Members of the tomato FRUITFULL MADS-box family regulate style abscission and fruit ripening

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Abstract

The tomato (Solanum lycopersicum) protein MADS-RIN plays important roles in fruit ripening. In this study, the functions of two homologous tomato proteins, FUL1 and FUL2, which contain conserved MIKC domains that typify plant MADS-box proteins, and which interact with MADS-RIN, were analysed. Transgenic functional analysis showed that FUL1 and FUL2 function redundantly in fruit ripening regulation, but exhibit distinct roles in the regulation of cellular differentiation and expansion. Over-expression of FUL2 in tomato resulted in a pointed tip at the blossom end of the fruit, together with a thinner pericarp, reduced stem diameter, and smaller leaves, but no obvious phenotypes resulted from FUL1 over-expression. Dual suppression of FUL1 and FUL2 substantially inhibited fruit ripening by blocking ethylene biosynthesis and decreasing carotenoid accumulation. In addition, the levels of transcript corresponding to ACC SYNTHASE2 (ACS2), which plays a key role in ethylene biosynthesis, were significantly decreased in the FUL1/ FUL2 knock-down tomato fruits. Overall, our results suggest that FUL proteins can regulate tomato fruit ripening through fine-tuning ethylene biosynthesis and the expression of ripening-related genes.

Key words: Development, fruit shape, FRUITFULL, MADS-box, ripening, tomato.

Introduction

Members of MADS-box transcription factor families, which are widely distributed among animal and plant taxa, contain a conserved domain of approximately 60 amino acids in the N-terminal region, named the MADS-box (M) domain. Plant MADS-box gene families have been studied in detail and are now associated with a wealth of genetic and evolutionary data (Ferrario et al., 2004). In addition to the M domain, most of the plant MADS-box proteins contain three other domains: the Intervening (I) domain, the Keratin (K) domain, and the C-terminal (C) domain (Kaufmann et al., 2005). The MADS-box domain is involved in DNA binding and dimerization, while the I domain is responsible for DNA-binding specificity during dimer formation and the K domain mediates protein–protein interactions. Compared with the other domains, the C domain shows less sequence conservation and it has been found to be essential for ternary complex formation and transcriptional activation (Davies et al., 1996; Riechmann et al., 1996; Riechmann and Meyerowitz, 1997; Kramer et al., 1998; Cho et al., 1999; Messenguy and Dubois, 2003; de Folter and Angenent, 2006).

Plant MADS-box genes were first investigated in the context of functional studies of Arabidopsis floral organ development and flowering time (Sommer et al., 1990; Yanovsky et al., 1999) and are the major modules in the well-known ABCDE model of floral organs. In Arabidopsis, the counterparts of MADS-box genes are: class A, APETALA1 (AP1); class B, PISTILATA (PI) and AP3; class C, AGAMOUS (AG); class D, SEEDSTICK/AGAMOUS-LIKE11 (STK/AGL11); and class E, SEPALLATA (SEP1, SEP2, SEP3, and SEP4). Moreover, some other MADS-box genes, including SOC1, FLC, AGL24, and SVP, have been confirmed to be involved in flowering time and flower initiation (Michaels and Amasino, 1999; Hartmann et al., 2000; Hepworth et al., 2002; Yu et al., 2002; Michaels et al., 2003; Searle et al., 2006; Kim et al., 2007;
Liu et al., 2008). MADS-box genes also function in seed pig-
mentation and endodermis development (TRANSPARENT
TESTA16) (Nesi et al., 2002), root development (AGL12 and
AGL17) (Rounsley et al., 1995; Tapia-Lopez et al., 2008), and
fruit formation (SHP1, SHP2, and FUL) (Ferrandiz et al.,
2000; Gu et al., 1998; Liljegren et al., 2000).

Other than these Arabidopsis genes, another well studied
MADS-box gene is MADS-RIN, which was identified after
screening a series of tomato mutants (Vrebalov et al., 2002).
Fruits from the loss-of-function rin (ripening inhibitor) mutant
exhibit perturbed ripening, as shown by the absence of a res-
piratory climacteric and associated ethylene evolution, as well
as reduced softening, carotenoid accumulation, and the pro-
duction of flavour compounds. While rin fruits are sensitive to
ethylene, their ripening is not induced by exogenous ethylene
(Vrebalov et al., 2002). In strawberry, a non-climacteric fruit,
silencing of a tomato MADS-RIN homologue, FaMADS9,
also leads to the inhibition of normal development and ripen-
ing (Seymour et al., 2011). These results suggest that MADS-
RIN genes play a conserved function in ripening regulation in
to climacteric and non-climacteric fruits (Seymour et al.,
2011).

The mechanism by which MADS-RIN proteins regulate
fruit ripening has been studied and it was reported that the
tomato MADS-RIN protein influences gene expression by
fruit ripening has been studied and it was reported that the
over-expression of FUL2, but not FUL1, affected fruit shape,
resulted in a thinner fruit pericarp, and changed the
number of layers of cells in the stem. These results demon-
strate that FUL MADS-box transcription factors have diverse
functions in growth and developmental regulation in tomato.

Materials and methods

Plant material and growth conditions

All tomato (Solanum lycopersicum cv. Ailsa Craig) plants, including
transgenic lines and a mutant line homozygous for the rin mutation
in the Ailsa Craig background, were grown in a greenhouse under
natural daylight and with 60–75% relative humidity and ambient
temperature (>20 °C). The tomato transgenic lines were advanced
to the T3 generation. Flowers were tagged at the full-bloom stage
to synchronize developmental comparisons. For analysis, 1, 7, and
14 days post-anthesis (DPA) corresponded to ovaries of 1, 7, and
DPA, respectively. The fruit stages used were immature green (IG),
mature green (MG), breaker (BR), yellow ripe (YR), and red ripe
(RR), which were picked at approximate 28, 35, 38, 41, and 44 DPA,
respectively.

Construct recombination and plant transformations

For the FUL1/FUL2 double-silencing construct, a 362bp fragment of
the FUL1-IK domain was amplified by FUL1 sequence-specific
primers (amplified with the primers in Supplementary Table S1 at
JXB online). Fragments were incorporated into the pDONR221 vectors using the Clonase BP reaction (Invitrogen). The LR reaction
(Invitrogen) was performed subsequently to incorporate the
fragments into the pHILLSGATE8 vector. For the FUL1 and
FUL2 over-expression constructs, the coding regions of FUL1 and
FUL2 were amplified and cloned into the XbaI and KpnI sites of the
pMV2 vector (modified from pHILLSGATE2). The expressions of
transgene were driven using the CaMV 35S promoter in both vector
systems.

For the ProFUL1::GUS construct, a genomic DNA (gDNA)
sequence (from –2518 to –24bp) upstream of the FUL2 coding
sequence was ampliﬁed using sequence-speciﬁc primers. The primers
were fused with the attB1 and attB2 sites for recombination. The resulting fragment was recombined into the pDONR221 vector
using BP recombinase and the LR reaction was performed to incor-
porate the fragment into the pV3P vector (modiﬁed from pHILLS-
GATE2) which contains the glucuronidase synthase (GUS) coding
sequences. All of the recombinant constructs were transformed into
the Agrobacterium strain C58 by electroporation, and subsequently
transformed into tomato cotyledon explants.

Ethylene assay

To measure ethylene production, BR stage fruits of approximately
the same size were harvested and kept in sealed containers for
4 h and left open for 20 h each day (16 d) at room temperature (each
biological replicate contained three fruits). Ethylene was meas-
ured in the headspace of the sealed containers by sampling with a
syringe. Measurements were performed as described by Vrebalov
et al. (2009). All samples involved three technical and three biologi-
cal replicates.

Gene expression analysis

RNA from various tissues of wild-type and transgenic plants
were isolated using TRIZol® 117 reagent (Invitrogen, USA). For
GAPDH synthesis, 3 μg RNA was used with M-MLV reverse

Furthermore, our data showed that FUL1 and FUL2 play distinc
t roles in regulating cellular differentiation and expansion.
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resulted in a thinner fruit pericarp, and changed the
number of layers of cells in the stem. These results demon-
strate that FUL MADS-box transcription factors have diverse
functions in growth and developmental regulation in tomato.
transcriptase (Toyobo, Japan) according to the manufacturer’s instructions. The cDNA concentrations were normalized to actin expression levels for RT-PCR analysis. Primers of the ripening-related genes are listed in Supplementary Table S1 at JXB online. The actin gene was used as an internal control for quantitative real-time PCR (qPCR), which was performed using the Power SYBR Premix Ex Taq kit and the TaKaRa two-step method (TaKaRa, Japan). PCR products were quantified using the Roche LightCycler 480 Real-Time PCR Detection System and the SYBR Green I Master Kit (Roche, Switzerland). The wild-type and transgenic plants were represented by three biological replicates for each sample.

Paraffin section

Light microscopic observation of paraffin sections was used to measure the number of cell layers and cell sizes in stems and pericarp of both wild-type and transgenic plants. For the fruit material, 14 DPA and BR stage fruits were harvested and at least nine pericarp sections were isolated. For the stem samples, the 3rd, 13th, and 17th internodes were selected and at least nine sections were measured and harvested for analysis. Paraffin sections were prepared as described by Yang et al. (2011) and the number of cell layers was counted manually. Photomicrographs were taken using an Olympus microscope.

GUS staining

Slices of fruits and stems from the transgenic lines transformed with the native promoter of FUL2-driving expression of the GUS gene (ProFUL2::GUS) were stained with a GUS staining solution (100 mM sodium phosphate buffer) to evaluate GUS activity. Staining was allowed to proceed for 5 h at 37 °C in the dark and then washed with a graded ethanol series at room temperature for decolorization and observation by light microscopy (OLYMPUS SZX12).

Results

FUL1 and FUL2 show distinct expression patterns in tomato tissues

Previous studies indicate that expression of MADS-RIN is induced coincident with ripening and peaks at the red ripe stage (Vrebalov et al., 2002). For comparative purposes, real-time quantitative PCR (qPCR) was performed to investigate the expression levels of FUL1, FUL2, and RIN in different tissues and fruit developmental stages of the tomato cultivar Ailsa Craig (Fig. 1). The results showed that the expression of FUL1 was similar to RIN, and was very low in roots, stems, leaves, and in the early stages of fruit development, but increased rapidly at the breaker (BR) stage, which marked the onset of ripening. This suggested that FUL1 might have a similar function to RIN and regulate fruit ripening. On the other hand, the expression of FUL2 was high in flowers and throughout fruit development, as well as in stems and leaves. Indeed, FUL2 was expressed in all tissues tested except roots, indicating that it functions in both reproductive and vegetative organs. It was noted that Bemer and colleagues reported similar expression patterns for FUL1 and FUL2 (Bemer et al., 2012). Taken together, the contrasting spatial and temporal expression patterns of FUL1 and FUL2 indicated that FUL1 and FUL2 may have distinct functions in the regulation of tomato plant growth.

Over-expression of tomato FUL2 inhibits the abscission of styles from fruit leading to a ‘pointed tip’ phenotype

To gain an insight into the functions of FUL1 and FUL2, vectors were constructed to mediate their suppression (RNAi) or over-expression and transformed into tomato (cv. Ailsa Craig). At least 15 independent transgenic lines were obtained corresponding to each vector. Nine of the 15 FUL2 over-expressing transgenic lines showed an obvious alteration in fruit shape with a prominent pointed tip at the blossom end of the fruit (Fig. 2A), of which three lines (FUL2-OE-3, 10, and 13) were selected for transcriptional analysis. Expression of FUL2 in fruit of these lines was 7–28-fold higher than that in the wild type (Fig. 2B). The developmental onset of the pointed tip phenotype was assessed using stereomicroscopy and at 1 DPA, an early stage of fruit development, styles and ovaries of the FUL2-OE and the wild type showed no visible differences. Subsequently, at 7 DPA, the style of the wild type typically wilted from the tip to the base and abscised while the FUL2-OE lines showed a similar abscission phenotype, but
with a visibly enlarged base. However, by 14 DPA, rather than showing abscission, as is typical of the wild type, the style of FUL2-OE was still connected to the ovary and the visibly enlarged basal region developed into a pointed tip (Fig. 2C), suggesting that this phenotype of FUL2-OE fruit is related to an abnormally persistent style.

To evaluate the abnormal style development further, a series of paraffin sections of ovaries at different developmental stages was prepared for microscopic observation. While the intact style typically abscised from the wild-type fruit, the style of FUL2-OE remained attached to the ovary and the visibly enlarged basal region developed into a pointed tip (Fig. 2C), suggesting that this phenotype of FUL2-OE fruit is related to an abnormally persistent style.

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division determines stem diameter. These results therefore support the idea that FUL2 regulates stem growth by repressing cell division in the cambium and, consequently, the number of cell layers. Taken together, our data indicate that FUL2 controls shoot growth by regulating both cell expansion and cell division.

Over-expression of FUL2 alters tomato pericarp structure

Shelf life is an important agronomic trait for many species of fleshy fruits, and one of the notable characteristics of the tomato rin mutant is substantially extending fruit shelf life (Giovannoni, 2007). To determine whether FUL2 potentially interacts with RIN to regulate tomato fruit shelf life, a storage experiment was performed with FUL2-OE fruits. Both wild-type and FUL2-OE fruits with peduncles were harvested at the BR stage and stored at room temperature for 45 d. As expected, FUL2-OE fruits dehydrated much more slowly than those of the wild type (Fig. 4A). A previous study suggested that silencing FUL1/FUL2 expression in MicroTom fruit influences the expression of genes related to cuticle biosynthesis and post-harvest water loss, but does not affect pericarp development (Bemer et al., 2012). Our results further suggest that FUL2, but not FUL1, functions in modulating post-harvest water loss.

In addition to the slower post-harvest dehydration, FUL2-OE fruits showed the thinner pericarp. The smaller size and the fewer number of pericarp cells were observed by using paraffin wax in the FUL2-OE pericarp (Fig. 4B, C, D). Moreover, this indicated that over-expressing FUL2 inhibited pericarp cell expansion, possibly through a similar mechanism to that in the stem. In accordance with the high expression of FUL2 in the stem, it was also highly expressed in parenchyma cells of the fruit pericarp (Fig. 4F). These results suggested that FUL2 functions in cell division and expansion in both vegetative and reproductive organs. Plant-cell-wall-modifying proteins known as expansins have been reported to play roles in fruit softening and organ abscission, as well as affecting organ size and morphology by regulating cell growth (Brummell et al., 1999; Cho and Cosgrove, 2000). Given the reduced cell size of the FUL2-OE fruit, it was investigated whether the expression of the SlEXP1, an expansin gene that is abundantly and specifically expressed during fruit ripening (Brummell et al., 1999), was influenced by FUL2. It was determined that, indeed, the transcript levels of SlEXP1 were significantly reduced in FUL2-OE fruit at the mature green (MG) and BR stages (Fig. 4E).

Double silencing of FUL1 and FUL2 suppressed tomato fruit ripening

Since FUL1 and FUL2 share a high degree of sequence similarity, and a high expression level in the pericarp during ripening, it was hypothesized that they might have similar functions. To test this, a sequence domain that is conserved between the FUL1 and FUL2 proteins was used as the basis to develop a strategy to doubly silence FUL1/FUL2 by RNAi. Screening of the resulting transgenic lines indicated that this approach effectively suppressed the expression of both FUL1 and FUL2 (Fig. 5A).

As previously described by Bemer et al. (2012) the FUL1/FUL2 doubly silenced transgenic lines showed bright yellow fruit during ripening (Fig. 5C). However, unlike the previous report, it was observed that fruits from the FUL1/FUL2 doubly silenced lines showed blocked ethylene production compared with the wild type, which had a typical peak (6 ml kg⁻¹ h⁻¹) at BR+3 d. The pattern of ethylene...
biosynthesis by the transgenic fruits was similar to that of the rin mutant, which produced <1 ml kg⁻¹ h⁻¹ (Fig. 5B). The discrepancy between our results and those of Bemer et al. (2012) may be explained by differences in genotype, since Ailsa Craig was used here, while Bemer et al. (2012) used the dwarf mutant MicroTom as experimental material.

The climacteric burst of ethylene production in tomato fruit is largely driven by the ethylene biosynthetic genes ACO1, ACS2, and ACS4 (Barry and Giovannoni, 2007), and their transcription is inhibited by the rin mutation (Martel et al., 2011). The transcript levels of these genes were evaluated in the FUL1/FUL2 doubly silenced fruit to establish whether this might explain the reduced ethylene production. Compared with wild-type fruit, the transcript abundance of ACS2 in the transgenic fruit was reduced by 10-fold and 5-fold at the BR and YR stages, respectively, and ACO1 showed a similar reduction (Fig. 5D). By contrast, transcript levels of ACS4 showed no significant suppression at the BR stage, but a sharp decrease at the YR stage. Recently, Shima and colleagues also confirmed that both FUL1 and FUL2 can bind to the CArG-box sites of the ACS2 promoter in a RIN-dependent manner (Shima et al., 2013). These results indicate that FUL proteins regulate ethylene biosynthesis through enhancing the expression of ACS2 and ACO1 during tomato fruit ripening.

**Discussion**

FUL1 and FUL2 regulate tomato fruit ripening in a functionally redundant manner

FUL1 was first identified in tomato as a floral identity gene (Pnueli et al., 1991), but the expression pattern of FUL1 suggests a possible role in fruit development and ripening (Busi et al., 2003; Eriksson et al., 2004). Moreover, it has previously been reported that MADS-RIN directly regulates the expression of the FUL1 gene (Fujisawa et al., 2012; Martel et al., 2011). These results suggested that FUL1 might be a candidate gene for regulating tomato fruit ripening, an idea that is supported by the observation that a FUL1 homologue plays a central role in bilberry fruit ripening (Jaakola et al., 2010). However, it was previously reported that suppressing
Fig. 5. FUL1/FUL2 regulated ethylene production. (A) The relative expression levels of FUL1 and FUL2 in the FUL1/FUL2 RNAi fruits compared with the wild type (n=3). (B) Ethylene produced by FUL1/FUL2 RNAi and wild-type fruits at different development stages after the BR stage. (C) Wild-type (left) and FUL1/FUL2 RNAi (right) fruits showed differences in fruit colour (BR+6 stage). (D) Quantitative real-time PCR analysis of ethylene biosynthesis related genes in FUL1/FUL2 RNAi and wild-type fruits at the BR and YR stages.
a single tomato FUL gene resulted in no obvious phenotype (see Supplementary Fig. S1 at JXB online), but the double knock-out of FUL1 and FUL2 led to obvious phenotypes, such as yellow ripe fruits (Bemer et al., 2012), and severely perturbed ethylene production. These results suggest that FUL1 and FUL2 contribute to tomato fruit ripening in a redundant manner. Our data also suggest that the functions of FUL1 and FUL2 have diversified since FUL2, but not FUL1, was observed to affect style abscission and cell expansion. Transgenic plants over-expressing either FUL1 or FUL2 were generated and, interestingly, FUL2 over-expressing plants showed pleiotropic effects, including altered fruit shape (Fig. 2), stem structure (Fig. 3), and pericarp structure (Fig. 4), but FUL1 over-expressing plants had no obvious phenotypic change.

In the Arabidopsis ful mutant, both cell expansion and differentiation were affected in the carpel valves which have broader and rounder cells and fewer cell layers than those of the wild type (Gu et al., 1998). These results imply that the FRUITFULL protein has conserved functions in different plant species with regard to regulating cell expansion, differentiation, and fruit ripening. Our data suggest that FUL1 has lost some of the functions that have been retained by FUL2. This functional loss may be related to differences in protein structure. Leseberg et al. (2008) analysed the protein–protein interactions of 16 tomato MADS-box proteins with the MADS-domain removed and showed that FUL2 could interact with RIN, TM3, JOINTLESS, MBP18, MBP24, MBP21, MADS1, TAG1, and TM5, but FUL1 could only interact with TM3, JOINTLESS, and RIN. The K domain of MADS-box transcription factors is responsible for the specificity of the DNA-binding dimer formation and it is proposed that structural differences in the K domains of FUL1 and FUL2 may be responsible for their functional divergence.

FUL2 regulates the abscission of styles from tomato fruit

Following pollination and fertilization, the style normally withers and abscises from the fruit. However, it was observed that this process was inhibited in fruit from the FUL2-OE transgenic lines. The whole style of FUL2-OE failed to abscise during fruit set, resulting in fruit with a pointed tip at the blossom end (Fig. 2). This suggests that FUL2 regulates style abscission from tomato fruit at an early developmental stage. Inhibition of style abscission in FUL2-OE plants led to a fruit with a pointed tip phenotype. Seven phenotypically similar mutants (nipple-tip (n), n-2, n-3, n-4, persistent style (pst), beaked (bk), and bk-2) have previously been reported in tomato (Barten et al., 1994). Of these, n has been assigned to tomato chromosome 5 (Mutschler et al., 1987), and pst and bk mapped to chromosomes 7 and 2, respectively (Rick and Butler, 1956), but none of the corresponding genes had been cloned at this time. Accordingly, FUL2 is not a candidate gene for the n, or bk genes as it is located on chromosome 3. These results also show that development of the tomato pistil is regulated by multiple genes but more experiments are needed to elucidate any potential relationship between FUL2 and other ‘nipple tip’ genes.

How does FUL2 regulate tomato cell development? Previous studies showed that expansin proteins may function not only in fruit softening and organ abscission, but may also regulate organ size and morphology. The expansin gene AtEXP10 has been shown to encode a cell-wall-loosening protein that regulates organ size, morphology, and abscission and suppression of AtEXP10 led to shorter petioles and leaf blades and a reduction in petiole cell size (Cho and Cosgrove, 2000). Indeed, a thinner pericarp with smaller cell sizes of FUL2-OE tomato fruits was observed compared with the wild type, as well as suppressed expression of the expansin gene SIEXP1 (Fig. 4E). Thus, FUL2 might affect tomato style abscission and cell growth through regulating the expression of expansin genes, and it is proposed that the repressed expression of different expansin genes might also contribute to the thinner pericarp, stem, and small leaf phenotypes of the FUL2-OE transgenic lines.

FUL proteins, TAGL1 and MADS-RIN may form higher order complexes to regulate tomato fruit ripening

MADS domains can bind to the CArG-box sites in the promoter region of many genes (Messenguy and Dubois, 2003), and in dimers of MADS-box proteins, both of the MADS domains take part in binding to a single DNA site (Pellegrini et al., 1995; Santelli and Richmond, 2000). The heterodimer of the MADS-box proteins MC and JOINTLESS show stronger CArG-box DNA motif binding ability than MC and JOINTLESS alone, and the DNA-binding specificities of the heterodimer may be different from the homodimers of MC and JOINTLESS. This suggests that the heterodimerization of MADS-box proteins may be important for their physiological functions. TAGL1 and FULs can interact with the MADS-RIN protein, and all of them play important roles in tomato fruit ripening. It may be that both TAGL1 and FULs can form heterodimers with MADS-RIN, or form higher order complexes to regulate fruit ripening through an enhanced CArG-box DNA motif binding ability or altered DNA-binding specificities, and defects in any one of them perturbs tomato fruit ripening.

Similar to the rin mutant, TAGL1-RNAi and FUL1/FUL2-RNAi transgenic fruit showed a complete block in ethylene production and repressed expression of ACS2 (Barry et al., 2000; Vrebalov et al., 2002). This suggests that TAGL1, the two FUL genes and RIN may regulate ethylene biosynthesis through a similar, or even an identical pathway. It has been confirmed that RIN, TAGL1 and FULs can bind to the ACS2 promoter directly (Ito et al., 2008; Itkin et al., 2009; Shima et al., 2013), and the heterodimerization of RIN with TAGL1 may regulate tomato fruit ripening by enhancing the ability of RIN and TAGL1 to bind to the ACS2 promoter. If this is true, FULs may also form heterodimers with RIN, and this heterodimerization may enhance the ability of the RIN and FULs proteins to interact with the ACS2 promoter directly and compensate for the functional defect caused by the failed heterodimerization of RIN and TAGL1. However, it is known
that knocking down the expression of either TAGL1 or the two FUL genes in tomato represses the ethylene burst during fruit ripening, which suggests that the interactions of RIN and TAGL1, or RIN and FULs, may not result in functional complementation. This raises the question, how do these three genes regulate autocatalytic ethylene production during tomato fruit ripening? One hypothesis is that RIN, TAGL1, and FULs can form heterodimers to regulate the expression of their own specific ripening related genes, and they can also form D-FUL-E higher order complexes to regulate the expression of ACS2 during tomato fruit ripening. Indeed, yeast three-hybrid assays have shown that TAGL1, RIN, and FUL1 can form D-FUL-E higher order complexes (Leseberg et al., 2008). Thus, the regulation of gene expression during fruit development and ripening by MADS box transcription factors is highly complex and much still remains to be learnt about the mode of action of RIN in regulating fruit ripening.

Supplementary data
Supplementary data can be found at JXB online.
Supplementary Table S1. Primers used in this study.
Supplementary Fig. S1. FUL1 and FUL2 regulated fruit development and ripening.

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