FRUITFULL-like genes regulate flowering time and inflorescence architecture in tomato

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Short title:

Tomato *FUL*-like genes regulate flowering

ABSTRACT

The timing of flowering and inflorescence architecture are critical for the reproductive success of tomato, but the gene regulatory networks underlying these traits are still hardly explored. Here we show that the tomato *FRUITFULL*-like (*FUL*-like) genes *FUL2* and *MADS-BOX PROTEIN 20 (MBP20)* induce flowering and repress inflorescence branching. *FUL1* fulfils a less prominent role and appears to depend on FUL2 and MBP20 for its upregulation in the inflorescence meristem. Our results demonstrate that *MBP10*, the fourth tomato *FUL*-like gene, has probably lost its function. The tomato FUL-like proteins cannot homodimerize, but heterodimerize with JOINTLESS (J), SlMBP21 (J2), ENHANCER OF JOINTLESS (EJ2/MADS1) and the SOC1-homolog TOMATO MADS-box gene 3 (TM3), which are co-expressed during inflorescence meristem maturation. Transcriptome analysis revealed various interesting downstream targets, including five repressors of cytokinin signaling, which are all upregulated during the vegetative-to-reproductive transition in *ful1/ful2/mbp10/mbp20* mutants. FUL2 and MBP20 can also bind *in vitro* to the upstream regions of these genes, thereby probably directly stimulating cell division in the meristem upon the transition to flowering. Our research reveals that the four tomato FUL-like genes have diverged functions, but together regulate the important developmental processes flowering time, inflorescence architecture and fruit development.

INTRODUCTION

The MADS-box transcription factor gene family is involved in almost every developmental process in plants (Smaczniak et al., 2012a), and the members of the angiosperm-specific APETALA1/FRUITFULL (AP1/FUL) subfamily play key roles in flower and fruit development (Litt and Irish, 2003; McCarthy et al., 2015). In the core eudicots, the AP1/FUL subfamily consists of three clades, euAP1, euFULI and euFULII (Litt and Irish, 2003). The Arabidopsis genome carries four *AP1/FUL*-clade genes, with *AP1* functioning as an A-class gene in the ABC model, promoting perianth identity (Mandel et al., 1992; Theissen and Saedler, 2001) and establishing meristem identity and floral initiation (Ferrándiz et al., 2000; Kaufmann et al., 2010). Its lower-expressed paralog CAULIFLOWER (*CAL)* functions to a large extent redundantly with *AP1* (Bowman et al., 1993; Ye et al., 2016). The euFULII-clade gene *AGL79* appears to have a minor function in roots (Gao et al., 2018), whereas the euFULI gene *FUL* is a pleiotropic gene with roles

in both vegetative and reproductive development. In addition to its well-known function during fruit development (Gu et al., 1998), *FUL* functions in induction and termination of flowering (Hempel et al., 1997; Gu et al., 1998; Wang et al., 2009; Balanzà et al., 2018), inflorescence architecture (Bemer et al., 2017), repression of secondary growth (Melzer et al., 2008) and cauline leaf development (Bemer et al., 2017). Functional evidence from other angiosperms suggests that *FUL* orthologs have a conserved role in reproductive development in many species, including poppy, tomato and cucumber (Jaakola et al., 2010; Bemer et al., 2012; Pabón-Mora et al., 2012; Pabón-Mora et al., 2013; Jia et al., 2015; Cheng et al., 2018; Li et al., 2019; Zhao et al., 2019).

While most core eudicots contain only one *FUL*-like gene in the euFULI clade and one in the euFULII clade, most Solanaceae have two genes in each clade (Maheepala et al., 2019). The tomato genome contains *Solanum lycopersicum FUL1* (*SlFUL1* / *FUL1* / *TM4* / *TDR4,* hereafter called *FUL1*) and *SlFUL2* (*FUL2* / *MBP7,* hereafter called *FUL2*) as euFULI clade members, probably originating through an early whole-genome duplication event. As the result of a more recent tandem duplication event, the tomato genome contains *MADS-BOX PROTEIN 10* (*MBP10*) and *MBP20* in the euFULII clade (Litt and Irish, 2003; Maheepala et al., 2019). In addition, tomato contains one *AP1* gene, *MACROCALYX* (*MC*) (Nakano et al., 2012; Liu et al., 2014; Yuste-Lisbona et al., 2016). Previous work has shown that *FUL1* and *FUL2* are crucial for tomato fruit ripening (Bemer et al., 2012; Shima et al., 2013; Fujisawa et al., 2014; Wang et al., 2019). Additional functions for the tomato *FUL*-like genes have been suggested based on overexpression studies, including the regulation of compound leaf development (Burko et al., 2013) and secondary stem growth (Shalit-Kaneh et al., 2019). However, the biological roles of *MBP10/20* and the unique and overlapping functions of all tomato *FUL*-like genes have remained elusive. Because of their importance for plant development, it is also possible that *FUL*-like genes have played a role during tomato domestication. This has already been unveiled for the MADS-box genes *JOINTLESS 2* (*J2*, also called *SlMBP21*), *ENHANCER OF JOINTLESS2* (*EJ2/MADS1*, hereafter called *EJ2*) and *SISTER OF TOMATO MADS-box gene 3* (*STM3*) (Roldan et al., 2017; Soyk et al., 2017; Soyk et al., 2019; Alonge et al., 2020). The expression of *FUL1*, *FUL2* and *MBP20* is strongly upregulated during the transition from shoot apical meristem (SAM) to inflorescence- and floral meristem (IM and FM) (Park et al., 2012), suggesting that these genes may function in inflorescence meristem development like their Arabidopsis homolog. However, it is yet

unclear how the *FUL*-like genes (*SlFUL*) would promote floral transition and meristem specification in tomato.

Tomato reproductive development begins with doming of the vegetative meristem (VM), which then becomes the transition meristem (TM). The TM develops into an FM, which forms an IM on its side before it terminates. In its turn, this IM generates a new IM on its side before ending into an FM. The successive IM-FM transitions are rapid and produce a zigzagged inflorescence with multiple flowers (Pnueli et al., 1998; Lippman et al., 2008). Delayed FM termination causes additional IM formations, resulting in a branched inflorescence, such as in the *compound inflorescence* (*s*) mutant (Park et al., 2012; Soyk et al., 2017). Meanwhile, vegetative growth continues through a sympodial shoot meristem (SYM) that develops from the axil of the youngest leaf and generates three leaves before maturing into the first flower of the new inflorescence. This process forms the compound shoot, where three leaves and an inflorescence comprise a sympodial unit, a pattern that is endlessly repeated in the indeterminate wild-type tomato. Although tomato inflorescence development differs fundamentally from that of monopodial species such as Arabidopsis, which develop a single inflorescence meristem, the orthologs of several important Arabidopsis flowering and inflorescence meristem genes are also essential in tomato.

The tomato vegetative-to-reproductive transition is modulated by the activity of the floral specification complex encoded by the F-box gene *ANANTHA* (*AN*, ortholog of *UNUSUAL FLORAL ORGANS*) and its transcription factor partner *FALSIFLORA* (*FA*, ortholog of *LEAFY*) (Chae et al., 2008; Souer et al., 2008). Precocious activation of the AN/FA complex leads to a quick SAM maturation and thus early flowering, while blocking its activity results in delayed flowering. *SINGLE FLOWER TRUSS* (*SFT*, ortholog of *FLOWERING LOCUS T*) promotes the transition in the primary shoot (Molinero-Rosales et al., 1999; Molinero-Rosales et al., 2004; Lifschitz et al., 2006), while *SELF PRUNING* (*SP*, ortholog of *TERMINAL FLOWER 1*) controls flowering time in the sympodial segments (Pnueli et al., 1998). In addition, *mc* and *jointless* (*j*) mutants both showed a delayed transition to flowering and reversion to the vegetative phase after the production of a few flowers. MC and J can probably form a complex in the inflorescence meristem, and act synergistically with SFT (Yuste-Lisbona et al., 2016). However, the understanding of the tomato flowering regulatory network is far from complete, and it is unclear whether *FUL*-like genes function similarly as in Arabidopsis.

Here, we investigated the developmental roles of the four *FUL*-like genes in tomato by CRISPR/Cas9-mutagenesis and transcriptome profiling. We demonstrate that subfunctionalization has occurred after duplication within the Solanaceae euFULI and euFULII clades, and that the *FUL1* sequence has undergone further divergence during tomato domestication and breeding. *FUL2* and *MBP20* are highly expressed in the meristem during the vegetative-to-reproductive transition to additively promote tomato flowering and to repress inflorescence branching together with *FUL1*. Several homologs of known Arabidopsis flowering and/or architecture genes, such as *VERNALIZATION 1* (*VRN1*) and *AT-HOOK MOTIF NUCLEAR-LOCALIZED PROTEIN 15* (*AHL15*) are differentially expressed in the mutants. We also identified a remarkable upregulation of the cytokinin signaling repressors *CYTOKININ OXIDASE 1 (CKX1), CKX2 and CKX6* and the *type-A ARABIDOPSIS RESPONSE REGULATORs* (*ARRs*) *ARR7* and *ARR16*, which can be bound by FUL2 and MBP20 proteins and can partially explain the observed phenotypes.

RESULTS

Expression patterns and protein-protein interaction profiles differ between the tomato FUL homologs

To investigate to what extent the different *FUL*-like genes may have overlapping functions in specific organs, we performed expression profiling using qRT-PCR in the cultivar Moneyberg (Figure 1A). The results show that *FUL1* and *FUL2* are expressed very weakly during vegetative growth and have increased expression in the inflorescence meristem. Their expression remains high throughout reproductive development, where both genes show considerable expression in all floral whorls and all stages of fruit development. *FUL2,* in particular, is strongly expressed in all floral organs, early fruits and ripening fruits, while *FUL1* expression is moderate until the fruit ripening phase, when it increases strongly as reported previously (Bemer et al., 2012). Our data also show the striking differences in the spatial expression of *MBP10* and *MBP20* that are both much weaker expressed than *FUL1* and *FUL2* in the reproductive tissues, except for the expression of *MBP20* in the inflorescence meristem. *MBP10* especially was extremely weakly expressed, with detectable levels in stem and flower bud only.

MADS-domain transcription factors regulate multiple developmental processes by forming higher-order protein complexes (De Folter et al., 2005). To investigate which protein complexes may be formed by the different FUL-like proteins, we performed a yeast-two hybrid (Y2H) assay to identify the interactions of FUL1, FUL2, MBP10 and MBP20 with other tomato MADS-domain family proteins (Figure 1B, Supplemental Figure 1). We chose a set of proteins homologous to MADS-domain proteins known to interact with Arabidopsis FUL (De Folter et al., 2005). While Arabidopsis FUL can form homodimers (Smaczniak et al., 2012b), none of the tomato FUL-like proteins possessed this capacity, nor could they heterodimerize with each other (Supplemental Figure 1). Of the tested MADS-domain proteins, MC, MBP9, MBP12, MBP13, MBP14, MBP22 and MBP24 did not interact with any of the tomato FUL homologs. Our screen showed that the SlFUL proteins could interact with nine other tomato MADS-domain proteins, and we observed clear differences between the interaction profiles of FUL1, FUL2, MBP10 and MBP20. All FUL-like proteins interacted with JOINTLESS (J), J2 (or SlMBP21, hereafter called J2), the fruit ripening regulator MADS-RIN, and TOMATO MADS 3 (TM3), the ortholog of the Arabidopsis flowering integrator SOC1 (Alonge et al., 2020). FUL2 exhibits the most extensive interaction network, interacting with nine proteins. It is the only protein to strongly interact with the SEPALLATA (SEP)-like proteins EJ2 and LeSEP1/TM29, and the AGAMOUS (AG) ortholog TAG1 (Figure 1B and Supplemental Figure 1). The latter interaction is especially interesting in the light of the high expression of FUL2 in carpels, where FUL2 may have a specific function in a complex with the co-expressed TAG1. In addition to these specific interactions, FUL2, FUL1 and MBP20 share interactions with the SEP-like protein TM5 and the AGL42-homolog FOREVER YOUNG FLOWER-LIKE (FYFL/SlMBP18) (Supplemental Figure 1), and FUL1 could also weakly interact with EJ2. Part of the interaction pairs we tested has been investigated before in a tomato MADS-domain interaction screen (Leseberg et al., 2008). We could reproduce all the previous results, except for interactions of FUL2 and MBP20 with MBP13, and FUL2 with MBP24. In conclusion, FUL2 can form most protein-protein interactions, which, together with its broad expression pattern, suggests that it can fulfil multiple functions in tomato, similar to FUL in Arabidopsis. FUL1 and MBP20 share a reduced set of interaction partners and MBP10 has the smallest set of interactors. The low number of interactors for MBP10 in combination with its weak overall expression pattern hints at relaxed selective pressure on this gene.

Different variants of the *FUL1* gene exist in tomato cultivars

Upon cloning and sequencing of the *FUL1* cDNA of tomato cv. Moneyberg, which was used in our experiments, we noticed a deletion of the 5th base (G) of the last exon as compared to the reference sequence (cv. Heinz). This deletion is predicted to result in a 205 amino acid protein, lacking the C-terminal 40 amino acids as compared to the reference (Figure 1C). Inspection of the genome sequence of 38 re-sequenced cultivated tomato varieties (Aflitos, et al., 2014) showed that approximately half (17) contain this deletion (Supplemental Figure 2A). The latter included the much-studied cv. 'Ailsa Craig'. The deletion was not detected in any of the re-sequenced wild accessions, suggesting that it may have first emerged after domestication. Although the deletion results in a C-terminally truncated protein, no differences in interactions were observed in a yeast two-hybrid assay (Supplemental figure 2B). This lack of *in vitro* interaction differences, together with the already described fruit ripening function for *FUL1* in the cultivars Moneyberg and Ailsa Craig (Wang et al., 2019; Bemer et al., 2012), indicates that the truncated protein is functional.

FUL2 and MBP20 promote flowering in the primary and sympodial shoots

To dissect the biological roles of the *SlFUL* genes *in planta*, we generated loss-of-function single- and higher-order mutants with the CRISPR/Cas9 method. The first coding exon of each gene was targeted with three single-guide RNAs. After stable tomato transformation of the cultivar Moneyberg, we screened several independent first-generation (T0) transgenic lines for the presence of insertion/deletion (indel) alleles by PCR and sequencing. Then we generated the progeny of the primary transgenics (T1) and selected two different homozygous indel alleles for each gene, encoding truncated proteins caused by frameshifts and premature stop codons (Supplemental Figure 3). T2 phenotyping of the selected mutants revealed that the plants with homozygous knockout alleles for either *FUL2* or *MBP20* exhibited delays in primary shoot flowering, switching to reproductive growth after 13 leaves, compared to 11 in the wild type (Figure 2A). The number of days to first flowering was also significantly delayed, but was more variable between individuals of the same genotype (Supplemental Figure 4A). No significant effect in the number of leaves was observed for the *ful1* or *mbp10* single mutants (Figure 2A, Supplemental Figure 4A). The observed delay in the transition to flowering was more pronounced in

higher-order mutants, with approximately three leaves extra in *ful2/mbp20*, *ful1/ful2/mbp20* and *ful1/ful2/mbp10/mbp20* compared to the wild type (Figure 2A). The fact that neither the *ful1* nor the *mbp10* mutations enhanced the mutant phenotype, suggests that *FUL2* and *MBP20* are the most important *FUL*-like genes for promoting the floral transition. In addition to a delay in primary shoot transition, we also observed late flowering in the sympodial shoots of the same set of mutants, increasing to an average of four leaves per sympodial shoot. At the same time, the wild type always has three (Figures 2B and 2C, Supplemental Figure 4B). Thus, *FUL2* and *MBP20* additively regulate the timing of flowering in both the primary shoot and the sympodial shoots. Later during development, *ful1* single mutants also showed an increase in leaf number (Figure 2C, Supplemental Figure 4B), suggesting that *FUL1* may play a minor role as well.

FUL1, FUL2 and MBP20 control inflorescence architecture

We observed that the mutant plants had more branched inflorescences than wild-type plants, which typically produced only non-branched inflorescences (Figure 2D). We quantified the branching events for the first seven inflorescences of each plant in the T2 generation. With the exception of *mbp10*, all mutants showed increased inflorescence complexity, ranging from bi-parous to quintuple-parous inflorescences (Figure 2E). Notably, 13.6% of the inflorescences from wild-type plants branched, while *ful1*, *ful2* and *mbp20* lines produced 43.1%, 56.1% and 50% branched inflorescences, respectively. In higher-order mutants, branching increased further to ~75% in both the *ful2/mbp20* and the *ful1/ful2/mbp20* mutants. *mbp10* mutants were hardly branching, similar to the wild type, while *mbp10/mbp20* mutants were identical to *mbp20* mutants. These results indicate no additional contribution of *mbp10* or *ful1* to the branching phenotype of the mutants. Surprisingly, the *ful1* mutant did exhibit enhanced branching, but its mutation did not further enhance the phenotype of the *ful2/mbp20* mutant, suggesting that *FUL1* function depends on *FUL2* and/or *MBP20* (Supplemental Figure 4C). Interestingly, all first inflorescences did not branch, except for the first inflorescence of *ful2/mbp20* (3 plants out of 11) and *ful1*/*ful2/mbp20* (2 plants out of 11). Higher order branching events (i.e. quintuple parous) were only observed in mutant lines where *ful2* was included, suggesting that *FUL2* has the most prominent role in the repression of inflorescence branching. We quantified the number of flowers on the second to fourth inflorescences. The more complex inflorescences developed more flowers, increasing from on average 10 in wild-

type inflorescences to approximately 20 in the higher order mutants (Figure 2F). These results show that mutations in *FUL2* and *MBP20,* individually or combined, result in increased branching during inflorescence development, and that both genes thus regulate inflorescence architecture in an additive manner. *FUL1* is also involved in this process as is shown by the *ful1* single mutant phenotype, but its role is masked in higher order mutants that contain *ful2* and *mbp20* alleles.

MBP10/20 **do not contribute to fruit development and ripening**

FUL1 and *FUL2* were reported as redundant regulators of tomato fruit ripening, and *FUL2* has an additional function in early fruit development (Bemer et al., 2012; Wang et al., 2019). Since *MBP20* is weakly expressed in carpels and early stages of fruit development (Figure 1A), we asked whether it could function in fruit development as well. We therefore examined fruit development and ripening in the different mutant lines. As reported before, the *ful2* mutant fruits were smaller with superficial stripes on the pericarp, while the *ful1lful2* mutant fruits were more severely impaired in ripening (Wang et al., 2019). *ful2/mbp20*, triple (*ful1/ful2/mbp20)* and quadruple mutant fruits had the same phenotype as *ful2* and *ful1/ful2* fruits in terms of width, Brix value, number of locules and overall appearance, indicating that *MBP10/20* do not contribute to fruit development and ripening (Supplemental Figures 5 and 6). Remarkable was the high Brix values of fruits that contained *ful2* mutant alleles, although this may to a large extent be due to the smaller size of *ful2* fruits. Interestingly, the number of locules was slightly, but significantly, enhanced in *ful2* single mutants, and in most mutant combinations that contained *ful2* (Supplemental Figure 5C), suggesting that *FUL2* is also involved in determining floral meristem size, and thereby carpel number.

Dynamic expression of MADS-box genes in the inflorescence meristem

The CRISPR mutant analysis revealed that *FUL2* and *MBP20* promote the transition from vegetative to reproductive development and control inflorescence architecture. To further unveil the role of the tomato FUL-like genes in flowering, we conducted RNA-seq to compare the transcriptome dynamics during three consecutive stages (VM, TM, FM) of meristem development between the wild type and quadruple mutant. Meristems at the same developmental stage were dissected and pooled for RNA extraction. To also increase our insight into the cause of the enhanced inflorescence branching, the FM and

neighboring IM were harvested together, which is further referred to as FM (Figure 3A). High-throughput sequencing yielded a minimum of 30M reads per sample. We first determined the expression of the *FUL*-like genes in the different stages of meristem maturation, revealing dynamic expression changes through the vegetative-to-reproductive transition for *FUL1, FUL2* and *MBP20* (Figure 3B). *FUL2* and *MBP20* are already considerably expressed in the VM stage, but their expression highly increased in the TM stage. *FUL1* is much weaker expressed in the VM, but also reaches high expression levels in the TM and FM stages. The higher expression of *FUL2* and *MBP20* in the VM stage is in line with their prominent role in the determination of flowering time. Of the MADS-box genes encoding SlFUL interactors, *J* (the *SVP*-homolog), was exceptionally highly expressed in all three stages. While the expression of the SEP-like genes *EJ2*, *TM29* and *J2* gradually increased from practically absent in VM to clearly expressed in FM. The *SOC1*-homologs *TM3* and *STM3* were also expressed in all three stages (Figure 3C). The other SlFUL interactors were only weakly expressed. To validate the RNA-seq data analysis, we confirmed the expression of these SlFUL interacting genes with qRT-PCR analysis (Supplemental Figure 7). Thus, based on expression patterns of the genes and interaction capacity, FUL1, FUL2 and MBP20 can probably form complexes with J and TM3 in all meristem stages and with J2 in the IM/FM, while FUL2 could additionally form complexes with EJ2 and TM29 in the IM/FM.

Identification of Differentially Expressed Genes

Comparison of WT and quadruple mutant transcriptomes revealed 167 differential expressed genes (DEGs) for the VM stage (152 up- and 15 down-regulated in the mutant), 162 for the TM stage (137 up- and 25 down-regulated), and 185 DEGs for the FM stage (162 up- and 23 down-regulated), using FDR-corrected p-value <0.05 as a threshold for significance and a Log_2 fold change >1.0. These genes significantly overlapped between stages, with 18 genes differentially expressed in all three stages (Figure 3D). Many more genes were upregulated in the mutant than there were down-regulated, pointing towards a general repressive function of SlFUL-containing complexes, in agreement with data from Arabidopsis FUL studies (Ferrándiz et al., 2000; Bemer et al., 2017; Balanzà et al., 2018). A large proportion of the DEGs are involved in metabolic processes, such as terpene synthesis or the phenylpropanoid pathway, but the corresponding genes were in general weakly expressed in the meristem (Supplemental Table 1). Notably, the phenylpropanoid

pathway is also controlled by FUL1/2 in tomato fruits (Bemer et al., 2012), indicating that the regulation of some identified DEGs reflects regulation in other tissues. The DEG lists also contained several interesting genes that are probably involved in the transition to flowering or inflorescence architecture (Table 1), although previously described tomato key regulators, such as *AN*, *FA*, *SFT*, *SP* and *S* were not among the DEGs. Instead, we identified *VRN1*- and CIS-CINNAMIC ACID-ENHANCED 1 (*ZCE1)*- like genes, which are in Arabidopsis involved in the regulation of flowering time (Levy et al., 2002; Guo et al., 2011) and a homolog of *AHL15* that is involved in inflorescence architecture and growth (Karami et al., 2020). Also, the MADS-domain factors *TM3*, *MC* and *SlMBP13* were significantly upregulated in the quadruple mutant in all three meristem stages. Most interestingly, however, is the identification of five cytokinin signaling genes as targets of the SlFULs.

The tomato FUL-like proteins repress negative regulators of cytokinin signaling

Compelling evidence shows that cytokinin (CK) is required for SAM activity and FM initiation, and that the interplay of transcription factor regulation and CK signaling controls the size and activity of the shoot apical meristem (Kurakawa et al., 2007; Bartrina et al., 2011; Han et al., 2014b). In our list of DEGs, we identified several genes involved in CK signaling, namely three *CYTOKININ OXIDASEs* (*CKXs*), *CKX1/2/6*, which degrade bioactive cytokinins, and two *type-A ARABIDOPSIS RESPONSE REGULATORs* (*ARRs*), *ARR7/16*. We verified their differential expression with qPCR (Supplemental Figure 8A). *CKX2* and *CKX6* were upregulated in the VM and TM stages of the mutant, but not in the FM stage, while *ARR16* and *CKX1* were only upregulated in the TM stage (Figure 4A, Table 1). CKXs irreversibly degrade active CKs and type-A ARRs function as negative regulators of the CK response (Brownlee et al., 1975; McGaw and Horgan, 1983; D'Agostino et al., 2000). Therefore, upregulation of *CKX1/2/6* and *ARR7/16* in the VM and TM stages will probably result in a reduced CK content and responsiveness. We further investigated whether FUL2 and MBP20 can directly repress *CKX* and *ARR* gene expression by binding to their promoters. We therefore scanned the up- and downstream regions of the *ARR* and *CKX* genes for CArG-box motifs, the binding sites for MADS-domain proteins (Kaufmann et al., 2009; Aerts et al., 2018). Putative CArG-boxes were present in all differentially expressed *ARR* and *CKX* genes (Supplemental figure 9A). To test whether FUL2 and MBP20 can bind to these, we performed electrophoretic mobility

shift assays (EMSAs) using fragments containing these CArG-boxes as native probes. Because MADS-domain proteins need to form a dimer to bind to the DNA, we tested TM3-FUL2 and TM3-MBP20 heterodimers, because these proteins form strong heterodimers in yeast and are expressed at equal levels in the VM (Figure. 4B, Supplemental Figure 8B). The shifts were clearly detected, suggesting that the FUL2-TM3 and MBP20-TM3 heterodimers can physically bind to the regulatory fragments. To investigate whether the CArG-box is essential for the binding, we also generated mutated probes, in which the CArG-box was mildly perturbed by a single-nucleotide mutation in the center of the motifs. This probe mutation abolished or reduced the binding in all cases except for *CKX1* (Figure 4B), confirming the importance of the CArG box for the binding of the heterodimers. These results suggest that FUL2 and MBP20 directly bind to the promoter of the *CKX1/2/6* and *ARR7/16* genes to repress their expression and thereby upregulate cytokinin signaling in the vegetative meristem at the start of the transition to flowering.

FUL1 expression is probably regulated via an autoregulatory loop

Despite the high expression of *FUL1* in the TM and FM and the branching phenotype in the *ful1* single mutants, the *ful1* mutation does not enhance the branching phenotype of the *ful2/mbp20* mutants. The distinct down-regulation of *FUL1* may explain this apparent discrepancy in the quadruple mutant (Figure 5A), indicating that the gene is induced by FUL2, MBP20 and/or by itself via a positive autoregulatory loop. There are four CArG-boxes in the upstream region of *FUL1* that can probably be bound by MADS-domain complexes (Supplemental Figure 9B). Because the expression of *FUL1* is low in the VM, FUL2- and/or MBP20-containing complexes may need to bind to the CArG-boxes in the *FUL1* regulatory region to upregulate its expression in TM and FM. To test whether FUL2 and MBP20 can bind, we performed EMSAs with TM3-FUL2 and TM3-MBP20 dimers and observed clear binding (Figure 5B) to the CArG-box containing probes, but not to the mutated versions, suggesting that *FUL1* depends on FUL2 and/or MBP20 for maximal expression in the TM and FM stages.

DISCUSSION

Subfunctionalization of the tomato *FUL*-like genes

Following segmental or whole-genome duplication events, genes with new molecular functions can arise through sub- or neofunctionalization, resulting in divergence of biological functions. We show here that functional divergence also occurred for the tomato *FUL*-like genes after their multiplication early in the Solanaceae lineage. In addition to their previously described roles in fruit development and ripening (Bemer et al., 2012; Wang et al., 2019), we unveil that *FUL1* and *FUL2* both regulate IM development, albeit at different levels. *MBP20* promotes IM development together with *FUL2*, but does not contribute to fruit development. We did not observe any phenotype for the *mbp10* mutant however, nor did the mutation enhance the phenotype in higher-order mutants. This suggests, together with its weak overall expression pattern and low number of protein-protein interactions, that *MBP10* may be losing its role in regulating plant developmental processes. In line with this, *MBP10* lacks regulatory sequences in its first intron (Maheepala et al., 2019), and has a three amino-acid mutation in the I-domain, a region important for protein-protein interactions (Van Dijk et al., 2010). The loss of *MBP10* in other Solanaceae genera such as *Fabiana*, *Petunia* and *Plowmania* also hints in this direction (Maheepala et al., 2019).

Although previous overexpression studies suggested that *MBP20* functions in leaf development and *FUL2* in stem development and secondary growth (Burko et al., 2013; Wang et al., 2014a; Shalit-Kaneh et al., 2019), we did not observe aberrant phenotypes in these tissues in our knockout mutants. The most probable explanation for this discrepancy is the use of the Cauliflower 35S promoter in the previous experiments (Wang et al., 2014a; Shalit-Kaneh et al., 2019), resulting in ectopic expression and mis-regulation of target genes at a position where *FUL2* and *MBP20* are usually not expressed. Overexpressing MADS-domain proteins or dominant-negative forms of MADS proteins can also perturb complexes that involve their interaction partners, and the phenotype may thus reflect the phenotype of mutants impaired in other, interacting tomato MADS proteins. However, another possibility is that *FUL2* and/or *MBP20* function redundantly with other MADS proteins in the investigated tissues. Arabidopsis *FUL* and *SOC1* act redundantly in the regulation of secondary growth (Melzer et al., 2008), and *FUL2* may thus function redundantly with *(S)TM3* in the tomato stem as well. In conclusion, the four *FUL*-like genes

in tomato underwent a functional divergence during evolution, but together retained functions in both inflorescence and fruit development. It is possible that some functions have remained unidentified due to redundancy with other MADS-box genes.

The position of *FUL1* in the flower regulatory network

FUL1 appears to act differently from *FUL2* and *MBP20* in the inflorescence meristem. It is only weakly expressed in the VM, and its high expression in TM and FM probably depends on FUL2 and MBP20, which are already expressed earlier in the VM and can bind to the *FUL1* promoter. The expression of *FUL2*, which has several CArG-boxes in its promoter, was also downregulated in the quadruple mutant, albeit at a more modest level (~1.5 fold, Supplemental Table 1). That suggests that both are regulated by a positive autoregulatory loop. However, we cannot rule out the possibility that their down-regulated expression is due to nonsense-mediated decay (NMD) as a result of the CRISPR mutations. Autoregulatory loops are a common phenomenon in MADS-box gene regulation. For example, Arabidopsis *AP1* contains a CArG-box in its promoter, which can be bound by its own protein as well as by its paralog (CAL) to achieve high expression levels throughout different stages. (Ye et al., 2016). In tomato, the more distantly related EJ2 protein may also be involved in upregulation of the *FUL*-like genes, because the expression level of *FUL2* is highly correlated with the gene dosage of *EJ2* (Soyk et al., 2019).

Interestingly, we found that FUL1 has a premature stop codon at the C-terminus in the cultivar Moneyberg and many other cultivars. Although this truncation does not alter *in vivo* dimer formation with other MADS-domain proteins (Supplemental Figure 2B), the C-terminus may be important for protein activity. It contains the highly conserved, but uncharacterized, FUL-specific PQWML motif (Litt and Irish, 2003). Arabidopsis *ful* mutants complemented with a FUL copy with a mutation in this motif, were less able to rescue the silique phenotype than those transformed with a wild-type copy, suggesting that the motif is important for protein activity (McCarthy et al., 2015). Interestingly, the truncated allele has not been observed in wild relatives of tomato, but probably occurred after domestication (Supplemental Figure 2A). It may thus have been selected during breeding, for example by allowing slightly larger inflorescences without severe branching. However, this will need to be further investigated.

The role of *FUL2* and *MBP20* in the tomato flowering network

Several previously identified tomato flowering genes were revealed to be functional homologs of Arabidopsis flowering genes, such as *SFT* (*FT*) and *FA* (*LFY*) (Molinero-Rosales et al., 1999; Molinero-Rosales et al., 2004; Lifschitz et al., 2006), indicating that at least part of the Arabidopsis flowering network is conserved in tomato. However, the knowledge of the regulatory network underlying the tomato sympodial flowering pathway is still fragmented and it is yet unclear whether homologs of many important players in Arabidopsis, such as *SOC1* and *FLC*, are important for tomato flowering as well. Here, we show that tomato *FUL*-like genes regulate flowering and inflorescence development in tomato, thereby adding a piece to the tomato flowering network puzzle. In Arabidopsis, *FUL* is a target of FLOWERING LOCUS D (FD) and SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) proteins in the photoperiodic pathway and the age pathway (Kardailsky et al., 1999; Wang et al., 2009; Jung et al., 2016), and functions partially redundantly with *AP1* in the promotion of flowering (Ferrándiz et al., 2000). We demonstrate here that *FUL2* and *MBP20* additively promote flowering similar to their homolog in Arabidopsis, but it is yet unclear whether they act downstream of SFT and the tomato SPLs as well. However, we did identify putative SPL and FT/FD binding sites *in silico* in both the *FUL2* and *MBP20* promoter sequences, suggesting that their expression may be regulated in a similar way. Within our set of DEGs, we did not identify any of the previously identified flowering regulators (e.g. FA, S, SFT, SP), further indicating that the tomato *FUL* homologs may act downstream of these factors.

Downstream of the *FUL*-like genes, we discovered several repressors of the cytokinin signaling pathway that are upregulated in the VM and TM stages of the quadruple mutant, probably resulting in reduced CK levels and signaling. In Arabidopsis, CK deficiency through overexpression of *CKXs* diminishes shoot meristem activity and retards flowering. It has also been shown that CK promotes flowering by transcriptional activation of the *FT* paralogue *TWIN SISTER OF FT* (*TSF*) (Werner et al., 2003; D'Aloia et al., 2011). In tomato, the switch from vegetative to transition meristem is accompanied by cell division in the meristem, which results in the doming of the TM. Because CK is a prominent inducer of cell proliferation (Miller et al., 1955), CK can probably positively regulate cell division during SAM maturation to allow doming of the meristem and the transition to flowering. In line with this hypothesis, the reduced CK levels may inhibit SAM doming and thereby delay flowering. In addition, initiation of both the axillary meristem and the FM revealed to require

a cytokinin signaling pulse (Han et al., 2014b; Wang et al., 2014b). Our data suggest that FUL2 and MBP20 might promote flowering through indirect regulation of CK levels and responsiveness by directly repressing *CKX* and *type-A ARR* expression, respectively (Figure 6).

Reduced *SlFUL* activity results in a branched inflorescence

We show that loss of function of *FUL1*, *FUL2* and/or *MBP20* results in a branched inflorescence that produces an increased number of flowers. One could speculate that this branching is due to reduced activity of *S*, or to delayed activation of the FA/AN complex (Lippman et al., 2008; Park et al., 2012), as *FUL* can activate the *FA* homolog *LFY* directly in Arabidopsis (Balanzà et al., 2014). However these genes were not differentially expressed in the meristems of the *slful* quadruple mutant, suggesting that *FUL* does not regulate inflorescence architecture via *S, AN* or *FA.* Since we sampled only the first inflorescences for transcriptome analysis and the inflorescence branching phenotype in the quadruple mutants became only apparent from the second inflorescence onwards, it is possible that genes involved in the branching phenotype are only differentially expressed in later SIMs. Alternatively, these known branching genes act upstream the *FUL*-like genes in tomato.

We identified several other genes in the list of DEGs that may influence inflorescence branching based on their functions in Arabidopsis. Most interesting are a *ZPR*-like gene, which counteracts in Arabidopsis the function of the SAM maintenance HD-ZIPIII factors (Emery et al., 2003), and an At-hook-like gene similar to *AHL15/17*. Arabidopsis *AHL15* was recently identified as a direct target of FUL in axillary meristems (Karami et al., 2020). It is upregulated in the *ful* single mutant and more drastically in the *ful/soc1* double mutant, which results in increased secondary growth and branching. Despite their different inflorescence types, the *FUL*/*SOC1-AHL15* regulatory module may be conserved and be the cause of increased branching in tomato. The differential expression of the *ZPR* and *AHL15* homologs may be more prominent in later developing inflorescences, and their role in tomato inflorescence architecture remains to be investigated. Moreover, it has been reported that cytokinin effects inflorescence branching through regulation of meristem activity in many species (reviewed by Han et al., 2014a). The reduction of cytokinin signaling in the quadruple mutant may also enhance inflorescence branching, as delay of FM termination (possibly through delayed cell division) allows additional IM formation.

Complex interactions between different MADS-domain proteins in the inflorescence meristem

The MADS-box proteins J, J2, EJ2 and TM3 can form heterodimers with the FUL-like proteins, and have been shown to regulate different aspects of flowering (Szymkowiak and Irish, 2006; Thouet et al., 2012; Yuste-Lisbona et al., 2016; Soyk et al., 2019; Alonge et al., 2020). MC, which does not interact with the FULs in a yeast two-hybrid assay, but is a member of the FUL/AP1-clade, displays flowering phenotypes as well (Yuste-Lisbona et al., 2016). Since MADS-box proteins often function in tetrameric complexes that are 'glued together' by SEPALLATA-like proteins such as J2 and EJ2 (Immink et al., 2009; Melzer et al., 2009), inflorescence meristem development is probably regulated by multimeric MADS-domain complexes that include the AP1/FUL-like proteins. *J* is highly expressed in the VM and its mutant shows delayed flowering (Thouet et al., 2012), suggesting that a complex of J and FUL2/MBP20/MC is responsible for the induction of the transition to flowering in the vegetative meristem. (S)TM3 may also be part of this complex, as lack of both *TM3* and *STM3* causes a mild delay in flowering (Alonge et al., 2020). In addition to delayed flowering, *mc* and *j* mutants both exhibit inflorescences that revert to vegetative growth after the formation of several flowers (Szymkowiak and Irish, 2006; Thouet et al., 2012). We observed this phenotype only occasionally in the *ful* mutants, suggesting that the promotion of IM fate is mainly regulated by *J* and *MC*. It is possible however, that the tomato *FUL*-like genes act redundantly with *MC*. This would reflect the situation in Petunia, where the *FUL* homologs *FLORAL BINDING PROTEIN 26* (*FBP26*), *FBP29* and *PETUNIA FLOWERING GENE* (*PFG*) function partially redundantly with the *AP1* homolog *PheuAP1* in the specification of IM identity (Morel et al., 2019).

The *j2 ej2* double mutant displays increased inflorescence branching, similar to the higher order *slful* mutants (Soyk et al., 2019), suggesting that the FUL-like proteins interact with these SEP-like proteins during later stages. Our observation that the *ful2* mutation appears to have the strongest effect on the branching phenotype fits the result that FUL2 can strongly interact with both J2 and EJ2, while MBP20 can only interact with J2. Obviously, more research is necessary to understand the complex genetic and molecular interactions between these MADS-domain transcription factors in inflorescence meristem development.

MATERIALS AND METHODS

Accession numbers

FUL1, Solyc06g069430; FUL2, Solyc03g114830; MBP10, Solyc02g065730; MBP20, Solyc02g089210, J, Solyc11g010570; J2, Solyc12g038510; EJ2, Solyc03g114840; TM29, Solyc02g089200; MADS-RIN, Solyc05g012020; TM5; Solyc05g015750; TM3, Solyc01g093965; STM3, Solyc01g092950; SlMBP18, Solyc03g006830; TAG1, Solyc02g071730; SlMBP24, Solyc01g105800; SlMBP13, Solyc08g080100; SlMBP14, Solyc12g056460; SlMBP9, Solyc04g076680; SlMBP12, Solyc12g088090; SlMBP22, Solyc11g005120; MADS-MC, Solyc05g056620. Supplementary Table 1 contains the accession numbers of the DEGs.

Plant materials and growing conditions.

Tomato cv. Moneyberg was used for the *Agrobacterium tumefaciens*-mediated transformation experiments (Van Roekel et al., 1993). Tissue culture was conducted in a growth chamber with 16 h light and 8 h dark at 25 °C. After rooting, the transformed plants were cultivated in a 21 °C growth chamber (16h light/8h dark). 25-day old plants were moved to the greenhouse and grown under ambient temperatures and natural light, supplemented with artificial sodium lights.

qRT-PCR analysis.

For qRT-PCR analysis of *SlFUL* expression, root, shoot, leaves, flower organs, and fruits of different stages were harvested from wild-type tomato plants. RNA was extracted with a CTAB/LiCl method (Porebski et al., 1997), DNase treated with Ambion Turbo DNase (AM1907) and cDNA was synthesized with the iScript cDNA synthesis kit (Bio-Rad). Real-time RT-PCR was performed with the iQ SYBR Green Supermix from Bio-Rad (all primer sequences, including reference primers, are listed in Supplemental Table 2).

Yeast two-hybrid

Protein-protein interaction assays in yeast were performed using the GAL4 System using Gateway vectors as described (De Folter and Immink, 2011). The coding sequences for bait proteins and prey proteins were cloned into the pDEST32 and pDEST22 vectors

respectively, and the vectors were transformed into the PJ69-4A and PJ69-4α yeast strains. The interaction screen was performed using -LWH dropout medium, supplemented with 3 mM 3-amino-1,2,4-triazole (3-AT) or -LWA dropout medium. Plates were incubated for 5 days at RT. All primer sequences used for cloning are listed in Supplemental Table 2.

CRISPR construct generation and stable tomato transformation

The *ful2* and *ful1/2* transgenic CRISPR lines have been previously generated (Wang et al., 2019). The constructs for all other lines were generated using GoldenGate cloning and the MoClo toolkit according to (Weber et al., 2011). Briefly, each gRNA was fused to the synthetic U6 promoter as U6p::gRNA, and ligated in a Level 1 vector. Level 1 constructs pICH47732-NOSpro::NPTII::OCST, pICH47742-35S::Cas9::NOST, pICH47751-35S::GFP::ter35S, pICH47761-gRNA1, pICH47772-gRNA2, pICH47781-gRNA3 and the linker pICH41822 were cut/ligated into the level 2 vector pICSL4723 as described. After confirming the constructs, the plasmids were transformed into Agrobacterium strain C58C1. All primers are listed in Supplemental Table 2. The above constructs were introduced into tomato cv Moneyberg by *Agrobacterium tumefaciens*-mediated transformation. Homozygous T1 or T2 transgenic plants were used for phenotypic and molecular characterization.

Meristem imaging

Shoot apices were dissected from seedlings using a forceps and older leaf primordia were removed to expose meristems under the stereomicroscope. Immediately after dissection, live meristems were imaged using an euromex scientific camera.

Meristem transcriptome profiling

The domesticated tomato (S. lycopersicum) cultivar Moneyberg and the isogenic mutant *ful1/ful2/mbp10/mbp20* generated in the Moneyberg background were used for transcriptome profiling. Meristems were dissected using a stereoscope, and tissue was processed for RNA stabilization using an acetone fixation technique (Park et al., 2012). RNA was extracted using the PicoPure RNA Extraction kit (Arcturus). More than 30 meristems were collected for each sample, yielding 1~3 µg RNA, which was enriched for mRNA and processed into cDNA libraries using the Illumina TruSeq Stranded Total RNA

LT Sample Prep Kit (Illumina). After quality control (Qubit and Fragment Analyzer), samples were sequenced using Illumina NovaSeq 2x150 nt Paired End sequencing. Samples were randomized across sequencing flow cells and lanes within flow cells, and two biological replicates were used for all library constructions. After quality control, all data were analyzed using the CLC work package. The raw data has been deposited in GEO under accession number GSE154419.

Electrophoretic mobility shift assays (EMSAs)

FUL2 and MBP20 coding sequences were amplified from wild-type Moneyberg cDNA and cloned into pSPUTK (see Supplemental Table 2 for all primer sequences). The pSPUTK promoter allowed *in vitro* protein synthesis using the TnT® SP6 High-Yield Wheat Germ Protein Expression System (Promega) according to manufacturer instructions. The probe fragments consisted of a region of 80-100 bp with the canonical CArG-box in the middle (Supplemental Figure 9), and were amplified from genomic DNA; The mutated probe fragments was generated by overlapping PCR using primers that replaced one base pair in the middle of the CArG-box (Supplemental Figure 9). EMSAs were performed essentially as described by Smaczniak *et al.* (2012) with minor modifications. Oligonucleotides were fluorescently labelled using DY-682. Labelling was performed by PCR using vector-specific DY-682-labelled primers followed by PCR purification with NucleoSpin® Gel and PCR Clean-up kit (MACHEREY-NAGEL). Gel shifts were visualized using a LiCor Odyssey imaging system at 700 nm.

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AUTHOR CONTRIBUTIONS

M.B. conceived the project and designed the experiments; X.J. performed the qPCRs, tomato CRISPR/cas9 mutagenesis and RNA-seq experiment; K.R, G.L. and J.H.L. did the Yeast Two Hybrid analyses; V.V. measured the fruit phenotypes; M.B. and X.J. performed the EMSAs; R.A.d.M. analyzed *FUL1* alleles in cultivars and assisted with the RNA-seq analysis; G.C.A. and M.B. supervised the project; M.B. and X.J analyzed the data, prepared the figures and wrote the article; all authors read and approved the final version.

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FIGURES

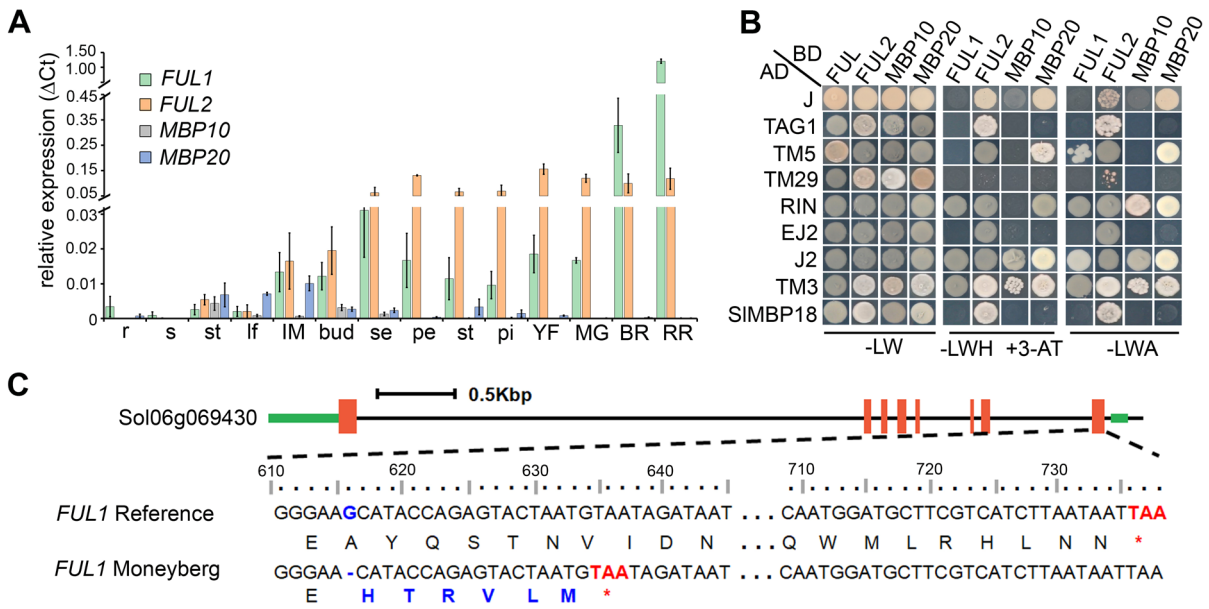

Figure 1. **Characterization of the tomato *FUL*-like genes**. **(A)** Relative expression (△Ct) profiles of *SlFUL* genes in different organs obtained by qRT-PCR. r: two-week-old root; s: two-week-old shoot; st: stem below apex; lf: young leaf; IM: inflorescence meristem (dissected apex); bud: closed flower bud; se: sepal; pe: petal; st: stamen; pi: pistil; YF: young fruit; MG: mature green fruit; BR: breaker stage fruit; RR: red ripe fruit. The error bars indicate ±SD based on three biological replicates. **(B)** Yeast two-hybrid assays showing the protein interactions of the FUL-like proteins with other tomato MADS-domain proteins. L, leucine; W, tryptophan; H, histidine; A, adenine; 3-AT, 3-amino-1,2,4-triazole. Only part of the extensive data set is shown here. Supplemental Figure 1 shows the complete set. **(C)** The location of the one-nucleotide deletion present at the 3' end of the *FUL1* gene in the cultivar Moneyberg, resulting in a protein lacking the C-terminus. The blue letters indicate the differences; the orange nucleotides the stop codons.

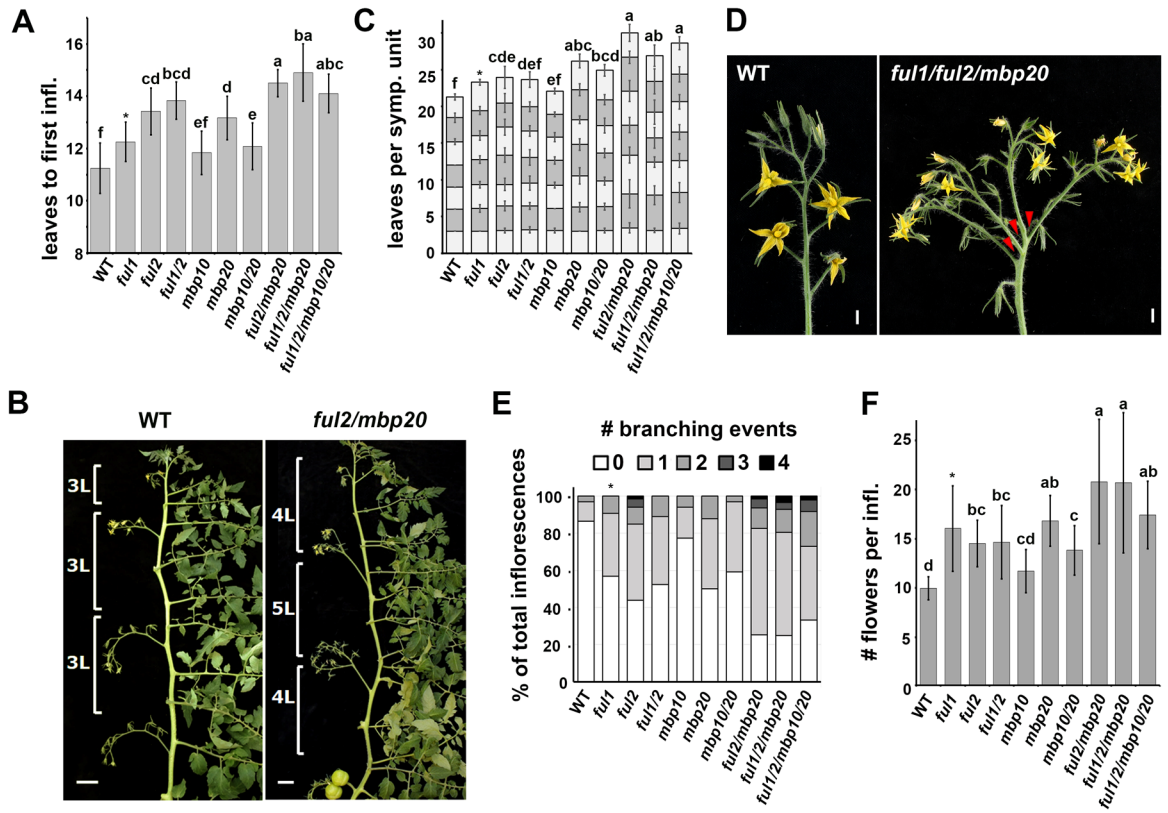

Figure 2. Disruption of the *FUL*-like genes results in delayed flowering and enhanced inflorescence branching. **(A)** Quantification of primary shoot flowering for WT and *slful* mutants. **(B)** Representative sympodial shoots from WT plants and the *ful2/mbp20* mutant. L: leaf; Scale bars: 5 cm. **(C)** Number of leaves per sympodial unit for the first seven units in WT and *slful* mutants. The sum of the leaves was used to test the significance. (**D)** Representative images of wild-type and mutant (branched) inflorescences. Red arrowheads indicate branching events. Scale bars: 2 cm. **(E)** Proportion of branched inflorescences in the indicated genotypes. **(F)** The quantification of the number of flowers per inflorescence. In A, C, and F, mean values (± SD) were compared between genotypes using one-way ANOVA followed by a post hoc LSD test, different letters indicate the difference at P<0.05 level, six individual T2 offspring plants were analyzed per line and the data from the two different genotypes were combined for each mutant (e.g. 2x6 individuals for *ful1* etc.). The asterisk in A, C, and F indicates that the *ful1* data is acquired from a second phenotyping experiment. The independent results from this second experiment are shown in Supplemental Figure 4 A, B and C. WT: wild-type plants.

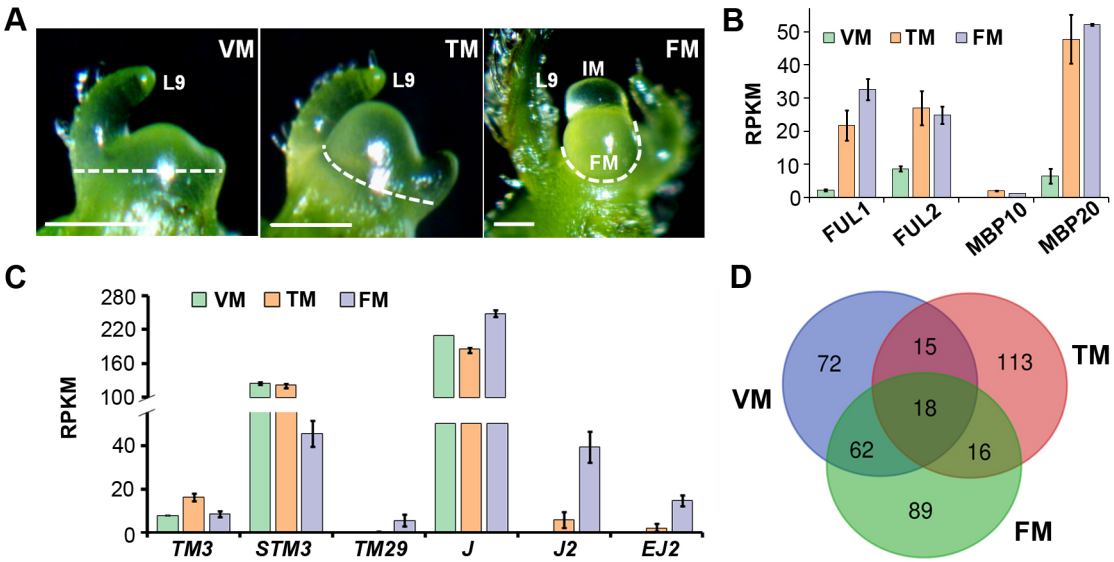

Figure 3. **Gene expression dynamics in the primary shoot meristem. (A)** manual microdissection of the three successive meristem stages of primary shoot meristems for transcriptome profiling. Dashed lines indicate the dissected tissues. White bar: 100 µm. **(B)** Normalized gene expression (RPKM) of the *FUL*-like genes in wild-type meristem stages. **(C)** RPKM values for *TM3, STM3*, *TM29*, *J, J2*, and *EJ2* during wild-type shoot inflorescence meristem maturation. **(D)** Venn diagram showing the overlap of differentially expressed genes between WT and the quadruple mutant in the three meristem stages. VM: vegetative meristem; TM: transition meristem; FM: floral meristem. The values shown (mean ± SD) are the average of two replicates. The qPCR validation of these results is shown in Supplemental Figure 7.

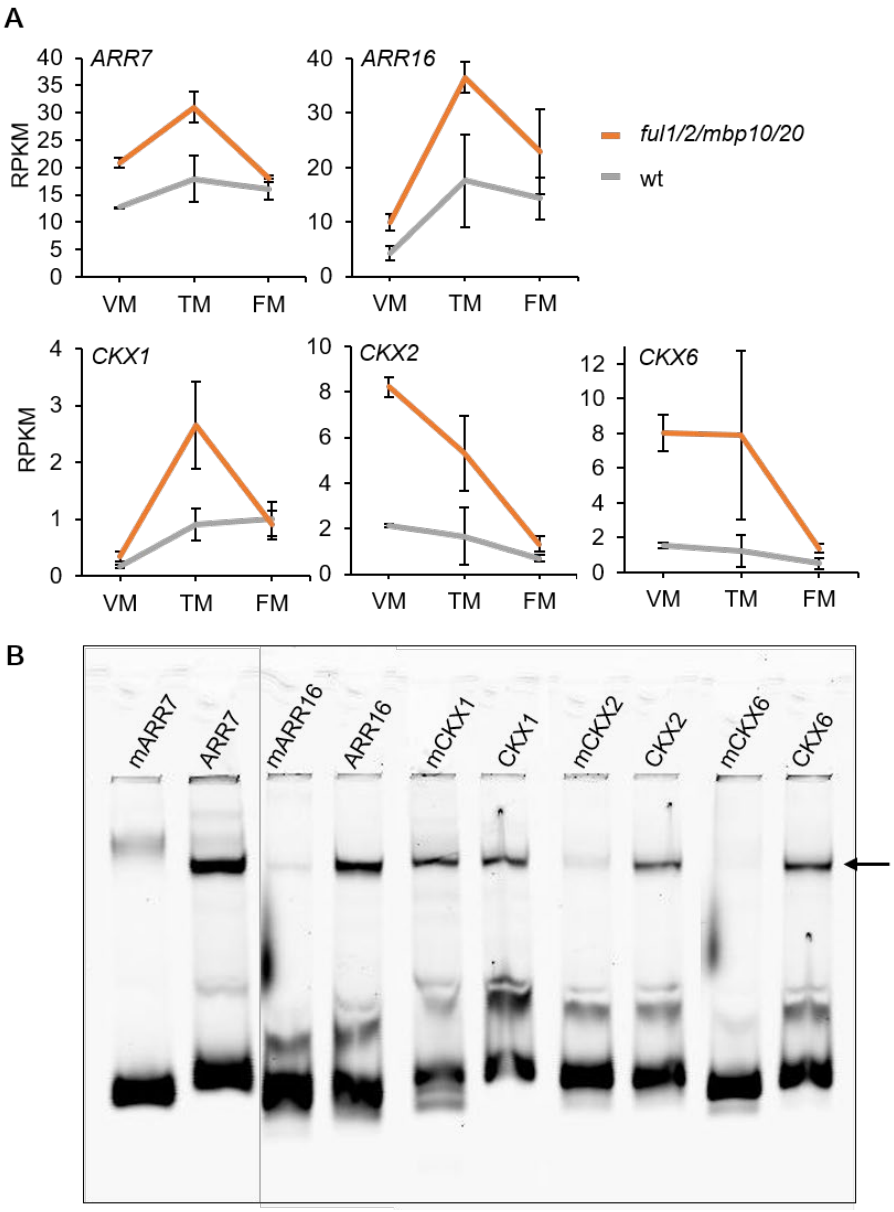

Figure 4. ***FUL2* and *MBP20* regulate *ARR7/16* and *CKX1/2/6* expression. (A)** Normalized gene expression (RPKM) for *ARR7/16* and *CKX1/2/6* across vegetative and reproductive meristem stages. VM: vegetative meristem; TM: transition meristem; FM: floral meristem. The values shown (mean ± SD) are the average of two replicates. **(B)** EMSA assays showing that FUL2-TM3 directly binds to promoter fragments of *ARR7/16*, *CKX1/2/6 in vitro*. The arrow indicates the shift of the probe caused by the binding of FUL2-TM3. The mutated versions of the promoter fragments are indicated with an 'm'. The EMSA with MBP20/TM3 is available as Supplemental Figure 8B.

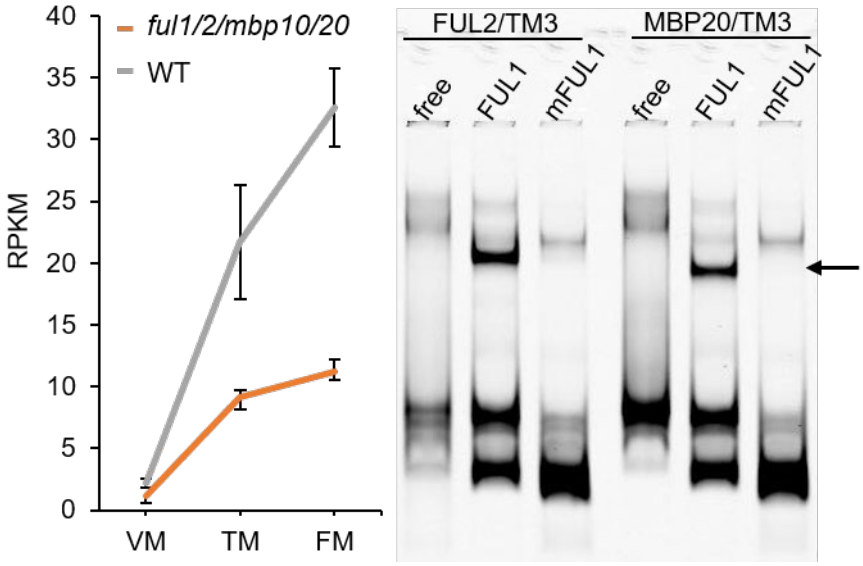

Figure 5. FUL2 and MBP20 regulate *FUL1* expression. (A) Normalized gene expression (RPKM) of *FUL1* across vegetative and reproductive meristem stages. VM: vegetative meristem; TM: transition meristem; FM: floral meristem. **(B)** EMSA assays showing that FUL2/TM3 and MBP20/TM3 directly bind to the promoters of *FUL1 in vitro*. Average and standard deviation of two independent replicates per stage. The arrow indicates the shift of the probe caused by the binding of FUL2-TM3 or MBP20/TM3. The mutated version of the CArG-box in the *FUL1* promoter fragment is indicated with mFUL1.

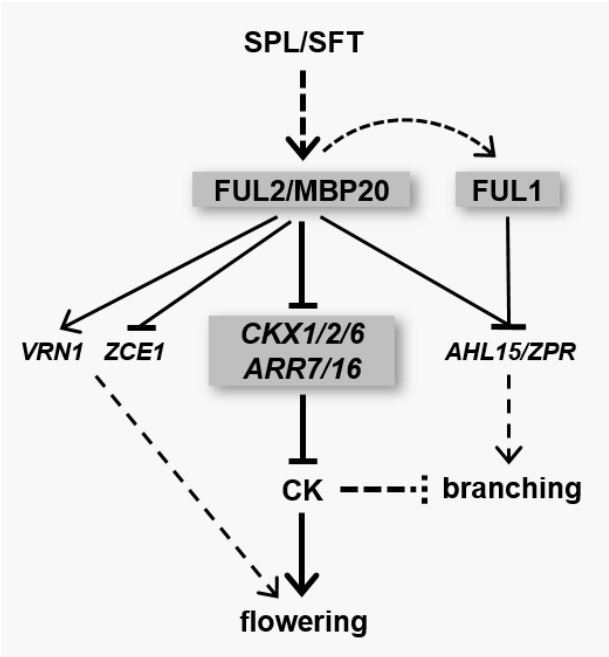

Figure 6. Model of SlFUL regulation of flowering time and inflorescence branching in tomato. The connections between the different regulators are based on the results of this study and other work described in the text. The genes studied in this work are shown in gray boxes. Solid lines display confirmed interactions, while dashed lines represent putative interactions. CK is cytokinin.

Table 1. Shortlist of up- and down-regulated genes in maturing meristems of *ful1/ful2/mbp10/mbp20* in comparison with wild type. Number: $\log_2$ fold change. Significant differences are represented by asterisks. (*) FDR P-value < 0.05; (**) FDR P-value < 0.01.

iTAG Locus	annotation	VM	TM	FM
Solyc01g007070	BLH1	2.83**	3.03**	2.60**
Solyc01g093965	TM3	1.59**	1.16**	1.38**
Solyc01g094070	BEN1	2.30*	1.13	0.18
Solyc03g114940	KLUH	-0.82**	-1.09*	-2.29
Solyc04g007820	ZCE1	2.39**	-0.55	1.91**
Solyc06g048930	ARR16	1.23	1.06**	0.55
Solyc06g048600	ARR7	0.78	0.81**	0.17
Solyc06g069430	FUL1	-0.79	-1.24**	-1.51**
Solyc07g054630	VRN1	-1.02**	-0.91**	-1.13**
Solyc08g007570	ZPR	1.67*	0.66	1.20**
Solyc09g092620	CYP83B1	2.35*	-0.67	2.01**
Solyc04g016430	CKX1	0.34	1.56**	-0.36
Solyc10g017990	CKX2	2.00**	1.69**	0.76
Solyc12g008900	CKX6	2.44**	2.70**	1.23
Solyc12g087950	AHL15/17	2.22*	1.93*	1.87*