



A phylogenetically conserved APETALA2/ETHYLENE RESPONSE FACTOR, ERF12, regulates *Arabidopsis* floral development

J. W. Chandler¹ · W. Werr¹

Received: 5 March 2019 / Accepted: 30 October 2019 / Published online: 5 December 2019
© The Author(s) 2019

Abstract

Key message *Arabidopsis* ETHYLENE RESPONSE FACTOR12 (ERF12), the rice MULTIFLORET SPIKELET1 orthologue pleiotropically affects meristem identity, floral phyllotaxy and organ initiation and is conserved among angiosperms.

Abstract Reproductive development necessitates the coordinated regulation of meristem identity and maturation and lateral organ initiation via positive and negative regulators and network integrators. We have identified ETHYLENE RESPONSE FACTOR12 (ERF12) as the *Arabidopsis* orthologue of MULTIFLORET SPIKELET1 (MFS1) in rice. Loss of ERF12 function pleiotropically affects reproductive development, including defective floral phyllotaxy and increased floral organ merosity, especially supernumerary sepals, at incomplete penetrance in the first-formed flowers. Wildtype floral organ number in early formed flowers is labile, demonstrating that floral meristem maturation involves the stabilisation of positional information for organogenesis, as well as appropriate identity. A subset of *erf12* phenotypes partly defines a narrow developmental time window, suggesting that ERF12 functions heterochronically to fine-tune stochastic variation in wild type floral number and similar to MFS1, promotes meristem identity. ERF12 expression encircles incipient floral primordia in the inflorescence meristem periphery and is strong throughout the floral meristem and intersepal regions. ERF12 is a putative transcriptional repressor and genetically opposes the function of its relatives DORNROSCHEN, DORNROSCHEN-LIKE and PUCHI and converges with the APETALA2 pathway. Phylogenetic analysis suggests that ERF12 is conserved among all eudicots and appeared in angiosperm evolution concomitant with the generation of floral diversity.

Keywords Angiosperm evolution · APETALA2 · ETHYLENE RESPONSE FACTOR 12 · Floral transition · Meristem identity · MULTIFLORET SPIKELET1 · Phyllotaxy · Supernumerary sepals

Introduction

Plant reproductive development involves successive phase changes, whereby vegetative apical meristems that initiate leaf primordia at their periphery become inflorescence meristems (IMs) and initiate floral meristems, which in turn,

generate floral organs. The gene regulatory networks that regulate these meristem identities have been elucidated in detail (Ó'Maoiléidigh et al. 2014), together with those involved in the floral transition in response to seasonal cues, for representative dicot and monocot species (Shrestha et al. 2014). Reproductive phase change also depends on endogenous age-related competencies that integrate into these genetic networks via miRNAs (Teotia and Tang 2015). Flower development thus proceeds from the complex coordinated specification of meristem identity, and floral organ initiation and identity, via positive and negative regulators and network integrators.

Instead of leaf initiation at the shoot apical meristem switching to flower production within a single plastochron, the acquisition and maintenance of floral meristem (FM) identity are not robust and can revert to IM identity in several species in response to environmental conditions

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11103-019-00936-5>) contains supplementary material, which is available to authorized users.

✉ J. W. Chandler
john.chandler@uni-koeln.de

W. Werr
werr@uni-koeln.de

¹ Developmental Biology, Institute of Zoology, Cologne Biocenter, University of Cologne, Zulpicher Straße 47b, 50674 Cologne, Germany

(McCullough et al. 2010; Tooke et al. 2005). Furthermore, floral organs do not initiate simultaneously within the FM in concentric whorls: instead, outer whorl organs initiate sequentially along an abaxial/adaxial axis, with a stereotypy that can be disrupted by bract founder cell recruitment, or gene mutations that lead to pentameric asymmetry (Chandler and Werr 2014). The floral transition and floral organ initiation therefore represent the outread of multiple components and incremental signals and consist of several distinct phases.

Threshold expression models have been invoked to explain the function of some master regulators of the floral transition, such as *LEAFY* (*LFY*) (Blázquez et al. 1997), and the control of *APETALA2* (*AP2*) by miRNAs (Chen 2004). Many master regulators such as *LFY*, function pleiotropically in both reproductive timing and floral identity (Schultz and Haughn 1991; Blázquez et al. 1997), and in addition to regulating the identity of the outer two floral organ whorls, *APETALA2* (*AP2*) represses the floral transition (Yant et al. 2010). *Arabidopsis* AP2 is the founding member of the large AP2 superfamily of transcription factors, which has been subdivided according to the presence of one or two AP2 domains (Nakano et al. 2006). AINTEGUMENTA (*ANT*) subclass AP2 members contain two AP2 domains and play developmental roles in *Arabidopsis* (Horstman et al. 2014) and also mediate resistance to salt stress (Meng et al. 2015). Most single AP2-domain proteins belong to the ethylene response element (ERF), also known as ethylene response element binding protein (EREBP) subgroup (Nakano et al. 2006). Although ERF proteins mediate biotic and abiotic stress responses in many taxa (Dey and Corina Vlot 2015; Müller and Munné-Bosch 2015), the group VIIIb members DORNROSCHE (DRN), DORNROSCHE-LIKE (DRNL), *LEAFY PETIOLE* and *PUCHI* also regulate development in diverse plant species (Chandler 2018). Given the high sequence conservation of the AP2 domain among AP2 family members, genetic redundancy is a common feature. For example, *ANT-like* genes provide distinct individual contributions towards flower development, but function redundantly with *ANT* (Krizek 2015), and *DRN*, *DRNL* and *PUCHI* redundantly regulate organ number and floral meristem identity (Chandler and Werr 2017). The subtle nature of individual mutant phenotypes and the ubiquity of higher orders of genetic redundancy, suggest that the full repertoire of developmental functions of AP2 family members might not yet have been elucidated.

The comparative analysis of AP2 transcription factor functions can inform their evolution and establish plesiomorphic functions. For example, AP2/ERF members in monocots have revealed evolutionary divergence and functional conservation. In rice, the AP2-type genes *SUPER-NUMERARY BRACT* and *OSINDETERMINATE SPIKELET1* synergistically determine inflorescence architecture

and floral meristem identity (Lee and An 2012). Similarly, *INDETERMINATE SPIKELET* (*IDS*) and *SISTER OF INDETERMINATE SPIKELET1* (*SIDI*) play multiple roles in regulating maize inflorescence architecture and although they share some gene targets and functions with *Arabidopsis* AP2, have also adopted novel functions (Chuck et al. 2008). Monocot counterparts of the clade VIIIb ERF subfamily in *Arabidopsis* have also neofunctionalised or retained ancestral functions: in contrast to a mild mutant phenotype in *Arabidopsis*, mutations in the *PUCHI* orthologues *BRANCHED SILKLESS* in maize (Colombo et al. 1998) and *FRIZZY PANICLE* in rice cause much more severe phenotypes (Komatsu et al. 2003). Inter-taxa studies are important not least because *Arabidopsis* inflorescence development is not representative of the whole plant kingdom, due to the different inflorescence structure of monocots versus dicots, including branching, and multiple meristem identities and transitions (Whipple 2017). Furthermore, grasses such as rice represent a specialised and derived lineage of monocots (Kellogg 2001).

Here, we describe the function of *ERF12* in *Arabidopsis*, the orthologue of rice *MULTIFLORET SPIKELET1*, which regulates floral organ identity and number, and the timing of spikelet initiation (Ren et al. 2013). Loss of *ERF12* function affects pleiotropic aspects of floral development in long days, including floral meristem phyllotaxy, organ merosity in the first-formed flowers, notably in sepal number, and delays the floral transition in long and short days. These phenotypes, which are partly restricted to a narrow developmental time window, suggest that *ERF12* possesses a heterochronic function, which regulates the timing of FM specification and fine-tunes the inherent wild type stochastic variation in floral organ merosity in first-formed flowers. Phylogenetic analysis of the *ERF12* AP2 domain suggests that *ERF12* represents a highly conserved angiosperm-specific innovation.

Materials and methods

Plant material and growth conditions

The mutant line SAIL_873_D11 (N877578) contains a T-DNA insertion in the open reading frame of the *ERF12* gene (*At1G28360*) after nucleotide 427 from the ATG start. For *erf9*, the line SALK_043407 contains a T-DNA insertion after nucleotide 314 from the ATG start in the *ERF9* (*At5G44210*) open reading frame. Both *erf12* and *erf9* alleles were obtained from NASC. Homozygosity was confirmed by genotyping using primers ERF12genoF/ERF12genoR to genomic DNA spanning the open reading frame, or ERF12F/LB3 for the presence of the insertion for *erf12*, or using primers ERF9F/ERF9R or Lba1/ERF9R for *erf9*. The mutant lines *drn-1*, *drnl-1* and *puchi* and their genotyping

have been described previously (Chandler et al. 2007; Chandler and Werr 2017). The *ap2-7* allele from NASC (N6241) is in Col-0 background. Plants were grown on soil in the greenhouse in long-day conditions (16 h light, 8 h dark) or in a controlled environment cabinet at 100 $\mu\text{M m}^{-2}$ light intensity under short days (8 h dark, 16 h light).

Constructs for expression analysis and complementation

For expression analysis, 2356 bp *ERF12* upstream promoter sequence from the stop codon of the upstream gene to the start codon of the *ERF12* open reading frame (ATIG28360) was amplified from *Arabidopsis* genomic DNA extracted from wild-type plants using the NucleoSpin® Plant II kit (Macherey–Nagel) and primers promERF12F and promERF12R. The resulting fragment was cloned into the TOPO™ TA Cloning™ vector (ThermoFisher Scientific). The primers included flanking *AscI* (5′) and *XmaI* (3′) sites, the latter allowing the introduction the coding region of the green fluorescent protein targeted to the endoplasmic reticulum (*erGFP*) sequence that terminates in the cauliflower mosaic virus 35S 3′UTR/polyadenylation signal in front of a second *AscI* restriction site (Comelli et al. 2016). The orientation of *erGFP* relative to the *ERF12* promoter was confirmed by sequencing, to produce *pERF12::erGFP*. Two genomic regions containing the *ERF12* open reading frame were used for complementation analysis. The shorter version (*gERF12Up*; 3527 bp) was amplified using primers promERF12F and gERF12UpR, to include the whole *ERF12* upstream promoter region from the stop codon of the upstream gene, the *ERF12* open reading frame and the 3′ untranslated region. Both primers included flanking *AscI* sites. A longer genomic region (*gERF12UpDown*; 6773 bp) was constructed, consisting of the whole genomic region, between the open reading frames of the up- and downstream genes, including the *ERF12*-coding region. This construct was made in two steps, by firstly reamplifying *gERF12Up* using primers promERF12F and gERF12UpRnew, the latter containing an *XmaI* site and cloning the product into TOPO™ TA Cloning™ vector (ThermoFisher Scientific) and then introducing a PCR fragment amplified using primers gERF12DSF and gERF12DSR at the 3′ end of *gERF12Up* via the flanking *XmaI* sites and confirming the correct orientation in relation to the *gERF12Up* fragment, to create *gERF12Up-Down*. The *pERF12::erGFP* expression or complementation cassettes were transferred into the binary pGPTV-Asc-Bar (*erGFP* expression construct) or pGPTV-Asc-Kan (complementation constructs) vectors (Überlacker and Werr 1996) via *AscI* and were introduced into *Agrobacterium tumefaciens* GV3101V. Mutant *erf12* plants (for complementation) or Col-0 wild type (*pERF12::erGFP*) were transformed via floral dipping (Clough and Bent 1998). For expression analysis, transgenic plants for *pERF12::erGFP* were selected with

BASTA and at least eight independent T_2 lines were analysed. For complementation, T_1 seeds were surface-sterilised with bleach (Lindsey et al. 2017). Plants were selected on sterile half-strength Murashige and Skoog medium for resistance to kanamycin (50 $\mu\text{g mL}^{-1}$) and were genotyped to confirm the presence of the appropriate transgene using pGPTV-specific pGPTVF/pGPTVR and gene-specific primers ERF12Fgeno/ERF12Rgeno. T_2 populations of several independent transgenic lines were analysed for complementation.

Confocal imaging

Imaging of *pERF12::erGFP* expression was performed with a Zeiss LSM 700 confocal laser scanning microscope (CLSM). GFP was excited at 488 nm and emission was analysed between 502 and 525 nm. Photoshop CS2 software (Adobe) was used to process the CLSM images and Imaris software (Bitplane, Zürich, Switzerland) converted Z-stacks into 3D images.

Phenotypic analysis

Organ numbers were counted for each whorl of the first five-formed flowers for 100 plants. Statistical analyses of organ numbers were performed using *t* tests.

Phylogenetic analysis and phylogenetic shadowing

Full-length protein sequences were obtained from GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) or the PLAZA databases (<https://bioinformatics.psb.ugent.be/plaza/>) using ERF12 or MFS1 sequences as a query. A phylogenetic tree was compiled using the phylogeny.fr platform (Dereeper et al. 2008); protein sequences were aligned using the MUSCLE program and tree construction employed the maximum likelihood method (PhyML programme) and the Bootstrap procedure (100 replicates). The phylogenetic tree was rendered with TreeDyn software and Bootstrap support values are indicated next to the branches. For phylogenetic shadowing, *ERF12* genomic regions of Brassicacea species (*Arabidopsis thaliana*, *Arabidopsis lyrata*, *Boechera stricta*, *Camelina sativa*, *Eutrema salsugineum* and *Capsella rubella*) were obtained from <http://phytozome.jgi.doe.gov/pz/portal.html> or <http://www.ncbi.nlm.nih.gov/>. Sequences were analysed with online mVista tool with LAGAN alignment (<http://genome.lbl.gov/vista/index.shtml>).

Results

ERF12 and MFS1 represent an invention that postdates basal angiosperms

A blast search using the MFS1 amino acid sequence revealed *Arabidopsis* ERF12 to be a putative orthologue. ERF12 belongs to sub-clade VIIIa of AP2/ERF proteins (Nakano et al. 2006), which contains eight members (ERF3, ERF4, ERF7–12). To address the evolutionary sequence conservation between rice MFS1 and *Arabidopsis* ERF12, a phylogenetic analysis of the closest available full-length-protein sequences to ERF12 and MFS1 was performed from a range of representative species from different extant phylogenetic groups. All members of clade VIIIa and VIIIb were included in the comparison, together with the closest one, or occasionally two sequences from blast searches made with ERF12 and MFS1 against genomes of mosses and liverworts (*Marchantia polymorpha*, *Physcomitrella patens*), basal angiosperms (*Amborella trichopoda* and the mangoliid *Cinamomum kanehirae*), gymnosperms (*Ginkgo biloba*, *Picea abies*, *Pinus sylvestris*), grass monocots (*Brachypodium distachyon*, *Phyllostachys edulis*, *Setaria italica*, *Triticum aestivum*, *Zea mays*), non-grass monocots (*Ananas comosum*, *Musa acuminata*, *Phalaenopsis equestris*, *Spirodela polyrhiza*, *Zostera marina*), basal eudicots (*Nelumbo nucifera*, *Papaver somniferum*), core eudicots (*Glycine max*, *Medicago truncatula*, *Populus trichocarpa*, *Solanum lycopersicum* and *Vitis vinifera*). These sequences formed monocot and eudicot ERF12 subclades, but the closest gymnosperm, moss and liverwort ERF12 and MFS1 homologues clustered more closely with *Arabidopsis* subclades ERF3 and ERF7 (Fig. 1). Furthermore, the closest ERF12/MFS1 homologue in the angiosperm sister taxon *Amborella trichopoda* aligned most closely with DRNL (Fig. 1), suggesting that ERF12 proteins represent an invention subsequent to the divergence of gymnosperms and basal angiosperms. The AP2 domain is highly conserved between ERF12 and MFS1 and both proteins share a C-terminal EAR-like domain (Fig. 2a). To assess the conservation of *ERF12* regulatory sequences, phylogenetic shadowing of the genomic *ERF12* locus among several Brassicaceae species revealed that upstream *ERF12* promoter sequences are more highly conserved than downstream regulatory sequences (Fig. 2b).

ERF12 promotes the floral transition in long and short days

To characterise the function of *ERF12*, an *erf12* mutant was analysed that contained a T-DNA insertion within the *ERF12* open reading frame (Fig. 2a). As a proxy

for the timing of the floral transition, the number of rosette and cauline leaves were all significantly higher ($0.001 > p < 0.0001$) for *erf12* than wild type in both long and short days (Fig. 3a, b).

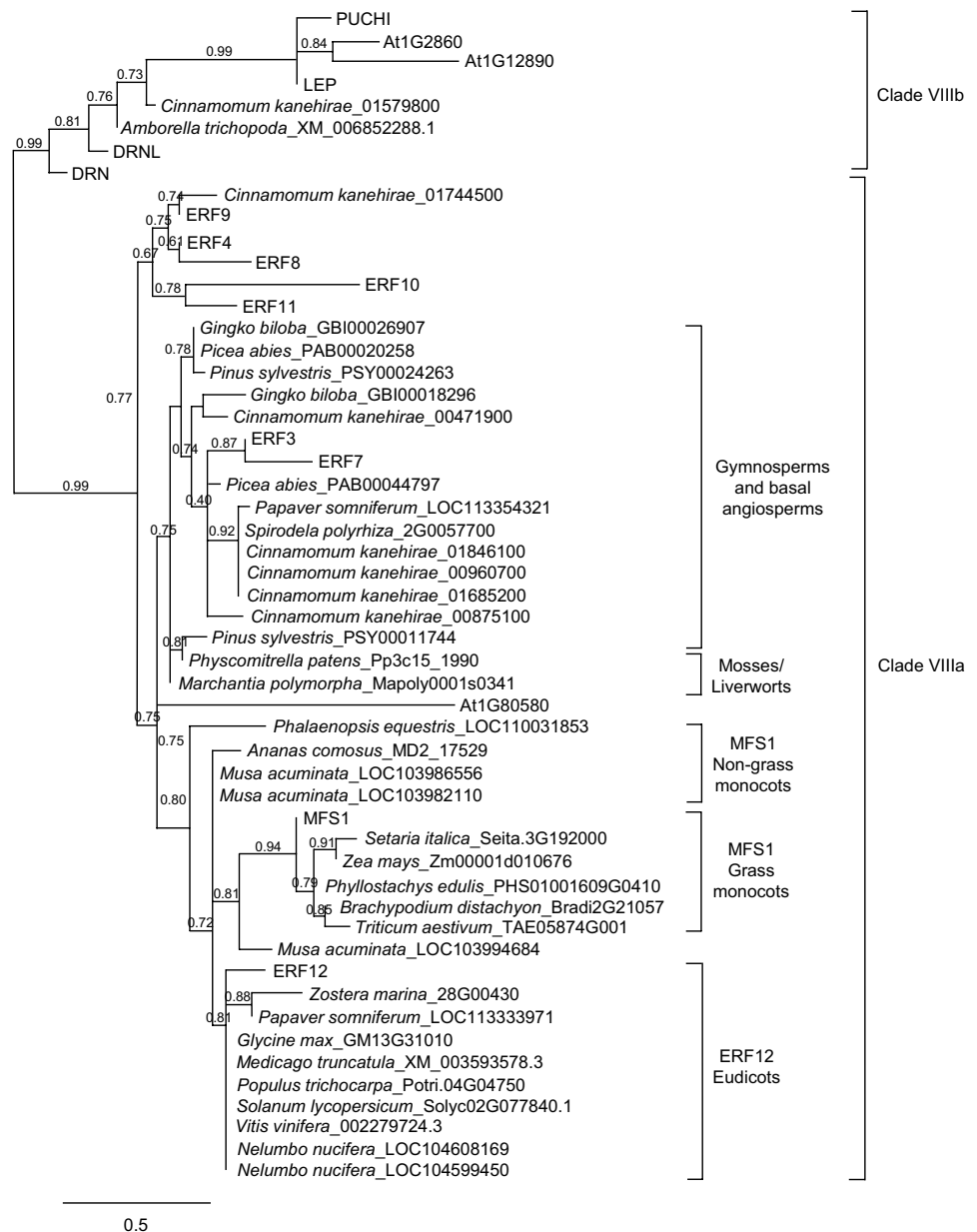
Loss of *ERF12* function preferentially affects sepal and stamen number in long days

Petal and stamen frequency in *erf12* mutants grown in long days showed the same qualitative trend across the first few flowers in the inflorescence as wild type, but differed quantitatively. The most pronounced *erf12* floral phenotype was significantly more sepals in the first two-formed flowers than wild type (Figs. 3c, e–g, 4a) at a penetrance that was highest in first-formed flowers (24%) and decreased to 10% in flower 2 and 1% in flower 3, but ectopic sepals were observed up to flower 6. Sepals were also occasionally fused (in 11% of first-formed flowers; Fig. 3d, e). Mean petal number in the first few *erf12* flowers was slightly but not significantly greater than in wild type (Fig. 4b), but more *erf12* flowers had either three or five petals than wild type (Fig. 3h, i; data not shown), which usually had four (Fig. 3d). *erf12* flowers had significantly more stamens than wild type (up to nine) in the first three flowers (Figs. 3j, 4c), preferentially due to significantly more lateral stamens (Fig. 4d); the number of medial stamens in flowers 1 to 5 did not differ between wild type and *erf12* plants (Fig. 4e), but reached seven in both genotypes. Three fused carpels were observed among the first five *erf12* flowers at a very low frequency (Fig. 3k; 1/200 plants). Thus, early *erf12* flowers possessed supernumerary organs in all four floral whorls.

Notably, floral organ number in earliest-formed wild-type flowers was non-stereotypic in long-day conditions and was not robust across the inflorescence. No significant variation in sepal number was observed in the earliest-formed flowers (Fig. 4a), but first-formed flowers had more petals than subsequent flowers (Fig. 4b). However, a significant increase total stamen number was observed, from 5.40 in the first-formed flower to 5.95 in flower 5 (Fig. 4c), which resulted from opposing trends in the number of lateral and medial stamens, with the former increasing to a greater extent than the decrease in the number of medial stamens (Fig. 4d, e). In addition to mean stamen number, approximately half (46.5%) of wild type flowers possessed more than six and up to nine stamens. These supernumerary stamens were always medial, on either or both ab- or adaxial sides of the flower.

Primary or secondary *erf12* inflorescences showed phyllotactic defects at almost complete penetrance (98%; N = 99 inflorescences), resulting from an aberrant divergence angle of siliques on the stem, or inhibited internode elongation, to give clustered siliques (Fig. 3l–n). These defects were maintained throughout inflorescence development and were not lost in later-initiated flowers (visible in Fig. 3n).

Fig. 1 Phylogenetic relationship between *Arabidopsis* class VIIIa and VIIIb ERF/AP2 transcription factors and ERF12/MFS1 homologues from diverse plant taxa. The tree was compiled using the maximum likelihood method (PhyML programme), via the phylogeny.fr platform (Dereeper et al. 2008)



In controlled short-day growth conditions, the number of wild type and *erf12* floral organs did not differ significantly (Fig. 4f for sepals).

ERF12 is expressed dynamically in the IM and FM

ERF12::erGFP was dynamically expressed in the IM and FM during inflorescence development (Fig. 5a–c). Strongest expression was at the IM periphery at the border between newly initiated FMs from about P1 stage onwards, around the basal circumference of the floral primordium, as soon as the buttresses physically extended from the IM (Fig. 5d, e). In buds from about stage 3 onwards, foci of *ERF12::GFP* expression marked the intersepal regions in floral buds

and diffuse expression was present internally to the sepals throughout the FM (Fig. 5f, g) and in the tips of sepals as they overgrew the FM (Fig. 5h). Strong expression was also observed at the abaxial underside flank of the developing bud (Fig. 5i, j) and in guard cells of cotyledons and leaves (Fig. 5k, l).

Complementation of the *erf12* mutant

Sepal number in the first-formed flower was used to demonstrate complementation by genomic *ERF12* sequences containing the *ERF12* open reading frame and either the upstream genomic region (*gERF12Up*) or all upstream and downstream sequences (*gERF12UpDown*). Five and

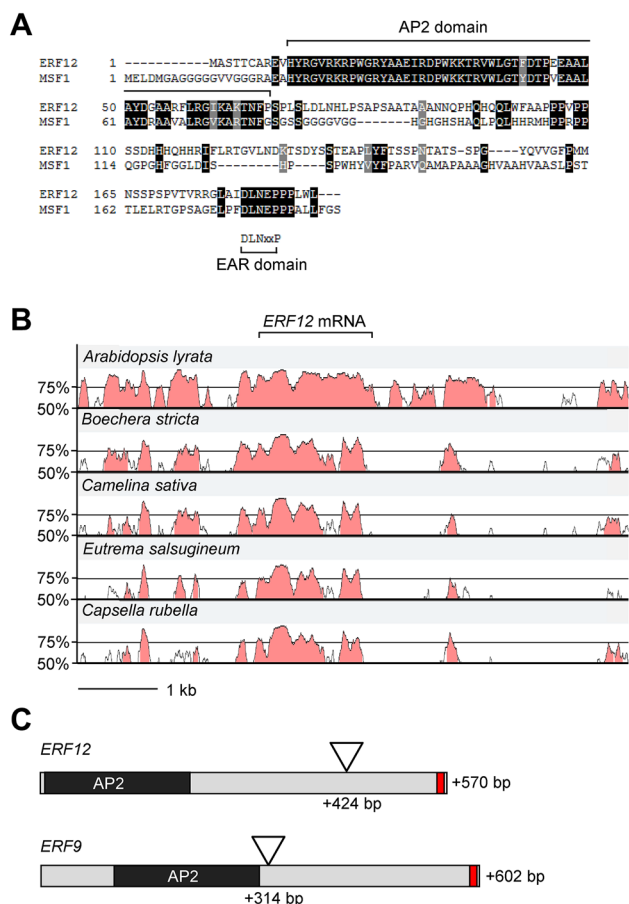


Fig. 2 Position of *erf12* mutation and conservation of ERF12 protein domains and regulatory regions. Amino acid alignment of MFS1 from rice and Arabidopsis ERF12 (a) showing high conservation in the AP2 and EAR domains. VISTA blots of the *ERF12* genomic locus, including the up- and downstream flanking genes compared between *Arabidopsis thaliana* and a range other Brassicaceae species (*Arabidopsis lyrata*, *Boechera stricta*, *Camelina sativa*, *Eutrema salsugineum* and *Capsella rubella*) (b). Schematic representation of the *ERF9* and *ERF12* open reading frames (c) showing the position of the T-DNA insertions and the encoded AP2 domain and EAR domain (red block)

seven independent transgenic lines containing *gERF12Up* or *gERF12Down*, respectively, all showed significantly fewer sepals in the first-formed flower than *erf12* mutants (Fig. 6), mostly to the wild-type number of four. The late flowering *erf12* phenotype was not complemented (data not shown).

Genetic interactions between *erf12*, *drn*, *drnl* and *puchi*

The related AP2/ERF genes *DRN*, *DRNL* and *PUCHI* redundantly regulate floral organ number in all whorls and *drn drnl puchi* mutants display trichomes at the base of floral pedicels at the position of the cryptic bract (Fig. 3o). We

therefore investigated potential genetic interactions between these genes and *ERF12* by creating a quadruple *drn drnl puchi erf12* mutant, which was not trivial, due to linkage between *DRN*, *DRNL* and *ERF12* loci on chromosome 1. Similar to wild type, *drn drnl puchi* triple mutant flowers showed a gradient of organ numbers throughout inflorescence development, with a decrease in the number of trichomes subtending the pedicels, fewer sepals and petals and more stamens, from the first to the fifth flower (Fig. 7a–e). The *erf12* phenotype was not additive, synergistic or epistatic when combined with *drn drnl puchi*. Instead, the frequency of trichomes subtending the first-formed flowers of *drn drnl puchi erf12* quadruple mutants was attenuated (Fig. 7e), and the number of floral organs in all whorls was significantly higher than in *drn drnl puchi* triple mutants, although remained lower than wild type (Fig. 7a–d), except for the frequency of carpels, which was restored to the wild type number of two (Fig. 7d). Sepal number in the first few *drn drnl puchi erf12* flowers was significantly lower than in single *erf12* mutants. The penetrance of cotyledon defects in *drn drnl puchi* mutants was 46.16% (N = 2110), and was attenuated in *erf12 drn drnl puchi* mutants to 30.94% (N = 1939).

erf12 enhances the *ap2* mutant phenotype

Because *APETALA2* also functions in the floral transition and floral organ specification, we analysed the genetic interaction between *ap2-7* and *erf12* mutants by creating a double *ap2-7 erf12* mutant. *ap2-7* mutant flowers consisted of carpelloid sepals and bract-like structures, some stamens and a relatively normal gynoecium (Fig. 8a, b). However, *erf12* enhanced the *ap2* mutant phenotype: stamens were absent in *ap2 erf12* double mutant flowers and whorl one organs had a stronger carpelloid identity than in *ap2* single mutants (Fig. 8c, d) and were often completely fused and resembled a carpel (Fig. 8e, f), with trichomes. Similar to *erf12* single mutants, the floral phyllotaxy of *ap2 erf12* inflorescences was also defective (Fig. 8g), but was not more severe than that of single *erf12* mutants.

Mutation of *ERF9* does not affect floral development

To characterise potential genetic redundancy between *ERF12* and other related AP2/clade VIIIa members, we analysed the phenotype of a T-DNA insertion mutant of *ERF9* (Fig. 2c). *erf9* showed wild type floral and inflorescence development (data not shown) and when combined with *erf12*, no additional or enhanced phenotypic defects were observed in *erf9 erf12* plants to those of *erf12* single mutants.

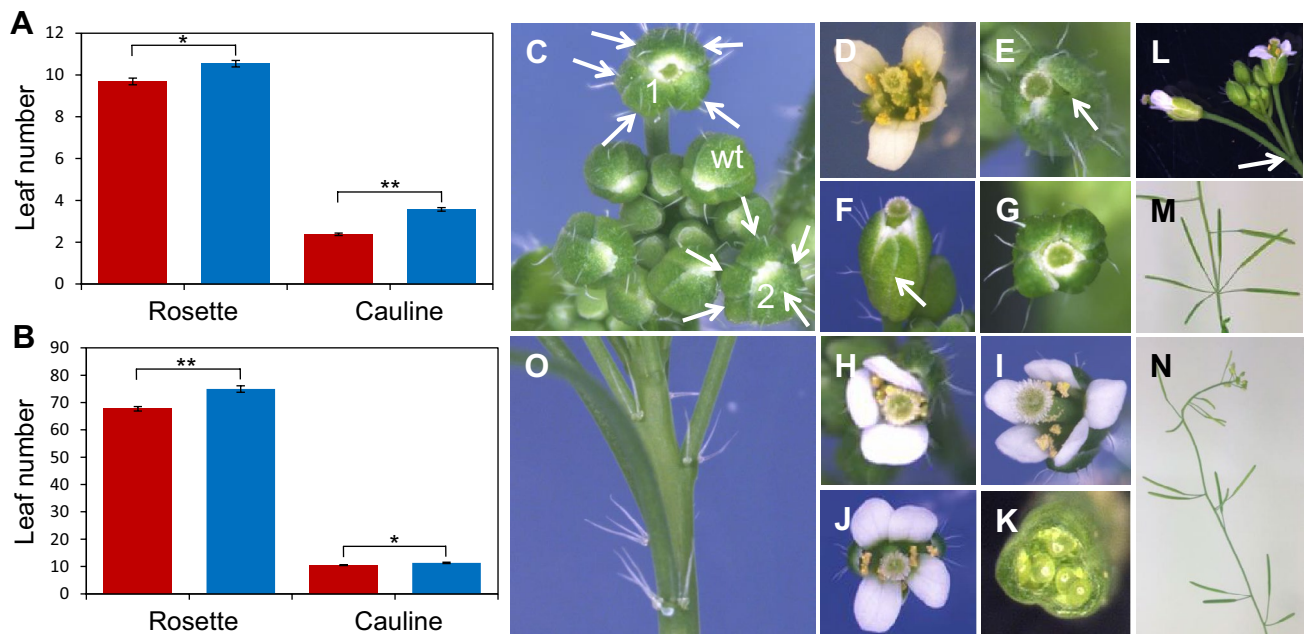


Fig. 3 *erf12* mutant phenotype. Flowering time as indicated by rosette and cauline and leaf number for wild type (red bars) and *erf12* plants (blue bars) in long day (a) and short day (b) photoperiods; $**p \leq 0.001$; $***p \leq 0.0001$. Sepal phenotypes of *erf12* mutants: top-view of young inflorescences (c) showing supernumerary sepals (all sepals marked by arrows) in the first two flowers (marked 1 and 2). A wild-type bud with four sepals is marked “wt”. A wild-type flower with four petals and six stamens clearly visible (d). Single buds showing fused supernumerary abaxial sepals (e; fusion marked with

arrows) or supernumerary sepals at various positions (f, g). *erf12* flowers with three (h) or five (i) petals; seven stamens (j) and three fused carpels (k). Floral phyllotaxy defects in inflorescences of *erf12* mutants: the first two flowers occupying the same stem node separated by an acute angle (l), or clustering of flowers due to aberrant phyllotaxy and internode elongation (m, n). Trichomes at the position of the cryptic bract, subtending floral pedicels of *drn drnl puchi* mutants (o)

Discussion

ERF12 and MFS1 are an angiosperm-specific invention

The MFS1 AP2 protein in rice, which determines floral meristem and organ identity, prompted a phylogenetic analysis, which identified the *Arabidopsis* orthologue as ERF12. The absence of ERF12/MFS1 homologues in plant radiations prior to the emergence of angiosperms and in *Amborella trichopoda*, the monotypic sister genus to all angiosperms, suggests that ERF12-type proteins represent a derived angiosperm trait subsequent to the divergence of eudicots from basal angiosperms that co-evolved with the diversification of angiosperm floral morphology. This contrasts with close AP2/ERF relatives belonging to clade VIIIb, which are more ancestral (Chandler 2018). This conclusion is supported by several other pieces of evidence. Firstly, no ERF12/MFS1 orthologue is present in the three gymnosperm genomes compared, nor in that of the magnoliid *Cinnamomum kanehirae*, another basal angiosperm, whose closest protein relatives group in the ERF3/7 clade. The robust and comprehensive analysis of plant taxa across plant kingdom (Fig. 1) show that an

ERF12/MFS1 homologue is clearly shared by all early non-grass monocots analysed (*Ananas comosum*, *Musa acuminata*, *Phalaenopsis equisetum*, *Spirodela polyrhiza*, *Zostera marina*), and in all grass monocots and evolutionarily young eudicots analysed. Furthermore, the closest *Amborella* relative does contain an ESR domain, confirming that it belongs to the clade VIIIb proteins (Chandler 2018). Another argument is that ERF12 predominantly affects sepals, which are a relatively recent morphological innovation, since they are absent in *Amborella*, the best extant reference for understanding the evolutionary molecular genetic basis of flower development (*Amborella* Genome Project 2013) and also the magnoliid *Cinnamomum kanehirae*. The tepals of *Amborella* flowers show a gradual morphological transition from outer to inner floral organs and floral trait modelling suggests that flowers of the most recent angiosperm ancestor had an undifferentiated perianth consisting of multiple tepals (Sauquet et al. 2017). *Phalaenopsis equestris* also possesses tepals, but contains a protein that more closely aligns with ERF12 than other group VIIIa proteins (Fig. 1). This might represent an intermediate ancestor in the evolution of an ERF12 protein associated with sepal development and flowers having a characteristic angiosperm floral body

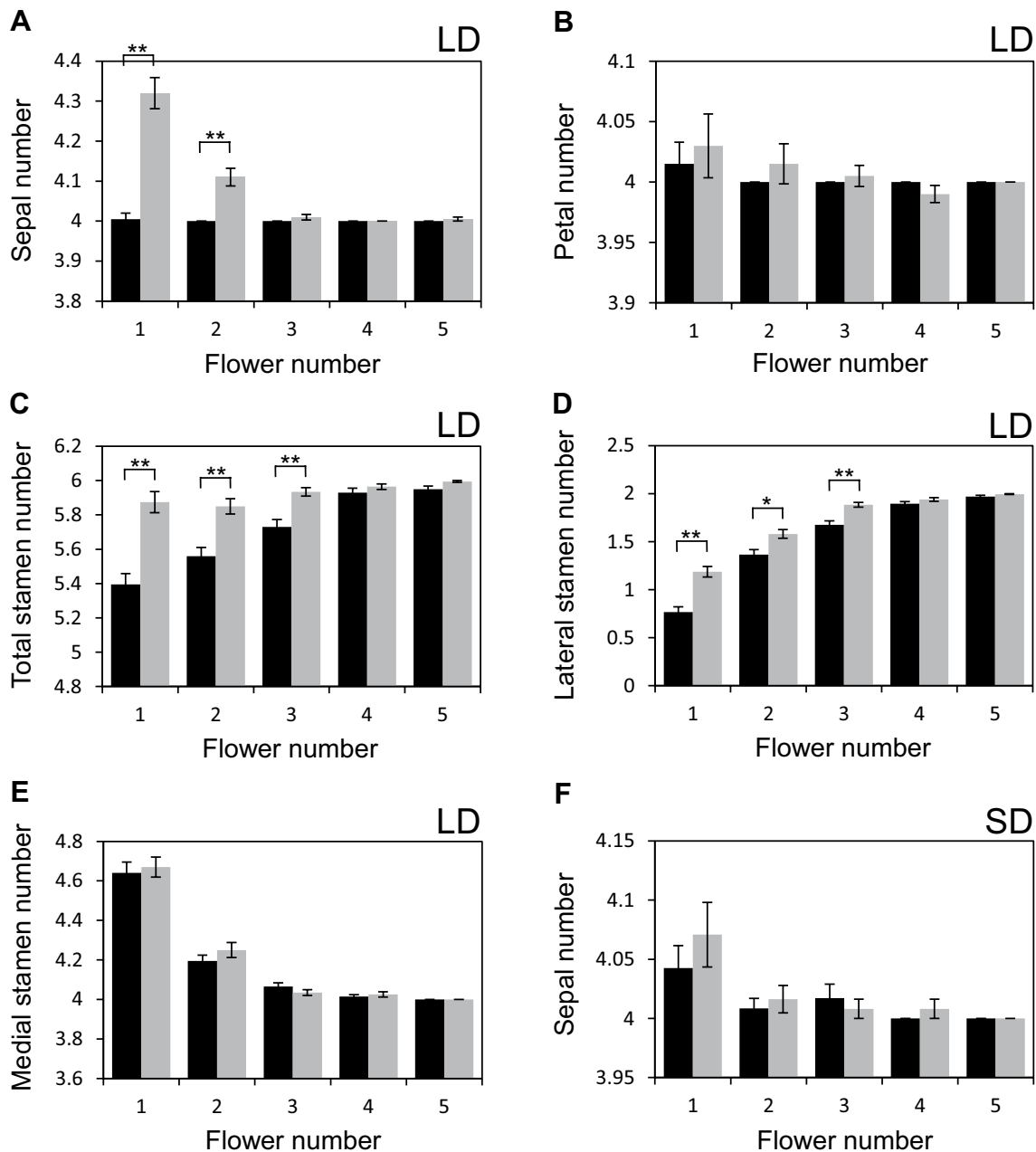


Fig. 4 Sepal, petal and stamen number is labile in earliest-formed wild type and *erf12* flowers. Frequency of sepals (a); petals (b); total stamens (c); lateral stamens (d); medial stamens (e) in the first five flowers of wild type and *erf12* mutants grown in long days. N=200; **p*<0.05; ***p*<0.0001. Frequency of sepals in the first five flowers

of wild type and *erf12* mutants grown in short days (F) (n=117 for wild type; 123 for *erf12*). Black and grey columns represent wild type and *erf12*, respectively. LD long days, SD short days. Flower number 1 represents the first-formed flower

plan. Widespread whole-genome duplication across angiosperms, leading to a diversification in gene family content, particularly in crucifers (Amborella Genome Project 2013), probably led to the coopting and neofunctionalisation of *ERF* genes such as *ERF12* for specific floral functions. During angiosperm evolution, perianth differentiation is thought to have multiple independent origins and many theories exist concerning the gene neo- and

subfunctionalisations that underlay these events (Irish 2009). One example is the conversion of tepals into sepals into *Phalaenopsis equestris* brought about by silencing *SEPALLATA3* (Pan et al. 2014). However, *ERF12* function is not associated with perianth organ identity, but organ merosity, and the phylogenetic analysis here that exploits the currently available sequenced genomes representing key plant evolutionary stages, robustly supports

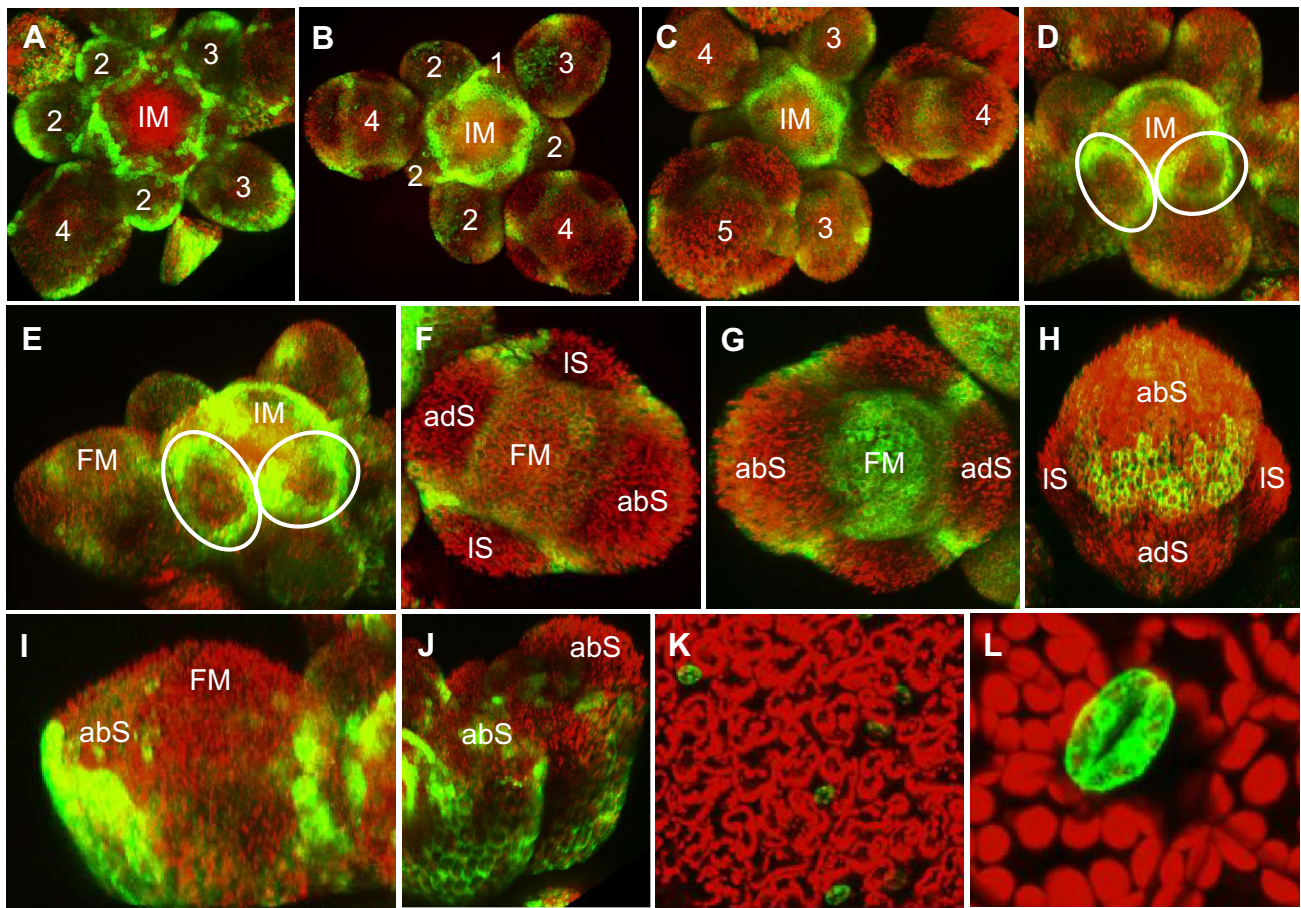


Fig. 5 *ERF12* is dynamically expressed in developing flowers and in guard cells. Expression of *pERF12::erGFP* in top views of inflorescence apices from independent transgenic lines showing flowers in different stages of development (a–c); oblique views of inflorescence apices showing rings of *pERF12::erGFP* expression surrounding the emerging floral primordia (marked by oblongs) (d, e); top views of

early (f) and late stage 4 (g) and stage 6 (h) floral buds; sagittal view of stage 4 floral buds (i, j); epidermis of a cotyledon (k); a leaf (l). *IM* inflorescence meristem, *FM* floral meristem, *abS* abaxial sepal, *adS* adaxial sepal, *IS* lateral sepal; numbers represent floral stages according to Smyth et al. (1990)

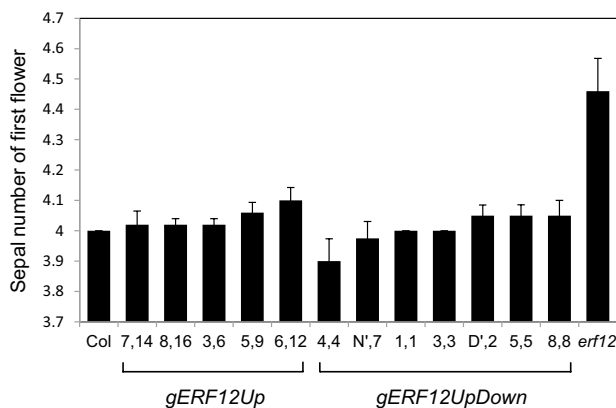


Fig. 6 Complementation of the *erf12* mutant. Sepal number in the first-formed flower of multiple independent *erf12* transgenic lines complemented with the *ERF12* locus containing upstream (*gERF12Up*) or up- and downstream regulatory sequences (*gERF12UpDown*)

the hypothesis that *ERF12* neofunctionalised concomitant with the appearance of angiosperm flowers with a differentiated perianth.

In *Arabidopsis*, clade VIIIa AP2/ERF members except *ERF8* and *ERF12*, phylogenetically associate as sister pairs or paralogues (*ERF3* and *ERF7*; *ERF4* and *ERF9*; *ERF10* and *ERF11*). The absence of redundant paralogues might explain why single *erf12* mutants display a phenotype, whereas redundancy between *ERF9* and *ERF4* might mask *erf9* phenotypes. Few clade VIIIa ERF proteins have been functionally characterised, but *ERF11*, which is encoded by the adjacent locus to *ERF12*, promotes internode elongation via gibberellin synthesis (Zhou et al. 2016) and represses ethylene synthesis (Li et al. 2011). *ERF9* is involved in plant defence against necrotic fungi via ethylene/JA pathways (Maruyama et al. 2013) and *ERF8* is a transcriptional repressor that negatively

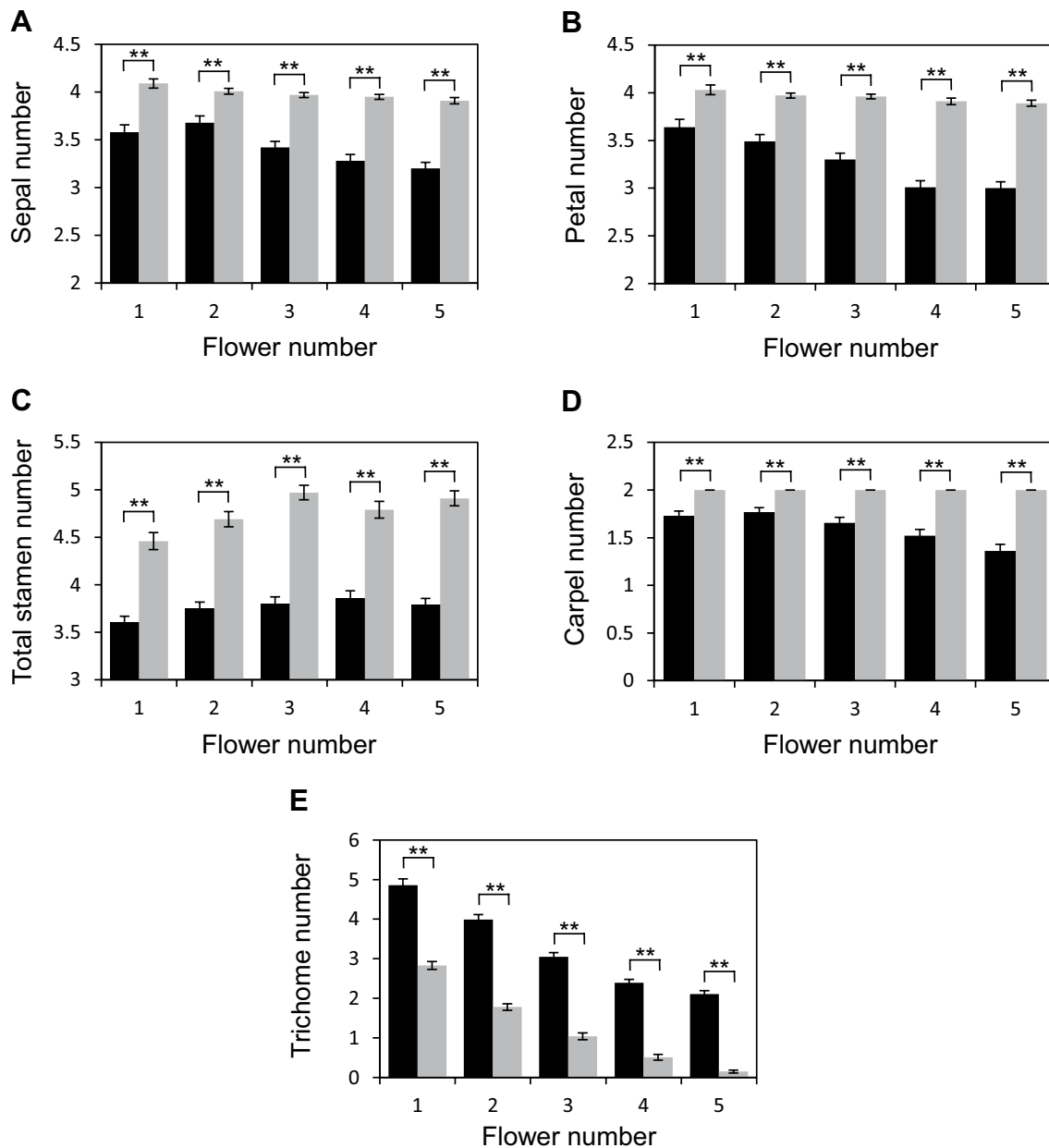


Fig. 7 *ERF12* interacts genetically with related AP2/ERF genes *DORNRÖSCHEN* (*DRN*), *DORNRÖSCHEN-LIKE* (*DRNL*) and *PUCHI*. Frequency of sepals (**a**); petals (**b**); total stamens (**c**); carpels (**d**); trichomes subtending the pedicels (**e**) in *drn drnl puchi* and *erf12*

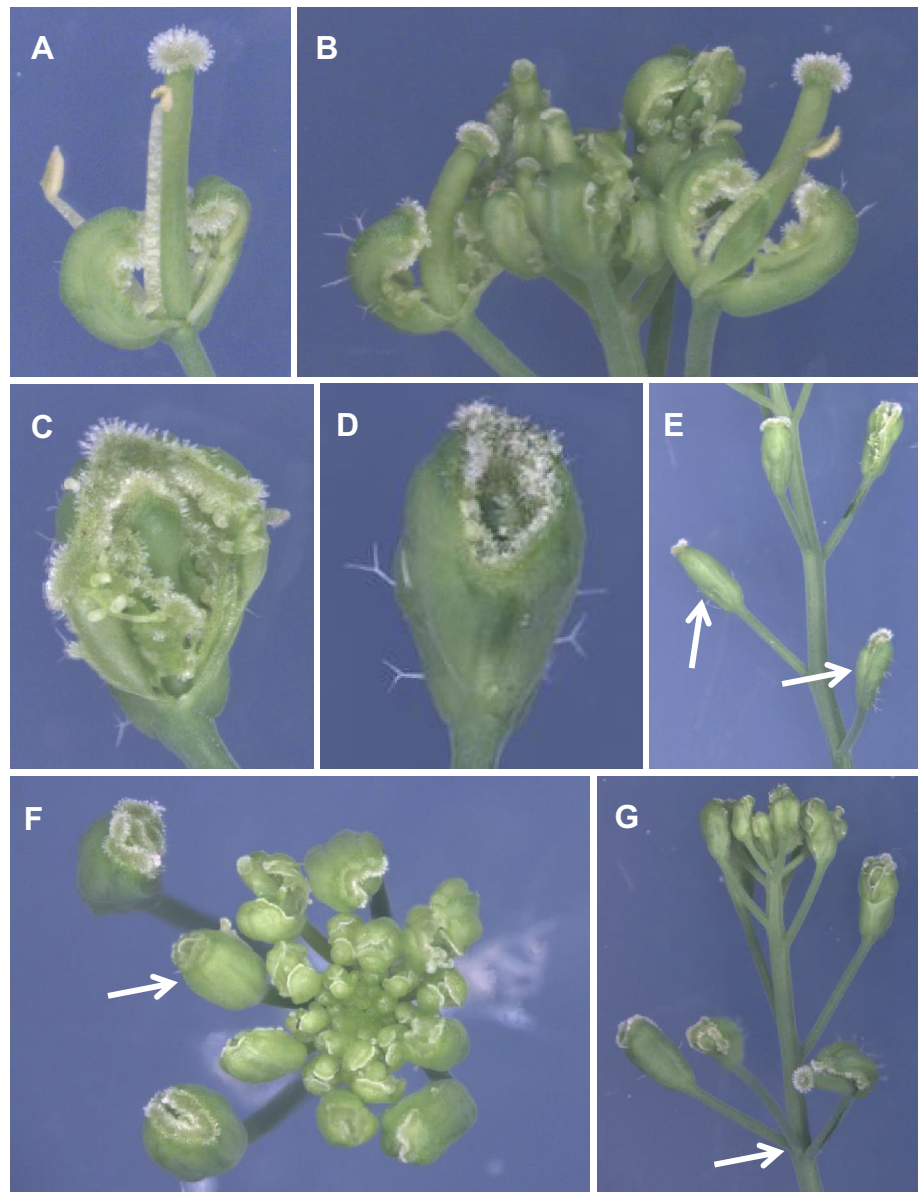
drn drnl puchi mutants. Black and grey columns represent *drn drnl puchi* and *erf12 drn drnl puchi* mutants, respectively. Flower number 1 represents the first-formed flower. N=200; ** $p \leq 0.0001$

regulates ABA-mediated responses and is involved in immune signalling (Cao et al. 2018) and water stress in kiwifruit (Zhang et al. 2017). Although *ERF12* has been functionally implicated in diverse hormone pathways, such as auxin (Lewis et al. 2013), gibberellin (Cao et al. 2006), salicylic acid and methyl jasmonate (Caarls et al. 2017), the data here implicate it as the first ERF VIIIa clade member with a developmental role.

Stereotypy in wild type floral organ number is initially unstable

Stochastic variation in floral organ number exists within populations or individuals of many species. For example, petal number in *Cardamine hirsuta* (Pieper et al. 2016) is naturally variable and together with stamen number, is regulated by seasonal temperature (Matsushashi et al. 2012; McKim et al. 2017) and tepal number varies among the

Fig. 8 *erf12* enhances the *ap2* phenotype. An exemplary *ap2* flower (a) and inflorescence (b). Flowers of *ap2 erf12* plants (c, d). Inflorescences of *ap2 erf12* plants (e–g). Note the complete fusion of outer whorl organs (arrows) in (e) and the aberrant phyllotaxy (arrow) in (g)



Ranunculaceae (Kitazawa and Fujimoto 2014). Stamens are numerically the most variable *Arabidopsis* organ, due to the frequent absence of lateral stamens (Anderson and Roberts 1998). In addition to an environmental and quantitative genetic basis for organ number variation, we show here that the number of wild type floral organs, especially of stamens, is initially developmentally labile and stabilises after the first few flowers. The floral transition thus involves variability in organ merosity, as well as meristem identity changes. The timing of stamen founder cell specification is unknown; auxin response maxima have been spatially associated with early developmental time points, but the founder-cell marker *DRNL* is expressed prior to *DR5* (Chandler et al. 2011) and non-robust positional

information might result from the imprecise resolution of initially diffuse expression domains of positional regulators. Floral organ number and increased organ fusion and homeosis in wild-type *Arabidopsis* are enhanced by GA treatment (Plackett et al. 2018) and the most common homeotic conversions are petaloid stamens (Chandler and Werr 2011). Variable organ numbers in first-formed wild type flowers probably represents variable molecular stochasticity in expression levels or boundaries of homeotic or other regulatory genes (Kitazawa and Fujimoto 2014), especially considering the close proximity of petal and lateral stamen founder cell populations (Chandler et al. 2011).

ERF12 promotes the floral transition and floral meristem identity

The late-flowering phenotype of *erf12* mutants in LD and SD establishes that *ERF12* promotes the IM-to-FM transition independently of photoperiod. This contrasts with mutation of several *Arabidopsis* AP2-type genes that repress the floral transition, including *AP2* (Ohto et al. 2005), *SCHLAFMÜTZE* (Mathieu et al. 2009), *TARGET OF EAT1 (TOE1)* and *TOE2* (Aukerman and Sakai 2003), and *GLOSSY15* in maize (Lauter et al. 2005). More cauline leaves in *erf12* than wild type in both photoperiods represents an extension of the meristem maturity phase, where phytomers transition from containing a secondary inflorescence meristem in the axil of a cauline leaf, to a floral meristem in the axil of a cryptic bract (Park et al. 2014). Despite repressing the floral transition, other AP2-type genes promote FM identity and simultaneously repress vegetative characteristics from flowers. Thus, sepals of *Arabidopsis ap2* mutants and *lip1 lip2* double mutants in *Antirrhinum* are often converted to leaf- or bract-like structures (Bowman et al. 1989; Keck et al. 2003) and *supernumerary bracts* in rice and double *indeterminate spikelet 1/sister of indeterminate spikelet 1* in maize display bracts instead of flowers (Lee et al. 2007; Chuck et al. 2008). Additionally, mutation of the *Arabidopsis* AP2/ERF gene *PUCHI* results in partial bract outgrowth (Karim et al. 2009), and of its orthologues *FRIZZY PANICLE* in rice (Komatsu et al. 2003) and *BRANCHED SILKLESS* in maize (Colombo et al. 1998), blocks the transition from spikelet to floral meristem identity. These mutant phenotypes can be interpreted as heterochronic effects, and the delayed or abolished transition from spikelet meristem or IM to FM produces ectopic bracts or leaf-like organs. The concept of the floral transition as a multidimensional process involving different spatiotemporal components is underlined by uncoupling of the floral transition and bolting by non-permissive conditions (Pouteau and Albertini 2011), which also causes the floral reversion of incompletely committed meristems in many species (Asbe et al. 2015). In *Petunia*, floral reversion has revealed a continuum of variation at the levels of meristem identity, primordium initiation and floral organ identity (Pouteau et al. 1998). Similarly, *Arabidopsis puchi* flowers partially spontaneously revert (Karim et al. 2009). Flower development is also a multistep process that invokes dynamic and competing ab-/adaxial polarity or centroradial models (Chandler and Werr 2014). Based on transcriptome analysis in pea where the *ERF12* homologue is expressed in the vegetative SAM (Wong et al. 2008), by analogy, *ERF12* might be present in the vegetative *Arabidopsis* meristem, raising questions whether it is upregulated in the IM either due to the endogenous age-related developmental programme or on the floral transition, and if this were the case, threshold models for its

function could be invoked, or its functional specificity might depend on appropriately expressed interaction partners.

Sepal initiation occurs independently of a stem cell population as marked by *CLAVATA3 (CLV3)* and *WUSCHEL (WUS)*, which are expressed in IM stem-cells and in the FM centre from late floral stage 2, but are absent during early FM development (Goldshmidt et al. 2008; Yadav et al. 2009). We speculate that in addition to a delayed floral transition, *erf12* mutants have an extended meristem maturation phase in which FM identity and autonomy are irreversibly acquired, and thus a prolonged sepal initiation phase prior to the centroradial initiation of inner floral organs, leading to more sepals. Genes such as *AP1* and *AP2* that regulate FM identity in *Arabidopsis*, are also required for sepal identity (Litt 2007), presumably because FM identity is acquired during the sepal initiation phase, which involves unidirectional polarity (Chandler and Werr 2014). The stochastic variation in wild-type floral inner organs is enhanced in *erf12*, suggesting that *ERF12* also contributes to organ merosity during the centroradial phase of inner organ initiation. Although *erf12* shows photoperiod-independent late-flowering, the increased frequency of sepals, petals and lateral stamens in *erf12* is dependent on long-days. This might reflect the photoperiodic regulation of *ERF12* transcription (Mantegazza et al. 2014), or be because short-day flowering as a default pathway, is potentially more robust than the long-day promotion of flowering, which involves the activation and more precise convergence of pathways at a temporally specific point and might be thus more inherently unstable and prone to disruption.

Phyllotaxis at the apical meristem is largely regulated by auxin response maxima (Reinhardt et al. 2000); however, disrupted phyllotaxy can also result from meristematic effects that cause delayed floral primordium outgrowth, as in *arabidopsis histidine phosphotransfer protein 6* mutants (Besnard et al. 2014), or post-meristematic mechanisms that perturb internode elongation due to the inappropriate regulation of *CUP-SHAPED COTYLEDON (CUC) 2* by miRNA164 (Peaucelle et al. 2007), as observed in higher-order *cuc* mutants (Burian et al. 2015). The basis of clustered siliques and disrupted phyllotaxis in *erf12* inflorescences is unknown, but appears at least partly to result from aberrant internode elongation.

Aspects of the *erf12* mutant phenotype are complemented by *ERF12*

Supernumerary sepals in the first-formed flower was used as the most easily quantifiable phenotype to assess *erf12* complementation by the genomic *ERF12* locus. Transformation by *gERF12Up* was sufficient to complement the *erf12* sepal phenotype. This might reflect the higher sequence conservation revealed by phylogenetic shadowing in the upstream

promoter region compared to downstream sequences. However, the late flowering phenotype of *erf12* was not complemented by the whole genomic sequence between the up- and downstream flanking genes, potentially reflecting the importance of the appropriate physical open chromatin context of *ERF12* within its native chromosomal environment, since epigenetic mechanisms are also major components of flowering time regulation (Ahmad et al. 2010).

***ERF12* expression spatiotemporally coincides with the observed mutant phenotypes**

Dynamic *pERF12::erGFP* expression in the IM and FMs throughout inflorescence development and not only in the first-formed flowers where the strongest floral mutant phenotypes were observed, suggests that these phenotypes also depend on the developmentally regulated expression of downstream targets. The ring of *pERF12::erGFP* expression at the base of young floral primordia as they emerge from the IM might mechanistically underlie a function for *ERF12* in the timing of primordium outgrowth and establishing phyllotaxy. Strong *pERF12::erGFP* expression in the abaxial side of floral stage 1 and 2 FMs, during the sepal initiation phase coincide with the *erf12* sepal phenotype and strong foci of *pERF12::erGFP* expression in the intersepal regions might be the basis for sepal fusion, and expression throughout the FM encompasses the sites of stamen and petal founder cells (Chandler et al. 2011). In summary, *pERF12* expression data spatiotemporally coincide with all aspects of the mutant phenotypes. Furthermore, *ERF12* expression in cotyledon and leaf guard cells shows that the gene is active in embryonic and postembryonic tissue in differentiated cell types as well as in meristematic tissue.

***ERF12* and *MFS1* are functionally similar**

A comparison between the functions of *ERF12* and rice *MFS1* must consider the different floral morphologies between both species. Wild-type rice spikelets contain two rudimentary glumes, two sterile lemmas, in addition to a terminal floret with a single lemma and palea in whorl 1, two lodicules in whorl 2, six stamens in whorl 3, and one carpel in whorl 4. The lodicules are considered by some to be modified petals (Whipple et al. 2007). The lemma and palea have historically been interpreted as extra-floral organs, as a bract and prophyll, respectively (Lombardo and Yoshida 2015). However, because they express floral genes and based on rice floral homeotic mutants, they are probably sepal analogues (reviewed in Lombardo and Yoshida 2015), with the palea potentially representing a differentiated lemma (Ambrose et al. 2000). In *mfs1* mutants, the sterile lemma is homeotically converted into the rudimentary glume and the majority of *mfs1* spikelets contain an extra lemma-like

organ, often two degenerated palea-like organs instead of one, and a variable number of stamens (Ren et al. 2013). Furthermore, an enlarged FM suggests that spikelets with an extra lemma might derive from two florets, due to a delay in FM determinacy acquisition. Thus, the pleiotropic *mfs1* phenotype consists of three components: a delayed spikelet meristem to FM transition, organ homeoses (sterile lemma/glume and palea) and increased organ merosity (supernumerary lemmas and paleae). Despite the absence of intermediate meristems in *Arabidopsis* equivalent to the rice spikelet meristem, delayed flowering of *erf12* mutants represents a similar delay in establishing FM identity to *mfs1*. No organ homeoses were observed in *erf12*, but this aspect of the *mfs1* phenotype is probably masked, because in contrast to lemma and palea, *Arabidopsis* sepals are morphologically similar and no analogues of sterile lemmas or rudimentary glumes are present. Conversely, rice has no analogous organs to petals, but the variable number of stamens and sepals in *erf12* phenocopies labile numbers of stamens, lemmas and paleae in *mfs1*. The largely congruent *erf12* and *mfs1* mutant phenotypes suggests that *MFS1* and *ERF12* are functionally equivalent, which is further supported by the high conservation of the AP2 domain and the shared C-terminal EAR-like motif. The *erf12* phenotype manifests within a narrow developmental time-window including the first-formed flowers, which suggests a heterochronic function for *ERF12*; but no evidence is available concerning changes in the severity of the *mfs1* phenotype along the rachis, the timing of the floral transition or defects in phyllotaxy.

ERF12* interacts genetically with closely related *ERF* factors and *AP2

Genetic interactions between *ERF12* and its closely related *ERF* clade VIIIb genes *DRN*, *DRNL* and *PUCHI* were revealed by higher-order mutant analysis. Loss of *ERF12* function in the *drn drnl puchi* triple mutant background led to attenuated phenotypes, suggesting that the phenotypic severity of *drn drnl puchi* plants requires *ERF12* function. Conversely, the supernumerary sepal phenotype of *erf12* single mutants was counteracted by loss of *DRN*, *DRNL* and *PUCHI* function. Thus, *ERF12* and the combined functions of *DRN*, *DRNL* and *PUCHI* are opposing in terms of the repression and promotion of organ initiation, respectively. *DRN*, *DRNL* and *PUCHI* are considered to be transcriptional activators (see Chandler 2018) and all contain a transcriptionally active ESR domain (Nomura et al. 2009). Conversely, *ERF12* contains a C-terminal EAR-like repressor domain, which might transcriptionally repress downstream targets at the timepoint of incipient FM development within the IM, which is associated with large-scale gene downregulation (Wellmer et al. 2006). *ERF12* also contains an AP2 transcriptional activation domain that might

facilitate protein–protein interactions (Chandler et al. 2007); thus, ERF12 might activate and repress distinct subsets of downstream targets, similar, to AP2 (Yant et al. 2010). The genetic interactions between *ERF12* and *DRN*, *DRNL* and *PUCHI* might represent the combined outread of cumulative activated and repressed targets. Although scant detailed knowledge concerning direct gene targets for these genes currently limits further interpretation of the interactions, it demonstrates that all genes converge on similar downstream targets. In rice, *MFS1* transcriptionally activates the *AP2* homologues *SNB* and *OsIDS1* (Ren et al. 2013). Here, the *ap2* phenotype is dependent on *ERF12* and is enhanced by *erf12*. In strong *ap2* alleles, sepals are converted into carpels, petals are absent and the number of stamens is reduced, whereas *erf12 ap2* mutants only produce carpels. This synergistic phenotype is similar to that of *ap2 ant* double mutants, which only produce carpels (Elliott et al. 1996). This potentially reflects the regulation of *AP2* transcription or its expression domain by *ERF12*. *AP2* possesses a cadastral role in regulating *AGAMOUS* expression (Drews et al. 1991) and potentially loss of *AP2* and *ERF12* function regulate downstream B-function genes to abolish stamens.

The absence of observable mutant phenotypes for *erf9* or genetic interactions in combination with *erf12* suggests that either it is not involved in development, or functions redundantly with other related *ERF* genes, which requires confirmation via a more systematic higher order mutant analysis of group VIIIa members.

In summary, we have characterised pleiotropic roles of the *ERF12* transcription factor in reproductive development (Fig. 9): firstly, it promotes the floral transition and specifies FM identity; secondly, dependent on long-day photoperiods, it represses floral organ initiation, particularly of sepals and stamens; supernumerary *erf12* sepals potentially arise from an extension of the FM maturity phase. Thirdly, as a putative repressor, *ERF12* counteracts the promotion of floral organ initiation by the combined activities of the related transcription factors *DRN*, *DRNL* and *PUCHI* and stabilises the inherent lability of organ number of the first-formed wild type flowers. Fourthly, *ERF12* converges with the *AP2* pathway to specify organ identity. Finally, *ERF12* is continuously required in the IM to maintain appropriate FM phyllotaxy. Alternatively, we can also speculate that the observed *erf12* phenotypes could result from the loss of a wild-type function that represses activators of the floral transition or growth, such that in the mutant, later flowering and overgrowth, including supernumerary organs arising under certain physiological conditions. This study is confined to a single mutant allele (also for *erf9*), because no others are currently available in public stock centres but the caveat exists that we cannot completely exclude that these alleles are null, especially *erf12*, because the T-DNA insertion is in the 3' region of the open reading frame. However, the

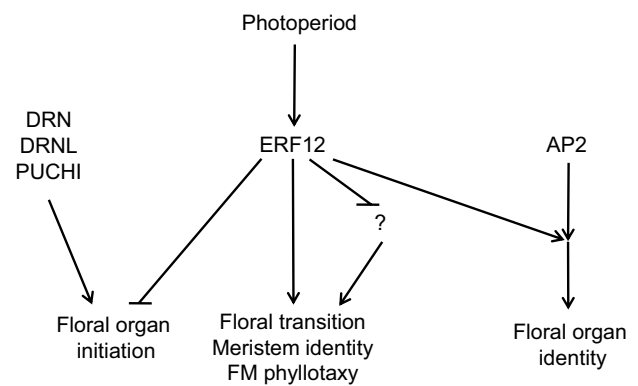


Fig. 9 Model for the integration of *ERF12* function into other genetic pathways and developmental processes. *ERF12* function is dependent on photoperiod and contributes pleiotropic roles to floral organ initiation and floral meristem identity. *ERF12* is a putative transcriptional repressor and opposes the functions of the related *AP2* transcription factors *DORNROESCHEN* (*DRN*), *DORNROESCHEN-LIKE* (*DRNL*) and *PUCHI*, and integrates into the *APETAL2* (*AP2*) pathway to regulate floral organ identity. Alternatively, *ERF12* might repress unknown growth or floral transition activators, leading to late flowering and organ overproliferation when this repressive function is lost in the mutant

successful complementation and conserved functions with rice *MFS1* convincing and unequivocally consolidate the interpretations and conclusions concerning *ERF12* functions in floral organogenesis. The discovery of a novel gene function that contributes to phase change and floral organogenesis suggests that the genetic network involved in these processes might not yet be fully elucidated, even at a non-redundant genetic level. *ERF12* also enhances our understanding concerning the incremental processes that comprise the floral transition, and the stochastic instability in wild-type floral organ merosity reveals that the floral transition also focuses positional information required to appropriately generate floral organ founder cells in the FM, in addition to establishing meristem identity.

Acknowledgements This work was supported by the Deutsche Forschungsgemeinschaft via Grant WE1262/11-2 to W. Werr.

Author contributions Experiments were designed and conducted by JWC. The data were analysed by JWC and the manuscript was written by JWC and edited by JWC and WW. All authors read and approved and final manuscript.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Ahmad A, Zhang Y, Cao XF (2010) Decoding the epigenetic language of plant development. *Mol Plant* 3:719–728
- Amborella Genome Project (2013) The *Amborella* genome and the evolution of flowering plants. *Science* 342:1241089
- Ambrose BA, Lerner DR, Ciceri P, Padilla CM, Yanofsky MF, Schmidt RJ (2000) Molecular and genetic analyses of the *silky1* gene reveal conservation in floral organ specification between eudicots and monocots. *Mol Cell* 5:569–579
- Anderson ML, Roberts JA (1998) *Arabidopsis*. CRC Press, Boca Raton, p 407
- Asbe A, Matsushita SC, Gordon S, Kirkpatrick HE, Madlung A (2015) Floral reversion in *Arabidopsis suecica* is correlated with the onset of flowering and meristem transitioning. *PLoS ONE* 10:e0127897
- Aukerman MJ, Sakai H (2003) Regulation of flowering time and floral organ identity by a microRNA and its *APETALA2*-like target genes. *Plant Cell* 15:2730–2741
- Besnard F, Refahi Y, Morin V, Marteaux B, Brunoud G, Chambrier P, Rozier F, Mirabet V, Legrand J, Lainé S et al (2014) Cytokinin signalling inhibitory fields provide robustness to phyllotaxis. *Nature* 505:417–421
- Blázquez MA, Soowal LN, Lee I, Weigel D (1997) *LEAFY* expression and flower initiation in *Arabidopsis*. *Development* 124:3835–3844
- Bowman JL, Smyth DR, Meyerowitz EM (1989) Genes directing flower development in *Arabidopsis*. *Plant Cell* 1:37–52
- Burian A, Raczynska-Szajgin M, Borowska-Wykręć D, Piatek A, Aida M, Kwiatkowska D (2015) The *CUP-SHAPED COTYLEDON2* and *3* genes have a post-meristematic effect on *Arabidopsis thaliana* phyllotaxis. *Ann Bot* 115:807–820
- Caarls L, Van der Does D, Hickman R, Jansen W, Verk MC, Proietti S, Lorenzo O, Solano R, Pieterse CM, Van Wees SC (2017) Assessing the role of ETHYLENE RESPONSE FACTOR transcriptional repressors in salicylic acid-mediated suppression of jasmonic acid-responsive genes. *Plant Cell Physiol* 58:266–278
- Cao D, Cheng H, Wu W, Soo HM, Peng J (2006) Gibberellin mobilizes distinct *DELLA*-dependent transcriptomes to regulate seed germination and floral development in *Arabidopsis*. *Plant Physiol* 2:509–525
- Cao FY, DeFalco TA, Moeder W, Li B, Gong Y, Liu XM, Taniguchi M, Lumba S, Toh S, Shan L, Ellis B, Desveaux D, Yoshioka K (2018) *Arabidopsis* ETHYLENE RESPONSE FACTOR 8 (ERF8) has dual functions in ABA signaling and immunity. *BMC Plant Biol* 18:211
- Chandler JW (2018) Class VIIIb *APETALA2* ethylene response factors in plant development. *Trends Plant Sci* 23:151–162
- Chandler JW, Werr W (2011) The role of *DORNROSCHEN-LIKE* in early floral organogenesis. *Plant Signal Behav* 6:1244–1246
- Chandler JW, Werr W (2014) *Arabidopsis* floral phytochrome development: auxin response relative to biphasic modes of organ initiation. *J Exp Bot* 65:3097–3110
- Chandler JW, Werr W (2017) *DORNROSCHEN*, *DORNROSCHEN-LIKE*, and *PUCHI* redundantly control floral meristem identity and organ initiation in *Arabidopsis*. *J Exp Bot* 68:3457–3472
- Chandler JW, Cole M, Flier A, Grewe B, Werr W (2007) The AP2 transcription factors *DORNROSCHEN* and *DORNROSCHEN-LIKE* redundantly control *Arabidopsis* embryo patterning via interaction with *PHAVOLUTA*. *Development* 134:1653–1662
- Chandler JW, Comelli P, Jacobs B, Cole M, Werr W (2011) *DORNROSCHEN-LIKE* expression marks *Arabidopsis* floral organ founder cells and precedes auxin response maxima. *Plant Mol Biol* 76:171–185
- Chen X (2004) A microRNA as a translational repressor of *APETALA2* in *Arabidopsis* flower development. *Science* 303:2022–2025
- Chuck G, Meeley R, Hake S (2008) Floral meristem initiation and meristem cell fate are regulated by the maize *AP2* genes *ids1* and *sid1*. *Development* 135:3013–3019
- Clough SJ, Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J* 16:735–743
- Colombo L, Marziani G, Masiero S, Wittich PE, Schmidt RJ, Gorla MS, Pè ME (1998) *BRANCHED SILKLESS* mediates the transition from spikelet to floral meristem during *Zea mays* ear development. *Plant J* 16:355–363
- Comelli P, Glowa D, Chandler JW, Werr W (2016) Founder-cell-specific transcription of the *DORNROSCHEN-LIKE* promoter and integration of the auxin response. *J Exp Bot* 67:143–155
- Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M et al (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res* 36:W465–W469
- Dey S, Corina Vlot A (2015) Ethylene responsive factors in the orchestration of stress responses in monocotyledonous plants. *Front Plant Sci* 6:640
- Drews GN, Bowman JL, Meyerowitz EM (1991) Negative regulation of the *Arabidopsis* homeotic gene *AGAMOUS* by the *APETALA2* product. *Cell* 65:991–1002
- Elliott RC, Betzner AS, Huttner E, Oakes MP, Tucker WQ, Gerentes D, Perez P, Smyth DR (1996) *AINTEGUMENTA*, an *APETALA2*-like gene of *Arabidopsis* with pleiotropic roles in ovule development and floral organ growth. *Plant Cell* 8:155–168
- Goldshmidt A, Alvarez JP, Bowman JL, Eshed Y (2008) Signals derived from *YABBY* gene activities in organ primordia regulate growth and partitioning of *Arabidopsis* shoot apical meristems. *Plant Cell* 20:1217–1230
- Horstman A, Willemsen V, Boutilier K, Heidstra R (2014) *AINTEGUMENTA-LIKE* proteins: hubs in a plethora of networks. *Trends Plant Sci* 19:146–157
- Irish V (2009) Evolution of petal identity. *J Exp Bot* 60:2517–2527
- Karim MR, Hirota A, Kwiatkowska D, Tasaka M, Aida M (2009) A role for *Arabidopsis* *PUCHI* in floral meristem identity and bract suppression. *Plant Cell* 21:1360–1372
- Keck E, McSteen P, Carpenter R, Coen E (2003) Separation of genetic functions controlling organ identity in flowers. *EMBO J* 22:1058–1066
- Kellogg EA (2001) Evolutionary history of the grasses. *Plant Physiol* 125:1198–1205
- Kitazawa MS, Fujimoto K (2014) A developmental basis for stochasticity in floral organ numbers. *Front Plant Sci* 5:545
- Komatsu M, Chujo A, Nagato Y, Shimamoto K, Kyojuka J (2003) *FRIZZY PANICLE* is required to prevent the formation of axillary meristems and to establish floral meristem identity in rice spikelets. *Development* 130:3841–3850
- Krizek BA (2015) *AINTEGUMENTA-LIKE* genes have partly overlapping functions with *AINTEGUMENTA* but make distinct contributions to *Arabidopsis thaliana* flower development. *J Exp Bot* 66:4537–4549
- Lauter N, Kampani A, Carlson S, Goebel M, Moose SP (2005) microRNA172 down-regulates *glossy15* to promote vegetative phase change in maize. *Proc Natl Acad Sci USA* 102:9412–9417
- Lee DY, An G (2012) Two AP2 family genes, supernumerary bract (*SNB*) and Osindeterminate spikelet 1 (*OsIDS1*), synergistically control inflorescence architecture and floral meristem establishment in rice. *Plant J* 69:445–461
- Lee DY, Lee J, Moon S, Park SY, An G (2007) The rice heterochronic gene *SUPERNUMERARY BRACT* regulates the transition from spikelet meristem to floral meristem. *Plant J* 49:64–78
- Lewis DR, Olex AL, Lundy SR, Turkett WH, Fetrow JS, Muday GK (2013) A kinetic analysis of the auxin transcriptome reveals cell wall remodeling proteins that modulate lateral root development in *Arabidopsis*. *Plant Cell* 25:3329–3346
- Li Z, Zhang L, Yu Y, Quan R, Zhang Z, Zhang H, Huang R (2011) The ethylene response factor *ATERF11* that is transcriptionally

- modulated by the bZIP transcription factor HY5 is a crucial repressor for ethylene biosynthesis in *Arabidopsis*. *Plant J* 68:88–99
- Lindsey BE, Rivero L, Calhoun CS, Grotewold E, Brkljacic J (2017) Standardized method for high-throughput sterilization of *Arabidopsis* seeds. *J Vis Exp* 128:56587
- Litt A (2007) An evaluation of A-function: evidence from the APETALA1 and APETALA2 gene lineages. *Int J Plant Sci* 168:73–91
- Lombardo F, Yoshida H (2015) Interpreting lemma and palea homologies: a point of view from rice floral mutants. *Front Plant Sci* 6:61
- Mantegazza O, Gregis V, Chiara M, Selva C, Leo G, Horner DS, Kater MM (2014) Gene coexpression patterns during early development of the native *Arabidopsis* reproductive meristem: novel candidate developmental regulators and patterns of functional redundancy. *Plant J* 79:861–877
- Maruyama Y, Yamoto N, Suzuki Y, Chiba Y, Yamazaki K, Sato T, Yamaguchi J (2013) The *Arabidopsis* transcriptional repressor ERF9 participates in resistance against necrotrophic fungi. *Plant Sci* 213:79–87
- Mathieu J, Yant LJ, Mürdter F, Küttner F, Schmid M (2009) Repression of flowering by the miR172 target SMZ. *PLoS Biol* 7:e1000148
- Matsuhashi S, Sakai S, Kudoh H (2012) Temperature-dependent fluctuation of stamen number in *Cardamine hirsuta* (Brassicaceae). *Int J Plant Sci* 173:391–398
- McCullough E, Wright KM, Alvarez A, Clark CP, Rickoll WL, Madlung A (2010) Photoperiod-dependent floral reversion in the natural allopolyploid *Arabidopsis suecica*. *New Phytol* 186:239–250
- McKim SM, Routier-Kierzkowska AL, Monniaux M, Kierzkowski D, Pieper B, Smith RS, Tsiantis M, Hay A (2017) Seasonal regulation of petal number. *Plant Physiol* 175:886–903
- Meng LS, Wang YB, Yao SQ, Liu A (2015) *Arabidopsis* AINTEGUMENTA mediates salt tolerance by trans-repressing SCABP8. *J Cell Sci* 128:2919–2927
- Müller M, Munné-Bosch S (2015) Ethylene response factors: a key regulatory hub in hormone and stress signaling. *Plant Physiol* 169:32–41
- Nakano T, Suzuki K, Fujimura T, Shinshi H (2006) Genome-wide analysis of the ERF gene family in *Arabidopsis* and rice. *Plant Physiol* 140:411–432
- Nomura Y, Matsuo N, Banno H (2009) A domain containing the ESR motif in ENHANCER OF SHOOT REGENERATION 1 functions as a transactivation domain. *Plant Biotechnol* 26:395–401
- Ohto MA, Fischer RL, Goldberg RB, Nakamura K, Harada JJ (2005) Control of seed mass by APETALA2. *Proc Natl Acad Sci USA* 102:3123–3128
- Ó'Maoiléidigh DS, Graciet E, Wellmer F (2014) Gene networks controlling *Arabidopsis thaliana* flower development. *New Phytol* 201:16–30
- Pan Z-J, Chen Y-Y, Du JS, Chen Y-Y, Chung M-C, Tsai W-C, Wang C-N, Chen H-H (2014) Flower development of *Phalaenopsis* orchid involves functionally divergent *SEPALLATA*-like genes. *New Phytol* 202:1024–1042
- Park SJ, Eshed Y, Lippman ZB (2014) Meristem maturation and inflorescence architecture — lessons from the Solanaceae. *Curr Opin Plant Biol* 17:70–77
- Peaucelle A, Morin H, Traas J, Laufs P (2007) Plants expressing a *miR164*-resistant *CUC2* gene reveal the importance of post-meristematic maintenance of phyllotaxy in *Arabidopsis*. *Development* 134:1045–1050
- Pieper B, Monniaux M, Hay A (2016) The genetic architecture of petal number in *Cardamine hirsuta*. *New Phytol* 209:395–406
- Plackett ARG, Powers SJ, Phillips AL, Wilson ZA, Hedden P, Thomas SG (2018) The early inflorescence of *Arabidopsis thaliana* demonstrates positional effects in floral organ growth and meristem patterning. *Plant Reprod* 31:171–191
- Pouteau S, Albertini C (2011) An assessment of morphogenetic fluctuation during reproductive phase change in *Arabidopsis*. *Ann Bot* 107:1017–1027
- Pouteau S, Tooke F, Battey N (1998) Quantitative control of inflorescence formation in *Impatiens balsamina*. *Plant Physiol* 118:1191–1201
- Reinhardt D, Mandel T, Kuhlemeier C (2000) Auxin regulates the initiation and radial position of plant lateral organs. *Plant Cell* 12:507–518
- Ren D, Li Y, Zhao F, Sang X, Shi J, Wang N, Guo S, Ling Y, Zhang C, Yang Z, He G (2013) *MULTI-FLORET SPIKELET1*, which encodes an AP2/ERF protein, determines spikelet meristem fate and sterile lemma identity in rice. *Plant Physiol* 162:872–884
- Sauquet H, von Balthazar M, Magallón S, Doyle JA, Endress PK, Bailes EJ, Barroso de Morais E, Bull-Hereñu K, Carrive L, Chartier M et al (2017) The ancestral flower of angiosperms and its early diversification. *Nat Commun* 8:16047
- Schultz EA, Haughn GW (1991) *LEAFY*, a homeotic gene that regulates inflorescence development in *Arabidopsis*. *Plant Cell* 3:771–781
- Shrestha R, Gómez-Ariza J, Brambilla V, Fornara F (2014) Molecular control of seasonal flowering in rice, *Arabidopsis* and temperate cereals. *Ann Bot* 114:1445–1458
- Smyth DR, Bowman JL, Meyerowitz EM (1990) Early flower development in *Arabidopsis*. *Plant Cell* 2:755–767
- Teotia S, Tang G (2015) To bloom or not to bloom: role of microRNAs in plant flowering. *Mol Plant* 8:359–377
- Tooke F, Ordidge M, Chiurugwi T, Battey N (2005) Mechanisms and function of flower and inflorescence reversion. *J Exp Bot* 56:2587–2599
- Überlacker B, Werr W (1996) Vectors with rare-cutter restriction enzyme sites for expression of open reading frames in transgenic plants. *Mol Breed* 2:293–295
- 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:e117
- Whipple CJ (2017) Grass inflorescence architecture and evolution: the origin of novel signaling centers. *New Phytol* 216:367–372
- Whipple CJ, Zanis MJ, Kellogg EA, Schmidt RJ (2007) Conservation of B class gene expression in the second whorl of a basal grass and outgroups links the origin of lodicules and petals. *Proc Natl Acad Sci USA* 104:1081–1086
- Wong CE, Bhalla PL, Ottenhof H, Singh MB (2008) Transcriptional profiling of the pea shoot apical meristem reveals processes underlying its function and maintenance. *BMC Plant Biol* 8:73
- Yadav RK, Girke T, Pasala S, Xie M, Reddy GV (2009) Gene expression map of the *Arabidopsis* shoot apical meristem cell niche. *Proc Natl Acad Sci USA* 106:4941–4946
- Yant L, Mathieu J, Dinh TT, Ott F, Lanz C, Wollmann H, Chen X, Schmid M (2010) Orchestration of the floral transition and floral development in *Arabidopsis* by the bifunctional transcription factor APETALA2. *Plant Cell* 22:2156–2170
- Zhang JY, Pan D-L, Wang G, Xuan J-P, Wang T, Guo Z-R (2017) Genome-wide analysis and expression pattern of the AP2/ERF gene family in kiwifruit under waterlogging stress treatment. *Int J Environ Agric Res* 3:83–90
- Zhou X, Zhang ZL, Park J, Tyler L, Yusuke J, Qiu K, Nam EA, Lumba S, Desveaux D, McCourt P et al (2016) The ERF11 transcription factor promotes internode elongation by activating gibberellin biosynthesis and signaling. *Plant Physiol* 171:2760–2770