multi-organism process	8	1820	0.0095	0.18
GO:0006952	P	defense response	7	1653	0.018	0.32
GO:0051707	P	response to other organism	6	1421	0.029	0.48
GO:0007275	P	multicellular organismal development	11	3864	0.048	0.75
GO:0003006	P	reproductive developmental process	7	2050	0.05	0.75
GO:0042221	P	response to chemical stimulus	19	3978	8.10E-06	0.0012

Table 11 REVIGO analysis of "47 genes"

Term ID	Description	Frequency in Db	log_{10} p-value	Uniqueness	Dispensability	Representative
GO:0042221	response to chemical	3.07%	-5.0915	0.499	0	response to chemical
GO:0009628	response to abiotic stimulus	0.57%	-2.4815	0.545	0.427	response to chemical
GO:0009414	response to water deprivation	0.02%	-4.1805	0.453	0.514	response to chemical
GO:0009743	response to carbohydrate	0.04%	-3.4318	0.45	0.65	response to chemical
GO:0006952	defense response	0.57%	-1.7447	0.532	0.427	response to chemical
GO:0006950	response to stress	4.58%	-2.284	0.488	0.562	response to chemical
GO:0051707	response to other organism	0.30%	-1.5376	0.561	0.397	response to chemical
GO:0010033	response to organic substance	0.90%	-5.301	0.439	0.451	response to chemical
GO:0009725	response to hormone	0.34%	-2.1427	0.435	0.692	response to chemical
GO:0010200	response to chitin	0.00%	-3.1739	0.492	0.571	response to chemical

3.6 Genes potentially involved in stamen development

Based on RNA-seq, *SUP* was expressed at normal levels in *crc* and, similarly, *CRC* expression was unaffected in *sup* (FDR < 0.05). Thus, it is unlikely that either transcription factor transcriptionally regulates the expression of the other (Figure 12). All of the 47 DEGs in *crc-1 sup-5* that overlapped with the list of DEGs from the stamen transcriptome dataset were categorized into two different clusters by K-means clustering (Figure 13A). Cluster 1 contained 28 genes, which were highly expressed in all mutant backgrounds, whereas cluster 2 contained genes that were downregulated in all mutant backgrounds (Figure 13A, B). Compared to the subtle difference in differential gene expression observed in either *crc-1* or *sup-5* single mutants, the expression levels of 47 genes were greatly affected in *crc-1 sup-5* double mutants.

The upregulated genes identified in *crc-1 sup-5* might be direct targets of SUP because SUP is a transcriptional repressor (Figure 13C–J). These genes could also be regulated by CRC, since CRC can act as a bifunctional transcription factor (Yamaguchi et al., 2017, 2018; Gross et al., 2018). Consistent with the GO term analysis, hormone-related genes such as *AHP6* and *IAA19* were highly expressed in the single mutants (Nakamura et al., 2003; Tatematsu et al., 2004; Mähönen et al., 2006; Besnard et al., 2014a, 2014b; Figure 14C, D). These two genes were also more highly expressed in *crc sup* than in either single mutant. The expression levels of *CELLULOSE SYNTHASE8* (*CESA8*), *REM25*, and *AT1G12180* (Turner and Somerville, 1997; Chen et al., 2005; Mantegazza et al., 2014) were also higher in *crc-1 sup-5* (Figure 13E–G). Among the potential downstream genes, the roles of *CESA8* and *REM25* have been well studied during cell wall organization and reproductive development, respectively.

By contrast, some of the genes related to hormonal regulation, such as *SMALL AUXIN UPREGULATED36* (*SAUR36*) and *DEHYDRATION-RESPONSIVE ELEMENT BINDING PROTEIN2A* (*DREB2A*) (Sakuma et al., 2006; Kim et al., 2011; Hou et al., 2013; Stamm and Kumar., 2013), were downregulated (Figure 13H–J). One sugar metabolism regulator, TREHALOSE-6-PHOSPHATE PHOSPHATASE I (TPPI) (Schluepmann et al., 2004), has also been identified as the downregulated target in the mutant backgrounds. The RNA-seq data were confirmed by RT-qPCR (Figure 14).

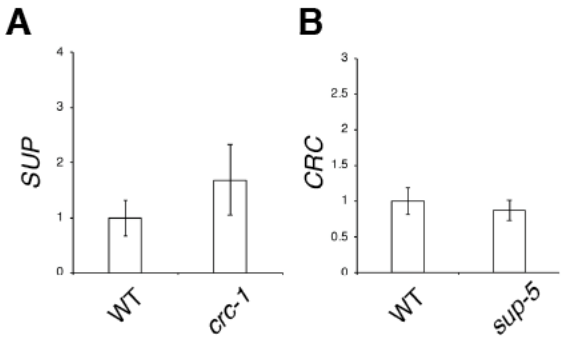


Figure 12 *SUP* and *CRC* expression in *crc* and *sup*, respectively. (A, B) Expression level of genes by RNA-seq. *SUP* **(A)** and *CRC* **(B)**. Mean ± SEM are shown. FDR < 0.05. N=5, error bars represent standard deviation.

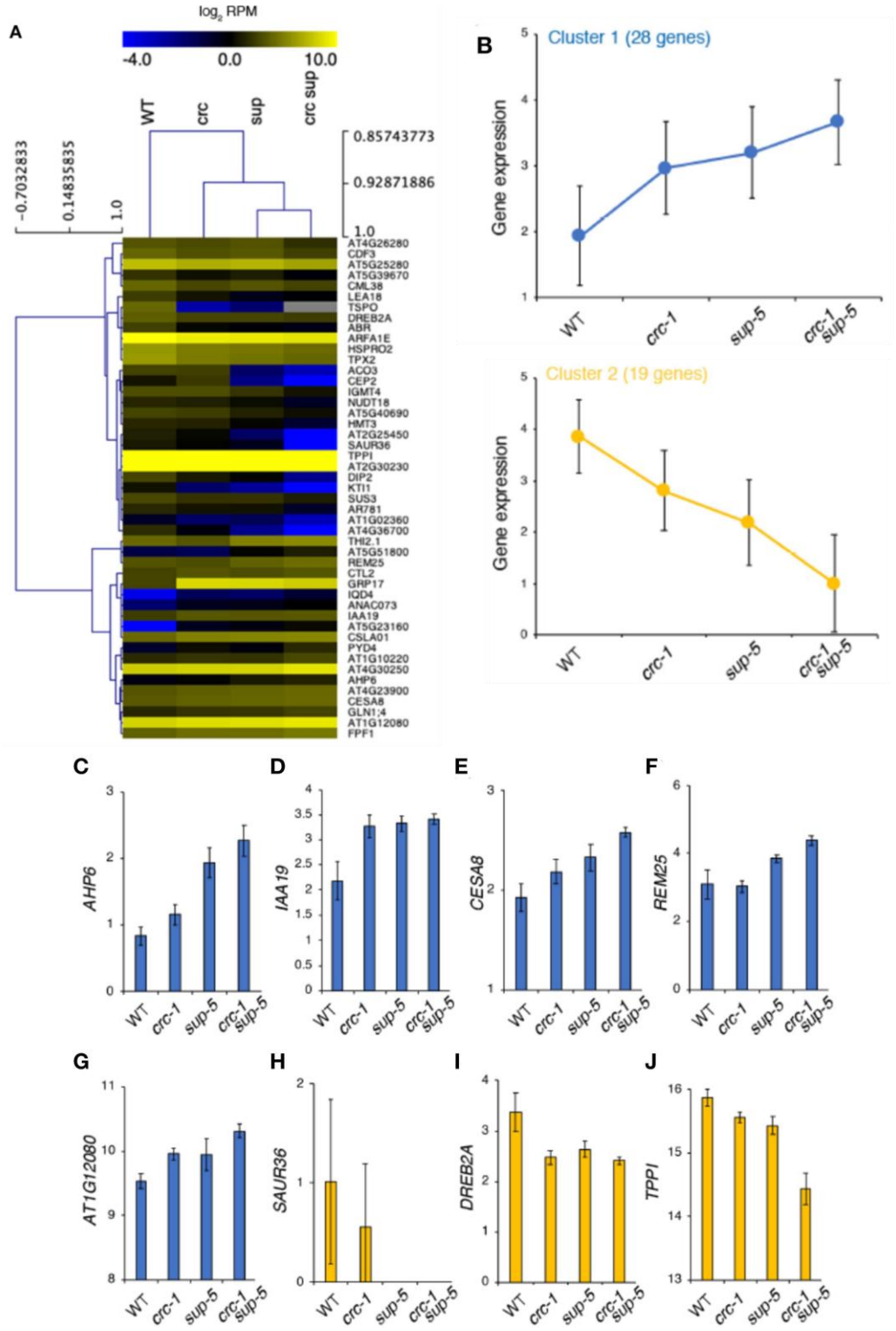


Figure 13 Clustering of high-confidence downstream targets regulated by CRC and SUP. **(A)** Clustering of 47 genes during stamen formation. **(B)** Gene expression from two different clusters: cluster 1 (left) and cluster 2 (right). **(C–J)** Expression level of genes according to RNA-seq. *AHP6* **(C)**, *IAA19* **(D)**, *CESA8* **(E)**, *REM25* **(F)**, *AT1G12080* **(G)**, *SAUR36* **(H)**, *DREB2A* **(I)**, and *TPPI* **(J)**. Reads per million mapped reads (RPM) are shown. N=5, error bars represent standard deviation.

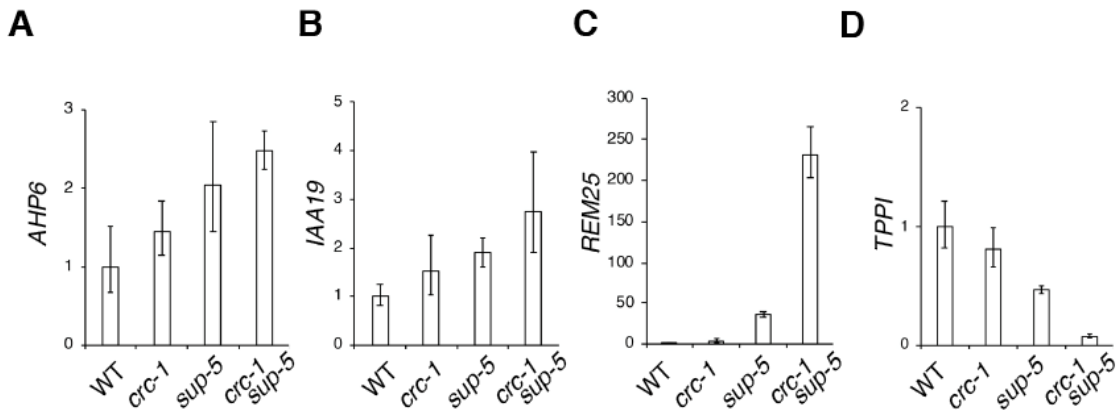


Figure 14 Verification of differentially expressed genes in the wild type and mutants by RT-qPCR. **(A–D)** Expression level of genes by RT-PCR. *AHP6* **(A)**, *IAA19* **(B)**, *REM25* **(C)**, and *TPPI* **(D)**. Mean ± SEM are shown. Expression levels of WT are set as 1.0. The fold changes are shown. N=5, error bars represent standard deviation.

3.7 Stage-specific cytokinin activities in *crc sup* mutants

Since both *CRC* and *SUP* are involved in the regulation of auxin homeostasis (Yamaguchi et al., 2017; 2018; Xu et al., 2018), it would be notable to examine the cytokinin activity in respective phenotypes. Among differentially expressed genes, AHP6 plays a key role in cytokinin signaling (Mähönen et al., 2006; Bartrina et al., 2011; Besnard et al., 2014a, 2014b). To visualize cytokinin signaling during floral meristem and stamen development, *TCSn::GFP* cytokinin marker was introduced into *crc-1*, *sup-5*, and *crc-1 sup-5* mutants by genetic crossing (Zürcher et al., 2013; Liu and Müller, 2017; Figure 15A-L). *TCSn::GFP* is a synthetic cytokinin reporter designed based on two-component signaling system (TCS) in plants to detect cytokinin response (Liu and Müller, 2017). Upon the cytokinin stimulus, the signal is transduced in the cytokinin-responsive cells to activate transcription via a phospho-relay signaling network and the binding of type-B nuclear RESPONSE REGULATOR (RR) proteins to TCS promoters is required (Dortay et al., 2006; Liu and Müller, 2017). Since stamen primordia are formed at stage 4 of flower development, it was first observed that *TCSn::GFP* signal from stage 4. Both the wild type and the *crc-1* mutant had the same levels of *TCSn* signals that were expressed at the center of FM at stage 4. Correlated with the expansion of presumptive medial region of gynoecium, the *TCSn* expression domain in the wild type and *crc-1* was gradually expanded toward lateral direction during stage 5 and 6. In *sup-5* mutants, *TCSn* was expressed slightly narrower region in the center of FM at stage 4 compared to that in the wild type. This cytokinin signaling reporter expression pattern is anti-correlated with auxin maxima (Xu et al., 2018). Consistent well with the presence of dome-like meristematic structure in *sup-5* mutant, *TCSn* expression domain was restricted to the center of FM during stage 5 and 6. In the *crc-1 sup-5* double mutants, the *TCSn::GFP* expression domain at stage 4 and 5 was even narrower than that in *sup-5* mutant. More non-*TCSn::GFP*-expressing cells in the *crc-1 sup-5* double mutants was observed at the location where stamens are developed, suggesting that inhibition of organ formation through cytokinin in the FM is controlled by genetic interaction between *CRC* and *SUP*.

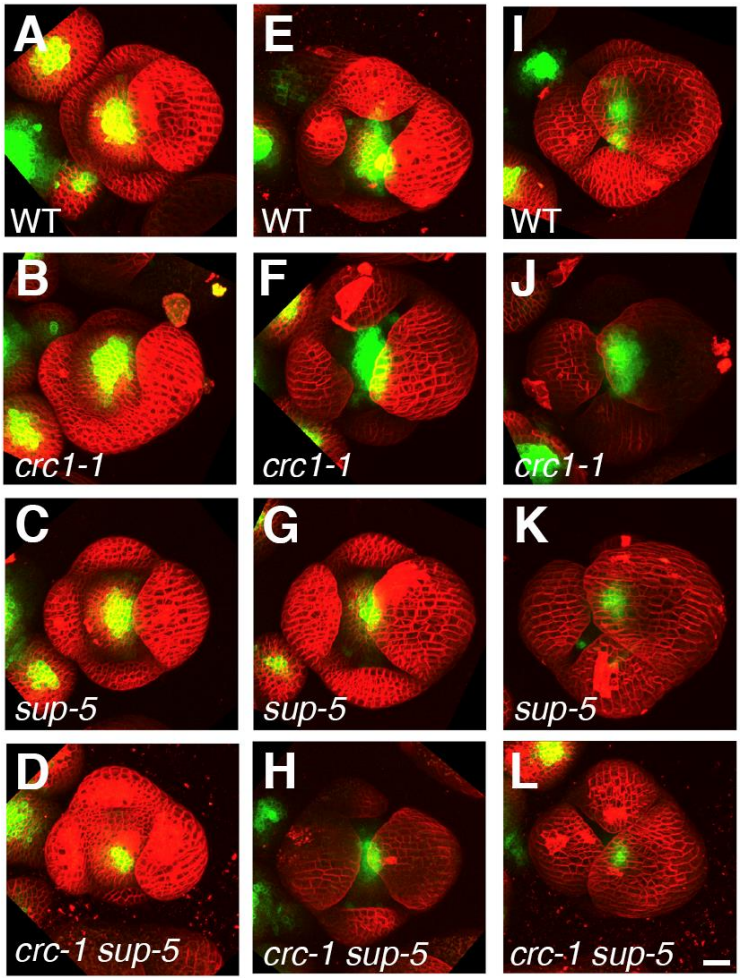


Figure 15 *TCSn::GFP* expression in wild type, *crc-1*, *sup-5* and *crc-1 sup-5* double mutant. **(A-D)** *TCSn::GFP* expression in floral meristems at stage 4. **(A)** Wild type, **(B)** *crc-1*, **(C)** *sup-5*, **(D)** *crc-1 sup-5*. **(E-H)** *TCSn::GFP* expression in floral meristems at stage 5. **(E)** Wild type, **(F)** *crc-1*, **(G)** *sup-5*, **(H)** *crc-1 sup-5*. **(I-L)** *TCSn::GFP* expression in floral meristems at stage 6. **(I)** Wild type, **(J)** *crc-1*, **(K)** *sup-5*, **(L)** *crc-1 sup-5*. Scale bar represents 50 μm. N=10.

4. DISCUSSION

4.1 *CRC* and *SUP* interact genetically during floral meristem and stamen development

Floral meristem determinacy and meristematic cell differentiation are two critical steps in flower development (Sablowski, 2015; Bommert and Whipple, 2017; Xu et al., 2019). Disruption in either or both of these processes caused by the misexpression of FM regulators eventually results in abnormal floral phenotypes (Lohmann et al., 2001; Ma, 2005). Both *SUP* and *CRC* are highly conserved genes in angiosperms and function as FM activity and floral organ identity genes in distinct spatio-temporal manners (Sun and Ito, 2015). Compared to *sup* mutants, *crc* mutants do not have altered floral organ number, despite the occasional presence of more than two carpels (Alvarez and Smyth, 1999; Bowman and Smyth, 1999). However, this study shows that combination of the *crc* mutation with *sup-5* resulted in the formation of sterile flowers with significantly more stamens and carpels. The *crc* mutation enhanced FM indeterminacy in *sup-5* mutants, leading to an increase in FM size. Because the increased number of stamens in *sup* mutants is at the expense of carpel tissues due to the expansion of the *APETALA3* gene expression domain into the fourth whorl, an increase in FM size, leading to the formation of more carpels, might increase the rate of floral organ identity conversion, coupled with hormonal changes (Prunet et al., 2017). The sterility of *crc sup* double mutant flowers was probably due to a failure in establishing carpel polarity caused by *crc* which in turn further causing more severe shape distortion in the carpel. The cells differentiation in developing carpel are aligned to the established abaxial–adaxial (abaxial is away from the meristem and adaxial is adjacent to the meristem) polarity at the axes of asymmetry (Eshed et al., 1999). Polarity changes further enhance the carpel defect phenotypes in *sup* single mutants, which produce apically open carpels with abnormal ovule integument development (Gaiser et al., 1995; Baker et al., 1997; Eshed et al., 1999; Breuil-Broyer et al., 2016).

Compared to the *Arabidopsis CRC* gene, its orthologs in other species play broader and prominent roles during plant growth and development (Nagasawa et al., 2003; Yamaguchi et al., 2004; Li et al., 2011). No clear defects in FM determinacy and no homeotic defects are observed in *crc* single mutants (Alvarez and Smyth, 1999; Bowman and Smyth, 1999; Baum et al., 2001; Nagasawa et al., 2003). By contrast, mutation of *CRC* orthologs in other plant species causes multiple phenotypic defects during the vegetative and reproductive stages. For example, one well-characterized *CRC* ortholog is *DROOPING LEAF* (*DL*) in rice (*Oryza sativa*). Molecular studies have identified the gene functions that specify carpel identity and regulate midrib leaf formation in rice (Yamaguchi et al., 2004). To date, no *crc* alleles that

show floral homeotic or leaf defects as strong as those in *dl* mutants have been identified in Arabidopsis. When combined with mutants of other key regulators of floral meristem activity or development, these higher-order *crc* multiple mutants show synergistic effects and produce flowers with extra floral whorls or floral organs with abnormal phenotypes (Eshed et al., 2001; Prunet et al., 2008; Zuniga-Mayo et al., 2012). In *crc sup* double mutants, aberrant FM indeterminacy and floral organ identity were observed. Although *CRC* is only expressed in the abaxial region of carpels prior to FM termination, the *crc* mutation enhances not only FM determinacy, but also the initiation of floral organs such as stamens or carpels. Thus, it is conceivable that phytohormones are involved in the genetic interaction between *CRC* and *SUP* because phytohormones exert multiple roles in a non-cell-autonomous manner. Recent studies have identified roles for *CRC* and *SUP* in hormone homeostasis (Yamaguchi et al., 2017, 2018; Xu et al., 2018). Hormonal regulation by CRC might also explain why its orthologs have various functions in different plant species.

4.2 *CRC* and *SUP* control shared downstream targets involved in stamen development

Based on RNA-seq, *SUP* was expressed at normal levels in *crc* and similarly, *CRC* expression was unaffected in *sup* (*FDR* < 0.05). Thus, it is unlikely that either transcription factor transcriptionally regulates the expression of the other (Figure 12).

Cytokinins maintain meristem activity by controlling cell division (Riou-Khamlichi et al., 1999; Werner et al., 2001; Yang et al., 2002; Zhang et al., 2013). Consistent with this finding, *AHP6* was identified as a common downstream gene of CRC and SUP. It has been reported that supernumerary stamen primordia in *sup* are formed at stage 7 (Prunet et al., 2017). Considering that *CRC* is expressed from floral stage 6 onwards (Bowman and Smyth, 1999; Lee et al., 2005), the effect of the *crc* mutation in the *sup* background and *AHP6* misexpression might be observed after stage 6. To further investigate the *SUP*/*CRC*-mediated spatio-temporal regulation of floral patterning and FM determinacy under auxin-cytokinin crosstalk, gene expression analysis (RT-qPCR of *AHP6* gene), auxin-cytokinin reporter assays using multiple phytohormone markers, and auxin-cytokinin quantitative measurement (HPLC or GC-MS) under stage-specific condition should be carried out. However, the architecture of the Arabidopsis inflorescence is in an indefinite growing pattern called "raceme" that is continuously producing floral meristems laterally which then develop into flowers or axillary meristems along a main axis (Benlloch et al., 2007; Teo et al., 2014, Figure 16). Hence, the main or axillary inflorescence consists of floral buds or primordia from different stages and a synchronized system (*apl cal 35S::AP1-GR*; Wellmer et al., 2006) which is capable of generating inflorescence with flowers all in same stage is needed to allow precise stage-specific study of gene expression and auxin-cytokinin crosstalk in Arabidopsis flower. Together with the expression of multiple marker genes, this will provide greater insight into how stamen number is defined by CRC and SUP via AHP6.

Subsequent floral organ growth and development might also be affected by CRC and/or SUP, potentially via the transcriptional regulation of downstream genes. These might include, for example, the auxin-responsive gene *Auxin/Indole-3-Acetic Acid 19* (*AUX/IAA19*), which regulates stamen elongation (Tashiro et al., 2009; Ghelli et al., 2018), and *REPRODUCTIVE MERISTEM* (*REM*) genes, which are potentially involved in the early stages of flower development and are often transcriptionally regulated by well-known key floral regulators (Mantegazza et al., 2014).

IAA19, which plays a key role in controlling stamen elongation (Tashiro et al., 2009; Ghelli et al., 2018), was also differentially expressed in *crc-1 sup-5* plants. This suggests that SUP might be important not only for early stamen formation, but also for subsequent stamen

growth. The *crc* mutation affects the expression of *IAA19*, which is expressed in stamens, even though *CRC* is not expressed in these organs. It is unclear how this regulation occurs, but might involve non-cell-autonomous effects.

The *REM25* and *TPPI* genes were also differentially expressed in *crc-1 sup-5* (Mantegazza et al., 2014). Based on previous *in situ* hybridization data, *REM25* is highly expressed in stamen and carpel primordia at floral stage 6. Since *SUP*, *CRC*, and *REM25* have overlapping expression domains (Sakai et al., 1995; Bowman and Smyth, 1999; Lee et al., 2005), SUP and CRC might be upstream regulators of *REM25*. Genetic redundancy and the physical linkage of *REM* loci hamper functional studies of *REM* family genes (Mantegazza et al., 2014); therefore, the generation of mutants via CRISPR/Cas9 might contribute to understanding the function of genes within this family. *TPPI* is required for the appropriate establishment of organ boundaries (Lor, 2014), which is consistent with the regulation of organ boundary genes, such as *CUC2* (Xu et al., 2018), by SUP. However, the exact function of *TPPI* during flower development is largely unknown and it is relevant to study the molecular function of *TPPI* during stamen/carpel boundary specification.

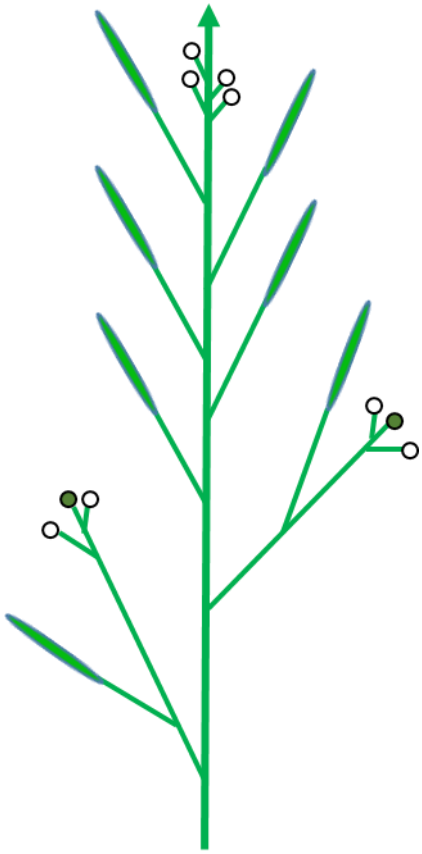


Figure 16 Illustration diagram of "raceme" type inflorescence architecture in Arabidopsis. Green arrow – growth direction of apical shoot; green circle – axillary meristem; white circle – flowers or floral primordia; green oval – siliques

4.3 The role of cytokinin signaling on floral meristem and stamen development

In wild type plants, cytokinin signaling is active in the center of FM at stage 4. This result indicates that cytokinin controls meristem activity not only in the shoot apical meristem, but also in the FM at earlier stages. Instead, cytokinin is responsible for the maintenance of stem cell pool pluripotency which acts as the positive regulator of *WUS* and *CLV* before the termination of FM activity (Wybouw B and De Rybel, 2019). From stage 5 onward, cytokinin accumulates at the medial region of gynoecium in wild type as reported previously (Marsch-Martinez et al., 2012; Reyes-Olalde et al., 2013; Sehra and Franks, 2015), suggesting that it promotes proper gynoecium differentiation at later stages. No differences in cytokinin response, and floral morphology were observed between wild type and *crc* mutants till stage 6, suggesting that CRC does not have a primary role in regulating cytokinin signaling till this stage (Figure 6, 13, 15). Results from these studies are consistent with the previous result that *CRC* starts to be expressed from stage 6 (Bowman and Smyth, 1999; Lee et al., 2005). After the initiation of gynoecium development and FM determinacy during stage 6, cytokinin plays two distinct roles in gynoecium and fruit patterning as well as morphogenesis at different stage which are stimulating proliferation of the medial tissues (early stage) and valve margin formation (late stage) (Marsch-Martinez et al., 2012).

On the other hand, the *sup* mutant has dome-shaped structure in the center of FM and possesses the *WUS* and *CLV3* expression there even after stage 6 (Prunet et al., 2017; Uemura et al., 2017; Xu et al., 2018). A fewer number of cells located at the center of FM of *sup* mutants at stage 6 contained *TCSn* expression (Figure 15). The previously published results and data from this study suggest that FMs are maintained for longer time in *sup* mutant flowers than in wild type and may be functional to produce extra floral organs. The effect of SUP on FM termination and organogenesis may be mediated by not only *WUS*, but also cytokinin. In rice, cytokinin deficient mutant have less stamens due to reduced meristem activity (Kurakawa et al., 2007; Yamaki et al., 2011). Cytokinin levels could be important to define appropriate number of stamens in higher plants. In *sup*-5 mutant, the *TCSn* was expressed slightly narrower region as compared to wild type plants during stage 5. The antagonistic activity between auxin might be the reason for this observation. As reported previously, stronger auxin reporter (*pDR5rev::2xGFP-N7*) activity could be detected at the whorl 3/4 boundary of *sup* mutants (Xu et al., 2018). Hence, the reduced cytokinin activity might be caused by the increased auxin activity due to the loss-of-function of SUP at the boundary between third and fourth whorls which might in turn reduces cytokinin response (Figure 16A). However, further investigation

which combines auxin and cytokinin reporter assay is needed to elucidate this antagonistic relationship of auxin-cytokinin at the boundary of whorls 3/4.

In *crc sup* double mutants, the *TCSn::GFP* expression domain at stage 4 and 5 was also narrower than either parental lines. More non-*TCSn::GFP*-expressing cells in the *crc sup* double mutants was observed at the location where stamens are developed (Figure 15). It has been reported that extra stamen primordia in *sup* mutant are formed at stage 7 (Prunet et al., 2017). Thus, increased number of cells without *TCSn* expression at stamen formation domain might contribute to extra stamen formation. The narrower activity domain was observed during stage 4 in *crc sup* double mutants (Figure 15) which was even earlier than in *sup* single mutant (stage 5), indicating that there might be systemic effect for cytokinin signaling during floral organs development in Arabidopsis since *CRC* expression is later than *SUP*. Previous reports have demonstrated the development of the flowers mediated by plant hormone is correlated to its position in the on the inflorescence. During plant development, the gene expressed after organ development could inhibit or affect the formation of new organs through plant hormone signaling (Stirnberg et al., 1999). Other studies also showed that mutants impaired in plant hormone gibberellin (GA) produced early flowers and later flowers with different degree of growth defects (Hu et al., 2008; Rieu et al., 2008). Hence, it is likely that the early flowers (develop first or earlier) with *crc* mutant background could affect the floral organs growth and hormone signaling of flowers in same inflorescence even before its expression (Figure 16B).

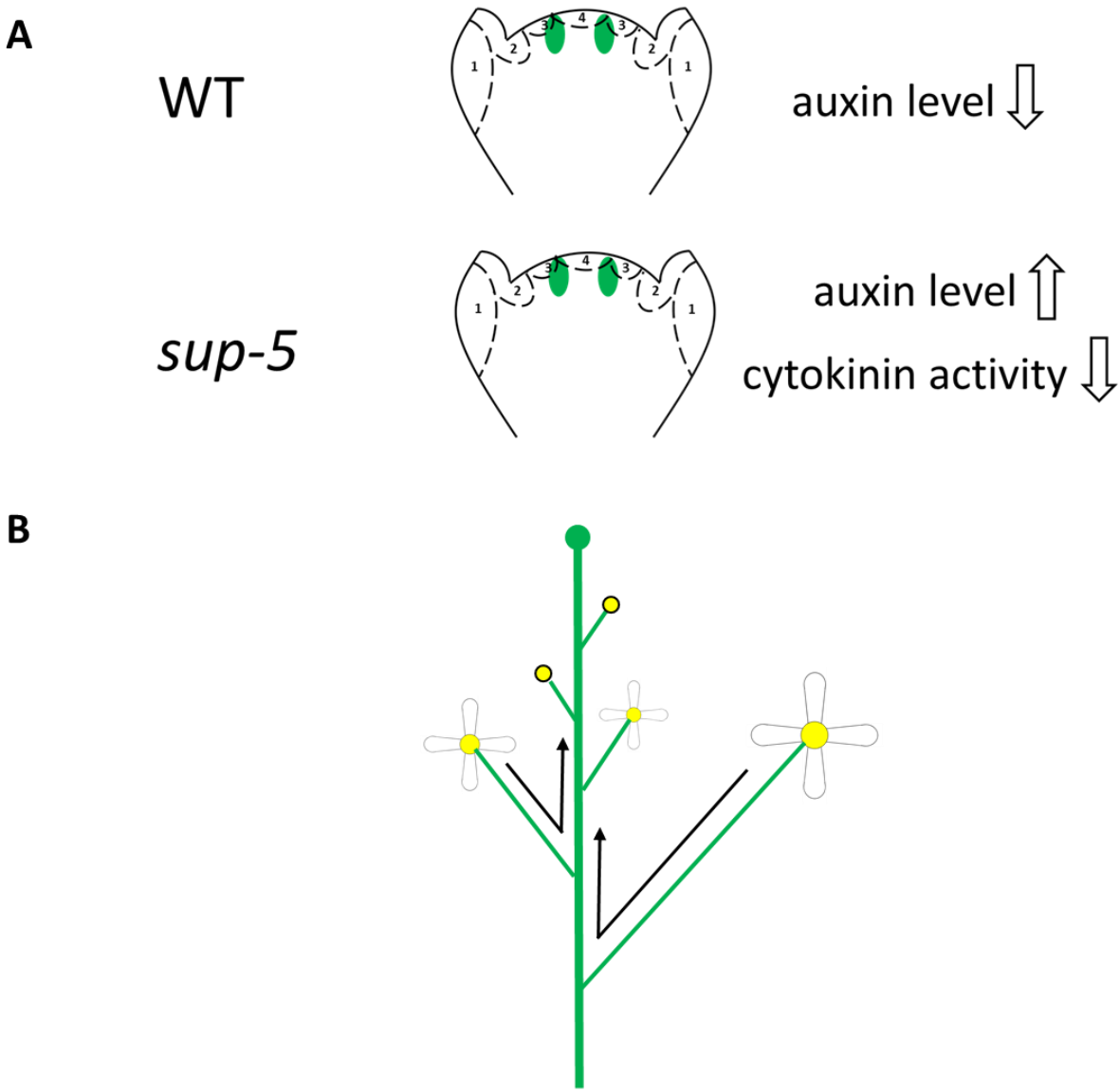


Figure 16 Hypothesized model of cytokinin activity domain in *sup -5* mutant and systemic effect in *crc-1 sup-5* double mutant. (A) Hypothesized cytokinin activity in *sup* mutant due to the disruption of auxin homeostasis at the boundary of whorls 3/4. Green colour indicates the expected hormonal activity domain. **(B)** Hypothesized model of systemic effect of hormone activity and floral patterning in late flowers (develop later) which are affected by early flowers (develop first or earlier).

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6. REFERENCES

Achard P, Baghour M, Chapple A, Hedden P, Van Der Straeten D, Genschik P, Moritz T, Harberd NP (2007) The plant stress hormone ethylene controls floral transition via DELLA dependent regulation of floral meristem-identity genes. *Proc Natl Acad Sci USA* 104: 6484–6489

Adibi M, Yoshida S, Weijers D, Fleck C (2016) Centering the organizing center in the *Arabidopsis thaliana* shoot apical meristem by a combination of cytokinin signaling and self-organization. *PLoS ONE* 11: e0147830

Aloni R, Aloni E, Langhans M, Ullrich CI (2006) Role of cytokinin and auxin in shaping root architecture: regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. *Ann Bot* 97:883–893

Alvarez J, Smyth DR (1999) *CRABS CLAW* and *SPATULA*, two *Arabidopsis* genes that control carpel development in parallel with *AGAMOUS*. *Development* 126: 2377–2386

Baker SC, Robinson-Beers K, Villanueva JM, Gaiser JC, Gasser CS (1997) Interactions among genes regulating ovule development in *Arabidopsis thaliana*. *Genetics* 145: 1109-1124

Barton MK, Poethig RS (1993) Formation of the shoot apical meristem in *Arabidopsis thaliana*: an analysis of development in the wild type and in the *shoot meristemless* mutant. *Development* 119: 823–831

Bartrina I, Otto E, Strnad M, Werner T, Schmülling T (2011) Cytokinin regulates the activity of reproductive meristems, flower organ size, ovule formation, and thus seed yield in *Arabidopsis thaliana*. *Plant Cell* 23: 69–80

Baum SF, Eshed Y, Bowman JL (2001) The *Arabidopsis* nectary is an ABC-independent floral structure. *Development* 128: 4657–4667

Benlloch R, Berbel A, Serrano-Mislata A, Madueno F (2007) Floral initiation and inflorescence architecture: A comparative view. *Ann Bot* 100: 659–676

Bennett SRM, Alvarez J, Bossinger G, Smyth DR (1995) Morphogenesis in pinoid mutants of *Arabidopsis thaliana*. *Plant J* 8: 505–520

Besnard F, Refahi Y, Morin V, Marteaux B, Brunoud G, Chambrier P, Rozier F, Mirabet V, Legrand J, Lainé S, Thévenon E, Farcot E, Cellier C, Das P, Bishopp A, Dumas R, Parcy F, Helariutta Y, Boudaoud A, Godin C, Traas J, Guédon Y, Vernoux T (2014a) Cytokinin signaling inhibitory fields provide robustness to phyllotaxis. *Nature* 505: 417–421

Besnard F, Rozier F, Vernoux T (2014b) The AHP6 cytokinin signaling inhibitor mediates an auxin-cytokinin crosstalk that regulates the timing of organ initiation at the shoot apical meristem. *Plant Signal Behav* 9: e28788

Boavida LC, Qin P, Broz M, Becker JD, McCormick S (2013) Arabidopsis tetraspanins are confined to discrete expression domains and cell types in reproductive tissues and form homo- and heterodimers when expressed in Yeast. *Plant Physiol* 163: 696–712

Bommert P, Whipple C (2017) Grass inflorescence architecture and meristem determinacy. *Semin Cell Dev Biol* 79: 37–47

Bowman JL, Smyth DR, Meyerowitz EM (1991) Genetic interactions among floral homeotic genes. *Development* 112: 1–20

Bowman JL, Smyth DR (1999) *CRABS CLAW*, a gene that regulates carpel and nectary development in *Arabidopsis*, encodes a novel protein with zinc finger and helix-loop-helix domains. *Development* 126: 2387–2396

Bowman JL, Eshed Y (2000) Formation and maintenance of the shoot apical meristem. *Trends Plant Sci* 3: 110–115

Breuil-Broyer S, Trehin C, Morel P, Boltz V, Sun B, Chambrier P, Ito T, Negrutiu I (2016) Analysis of the Arabidopsis *superman* allelic series and the interactions with other genes demonstrate developmental robustness and joint specification of male–female boundary, flower meristem termination and carpel compartmentalization. *Ann Bot* 117: 905–923

Carles CC, Fletcher JC (2003) Shoot apical meristem maintenance: The art of dynamic balance. *Trends Plant Sci* 8: 394–401

Chahtane H, Vachon G, Le Masson M, Thévenon E, Périgon S, Mihajlovic N, Kalinina A, Michard R, Moyroud E, Monniaux M, Sayou C, Grbic V, Parcy F, Tichtinsky G (2013) A variant of LEAFY reveals its capacity to stimulate meristem development by inducing *RAX1*. *Plant J* 74: 678–689

Chen ZZ, Hong XH, Zhang HR, Wang YQ, Li X, Zhu JK, Gong Z (2005) Disruption of the cellulose synthase gene, *AtCesA8*/*IRX1*, enhances drought and osmotic stress tolerance in Arabidopsis. *Plant J* 43: 273–283

Cheng Y, Dai X, Zhao Y (2006) Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in *Arabidopsis*. *Genes Dev* 20: 1790–1799

Cheng Y, Dai X, Zhao Y (2007). Auxin synthesized by the YUCCA flavin monooxygenases is essential for embryogenesis and leaf formation in *Arabidopsis*. *Plant Cell* 19: 2430–2439

Chiu WH, Chandler J, Cnops G, Van Lijsebettens M, Werr W (2007) Mutations in the *TORNADO2* gene affect cellular decisions in the peripheral zone of the shoot apical meristem of *Arabidopsis thaliana*. *Plant Mol Biol* 63: 731–744

Cnops G, Neyt P, Raes J, Petrarulo M, Nelissen H, Malenica N, Luschnig C, Tietz O, Ditengou F, Palme K, Azmi A, Prinsen E, Van Lijsebettens M (2006) The *TORNADO1* and *TORNADO2* genes function in several patterning processes during early leaf development in *Arabidopsis thaliana*. *Plant Cell* 18: 852–866

Coen ES, Meyerowitz EM (1991) The war of the whorls: Genetic interactions controlling flower development. *Nature* 353: 31–37

Denay G, Chahtane H, Tichtinsky G, Parcy F (2017) A flower is born: an update on Arabidopsis floral meristem formation. *Curr Opin Plant Biol* 35: 15–22

Dortay H, Mehnert N, Burkle L, Schmülling T, Heyl A (2006) Analysis of protein interactions within the cytokinin-signaling pathway of *Arabidopsis thaliana*. *FEBS Journal* 273: 4631–4644

El-Showk S, Ruonala R, Helariutta Y (2013) Crossing paths: Cytokinin signalling and crosstalk. *Development* 140: 1373–1383

Eshed Y, Baum SF, Bowman JL (1999) Distinct mechanisms promote polarity establishment in carpels of *Arabidopsis*. *Cell* 99: 199–209

Eshed Y, Baum SF, Perea JV, Bowman JL (2001) Establishment of polarity in lateral organs of plants. *Curr Biol* 11: 1251–1260

Fletcher JC (2002) Shoot and floral meristem maintenance in Arabidopsis. *Annu Rev Plant Biol* 53: 45–66

Gaiser JC, Robinson-Been K, Gasser CS (1995) The Arabidopsis *SUPERMAN* gene mediates asymmetric growth of the outer integument of ovules. *Plant Cell* 7: 333-345

Ghelli R, Brunetti P, Napoli N, De Paolis A, Cecchetti V, Tsuge T, Serino G, Matsui M, Mele G, Rinaldi G, Palumbo GA, Barozzi F, Costantino P, Cardarelli M (2018) A newly identified flower-specific splice variant of *AUXIN RESPONSE FACTOR8* regulates stamen elongation and endothecium lignification in Arabidopsis. *Plant Cell* 30: 620–637

Goto K, Meyerowitz EM (1994) Function and regulation of the *Arabidopsis* floral homeotic gene *PISTILLATA*. *Genes Dev* 8: 1548–1560

Gross T, Broholm S, Becker A (2018) CRABS CLAW Acts as a Bifunctional Transcription Factor in Flower Development. *Front Plant Sci* 9: 835

Guo S, Sun B, Looi LS, Xu Y, Gan ES, Huang J, Ito T (2015) Co-ordination of Flower Development Through Epigenetic Regulation in Two Model Species: Rice and Arabidopsis. *Plant Cell Physiol* 56: 830–842

Honma T, Goto K (2001) Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. *Nature* 409: 525–529

Hou K, Wu W, Gan SS (2013) *SAUR36*, a small auxin up RNA gene, is involved in the promotion of leaf senescence in Arabidopsis. *Plant Physiol* 161: 1002–1009

Hu J, Mitchum MG, Barnaby N, Ayele BT, Ogawa M, Nam E, Lai WC, Hanada A, Alonso JM, Ecker JR, Swain SM, Yamaguchi S, Kamiya Y, Sun TP (2008) Potential sites of bioactive gibberellin production during reproductive growth in *Arabidopsis*. *Plant Cell* 20:320–336

Ichihashi Y, Fukushima A, Shibata A, Shirasu K (2018) High Impact Gene Discovery: Simple Strand-Specific mRNA Library Construction and Differential Regulatory Analysis Based on Gene Co-Expression Network. In: Yamaguchi N. (eds) Plant Transcription Factors. *Methods in Molecular Biology* vol 1830. Humana Press, New York, NY

Jack T, Brockman LL, Meyerowitz EM (1992) The homeotic gene *APETALA3* of *Arabidopsis thaliana* encodes a MADS box and is expressed in petals and stamens. *Cell* 68: 683–697

Jacobsen SE, Sakai H, Finnegan EJ, Cao X, Meyerowitz EM (2000) Ectopic hypermethylation of flower-specific genes in *Arabidopsis*. *Curr Biol* 10: 179–186

Jetha K, Theissen G, Melzer R (2014) *Arabidopsis* SEPALLATA proteins differ in cooperative DNA-binding during the formation of floral quartet-like complexes. *Nucleic Acids Res* 42: 10927–10942

Kim JS, Mizoi J, Yoshida T, Fujita Y, Nakajima J, Ohori T, Todaka D, Nakashima K, Hirayama T, Shinozaki K, Yamaguchi-Shinozaki K (2011) An ABRE promoter sequence is involved in osmotic stress-responsive expression of the *DREB2A* gene, which encodes a transcription factor regulating drought-inducible genes in Arabidopsis. *Plant Cell Physiol* 52: 2136–2146

Kurakawa T, Ueda N, Maekawa M, Kobayashi K, Kojima M, Nagato Y, Sakakibara H, Kyozuka J (2007) Direct control of shoot meristem activity by a cytokinin-activating enzyme. *Nature* 445: 652–655

Kyozuka J (2007) Control of shoot and root meristem function by cytokinin. *Curr Opin Plant Biol* 10: 442–446

Laux T, Mayer KFX, Berger J, Jürgens G (1996) The *WUSCHEL* gene is required for shoot and floral meristem integrity in *Arabidopsis*. *Development* 122: 87–96

Leibfried A, To JPC, Busch W, Stehling S, Kehle A, Demar M, Kieber JJ, Lohmann JU (2005) WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators. Nature 438: 1172–1175

Lenhard M, Bohnert, A, Jürgens, G, Laux T (2001) Termination of stem cell maintenance in *Arabidopsis* floral meristems by interactions between *WUSCHEL* and *AGAMOUS*. *Cell* 105: 805–814

Lee JY, Baum SF, Alvarez J, Patel A, Chitwood DH, Bowman JL (2005) Activation of *CRABS CLAW* in the nectaries and carpels of *Arabidopsis*. *Plant Cell* 17: 25–36

Lee ZH, Hirakawa T, Yamaguchi N, Ito T (2019a) The Roles of Plant Hormones and Their Interactions with Regulatory Genes in Determining Meristem Activity. *Int J Mol Sci* 20: 4065

Lee ZH, Tatsumi Y, Ichihashi Y, Suzuki T, Shibata A, Shirasu K, Yamaguchi N, Ito T (2019b) CRABS CLAW and SUPERMAN coordinate hormone-, stress- and metabolic-related gene expression during Arabidopsis stamen development. *Front Ecol Evol* 7: 437

Li HF, Liang WQ, Yin CS, Zhu L, Zhang DB (2011) Genetic interaction of *OsMADS3*, *DROOPING LEAF*, and *OsMADS13* in specifying rice floral organ identities and meristem determinacy. *Plant Physiol* 156: 263–274

Li SB, Xie ZZ, Hu CG, Zhang JZ (2016) A review of auxin response factors (ARFs) in plants. *Front Plant Sci* 7: 47

Liu X, Dinh TT, Li D, Shi B, Li Y, Cao X, Guo L, Pan Y, Jiao Y, Chen X (2014) *AUXIN RESPONSE FACTOR 3* integrates the functions of *AGAMOUS* and *APETALA2* in floral meristem determinacy. *Plant J* 80: 629–641

Liu J, Müller B (2017) Imaging TCSn::GFP, a synthetic cytokinin reporter, in *Arabidopsis thaliana*. *Methods Mol Biol* 1497: 81–90

Lohmann JU, Hong RL, Hobe M, Busch MA, Parcy F, Simon R, Weigel D (2001) A molecular link between stem cell regulation and floral patterning in *Arabidopsis*. *Cell* 105: 793–803

Lor J L (2014) Function of TPPI and TPPJ in Arabidopsis Boundary Regions (Unpublished masters dissertation) University of California, Riverside, USA

Ma H (2005) Molecular genetic analyses of microsporogenesis and microgametogenesis in flowering plants. *Annu Rev Plant Biol* 56: 393–434

Mähönen AP, Bishopp A, Higuchi M, Nieminen KM, Kinoshita K, Törmäkangas K, Ikeda Y, Oka A, Kakimoto T, Helariutta Y (2006) Cytokinin signaling and its inhibitor AHP6 regulate cell fate during vascular development. *Science* 311: 94-98

Mandel MA, Gustafson-Brown C, Savidge B, Yanofsky MF (1992) Molecular characterization of the Arabidopsis floral homeotic gene *APETALA1*. Nature 360: 273–277

Mantegazza O, Gregis V, Mendes MA, Morandini P, Alves-Ferreira M, Patreze CM, Nardeli SM, Kater MM, Colombo L (2014) Analysis of the Arabidopsis *REM* gene family predicts functions during flower development. *Ann Bot* 114: 1507–1515

Marsch-Martinez N, Ramos-Cruz D, Reyes-Olalde, JI, Lozano-Sotomayor P, Zuniga-Mayo VM, De Folter S (2012) The role of cytokinin during *Arabidopsis* gynoecia and fruit morphogenesis and patterning. *Plant J* 72: 222–234

Mayer KF, Schoof H, Haecker A, Lenhard M, Jürgens G, Laux T (1998) Role of *WUSCHEL* in regulating stem cell fate in the *Arabidopsis* shoot meristem. *Cell* 95: 805–815

Mendes MA, Guerra RF, Berns MC, Manzo C, Masiero S, Finzi L, Kater MM, Colombo L (2013) MADS domain transcription factors mediate short-range DNA looping that is essential for target gene expression in *Arabidopsis*. *Plant Cell* 25: 2560–2572

Miwa H, Kinoshita A, Fukuda H, Sawa S (2009) Plant meristems: *CLAVATA3*/*ESR*-related signaling in the shoot apical meristem and the root apical meristem. *J Plant Res* 122: 31–39

Mizukami Y, Ma H (1992) Ectopic expression of the floral homeotic gene *AGAMOUS* in transgenic *Arabidopsis* plants alters floral organ identity. *Cell* 71: 119–131

Mizukami Y, Ma H (1997) Determination of Arabidopsis floral meristem identity by *AGAMOUS*. *Plant Cell* 9: 393–408

Moreira S, Bishopp A, Carvalho H, Campilho A (2013). AHP6 inhibits cytokinin signaling to regulate the orientation of pericycle cell division during lateral root initiation. *PLoS One* 8: e56370

Moyroud E, Kusters E, Monniaux M, Koes R, Parcy F (2010) LEAFY blossoms. *Trends Plant Sci* 15: 346–352

Müller CJ, Larsson E, Spichal L, Sundberg E (2017) Cytokinin-auxin crosstalk in the gynoecial promodium ensures correct domain patterning. *Plant Physiol* 175: 1144–1157

Nagasawa N, Miyoshi M, Sano Y, Satoh H, Hirano H Y, Sakai H, Nagato Y (2003) *SUPERWOMAN 1* and *DROOPING LEAF* genes control floral organ identity in rice. *Development* 130: 705–718

Nakamura A, Higuchi K, Goda H, Fujiwara MT, Sawa S, Koshiba T, Shimada Y, Yoshida S (2003) Brassinolide induces *IAA5*, *IAA19*, and DR5, a synthetic auxin response element in Arabidopsis, implying a cross talk point of brassinosteroid and auxin signaling. *Plant Physiol* 133: 1–11

Nibau C, Stilio VSD, Wu HM, Cheung AY (2010) Arabidopsis and tobacco *SUPERMAN* regulate hormone signalling and mediate cell proliferation and differentiation. *J Exp Bot* 62: 949–961

Okada K, Ueda J, Komaki MK, Bell CJ, Shimura Y (1991) Requirement of the auxin polar transport system in early stages of Arabidopsis floral bud formation. *Plant Cell* 3: 677–684

O'Maoileidigh DS, Wuest SE, Rae L, Raganelli A, Ryan PT, Kwasniewska K, Das P, Lohan AJ, Loftus B, Graciet E, Wellmer F (2013) Control of reproductive floral organ identity specification in Arabidopsis by the C function regulator AGAMOUS. *Plant Cell* 25: 2482–2503

Payne T, Johnson SD, Koltunow AM (2004) *KNUCKLES* (*KNU*) encodes a C2H2 zinc-finger protein that regulates development of basal pattern elements of the Arabidopsis gynoecium. *Development* 131: 3737–3749

Poethig RS (2003) Phase change and the regulation of developmental timing in plants. *Science* 301: 334–336

Prunet N, Morel P, Thierry AM, Eshed Y, Bowman JL, Negrutiu I, Trehin C (2008) *REBELOTE*, *SQUINT*, and *ULTRAPETALA1* function redundantly in the temporal regulation of floral meristem termination in *Arabidopsis thaliana*. *Plant Cell* 20: 901–919

Prunet N, Morel P, Negrutiu I, Trehin C (2009) Time to stop: Flower meristem termination. *Plant Physiol* 150: 1764–1772

Prunet N, Yang W, Das P, Meyerowitz EM, Jack TP (2017) *SUPERMAN* prevents class B gene expression and promotes stem cell termination in the fourth whorl of *Arabidopsis thaliana* flowers. *Proc Natl Acad Sci USA* 114: 7166–7171

Przemeck GK, Mattsson J, Hardtke CS, Sung ZR, Berleth T (1996) Studies on the role of the *Arabidopsis* gene *MONOPTEROS* in vascular development and plant cell axialization. *Planta* 200: 229-237

Reyes-Olalde JI, Zuniga-Mayo VM, Montes RAC, Marsch-Martinez N, de Folter S (2013) Inside the gynoecium: at the carpel margin. *Trends Plant Sci* 18: 644–655

Reyes-Olalde JI, Zuniga-Mayo VM, Marsch-Martinez N, de Folter S (2017a) Synergistic relationship between auxin and cytokinin in the ovary and the participation of the transcription factor SPATULA. *Plant Signal Behav* 12: e1376158

Reyes-Olalde, J. I., Zúñiga-Mayo, V. M., Serwatowska, J., Chavez Montes, R. A., Lozano-Sotomayor, P., Herrera-Ubaldo, H., Gonzalez-Aguilera K, Ballester P, Ripoll JJ, Ezquer I, Paolo D, Heyl A, Colombo L, Yanofsky MF, Ferrandiz C, Marsch-Martínez N, de Folter

S (2017b) The bHLH transcription factor SPATULA enables cytokinin signaling, and both activate auxin biosynthesis and transport genes at the medial domain of the gynoecium. *PLoS Genet* 13: e1006726

Rieu I, Ruiz-Rivero O, Fernandez-Garcia N, Griffiths J, Powers SJ, Gong F, Linhartova T, Eriksson S, Nilsson O, Thomas SG, Phillips AL, Hedden P (2008) The gibberellin biosynthetic genes *AtGA20ox1* and *AtGA20ox2* act, partially redundantly, to promote growth and development throughout the Arabidopsis lifecycle. *Plant J* 53:488–504

Riou-Khamlichi C, Huntley R, Jacqmard A, Murray JAH (1999) Cytokinin activation of *Arabidopsis* cell division through a D-type cyclin. *Science* 283: 1541–1544

Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26: 139–140

Sablowski R (2007) Flowering and determinacy in *Arabidopsis*. *J Exp Bot* 58: 899–907

Sablowski R (2015) Control of patterning, growth, and differentiation by floral organ identity genes. *J Exp Bot* 66: 1065–1073

Sakai H, Medrano LJ, Meyerowitz EM (1995) Role of *SUPERMAN* in maintaining *Arabidopsis* floral whorl boundaries. *Nature* 378: 199–203

Sakai H, Krizek BA, Jacobsen SE, Meyerowitz EM (2000) Regulation of *SUP* expression identifies multiple regulators involved in *Arabidopsis* floral meristem development. *Plant Cell* 12: 1607–1618

Sakuma Y, Maruyama K, Qin F, Osakabe Y, Shinozaki K, Yamaguchi-Shinozaki K (2006) Dual function of an *Arabidopsis* transcription factor DREB2A in water-stress-responsive and heat-stress-responsive gene expression. *Proc Natl Acad Sci USA* 103: 18822–18827

Sassi M, Vernoux T (2013) Auxin and self-organization at the shoot apical meristem. *J Exp Bot* 64: 2579–2592

Schaller GE, Bishopp A, Kieber JJ (2015) The Yin-Yang of Hormones: Cytokinin and Auxin interactions in plant development. *Plant Cell* 27: 44–63

Schluepmann H, van Dijken A, Aghdasi M, Wobbes B, Paul M, Smeekens S (2004) Trehalose mediated growth inhibition of Arabidopsis seedlings is due to trehalose-6-phosphate accumulation. *Plant Physiol* 135: 879–90

Schoof H, Lenhard M, Haecker A, Mayer KFX, Jürgens G, Laux T (2000) The stem cell population of *Arabidopsis* shoot meristems is maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes. *Cell* 100: 635–644

Schuetz M, Berleth T, Mattsson J (2008) Multiple MONOPTEROS-dependent pathways are involved in leaf initiation. *Plant Physiol* 148: 870–880

Sehra B, Franks RG (2015) Auxin and cytokinin act during gynoecial patterning and the development of ovules from the meristematic medial domain. *Wiley Interdiscip Rev Dev Biol* 4: 555–571

Shani E, Yanai O, Ori N (2006) The role of hormones in shoot apical meristem function. *Curr Opin Plant Biol* 9: 484–489

Sharma VK, Fletcher JC (2002) Maintenance of shoot and floral meristem cell proliferation and fate. *Plant Physiol* 129: 31–39

Smyth DR, Bowman JL, Meyerowitz EM (1990) Early flower development in Arabidopsis. *Plant Cell* 2: 755–767

Stals H, Inzé D (2001) When plant cells decide to divide. *Trends Plant Sci* 6: 359–364

Stamm P, Kumar PP (2013) Auxin and gibberellin responsive *Arabidopsis SMALL AUXIN UP RNA36* regulates hypocotyl elongation in the light. *Plant Cell Rep* 32: 759–769

Stirnberg P, Chatfield SP, Leyser HMO (1999) *AXR1* acts after lateral bud formation to inhibit lateral bud growth in Arabidopsis. *Plant Physiol* 121: 839–847.

Sun B, Xu Y, Ng KH, Ito T (2009) A Timing Mechanism for Stem Cell Maintenance and Differentiation in The *Arabidopsis* Floral Meristem. *Genes Dev* 23(15): 1791–1804

Sun B, Looi LS, Guo S, He Z, Gan ES, Huang J, Xu Y, Wee WY, Ito T (2014) Timing mechanism dependent on cell division is invoked by Polycomb eviction in plant stem cells. *Science* 343: 1248559

Sun B, Ito T (2015) Regulation of floral stem cell termination in *Arabidopsis*. *Front Plant Sci* 6: 17

Supek F, Bosnjak M, Skunca N, Smuc T (2011) REVIGO summarizes and visualizes long lists of gene ontology terms. *PLoS ONE* 6: e21800

Tashiro S, Tian CE, Watahiki MK, Yamamoto KT (2009) Changes in growth kinetics of stamen filaments cause inefficient pollination in *massugu2*, an auxin insensitive, dominant mutant of *Arabidopsis thaliana*. *Physiol Plant* 137: 175–187

Tatematsu K, Kumagai S, Muto H, Sato A, Watahiki MK, Harper RM, Liscum E, Yamamoto KT (2004) *MASSUGU2* encodes Aux/IAA19, an auxin-regulated protein that functions together with the transcriptional activator NPH4/ARF7 to regulate differential growth responses of hypocotyl and formation of lateral roots in *Arabidopsis thaliana*. *Plant Cell* 16: 379–393

Teo ZW, Song S, Wang YQ, Liu J, Yu H (2014) New insights into the regulation of inflorescence architecture. *Trends Plant Sci* 19: 158–165

Tian T, Liu Y, Yan H, You Q, Yi X, Du Z, Xu W, Su Z (2017) agriGO v2.0: a GO analysis toolkit for the agricultural community, 2017 update. *Nucleic Acids Res.* 45: W122–W129

Turner SR, Somerville CR (1997) Collapsed xylem phenotype of *Arabidopsis* identifies mutants deficient in cellulose deposition in secondary cell wall. *Plant Cell* 9: 689–701

Uemura A, Yamaguchi N, Xu Y, Wee WY, Ichihashi Y, Suzuki T, Shibata A, Shirasu K, Ito T (2017) Regulation of floral meristem activity through the interaction of *AGAMOUS*, *SUPERMAN*, and *CLAVATA3* in *Arabidopsis*. *Plant Reprod* 31: 89–105.

Veit B (2009) Hormone mediated regulation of the shoot apical meristem. *Plant Mol Biol* 69: 397–408

Vernoux T, Kronenberger J, Grandjean O, Laufs P, Traas J (2000) *PIN-FORMED 1* regulates cell fate at the periphery of the shoot apical meristem. *Development* 127: 5157–5165

Wakeel A, Ali I, Khan AR, Wu M, Upreti S. Liu D, Liu B, Gan Y (2018) Involvement of histone acetylation and deacetylation in regulating auxin responses and associated phenotypic changes in plants. *Plant Cell Rep* 37: 51–59

Wang F, Muto A, Van de Velde J, Neyt P, Himanen K, Vandepoele K, Van Lijsebettens M (2015) Functional analysis of the Arabidopsis *TETRASPANIN* gene family in plant growth and development. *Plant Physiol* 69: 2200–2214

Wang Y, Jiao Y (2018) Axillary meristem initiation-a way to branch out. *Curr Opin Plant Biol* 41: 61–66

Wellmer F, Alves-Ferreira M, Dubois A, Riechmann JL, Meyerowitz EM (2006) Genome-wide analysis of gene expression during early *Arabidopsis* flower development. *PLoS Genet* 2: 1012– 1024

Wellmer F, Graciet E, Riechmann JL (2014) Specification of floral organs in *Arabidopsis*. J Exp Bot 65: 1–9

Werner T, Motyka V, Strnad M, Schmülling T (2001) Regulation of plant growth by cytokinin. *Proc Natl Acad Sci USA* 98: 10487–10492

Williams L, Fletcher JC (2005) Stem cell regulation in the *Arabidopsis* shoot apical meristem. Curr Opin Plant Biol 8: 582–586

Wolters H, Jurgens G (2009) Survival of the flexible: Hormonal growth control and adaptation in plant development. *Nat Rev Genet* 10: 305–317

Wuest SE, O'Maoileidigh DS, Rae L, Kwasniewska K, Raganelli A, Hanczaryk K, Lohan AJ, Loftus B, Graciet E, Wellmer F (2012) Molecular basis for the specification of floral organs by APETALA3 and PISTILLATA. *Proc Natl Acad Sci USA* 109: 13452–13457

Wybouw B, De Rybel B (2019) Cytokinin—a developing story. *Trends Plant Sci* 24: 177–185

Xu Y, Prunet N, Gan ES, Wang Y, Stewart D, Wellmer F, Huang J, Yamaguchi N, Tatsumi Y, Kojima M, Kiba T, Sakakibara H, Jack TP, Meyerowitz EM, Ito T (2018) SUPERMAN regulates floral whorl boundaries through control of auxin biosynthesis. *EMBO J* 37: e97499

Xu Y, Yamaguchi N, Gan ES, Ito T (2019) When to stop: an update on molecular mechanisms of floral meristem termination. *J Exp Bot* 70: 1711–1718

Yamaguchi N, Wu MF, Winter CM, Berns MC, Nole-Wilson S, Yamaguchi A, Coupland G, Krizek BA, Wagner D (2013) A molecular framework for auxin-mediated initiation of flower primordia. *Dev Cell* 24: 271–282

Yamaguchi T, Nagasawa N, Kawasaki S, Matsuoka M, Nagato Y, Hirano HY (2004) The YABBY gene *DROOPING LEAF* regulates carpel specification and midrib development in *Oryza sativa*. *Plant Cell* 16: 500–509

Yamaguchi N, Winter CM, Wu MF, Kanno Y, Yamaguchi A, Seo M, Wagner D (2014a) Gibberellin acts positively then negatively to control onset of flower formation in Arabidopsis. *Science* 344: 638–641

Yamaguchi N, Wu MF, Winter CM, Wagner D (2014b) *LEAFY* and polar auxin transport coordinately regulate *Arabidopsis* flower development. *Plants* 3: 251–265

Yamaguchi N, Huang J, Xu Y, Tanoi K, Ito T (2017) Fine-tuning of auxin homeostasis governs the transition from floral stem cell maintenance to gynoecium formation. *Nat Commun* 8: 1125

Yamaguchi N, Huang J, Tatsumi Y, Abe M, Sugano SS, Kojima M, Takebayashi Y, Kiba T, Yokoyama R, Nishitani K, Sakakibara H, Ito T (2018) Chromatin-mediated feed-forward regulation of *YUCCA4* expression by AGAMOUS and CRABS CLAW directs gynoecium formation. *Nat Commun* 9: 5290

Yamaki S, Nagato Y, Kurata N, Nonomura K (2011) Ovule is a lateral organ finally differentiated from the terminating floral meristem in rice. *Dev Biol* 351: 208–216

Yang J, Zhang J, Huang Z, Wang Z, Zhu Q, Liu L (2002) Correlation of cytokinin levels in the endosperms and roots with cell number and cell division activity during endosperm development in rice. *Ann Bot* 90: 369–377

Yanofsky MF, Ma H, Bowman JL, Drews GN, Feldmann KA, Meyerowitz EM (1990) The protein encoded by the *Arabidopsis* homeotic gene *AGAMOUS* resembles transcription factors. *Nature* 346: 35–39

Yoshida S, Mandel T, Kuhlemeier C (2011) Stem cell activation by light guides plant organogenesis. *Genes Dev* 25: 1439–1450

Zhang W, Swarup R, Bennett M, Schaller GE, Kieber JJ (2013) Cytokinin induces cell division in the quiescent center of the *Arabidopsis* root apical meristem. *Curr Biol* 23: 1979–1989

Zhang K, Wang R, Zi H, Li Y, Cao X, Li D, Guo L, Tong J, Pan Y, Jiao Y, Liu R, Xiao L, Liu X (2018) AUXIN RESPONSE FACTOR3 regulates floral meristem determinacy by repressing cytokinin biosynthesis and signaling. *Plant Cell* 30: 324–346

Zhao Y, Christensen SK, Fankhauser C, Cashman JR, Cohen JD, Weigel D, Chory J (2001) A role for flavin monooxygenase-like enzymes in auxin biosynthesis. *Science* 291: 306–309

Zhao Z, Andersen SU, Ljung K, Dolezal K, Miotk A, Schultheiss SJ, Lohmann JU (2010) Hormonal control of the shoot stem-cell niche. *Nature* 465: 1089–1092

Zuniga-Mayo VM, Marsch-Martinez N, de Folter S (2012) JAIBA, a class-II HD-ZIP transcription factor involved in the regulation of meristematic activity, and important for correct gynoecium and fruit development in *Arabidopsis*. *Plant J* 71: 314–326

Zürcher E, Tavor-Deslex D, Lituiev D, Enkerli K, Tarr PT, Müller B (2013) A robust and sensitive synthetic sensor to monitor the transcriptional output of the cytokinin signaling network in planta. *Plant Physiol* 161: 1066–1075

7. LIST OF PUBLICATIONS

Lee ZH, Yamaguchi N, Ito T (2018) Using CRISPR/Cas9 System to Introduce Targeted Mutation in Arabidopsis. In: Yamaguchi N. (eds) Plant Transcription Factors. *Methods in Molecular Biology* vol 1830, Humana Press, New York, NY

Lee ZH, Hirakawa T, Yamaguchi N, Ito T (2019) The Roles of Plant Hormones and Their Interactions with Regulatory Genes in Determining Meristem Activity. *Int J Mol Sci* 20: 4065

Lee ZH, Tatsumi Y, Ichihashi Y, Suzuki T, Shibata A, Shirasu K, Yamaguchi N, Ito T (2019) CRABS CLAW and SUPERMAN coordinate hormone-, stress- and metabolic-related gene expression during Arabidopsis stamen development. *Front Ecol Evol* 7: 437