The ERECTA receptor kinase regulates *Arabidopsis* shoot apical meristem size, phyllotaxy and floral meristem identity

Tali Mandel¹, Fanny Moreau², Yaarit Kutsher¹, Jennifer C. Fletcher³, Cristel C. Carles² and Leor Eshed Williams¹,*

**ABSTRACT**

In plants, the shoot apical meristem (SAM) serves as a reservoir of pluripotent stem cells from which all above ground organs originate. To sustain proper growth, the SAM must maintain homeostasis between the self-renewal of pluripotent stem cells and cell recruitment for lateral organ formation. At the core of the network that regulates this homeostasis in *Arabidopsis* are the WUSCHEL (WUS) transcription factor specifying stem cell fate and the CLAVATA (CLV) ligand-receptor system limiting *WUS* expression. In this study, we identified the ERECTA (ER) pathway as a second receptor kinase signaling pathway that regulates *WUS* expression, and therefore shoot apical and floral meristem size, independently of the CLV pathway. We demonstrate that reduction in class III HD-ZIP and ER expression, and therefore *WUS* expression, and therefore shoot apical and floral meristem size requires tightly controlled balance between cell proliferation in the central zone (CZ) and cell recruitment for lateral organ formation. At the core of the network is the WUSCHEL (WUS) transcription factor, which specifies the pluripotent state in the CZ, and the CLAVATA (CLV) ligand-receptor system that limits *WUS* expression to maintain a stable stem cell population size (Brand et al., 2000; Carles and Fletcher, 2003; Schoof et al., 2000). This negative-feedback loop is further fine-tuned by many other signaling pathways and molecular mechanisms such as epigenetic factors and other receptor systems (Perales and Reddy, 2012). Extensive genetic screens have identified numerous mutants with abnormal shoot and/or floral meristem size, uncovering many genes that participate in the regulation of meristem size and function (DeYoung et al., 2006; Fletcher, 2001; Furner and Pumfrey, 1992; Kaya et al., 2001; Laux et al., 1996; Long and Barton, 1998; Vidaurre et al., 2007; Wu et al., 2005). Nevertheless, some genes may act in partially redundant parallel pathways and therefore have not yet been uncovered.

Redundancy in SAM regulation is illustrated by the three members of the *Arabidopsis* ERECTA (ER) gene family that encode leucine-rich-repeat receptor kinases (Torigai et al., 1996), which are broadly expressed and play redundant roles in diverse aspects of plant development (Shpak et al., 2004; Uchida et al., 2012; Yokoyama et al., 1998). Mutations in the ER gene enhance the SAM phenotypes of other mutants (Durbak and Tax, 2011; Uchida et al., 2011), and it has been suggested that the ER family regulates meristem homeostasis by buffering cytokinin responsiveness in the SAM (Uchida et al., 2013).

In the SAM peripheral zone, leaves initiate with a regular spacing that determines the phyllotaxis (Sussex, 1998; Traas, 2013). An accepted model for phyllotaxis regulation is that high auxin level accumulation at a precise position in the PZ triggers organ initiation. Local auxin maxima are achieved by auxin polar transporters that actively deplete the auxin from nearby cells (Vieten et al., 2007), resulting in low concentrations in the vicinity of the existing organ primordia. This allows a new auxin maximum, and therefore a new organ primordium, to develop only at a specific minimum distance from the pre-existing primordia (Jönsson et al., 2006; Lohmann et al., 2010; Smith et al., 2006).

Once the SAM switches from vegetative to reproductive phase, lateral organ identity changes and the inflorescence meristem (IM) produces floral meristems (FM) on its flanks. Each FM provides all the cells to form the four whorls of floral organs – sepals, petals, stamens and carpels – before ceasing its meristematic activity. The identity of each floral organ is specified by different combinations of transcription factors encoded by several classes of genes that form the basis for the ABCDE[D] model of flower development (Bowman et al., 2012; Krizek and Fletcher, 2005). In *Arabidopsis*, the C-class floral homeotic gene *AGAMOUS* (*AG*) is expressed in the center of the FM and specifies carpel identity in whorl four, while also acting

**KEY WORDS:** AGAMOUS (AG), Cell fate, WUSCHEL (WUS)

**INTRODUCTION**

In higher plants, all the aerial organs are produced post-embryonically through the activity of a pluripotent stem cell reservoir that resides in the apex of the meristem at the shoot tip (Carles and Fletcher, 2003; Sablowski, 2004). These cells divide slowly to renew themselves and to supply cells to the interior or to the periphery of the shoot apical meristem (SAM), where their progeny divide more rapidly to provide cells for the stem and for lateral organ primordia (Williams and Fletcher, 2005). The ability of the SAM to produce lateral organs while maintaining the appropriate size requires tightly controlled balance between cell proliferation in the central zone (CZ) and cell recruitment for primordium initiation

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Received 23 October 2013; Accepted 8 December 2013
with B-class genes to specify stamen identity in whorl three (Coen and Meyerowitz, 1991). During wild-type flower development, WUS is expressed in stage 1 floral primordia (stages according to Smyth et al., 1990) and is repressed once the carpel primordia form at stage 6, which results in FM termination (Lenhard et al., 2001). WUS binds directly to the second intron of AG and together with the LEAFY transcription factor, activates AG expression at floral stage 3 (Busch et al., 1999).

We have previously reported on the role of the Arabidopsis microRNA miR166g in regulating WUS-dependent SAM activity (Williams et al., 2005). We demonstrated that in jaba-1D (jba-1D) plants, overexpression of miR166g causes a decrease in the transcript levels of several class III homeodomain-leucine-zipper (HD-ZIPIII) target genes, which leads to a dramatic increase in WUS transcript levels and expansion of its expression domain, resulting in SAM enlargement (Williams et al., 2005).

To uncover novel genes that act redundantly with the HD-ZIPIII pathway in meristem regulation, we conducted a genetic screen to identify second-site mutations that modified the jba-1D enlarged SAM phenotypes and identified a mutation in the ER gene as an enhancer. jba-1D plants carrying the mutated ER modifier exhibit extremely enlarged SAMs with altered leaf phyllotaxis and ectopic carpels forming directly from the inflorescence meristem. We demonstrate that, owing to aberrant ER function, the WUS gene is upregulated, leading to ectopic AG expression and subsequent ectopic carpel formation. Our results indicate that the ER gene regulates SAM size in a genetic pathway parallel to those of the HD-ZIPIII and CLV pathways, and that ER has a similar function in the floral meristem, where it plays a role in regulating meristem size and homeostasis.

RESULTS

A mutation in the ERECTA gene enhances the jba-1D/+ enlarged meristem phenotype

Arabidopsis jba-1D plants display pleiotropic developmental phenotypes, in which the most prominently is a dramatic enlargement of the SAM that results in extremely fasciated stems and enlarged IMs (Williams et al., 2005). We demonstrated that in jaba-1D/+ parental background and the modifier was shown to act as a single nuclear trait. jM1 mutant was backcrossed twice into the jba-1D/+ parental background and the modifier was shown to act as a single nuclear trait. jM1 homozygous single mutant plants display round leaves with short petioles and a compact inflorescence with flowers clustering at the top (supplementary material Fig. S2). This phenotype closely resembles those of Landsberg erecta (Ler) plants (Tori et al., 1996), although jM1 plants are in the Col-0 genetic background. This resemblance led us to investigate whether the modifier mutation in jM1 was in the ER gene. Sequencing the ER gene from jM1 individuals confirmed the presence of a missense mutation that causes an isoleucine to threonine substitution at amino acid 750 in the cytoplasmic serine/threonine protein kinase domain (Lease et al., 2001). We performed a complementation assay (supplementary material Fig. S3), demonstrating that the missense mutation in the ER gene causes the strong enhancement of the jba-1D/+ phenotype. We therefore named this recessive allele er-20.

Analysis of jba-1D/+ er-20 plants revealed two distinct morphological phenotypes (Figs 1, 3). At the vegetative stage, jba-1D/+ er-20 seedlings lack the typical spiral phyllotaxis displayed by wild-type and jba-1D/+ seedlings (Fig. 1A-C,H,I), and the SAMs of jba-1D/+ er-20 plants show additional enlargement relative to jba-1D/+ plants (Fig. 1J-L). In wild-type and jba-1D/+ seedlings (Fig. 1A-C), the first pair of true rosette leaves emerges simultaneously in a decussate pattern, and all subsequent leaves arise following a spiral phyllotaxy characterized by the ‘golden angle’ of about 137.5°. Sixteen-day-old jba-1D er-20 seedlings exhibit similar phenotype to jba-1D seedlings in their first two radial leaves and successive curled leaves, although somewhat more severe (Fig. 1D,F). By contrast, jba-1D/+ er-20 seedlings exhibit a decussate pattern for the first pair of leaves followed by a transition to a whorled phyllotaxis (Fig. 1H,I). Four rosette leaves arise at the same time in an iterative manner throughout the vegetative phase, as do the cauline leaves (supplementary material Fig. S1). We examined the leaf primordia initiation by performing scanning electron microscopy (SEM) on jba-1D/+ er-20 seedlings and found that the four primordia emerge simultaneously at the SAM periphery (Fig. 1G), suggesting the concurrent establishment of auxin maxima at four distinct locations around the SAM (Fig. 1N).

Next, we examined at the histological level the SAM of wild-type, jba-1D/+ and jba-1D/+ er-20 plants. Sections through 9-day-old seedlings and found that the four primordia emerge simultaneously at the SAM periphery (Fig. 1G), suggesting the concurrent establishment of auxin maxima at four distinct locations around the SAM (Fig. 1N).
seedlings revealed that the er-20 mutation enhances the jba-1D/+ enlarged SAM phenotype (Fig. 1K) leading to an extremely enlarged meristem (Fig. 1L). In some cases, the huge meristem can be seen easily with the naked eye (Fig. 1M). The jba-1D/+ er-20 SAM enlargement and its altered phyllotaxis are consistent with reports showing that altered SAM size can lead to changes in phyllotaxis (Clark et al., 1993; Giulini et al., 2004; Laufs et al., 1998a; Medford et al., 1992; Leyser and Furner, 1992; Takahashi et al., 2002).

To further test the hypothesis that meristem enlargement is accompanied by simultaneous establishment of several auxin maxima (Fig. 1N), we performed RT-qPCR on 8-day-old whole seedlings using gene specific primers for SHOOT MERISTEMLESS (STM) and GH3.3 (Fig. 2). STM encodes a class I KNOX transcription factor that is expressed throughout the SAM and promotes meristem cell proliferation (Long et al., 1996). The GH3.3 gene is an early auxin-responsive gene that is expressed during primordium initiation, from the first sign of cell bulging at the SAM periphery to leaf plastochron 4 (Efroni et al., 2008; Mallory et al., 2005). The results showed an approximately twofold increase in STM expression levels in jba-1D/+ er-20 compared with jba-1D/+ seedlings, consistent with the meristem enlargement phenotype (Fig. 1L). The moderate increase in STM levels reflects the small fraction of meristematic cells within the whole seedling samples. With a higher proportion of meristematic cells in jba-1D/+ er-20, we would expect a reduction in GH3.3 transcript levels due to dilution of the GH3.3-expressing primordia cells within the whole seedlings. Yet RT-qPCR analysis showed an approximately twofold increase in GH3.3 expression in jba-1D/+ er-20 plants (Fig. 2). This indicates that the jba-1D/+ er-20 SAM contains more cells expressing the early auxin-responsive GH3.3 gene, consistent with the formation of additional auxin maxima for organ initiation. Our results therefore indicate that ER restricts SAM size during vegetative development, and that restricting SAM size may prevent the production of more than one auxin maximum at a time.

It has recently been suggested that the SAM possesses a functional buffering mechanism regulated by the ER receptor kinase family to maintain CLV3 homeostasis regardless of an increase in WUS expression induced by cytokinin treatment (Uchida et al., 2013). We therefore assessed the WUS and CLV3 expression levels by RT-qPCR. We observed a dramatic increase in WUS expression in jba-1D/+ er-20 compared with jba-1D/+ seedlings, whereas CLV3 expression levels were not significantly different (Fig. 2). In the absence of functional ER we would expect increased levels of CLV3 transcripts because of the increase in WUS expression. Therefore, this result raises the possibility that the jba-1D/+ er-20 meristem contains more cells of all types and that the ratio between the whole meristem tissue and the CLV3-expressing stem cells is maintained.

To test whether other ER family members compensate for the mutation in ER, we analyzed the expression of ER, ERECTA LIKE 1 and ERECTA LIKE 2 (ERL1 and ERL2). We found no differential expression for ER and ERL1 but a 1.7-fold increase in ERL2 expression levels in jba-1D/+ er-20 relative to jba-1D/+ plants. These results are consistent with the view that ER genes redundantly regulate the vegetative SAM (Uchida et al., 2013) and imply that
ERL2 upregulation compensates for the mutation in ER in maintaining CLV3 homeostasis.

**jba-1D/+ er-20 inflorescence meristems ectopically produce multi-fused carpels**

During reproductive development, mature *jba-1D/+ er-20* plants form ectopic, multi-fused carpels around the IM (Fig. 3). Like *jba-1D* plants, *jba-1D/+ er-20* form fasciated IMs that produce numerous flowers (Fig. 3A). However, 4-5 weeks after bolting, the inflorescence starts to form carpels at the periphery in place of complete flowers (Fig. 3A,D). Initially, a single or a few fused carpels emerge in a spiral phyllotaxy around the inflorescence (Fig. 3D) and the structures gradually develop as multi-fused carpels that encircle the IM almost as one piece (Fig. 3G,F). Iterations of whorls of ectopic multi-carpels form internal to one another, ultimately resulting in a massive structure of carpels within carpels at the top of the inflorescence stem (Fig. 3C,F; supplementary material Fig. S4C). The IM continues to produce carpels even 16 weeks after bolting, after the rest of the plant has senesced (supplementary material Fig. S5).

In *Arabidopsis*, each flower primordium initiates at the IM periphery as a small bulge of cells that divide and expand in three dimensions, generating a hemispherical primordium that becomes separated from the IM by a small groove (Kwiatkowska, 2006; Smyth et al., 1990). This primordium represents a *de novo* FM from which the four types of floral organs will arise in concentric rings called whorls. To determine whether the *jba-1D/+ er-20* ectopic carpels develop from FMs or arise directly from the IM, we performed SEM analysis (Fig. 3G-M). Initially, the *jba-1D/+ er-20* IM develops normal flowers, until a sharp transition to the formation of carpels as lateral organs occurs (Fig. 3G). Each carpel primordium arises directly from the meristem as a bulge on its edge that gradually becomes delineated from the IM (Fig. 3G,J,K; supplementary material Fig. S4A). We conclude that the ectopic carpels initiate directly from the *jba-1D/+ er-20* IM, indicating a change in cell identity from FM fate to carpel fate at the IM periphery.

The wild-type *Arabidopsis* gynoecium initiates as a raised rim around the center of the FM that grows and forms a hollow cylinder (Alvarez-Buylla et al., 2010). Very rarely the *jba-1D/+ er-20* inflorescence forms typical cylindrical gynoecium structures in the center of the huge IM, in addition to ectopic carpels on the flanks (supplementary material Fig. S4B). All the ectopic carpels on the *jba-1D/+ er-20* IM consist entirely of carpel-specific tissues, but they fail to form an open-ended tube that will fuse to generate the seed-bearing ovaries of a mature gynoecium. In each ectopic carpel, a valve flanked with valve margin, style bearing stigmatic papillae, replum and septum tissues are visible (Fig. 3H,I,L,M; supplementary material Fig. S4C). The adaxial side of the fused carpels shows reduced septum tissues with two rows of ovule primordia growing on opposite sides (Fig. 3L) and in the unfused mature gynoecium the ovule funiculus, inner and outer integuments are observed (Fig. 3M).

In summary, these observations indicate that *miR166g* overexpression and aberrant ER function in *jba-1D/+ er-20* plants lead to an extreme IM enlargement and to the formation of ectopic carpel structures in place of functional FMs. Therefore, *miR166g*
and ER function are together required to restrict IM size and specify floral meristem identity.

**Many gynoecium development genes are upregulated in jba-1D/+ er-20 IMs**

To further explore the hypothesis that jba-1D/+ er-20 IMs develop carpels on the periphery due to a switch in cell identity, we carried out an RNA-Seq analysis on jba-1D/+ and jba-1D/+ er-20 IMs. We collected 30 dissected inflorescences from each genotype at 4 weeks after bolting, once the first plant began to exhibit ectopic carpel formation. All the flowers were removed, leaving either bare meristems or bare meristems with undetachable primordia for jba-1D/+ IMs, or bare meristems with a mixture of un-detachable floral and carpel primordia for jba-1D/+ er-20 IMs. The meristems were independently pooled for mRNA isolation, sequencing and differential expression analysis (supplementary material Table S1).

As expected, WUS expression levels are fourfold higher in jba-1D/+ er-20 IMs compared with jba-1D/+ IMs, even though in jba-1D/+ the remaining floral primordia on the IMs contributed to the total WUS transcript levels, whereas in jba-1D/+ er-20 the source was mainly cells from the IMs themselves. This result indicates a much higher level of WUS expression in jba-1D/+ er-20 IMs, and demonstrates that ER negatively regulates WUS in the IM. As in the vegetative SAM, the GH3.3 gene is upregulated in jba-1D/+ er-20 IMs (Table 1), again suggesting the occurrence of additional auxin maxima around the IM. For the ER genes, although ER and ERL2 are not differentially expressed, ERL1 expression levels were threefold higher in jba-1D/+ er-20 IMs.

In addition, many of the CE[D] class floral organ identity genes are upregulated in jba-1D/+ er-20 plants (Table 1). For example, carpels are specified by the class C MADS-box gene AG, along with the related SHATTERPROOF1 (SHP1), SHP2 and SEEDSTICK (STK) genes (Becker and Theissen, 2003; Pinyopich et al., 2003). STK also acts redundanty with SHP1 and SHP2 to promote ovule identity determination (Colombo et al., 2010). Because ectopic carpels develop directly from the jba-1D/+ er-20 IMs, we expected these four genes to be highly expressed. Indeed, AG and SHP1 are both upregulated sevenfold in jba-1D/+ er-20 compared with jba-1D/+ IMs, SHP2 is elevated 65-fold, and STK is elevated 40-fold (Table 1). In addition, three SEPALLATA (SEP) genes that function redundantly as class E genes are upregulated in jba-1D/+ er-20 IMs: SEP1 by fourfold, SEP2 by threefold and SEP3 by 2.8-fold. The AG transcription factor orchestrates the expression of numerous downstream genes (Gómez-Mena et al., 2005; Ito et al., 2007; Ito et al., 2004). We found that many AG immediate targets and directly bound genes are upregulated in jba-1D/+ er-20 IMs, including CRC, SEP3 and SHP1 (Gómez-Mena et al., 2005) (supplementary material Table S2). In summary, many early gynoecium specification genes are upregulated in jba-1D/+ er-20 plants.

Together, our morphological and molecular datasets indicate that the ER gene is required to constrain WUS mRNA expression in jba-1D/+ meristems. We also find that it limits the transcription levels of floral organ identity genes, such as AG. We propose that in jba-1D/+ er-20 IMs, high levels of WUS transcripts lead to ectopic activation of AG expression. At the IM periphery, where the cells are competent to develop into flowers, this ectopic AG expression activates its downstream genes, leading to a switch in cell identity and the initiation of carpel formation.

**jba-1D/+ er-20 IMs show ectopic expression of WUS and AG**

In jba-1D/+ er-20 plants, the vegetative SAM and IM exhibit elevated levels of WUS expression, indicating that ER negatively regulates WUS. Yet, it is unclear whether ER tunes WUS transcription levels or limits its expression domain. To analyze this, we performed RNA in situ hybridization with a WUS probe (Fig. 4).

In wild-type Col IMs, WUS is expressed in the central cells of the IM and the FM, a region termed the organizing center (OC) (Fig. 4A). In jba-1D/+ IMs, the WUS expression pattern is similar to that of clv3-2 IMs (Fig. 4B), in which the domain expands laterally and upward into the outermost two to three layers across the meristem (Brand et al., 2000). In jba-1D/+ er-20 IMs, not only is the WUS signal is more intense than in jba-1D/+ IMs, it expands further into the interior cell layers (Fig. 4C). Thus, ER is a regulator that contributes both to limiting WUS transcript levels and to restricting the WUS expression domain to the OC. We have previously reported that wus-1 jba-1D meristems are indistinguishable from wus-1 meristems (Williams et al., 2005), indicating that WUS activity is absolutely required to obtain the jba SAM phenotypes. As wus-1 suppresses the jba-1D/+ phenotypes, it will also suppress the jba-1D/+ er-20 phenotypes, as er-20 single mutants do not exhibit ectopic carpel formation.

To further investigate the hypothesis that ectopic AG expression can lead to a switch in cell identity at the IM periphery, we tested whether ectopic AG expression accompanies the initiation of ectopic carpels in jba-1D/+ er-20 IMs using RNA in situ hybridization. In wild-type plants, AG mRNA is absent from the IM but is first detected in the center of stage 3 floral primordia prior to stamen and carpel initiation (Fig. 4D,E,H). AG expression is later confined to the inner two whorls of the flower. At stage 9, AG mRNA is detected at low levels in the valves of the developing carpels and at high levels in ovule primordia (Drews et al., 1991; Ito et al., 2004; Yanofsky et al., 1990). AG expression is unaltered in jba-1D/+ floral primordia, but unexpectedly we also detect a faint signal in the L1, L2 and uppermost L3 cell layers across the IM (Fig. 4F,G), indicating that AG is ectopically expressed in jba-1D/+ IMs. However, in jba-1D/+ Ler and jba-1D/+ er-20 IMs, AG expression is much more intense in the outer cell layers (Fig. 4I-M) than in jba-1D/+ IMs, and spreads to more layers inwards. Furthermore, jba-1D/+ er-20 ectopic carpels show strong AG expression on their adaxial side and in ovule primordia (Fig. 4K,M). These results are consistent with our proposed scenario that strong ectopic AG expression at the IM periphery leads to a cell identity switch from flower founder cell identity to carpel cell identity.

Next, we tested whether AG is required for the ectopic carpel phenotype by crossing jba-1D/+ er-20 to ag-1-null mutant plants. The stems of jba-1D/+ er-20 ag-1 plants are extremely fasciated and the IMs develop flowers lacking stamens and carpels (Fig. 5K,L). However, the IMs never generate ectopic carpel structures on their flanks, even late in their life cycles (Fig. 5K,L), indicating that AG is required to confer the jba-1D/+ er-20 ectopic carpel phenotype.

**Effect of ER gene family mutations on the jba-1D/+ phenotype**

To determine whether other mutations in ER could generate phenotypes like those of jba-1D/+ er-20 plants, we analyzed the genetic interactions between jba-1D/+ and either the Ler accession or the Col accession carrying the er-2 allele. Ler contains a point mutation in the ER kinase domain (Torii et al., 1996) and is likely to be a null allele (Lease et al., 2001). jba-1D/+ Ler plants display two types of phenotypes. The first is a less severe version of the jba-1D/+ er-20 carpel phenotype, where ectopic carpel formation ceases as the plants senesce (Fig. 5A,B). The second is the formation of a macroscopic IM with several whorls of ectopic carpels that encircle the IM but do not form reiterative multi-fused carpels structures.
### Table 1. Selected upregulated genes in jba-1D/+ er-20 compared with jba-1D/+ IMs

<table>
<thead>
<tr>
<th>Accession</th>
<th>Gene name and description</th>
<th>jba+/− (RPKM)</th>
<th>jba-1D/+ er-20 (RPKM)</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT2G21650</td>
<td>MATERNAL EFFECT EMBRYO ARREST 3, MEE3 (involved during early morphogenesis)</td>
<td>0.03</td>
<td>15.5</td>
<td>512</td>
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<tr>
<td>AT5G09750</td>
<td>HEC3, AGL5 (involved in transmitting tissue development, carpel formation, regulation of transcription)</td>
<td>0.17</td>
<td>41</td>
<td>223</td>
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<tr>
<td>AT2G01500</td>
<td>WUSCHEL RELATED HOMEOBOX, WOX6 (gene that delays differentiation and maturation of primordia and regulates ovule patterning)</td>
<td>0.03</td>
<td>6.3</td>
<td>194</td>
</tr>
<tr>
<td>AT2G21450</td>
<td>CHROMATIN REMODELING 34, CHR34</td>
<td>0.34</td>
<td>9.78</td>
<td>28</td>
</tr>
<tr>
<td>AT1G25330</td>
<td>CES, CESTA (a positive regulator of brassinosteroid biosynthesis)</td>
<td>0.28</td>
<td>22.4</td>
<td>78</td>
</tr>
<tr>
<td>AT2G42830</td>
<td>SHATTERPROOF 2, SHP2, AGL5 (involved in fruit development and a putative direct target of AG)</td>
<td>0.54</td>
<td>35.7</td>
<td>65</td>
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<tr>
<td>AT5G65080</td>
<td>AGL68 (regulates flowering time)</td>
<td>0.22</td>
<td>13.3</td>
<td>56</td>
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<td>AT4G09960</td>
<td>AGL11, STK (TF expressed in the carpel and ovules)</td>
<td>0.24</td>
<td>46</td>
<td>40</td>
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<tr>
<td>AT2G33880</td>
<td>WUSCHEL RELATED HOMEOBOX, WOX9, STIP (required for meristem growth and development and acts through positive regulation of WUS)</td>
<td>1.93</td>
<td>75.5</td>
<td>39</td>
</tr>
<tr>
<td>AT3G62820</td>
<td>Plant invertase/pectin methylesterase inhibitor superfamily protein</td>
<td>1.9</td>
<td>49.4</td>
<td>26</td>
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<tr>
<td>AT5G18000</td>
<td>VERDANDI, VOD (a direct target of the MADS domain ovule identity complex)</td>
<td>0.78</td>
<td>17.3</td>
<td>22</td>
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<tr>
<td>AT5G23260</td>
<td>AGAMOUS-LIKE 32, AGL32 (shown to be necessary for determining the identity of the endothelial layer within the ovule)</td>
<td>0.09</td>
<td>2.15</td>
<td>23</td>
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<tr>
<td>AT3G50330</td>
<td>HEC2 (involved in ovary septum development, transmitting tissue development, carpel formation, regulation of transcription)</td>
<td>0.89</td>
<td>15.3</td>
<td>17</td>
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<tr>
<td>AT5G21150</td>
<td>ARGAONTE 9 (AG9-dependent sRNA silencing is crucial to specify cell fate in the Arabidopsis ovule)</td>
<td>5.98</td>
<td>98.6</td>
<td>16</td>
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<tr>
<td>AT5G41410</td>
<td>BELL 1 (homeodomain protein required for ovule identity)</td>
<td>0.83</td>
<td>12.5</td>
<td>15</td>
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<tr>
<td>AT2G26440</td>
<td>Plant invertase/pectin methylesterase inhibitor superfamily</td>
<td>5.5</td>
<td>53.7</td>
<td>8.2</td>
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<tr>
<td>AT3G51060</td>
<td>STYLISH 1 (promotes gynoecium, stamen and leaf development)</td>
<td>1.2</td>
<td>11.3</td>
<td>9</td>
</tr>
<tr>
<td>AT1G23420</td>
<td>INNER NO OUTER, INO (may be required for polarity determination in the central part of the ovule)</td>
<td>0</td>
<td>5.4</td>
<td>5</td>
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<tr>
<td>AT4G18960</td>
<td>AGAMOUS, AG (specifies floral meristem and carpel and stamen identity)</td>
<td>9.4</td>
<td>70</td>
<td>7</td>
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<tr>
<td>AT3G58780</td>
<td>SHATTERPROOF 1, SHP1 (required for fruit dehiscence. Controls dehiscence zone differentiation)</td>
<td>7.2</td>
<td>49</td>
<td>7</td>
</tr>
<tr>
<td>AT1G69180</td>
<td>CRABS CLAW, CRC (putative TF involved in specifying abaxial cell fate in the carpel)</td>
<td>22</td>
<td>142.3</td>
<td>7</td>
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<tr>
<td>AT3G18010</td>
<td>WUSCHEL RELATED HOMEOBOX, WOX1 (encodes a WUSCHEL-related homeobox gene family member)</td>
<td>1.78</td>
<td>10.7</td>
<td>6</td>
</tr>
<tr>
<td>AT1G66350</td>
<td>RGL1 (negative regulator of GA responses, member of GRAS family of transcription factors; involved in flower and fruit development)</td>
<td>10.5</td>
<td>53</td>
<td>5</td>
</tr>
<tr>
<td>AT3G23130</td>
<td>SUPERMAN, SUP (flower-specific gene controlling the boundary of the stamen and carpel whorls)</td>
<td>2.15</td>
<td>9.25</td>
<td>5</td>
</tr>
<tr>
<td>AT3G22880</td>
<td>ARLIM15 (expression is restricted to pollen mother cells in anthers and to megaspore mother cells in ovules)</td>
<td>6.5</td>
<td>28.3</td>
<td>5</td>
</tr>
<tr>
<td>AT5G15800</td>
<td>SEPALLATA1, SEP1 (encodes a MADS box transcription factor involved flower and ovule development)</td>
<td>22.3</td>
<td>96</td>
<td>4</td>
</tr>
<tr>
<td>AT5G11320</td>
<td>YUCCA4, YUC4 (belongs to the YUC gene family. Involved in auxin biosynthesis and plant development)</td>
<td>2.2</td>
<td>8.8</td>
<td>4</td>
</tr>
<tr>
<td>AT3G57670</td>
<td>NO TRANSMITTING TRACT, NTT (TF specifically expressed in the transmitting tract and involved in transmitting tract development and pollen tube growth)</td>
<td>4.9</td>
<td>19.8</td>
<td>4</td>
</tr>
<tr>
<td>AT2G17950</td>
<td>WUSCHEL, WUS (homeobox gene controlling the stem cell pool; required to keep the stem cells in an undifferentiated state)</td>
<td>1.2</td>
<td>4.9</td>
<td>4</td>
</tr>
<tr>
<td>AT1G24260</td>
<td>SEPALLATA3, SEP3 (member of the MADS box transcription factor family; SEP3 is redundant with SEP1 and 2)</td>
<td>28.7</td>
<td>103.7</td>
<td>4</td>
</tr>
<tr>
<td>AT5G66350</td>
<td>SHORT INTERNODES, SHI (function synergistically with other SHI-related genes promote gynoecium, stamen and leaf development)</td>
<td>1.8</td>
<td>5.8</td>
<td>3.3</td>
</tr>
<tr>
<td>AT5G62230</td>
<td>ERECTA-LIKE 1, ERL1 (encodes a receptor-like kinase; along with ERL2 functionally compensates for loss of ER during integument development)</td>
<td>16.6</td>
<td>48.2</td>
<td>3</td>
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<tr>
<td>AT4G36920</td>
<td>APETALA 2, AP2 (involved in the specification of floral organ identity, establishment of floral meristem identity, suppression of floral meristem indeterminacy, and development of the ovule and seed coat)</td>
<td>7.7</td>
<td>29.9</td>
<td>3.9</td>
</tr>
<tr>
<td>AT2G23170</td>
<td>GH3.3 (encodes an IAA-amido synthase that conjugates Asp and other amino acids to auxin in vitro)</td>
<td>10.6</td>
<td>26.7</td>
<td>2.5</td>
</tr>
<tr>
<td>AT5G17810</td>
<td>WUSCHEL RELATED HOMEOBOX, WOX12 (encodes WUSCHEL RELATED HOMEOBOX gene family member with 65 amino acids in its homeodomain)</td>
<td>2.8</td>
<td>9.7</td>
<td>3.5</td>
</tr>
<tr>
<td>AT3G02310</td>
<td>SEPALLATA 2, SEP2 (MADS-box protein, binds K domain of AG in vivo)</td>
<td>56.6</td>
<td>155.7</td>
<td>2.8</td>
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<tr>
<td>AT1G68640</td>
<td>PERIANTHIA, PAN (encodes bZIP-transcription factor, and is essential for AG activation in early flowers of short-day-grown plants)</td>
<td>15.1</td>
<td>32</td>
<td>2</td>
</tr>
</tbody>
</table>

*Gene names and descriptions are from TAIR (http://www.arabidopsis.org/index.jsp). RPKM, reads per gene kilobase per million reads.*
The er-2 allele is an X-ray-induced frameshift mutation (Hall et al., 2007). jba-1D/+ er-2 IMs showed the same ectopic carpel formation phenotype as jba-1D/+er-20 IMs (Fig. 5D,E). These results indicate that loss of ER function is responsible for the jba-1D/+ modifier phenotypes.

The ER genes redundantly regulate SAM function (Shpak et al., 2004; Uchida et al., 2013). Therefore, we asked whether ERL1 upregulation in jba-1D/+ er-20 IMs partially compensates for the mutation in ER. We generated triple mutants by crossing jba-1D/+er-20 to the erl1-null mutant (SALK_081669) and observed two types of enhancement according to the jba-1D background (hemi-or homozygous) (Fig. 5F-J). In jba-1D er-20 erl1 plants, the ectopic carpels are evident after only four or five normal flowers have formed (Fig. 5F-H). jba-1D/+ er-20 erl1 plants display enhanced fasciation of the stems, which appear to be flattened with more multi-fused carpels, suggesting the presence of an enlarged wider IM (Fig. 5I,J). Consistent with the expression of ER genes in the shoot apex (Shpak et al., 2004; Uchida et al., 2013; Yokoyama et al., 1998), these results implicate the ER family members in IM size regulation.

The ER gene regulates meristem development in a CLV-independent pathway

Several er mutations enhance the SAM enlargement and gynoecium phenotypes of the CLV pathway mutants (Durbak and Tax, 2011). To test whether the er-20 mutation acts in the CLV pathway, we generated jba-1D/+ er-20 clv3-2 plants (Fig. 6). Compared with wild-type Col and Ler (Fig. 6A,B) inflorescences, both clv3-2-null and jba-1D/+ er-20 inflorescences are enlarged and produce supernumerary flowers (Fig. 6C,E,L). jba-1D/+ er-20 clv3-2 plants, however, form enormously enlarged IMs that produce few flowers before shifting to carpel formation at the periphery (Fig. 6O). This synergistic interaction between clv3-2 and jba-1D/+ er-20 indicates that CLV3, HD-ZIPIII and ER regulate SAM size in separate genetic pathways (supplementary material Fig. S6).

The jba-1D/+ er-20 clv3-2 gynoecium phenotypes also show a synergistic effect. Wild-type, jba-1D/+ and jba-1D/+ er-20 gynoecia consist of two fused carpels, whereas clv3-2 gynoecia are composed of four to six carpels (Fig. 6H). jba-1D/+ er-20 clv3-2 gynoecia are short and consist of 10 to 14 carpels, causing the fruit to have a sphere-like appearance (Fig. 6H,I). Because carpel number is tightly associated with FM size (Clark et al., 1993), these results indicate a direct role for ER in FM size regulation. In wild-type plants, stem cell termination in the FM is tightly coupled to the formation of carpel primordia, resulting in a mature gynoecium with two fused carpels (Fig. 6H) (Lenhard et al., 2001). By contrast, jba-1D/+ er-20 clv3-2 flowers not only form multi-carpel gynoecia, but their FMs fail to terminate and continue to produce numerous additional carpels, causing the gynoecia to burst (Fig. 6J,K). Thus, each jba-1D/+ er-20 clv3-2 gynoecium develops into a structure that

Fig. 4. Ectopic WUS and AG expression in jba-1D/+er-20 IM. Inflorescence tissues hybridized (purple staining) with a (A-C) WUS antisense or an (D-M) AG antisense RNA probe. (A,D,E) Col IM and flower primordia. (H) Ler inflorescence. (B,C) Ectopic WUS expression in the outer layers throughout jba-1D/+ and jba-1D/+er-20 IMs. (F) Weak AG signal in the three uppermost cell files of the jba-1D/+ IM. (G) AG expression in jba-1D/+ floral primordia. (L) Strong AG expression in jba-1D/+ Ler floral primordia and in the three or four uppermost cell files of the IM. (K-M) Ectopic AG expression in jba-1D/+er-20 IMs. Intense AG signal in the four outer cell layers of the splitting IMs, as well as in the adaxial region of the ectopic carpels and in their ovules. (L) Magnified view of the boxed area in K. Scale bars: 50 μm.
resembles the multifused carpel structure of the jba-1D/+ er-20 IM. These gynoecium phenotypes are consistent with a role for ER in regulating FM size and FM termination through a separate genetic pathway from the CLV pathway.

DISCUSSION

In this study we show that interference with SAM homeostasis between cell proliferation and cell recruitment for lateral organ formation by reducing HD-ZIPIII and ER gene function results in abnormal development, characterized by phyllotaxis alteration at the vegetative stage and ectopic carpel formation at the reproductive stage.

ER regulates WUS transcription and vegetative phyllotaxis

The ER gene is expressed in the SAM and throughout the FM at stages 1-3 (Roeder and Yanofsky, 2006; Shpak et al., 2004; Uchida

Fig. 6. Genetic interactions between jba-1D/+ er-20 and clv3 plants. (A-G) Top view of inflorescences 2 weeks after bolting. (A) Col, (B) Ler, (C) clv3-2, (D) jba-1D/+ (E) jba-1D/+ er-20, (F) jba-1D/+ clv3-2 and (G) jba-1D/+ er-20 clv3-2 plants display huge meristems. (H) Gynoecia from the same genotypes as A-G in the same order. (I-K) Short sphere-shaped jba-1D/+ er-20 clv3-2 gynoecia (I) at an early stage containing 12 valves, (J) with an indeterminate floral meristem (red arrow) producing numerous carpels and (K) bursting due to prolonged meristem activity that continuously produces carpels. (L-O) Side views of jba-1D/+ er-20 (L,N) and jba-1D/+ er-20 clv3-2 (M,O) plants at 2 (L,M) and 14 (N,O) weeks after bolting. Scale bars: 1 mm.
et al., 2013; Yokoyama et al., 1998). Here, we show that jba-1D/+ plants carrying a mutation in ER develop enormous SAMs that are wider and taller than jba-1D/+ meristems, and contain more cells. These data fit with previous work showing that mutations in ER enhance meristem-derived phenotypes (Dièvet et al., 2003; Durbak and Tax, 2011; Uchida et al., 2013). An increase in meristem size can occur via several pathways: a higher rate of cell division within the meristem, an increase in the size of the stem cell reservoir due to expansion of the OC that specifies their identity, or an accumulation of meristematic cells at the SAM periphery due to reduced cell incorporation into organ primordia (Kirch et al., 2003; Laufs et al., 1998b; Seidlová, 1980). The ER genes play overlapping roles in promoting cell proliferation in stems, leaves and floral organs (Torii et al., 1996; Shpak et al., 2003). If ER has the same function in the SAM, then mutations in ER should lead to a reduction in SAM cell number. However, we observe that loss of ER activity in jba-1D/+ leads to a dramatic increase in SAM size and cell number (Figs 1,3,4; supplementary material Fig. S1), suggesting that ER does not directly promote cell division in the SAM.

In jba-1D plants, a reduction in HD-ZIPIII transcript levels results in WUS upregulation and expression domain expansion (Williams et al., 2005). The er-20 mutation causes a further significant increase in WUS transcript levels, revealing a function for ER as a negative regulator of WUS expression. Furthermore, the enlarged jba-1D/+er-20 SAM produces supernumerary organ primordia, indicating that the increase in meristem size is not due to a reduced rate of primordium initiation but is the result of defective ER regulation of WUS.

It has been suggested that meristem size could have a major influence on phyllotaxis (Bainbridge et al., 2008). Our observations that jba-1D/+er-20 plants have extremely enlarged SAMs from which four primordia initiate simultaneously demonstrates the impact of meristem size on phyllotactic pattern. An accepted model for controlling phyllotaxis is that new primordia initiate at auxin maxima points on the SAM periphery (Benková et al., 2003; Heisler for controlling phyllotaxis is that new primordia initiate at auxin maxima points on the SAM periphery (Benková et al., 2003; Heisler 2008). The ER genes play overlapping roles in promoting cell proliferation in stems, leaves and floral organs (Torii et al., 1996; Shpak et al., 2003). If ER has the same function in the SAM, then mutations in ER should lead to a reduction in SAM cell number. However, we observe that loss of ER activity in jba-1D/+ leads to a dramatic increase in SAM size and cell number (Figs 1,3,4; supplementary material Fig. S1), suggesting that ER does not directly promote cell division in the SAM.

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This idea is consistent with the elevated expression levels of the GH3.3 early auxin-responsive gene in jba-1D/+ er-20 plants, which implies that additional auxin is present to stimulate its transcription. In conclusion, our observations indicate that ER negatively regulates WUS transcription, such that loss of ER function in the jba-1D/+ background leads to a massive increase in SAM size that affects phyllotaxis rather than to direct regulation of primordia initiation.

ER and HD-ZIPIII genes link SAM size maintenance with floral meristem identity specification

Upon bolting, the fasciated jba-1D/+ er-20 IMs produce numerous normal flowers until a cell fate switch occurs at the IM periphery, leading to ectopic carpel formation. Two important questions raised are why does this cell identity switch occur, and why does it take place around 4 weeks after bolting rather than exactly at the reproductive transition? Because the phenotype occurs in the context of high levels of ectopic WUS and AG mRNA expression across the IMs, which is more dramatic in jba-1D/+ er-20 than in jba-1D/+ , we propose that it is a matter of reaching a threshold of AG ectopic expression during inflorescence development.

In wild-type FMs, AG transcription is activated at stage 3 by WUS and LEAFY (Hong et al., 2003). In both jba-1D/+ and jba-1D/+ er-20 plants, the inflorescence stems gradually increase in width (supplementary material Fig. S1), indicating a progressively enlarging IM as a result of a gradual increase in the size of the WUS expression domain (Fig. 4). A similar gradual increase in WUS expression is detected in CLV mutants during their development (Clark et al., 1993; Würschum et al., 2006). In jba-1D/+ plants, WUS is upregulated to a level that leads to AG ectopic activation across the IM (Fig. 4; yet the amount of AG mRNA is not sufficient to induce bolting from FM to carpel identity. We propose that once jba-1D/+ er-20 plants bolt, WUS mRNA levels are high enough to activate AG across the IM, but not to the level required for carpel specification. During the next 4 weeks, WUS and AG expression continues to increase throughout the jba-1D/+ er-20 IMs (Figs 2,4; Table 1), with the gradual increase in WUS transcription ultimately inducing sufficient AG ectopic expression to surpass a threshold that triggers carpel formation.

Consistent with this concept, early studies indicate that AG likely functions in a dose-dependent fashion (Mizukami and Ma, 1995). In addition, in 35S:AG lines that constitutively express AG, the development of carpeloid structures correlates with high levels of AG expression, whereas lines with low levels of AG transcription show no visible phenotypes (C.C.C. and J.C.F., unpublished). This model is also consistent with evidence from jba-1D er-20 er11 and jba-1D/+ er-20 clv3-2 plants, in which the IMs form carpeloid structures much earlier. The meristems of these mutants appear larger than those of jba-1D/+ er-20 plants, implying that WUS transcript levels are higher, as they are in er er11 erl2 meristems (Uchida et al., 2013). We propose that in the triple mutant IMs, the extremely elevated WUS transcription levels leads to the activation of AG to the required threshold much earlier than in jba-1D/+ er-20 IMs, and therefore to the appearance of carpeloid carpels shortly after bolting.

Although AG is expressed in the upper cell layers across the jba-1D er-20 IM, ectopic carpel initiation is restricted to the periphery. One possible explanation for this is the presence of factors that specify pluripotent fate and preventing cell differentiation at the SAM center. These factors would be absent or inhibited at the periphery, allowing cell differentiation and thus permitting AG function to promote carpel formation. Supporting evidence is the phenotype of jba-1D/+ er-20 clv3-2 plants, which exhibit giant IMs with reduced ectopic carpel formation at the periphery. Mutations in CLV3 result in WUS domain expansion and a consequent increase in stem cell accumulation in shoot and floral meristems (Brand et al., 2000). We propose that in jba-1D/+ er-20 clv3-2 IMs, homeostasis is shifted towards stem cell identity, leaving fewer cells with the ability to differentiate. As a consequence, the triple mutant has a gigantic meristem with fewer carpeloid structures. Another explanation may be that other factors necessary for AG-mediated specification of carpel identity are absent from the center of the SAM. The need for additional factors is consistent with overexpression studies showing that ectopic AG activation is not sufficient to fully induce gynoecium formation outside of the flower (Lenhardt et al., 2001; Mizukami and Ma, 1992), and with mutants such as clf in which ectopic AG expression in leaves does not cause ectopic gynoecium formation (Goodrich et al., 1997; Laufs et al., 1998b).
Our results indicate that ER and the miR166g-regulated HD-ZIP III genes play an indirect role in specifying Arabidopsis FM identity. By negatively regulating WUS levels and restricting the WUS domain they prevent ectopic AG activation across the inflorescence meristem after the transition to flowering. This in turn enables the specification of floral meristem identity and precludes a switch to carpel cell identity on the IM flanks. Consistent with our data, a recent study (Bemis et al., 2013) independently confirms a function for the ER family genes in flower meristem development.

ER plays a role in meristem regulation independently from the CLV pathway

*jba-1D/+* gynoecia have two carpels with no detectable fifth whorl, indicating that FM size is not significantly increased and FM termination is unaffected. This suggests that repression of WUS expression in the center of the FM is not delayed in a miR166g overexpression background. The gynoecia of *jba-1D/+ er-20* flowers also show no morphogenetic defects, suggesting either that ER and the HD-ZIP III genes play a minor role in regulating FM size and determinacy or that FM size and termination are more tightly controlled by other factors, such as CLV3 (Clark et al., 1997), than the SAM size.

*jba-1D/+ er-20 clv3-2* plants show strong synergistic phenotypes in both the IMs and flowers. When these three pathways are non-functional, the plants exhibit severe phenotypes corresponding to four distinct morphological changes. The IMs display a macroscopic increase in size, with ectopic carpel formation around the periphery, the flowers form more carpels, and the FM indeterminacy defect is enhanced. None of the single mutants or double mutant combinations shows any resemblance to the CLV pathway – the ER pathway – in both the IMs and flowers. When these three pathways are non-functional, the plants exhibit severe phenotypes corresponding to four distinct morphological changes. The IMs display a macroscopic increase in size, with ectopic carpel formation around the periphery, the flowers form more carpels, and the FM indeterminacy defect is enhanced. None of the single mutants or double mutant combinations shows any resemblance to the *jba-1D/+ er-20 clv3-2* flowers; therefore, we conclude that all three pathways have an important role in regulating FM size and determinacy and that FM size and termination are more tightly controlled by other factors, such as CLV3 (Clark et al., 1997), than the SAM size.

**Plant materials**

The plant materials used in this study were: Columbia (Col-0), Landsberg erecta (Ler), *jba-1D* (Williams et al., 2005), *clv3-2* (Brand et al., 2000), *agl-1* (SALK_014999), *er-2* (CS3401) and *erl1* (SALK_081669). Double mutants were generated by crossing *jba-1D/+* plants to Ler, *clv3-2* and *er-2* plants, and by crossing *jba-1D/+ er-20* plants to *clv3-2* and *erl1* plants. Double mutant plants were identified phenotypically and by PCR genotyping the F2 progeny.

**Construction of transgenic lines**

A 9 kb genomic fragment corresponding to the ER promoter and gene, spanning from the 3’ end of the ER upstream gene to the 5’ UTR side of the ER downstream gene was amplified from *jba-1D/+* DNA, cloned into pENTR/D-TOPO (Invitrogen) and recombined into a modified pK2GW7 binary vector (Gent University) using the Gateway LR Clonase enzyme (Invitrogen). Transgenic lines were generated by the Agrobacterium-mediated floral dip method (Clough and Bent, 1998) into *jba-1D/+ er-20* and selected on MS plates containing kanamycin to select for the ER transgene and glufosinate ammonium (Fluka 45520) to select for the *jba-1D* allele.

**Microscopy and histology**

Plant images were captured using an Olympus SZX7 Stereomicroscope. Scanning electron microscopy was performed as described previously (Bowman et al., 1989) using a Hitachi 4700 scanning electron microscope. For histology analyses, 9-day-old seedlings were fixed, embedded and sectioned as described previously (Carles et al., 2004), and stained in Toluidine Blue solution.

**Quantitative RT-PCR analysis**

Total RNA was isolated from 8-day-old seedlings growing on MS plates using the Qiagen RNasey Mini-kit. cDNA synthesis was performed with the Invitrogen SuperScript II Reverse Transcriptase, using 1 μg of RNA. RT-qPCR analysis was carried out using a Rotor-Gene-Q instrument (Qiagen), with Absolute-Blue-qPCR-SYBR-Green Mix (Thermo). Three biological replicates were used for each genotype and three independent technical replicates were performed for each cDNA sample. *ACTIN2* (AT3G18780) was used as control and relative expression analysis was calculated using the 2−dΔCT method (Livak and Schmittgen, 2001). Statistical analysis was performed using JMP software (SAS Institute). Student’s t-test was used for comparison of means (significantly different at *P*<0.05). Primers used for RT-qPCR analysis are as follows: *ACT2-AT3G18780*, GGATCTGT-AACGTAACATGTTGC (forward) and CCACCGATCAGACACTGTAC (reverse); *WUS-AT2G17950*, CCACGTCTCAATACCGGAAGATTATGATGCA (forward) and CATGATGCATGCAAGATGAT (reverse); *STM-AT1G62360*, GATAGGAACAAATGAGGCCATCCATCCG (forward) and AACCACCTGTACGTCCGCAAGAG (reverse); *GH3.3-AT2G23170*, GTCGCCGTGCTCCACAGTGTAT (forward) and ATGCCTTTGGGATGAGTCTGG (reverse); *ERL2-AT2G62330*, AGACGGGGAACATATAGGT (forward) and GGTCGATAGGAGTGCC (reverse); *ERL1-AT3G18780*, GGATCTGT-AACGTAACATGTTGC (forward) and CCACCGATCAGACACTGTAC (reverse); *GAAG (forward) and TCACCCATGTGCA (reverse) and* ACTIN2 (AT3G18780)

**Differential expression analysis by mRNA-seq**

Four weeks after bolting, 30 *jba-1D/+* and 30 *jba-1D/+ er-20* IMs were collected by immersing the IM in liquid nitrogen and detaching the FM under a stereomicroscope. Bare meristems were pooled and total RNA isolated using Qiagen RNasey Mini-kit. mRNA was used to prepare libraries using TruSeqTM-RNA and single-end sequencing was performed by multiplexing on Illumina HiSeq-2000 at the Technion Genome Center. Data analysis was carried out at the Bioinformatics-Core-Facility in Ben-Gurion University. Raw sequence reads (Fastq files) were quality assessed using the FastQC software, and then aligned to the Arabidopsis genome using TopHat (70M for *jba-1D/+* and 50M for *jba-1D/+ er-20*). TAIR10 Genome reference sequence and gene model annotations for TopHat analysis were obtained from Illumina iGenomes website (http://tophat.cbcb.umd.edu/).
igenomes.html). Estimation of transcript and gene abundances in each sample as FPKM values (fragments per kilobase of exon per million fragments mapped) and differential expression analysis were carried out using CuffDiff (parameters –c 5 –b –u –M). Genes with P<0.01 and [fold of change]≥2 (total of 3524 genes) were considered differentially expressed. Genes are annotated with TAIR AGI locus names ("TAIR genes").

**In situ RNA hybridization**

Inflorescences were harvested 4 weeks after bolting. Tissue fixation and in situ hybridization were performed as described previously (Seidlová, 1980). Probes were transcribed using a digoxigenin-labeling mix (Roche). WUS antisense probe was generated as described previously (Mayer et al., 1998). AG antisense probe was generated by T7 RNA polymerase activity from a 1 kb insert cloned into the pBS KS+ vector (Seidlová, 1980).

**Acknowledgements**

We thank Hanita Zemach (Volcani center) for technical assistance and the Arabidopsis Biological Resource Center for insertion lines.

**Competing interests**

The authors declare no competing financial interests.

**Author contributions**

L.E.W. developed the concepts, T.M., F.M. and Y.K. performed experiments, L.E.W. wrote the manuscript, C.C.C. and J.C.F. edited and prepared the manuscript prior to submission.

**Funding**

This work was supported by the Israel Science Foundation [1351/10 to T.M.], by Vaadia-BARD [IS-4336-10R to Y.K.], by the US Department of Agriculture [5335-11710-65000-029-00D to J.C.F], by the Centre National de la Recherche Scientifique [IS-4336-10R to Y.K.], by the Israel Science Foundation [1351/10 to T.M.], by the Alpes county [21000-029-00D to J.C.F], and by the US Department of Agriculture [G5335–11710-65000-029-00D to J.C.F].

**Supplementary material**

Supplementary material available online at http://dev.biologists.org/cgi/lookup/suppl?doi=10.1242/dev.104687/-/DC1

**References**


