Supplemental Data

Overexpression of a novel MADS-box gene *SlFYFL* delays senescence, fruit ripening and abscission in tomato

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Results

Generation of transgenic tomato plants. The pBI121 binary vector containing *SlFYFL* was created and transformed into wild type tomato plants via *Agrobacterium*-mediated T-DNA transformation. Twenty independent transgenic lines were confirmed to be positive transgenic lines by PCR analysis using specific primers for the reporter gene *NPTII*. Quantitative real-time PCR results showed that *SlFYFL* was successfully overexpressed in these transgenic plants, and expression levels of *SlFYFL* in three transgenic lines: OE-1, OE-7 and OE-17, were approximately 25- to 42-fold higher than in wild type leaves (Fig. 1S B). Subsequently these three transgenic lines were selected for further investigation. In all stages of transgenic fruits, *SlFYFL* was significantly overexpressed, especially in B+7 stage fruit, which showed more than a 10-fold increase in *SlFYFL* expression level compared with wild type (Fig. 1S A). In fruit AZs of transgenic lines, *SlFYFL* expression was increased approximately 8-fold than wild type (Fig. 1S C). Furthermore, *SlFYFL* was also remarkably upregulated in transgenic flower sepals (Fig. 1S D).

The sepals of *35S:FYFL* flower were longer than those of wild type. In our study, we found that the *35S:FYFL* lines represented longer sepals than wild type

(Fig. S3 A, B). Sepal length statistics showed that sepals of *35S:FYFL* lines were 4-5 mm longer than that of wild type (Fig. S3 C). *SlMADS-MC* was reported to influence sepal development [1]. Quantitative PCR results displayed that *SlMADS-MC* was down-regulated in sepal of *35S:FYF* lines (Fig. S3 D). Moreover, expression pattern of *SlFYFL* in sepal indicated that *SlFYFL* might play an important role in development of sepal (Fig.1 C). These results hint that overexpression of *SlFYFL* might surpress the expression of *SlMADS-MC*, thus *35S:FYFL* plants produce longer sepals.

Method

Statistical analysis. The mean values of data were from the measurements of replicates and 'Standard Error' of the means were calculated. Data were analyzed by Origin 8.6 software, using *t* test to assess significant differences among the means.

Figure caption
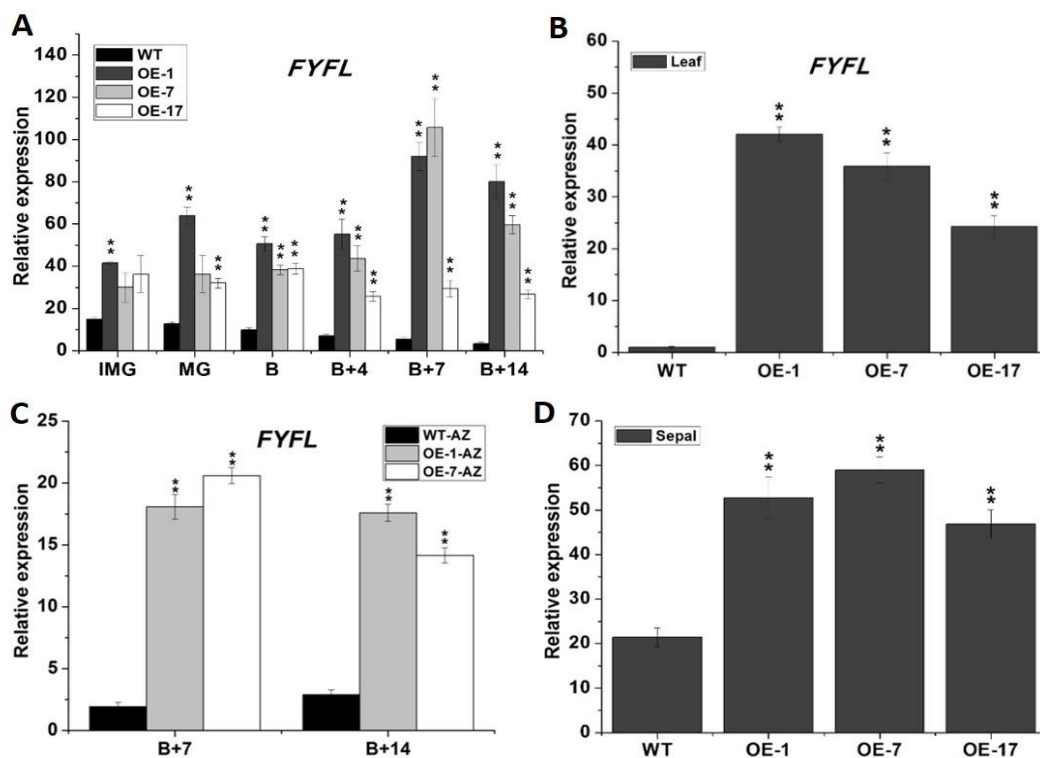
Fig. 1 S Expression of *FYFL* in WT and *35S:FYFL* lines. The relative overexpression of *FYFL* in fruit **(A)**, leaf **(B)**, AZ **(C)** and sepal **(D)** of wild type and *35S:FYFL* lines. IMG, immature green fruit; MG, mature green fruit; B, breaker fruit; B+4, 4 days after breaker fruit; B+7, 7 days after breaker fruit; B+14, 14 days after breaker fruit; AZ, abscission zone of flower in anthesis. WT, wild type; OE-1, OE-7, OE-17, different transgenic lines. The data represent mean from three replicates with three biological repeats. *, indicate P <0.05, **, indicate P <0.01 between the wild type and others by *t*-test. Error bars indicate SE.
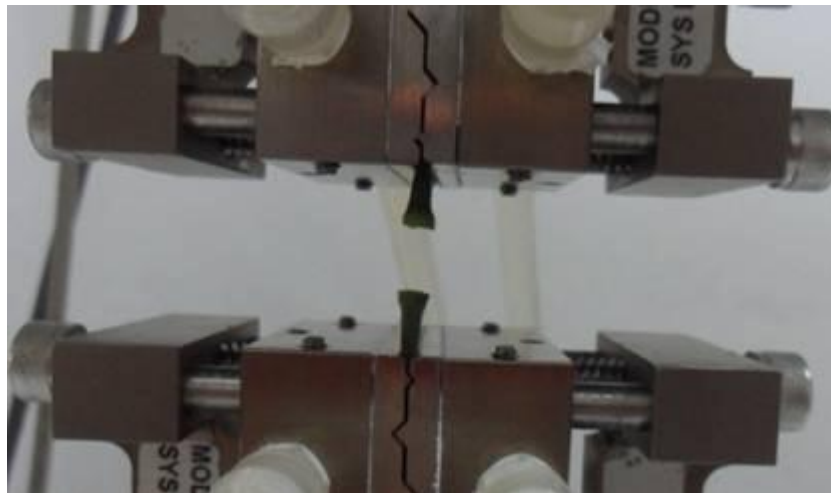
Fig. 2 S Breakstrength test Meter
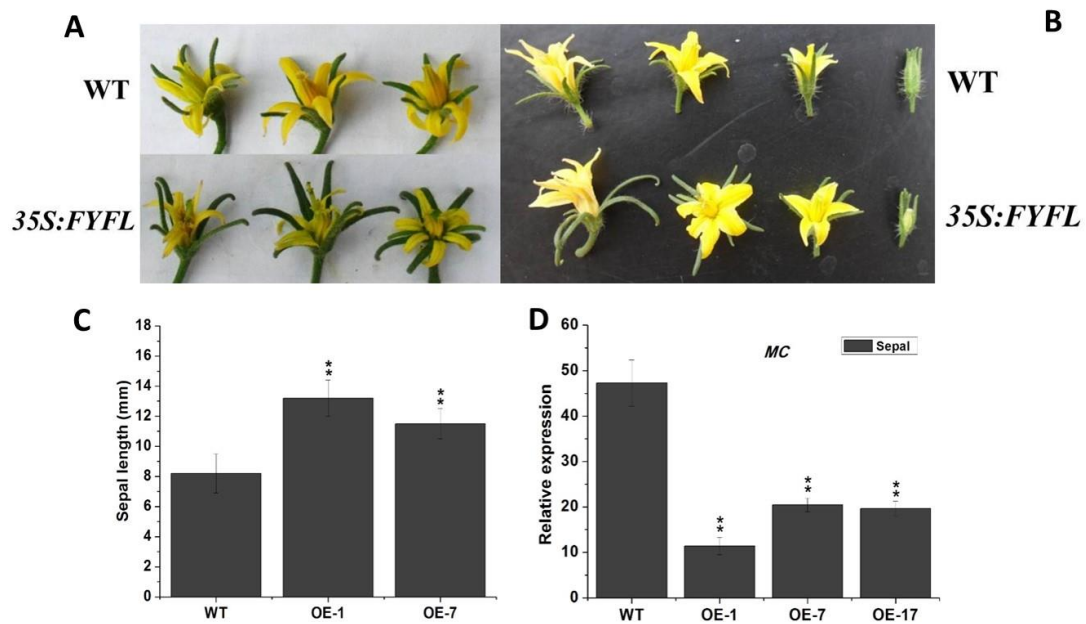
Fig. 3 S Sepals phenotype of wild type and transgenic lines and analysis of related gene. Phenotype **(A)** and **(B)** of sepals in different stages of flower development of wild type and transgenic lines. **(C)** Sepal length statistics in anthesis of wild type and transgenic lines. n=60. **(D)** Expression analysis of *MC* of sepals in anthesis of wild type and transgenic lines.WT, wild type; OE-1, OE-7, OE-17, different transgenic lines. The data represent mean from three replicates with three biological repeats. *, indicate P <0.05, **, indicate P <0.01 between the wild type and others by *t*-test. Error bars indicate SE.

1   Vrebalov, J. *et al.* A MADS-box gene necessary for fruit ripening at the tomato ripening-inhibitor (rin) locus. *Science* **296**, 343-346 (2002).

Table 1S. Primers used for PCRs and qRT-PCRs in this study

Primers	Primer sequence: forward primer (5'–3' top), reverse primer (5'–3' bottom)	Produncts(/bp)
SlFYFL	CTATTTTCTTTTCCATCCTCTT	1074
	GGTATTATTTATTGAATTTTGC	
SlFYFL (F+X)	TCTAGACTATTTTCTTTTCCATCCTCTT	1092

SlFYFL(R+S)	<u>GAGCTC</u>GGTATTATTTATTGAATTTTGC	
SlFYFL(RT)	CAGCCAACTGGAGAGGACCC	138
	CTAAGCCCGCACTTTTCACG	
SlFYFL(Y)	CCG<u>GAATTC</u>ATGAAAAGAATAGAAAATTCAACG	708
	GAAA<u>CTGCAG</u>CTATAAGCAGCGCGTTTGAG	
SlRIN(Y)	CCG<u>GAATTC</u>ATGGGTAGAGGGAAAGTAGA	1212
	CGC<u>GGATCC</u>TCATAGATGTTTATTCAT	
SlMADS1(Y)	CCG<u>GAATTC</u>ATGGGAAGAGGAAGAGTTG	760
	CGC<u>GGATCC</u>TTAAAGCATCCATCCATGAATA	
SlJ (Y)	CCG<u>GAATTC</u>ATGGCTAGAGAAAAAATTCAGATC	816
	CGC<u>GGATCC</u>ATCAGCCTGAGTAAGGTAGCCC	
SlCAC(RT)	CCTCCGTTGTGATGTAACTGG	173
	ATTGGTGGAAAGTAACATCATCG	
SlEF1(RT)	ACCTTTGCTGAATACCCTCCATTG	150
	CACACTTCACTTCCCCTTCTTCTG	
E4(RT)	AGGGTAACAACAGCAGTAGCA	167
	CCCAACCTCCGTCTTCAC	
E8(RT)	GGCACCATTCAACATACCG	242
	CTTTCACCGAAGAAGCACG	
PSY1(RT)	AGAGGTGGTGGAAAGCAA	298
	TCTCGGGAGTCATTAGCAT	
PDS(RT)	GCTTTACCCGCTCCTTTA	174
	ACCTTGCTTTCTCATCCA	
ZDS(RT)	GGTGGGTGCTGAAAAAAAT	93
	GGAAAGCGGAAATCAAGTT	
RIN(RT)	GGAACCCAAACTTCATCAGA	117
	TTGTCCCAAATCCTCACCTA	
ACO1(RT)	ACAAACAGACGGGACACGAA	181
	CTCTTTGGCTTGAAACTTGA	
ACO3(RT)	CAAGCAAGTTTATCCGAAAT	113
	CATTAGCTTCCATAGCCTTC	
ACS1A(RT)	AGAATAGTAATGAGTGGAGGAGCAA	171
	ATTACAACTTTCACAAACAACTGGA	
ACS2(RT)	GAAAGAGTTGTTATGGCTGGTG	107

Gene	Primer sequences	Product (bp)
	GCTGGGTAGTATGGTGAAGGT	
ACS6(RT)	TGATCCTGGTGATGCATTTCTAGTTC	146
	CTTCTTCTAAGGCTTCTTTTGTTACC	
PG(RT)	ATACAACAGTTTTCAGCAGTTCAAGT	188
	GGTTTTCCACTTTCCCCTACTAA	
ERF1(RT)	TTTTAGTATCGGATGGACG	102
	GGCGGAGAAACAGAAGTA	
J(RT)	CTCTTGAAACTGGATTGAGCCG	86
	CATACCCTTTTGTTGGAGTTGG	
MC(RT)	AAGTAGCAGAAGCAAGGAGGA	113
	CAAGCGATTAGCAAAGAGTGA	
GOB(RT)	CTGAACTTGACTGTATGTGGAGC	158
	GAACGTTTACAACAAAGTGACAAT	
WUS(RT)	TTTGGCTGCTCTTGAACTTACTCT	128
	ACATTACCATAAGGTCCAAATAGC	
Cel2(RT)	TTTTCACCAAACCCAAACCCA	98
	TCTTGAAAATAGGGTCTGGCGTCT	
TAPG1(RT)	TTGGAGTAAATGGGAAGAGGAA	98
	ATCACATGGAGTAGGCAATGGT	
Ls(RT)	TGTTTTCTACCTCCACCGCCT	115
	ATGATTTGCTTCCTTCTCCGC	
Bl(RT)	GGAAGATTTGATAGTTGTGTTG	91
	CAAAAATAGAGCTACACAAAACC	

Table 2S. Days from pollination to B stage for wild-type and transgenic lines (n=8)

tomato lines	Days
wild-type	36.0±0.50
OE-1	41.5±0.18
OE-7	40.2±0.38
OE-17	39.3±0.40