Bangladesh J. Bot. **44**(5): 827-831, 2015 (December), Supplementary

PREDICTION OF FRUIT WEIGHT BY ANALYSIS *FASCINATED* (*FAS*) LOCUS IN TOMATO (*SOLANUM LYCOPERSICUM* MULLER)

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Keywords: Tomato, *Fascinated* (*fas*), Fruit weight, Gene expression

Abstract

The function of *fascinated* (*fas*) gene was related to control fruit weight in tomato, and mutated in *fas* locus resulting in open reading frame alteration and created novel transcript which led to dramatically increased fruit weight. To establish a fast and reliable test method for fruit weight, identified *fas* gene whether wild or mutant is an available choice. This study showed that two novel transcripts were created as the result of inversion event occurred at *fas* locus. Based on *fas* gene and novel transcripts, two pairs of primers were specially designed to detect *fas* and novel transcripts, respectively. According to the test of genome and expression of *fas*, it provided an efficient method to identify *fas* type to screen the different fruit weight tomato without waiting long time for tomato fruit formation.

Introduction

Tomato (*Solanum lycopersicum* Muller), due to its nutritive and commercial value, is popular in studying fleshy fruit development and mature (Klee *et al*. 2011), compound leaf development, floral system and plant architecture (Kimura *et al*. 2008). Fruit weight is an important selection criterion in tomato breeding programs. The *locule* (*lc*) and *fas*, both affect fruit weight, but the *fas* has the larger effect. The main function of *fas* gene was related to control fruit weight in tomato (Lippman and Tanksley 2001, Barrero *et al*. 2004, Cong *et al*. 2008). However, evolution for thousands of years lead to mutation in *fas* and created novel transcript which led to dramatically increased fruit weight compared with fruits found in *fas* wild relatives (Cong *et al*. 2008). Till now, there are some hypothesis about *fas* mutation. One is that the mutation underlying the *fas* locus is due to a 6 to 8 kb insertion in the first intron of *fas* resulting in low level expression (Cong *et al*. 2008). Although *fas* expression was still detected in *fas* mutants, maybe it was not really from *fas* gene. The other is that the mutation is due to a 249 kb inversion disrupting the gene underlying the *fas* locus, and thus it is knocked in *fas* mutants (Huang 2011).

The goals of this study were to establish a sensitive and efficient method to identify *fas* type whether wild or mutant type in tomato. This result would offer a reliable and rapid method to predict fruit size before fruit formation in tomato. Two cultivars, FL1 tomato (*fas* gene wild-type) and MLK1 tomato (*fas* mutant-type), were selected in our study. First of all, two novel full length transcripts were got by 5' RACE and 3' RACE at *fas* locus in MLK1. Analysis between *fas* gene and novel transcripts showed that they were absolutely different after recombination. Then semi-quantitative RT-PCR primers were designed to detect the expression of *fas* gene and novel transcripts with great specificity. Moreover, our data suggested that *fas* gene expression was only detected in FL1, and novel transcripts were especially expressed in MLK1 by semi-quantitative RT-PCR. Our studies offered a valuable method to identify *fas* type to predict fruit weight among

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different cultivars by the analysis of genome and expression of *fas* locus in tomato. This information would offer important insights for further research on fruit weight.

Materials and Methods

Solanum lycopersicum Muller, MLK1 and FL1, were kept in Department of Horticulture, Shenyang Agricultural University. MLK1 fruits (about 500 g) were about 15 locule numbers, and FL1 fruits (about 30 g) were 4 to 6 locule numbers. F1 derived from a cross between MLK1 and FL1. Six different tissue types from MLK1 and FL1 tomato (*Solanum lycopersicum*) were collected in a greenhouse between 9:00 a.m. and 10:00 a.m. The tissues collected were root, stem, leaf, apical meristem around 5 mm long, flower buds younger than or equal to 5 days before anthesis (DBA), 15 days post anthesis (DPA) fruit, respectively. All the samples were frozen in liquid nitrogen immediately and stored at –80°C until used.

In this study, EP1069 was replaced with SF1 in our materials. fas-WT-1 (primer pair SF1 and EP1617) and fas-WT-2 (primer pair EP1070 and EP1071) were used to check the *fas* wild type genome structure; fas-I-1 (primer pair SF1 and EP1071) and fas-I-2 (primer pair EP1070 and EP1617) were used to check *fas* inversion. The primers SF1: 5'-TATTGGGTTGGGCAGGTG-3', EP1617, EP1070, and EP1071 were referred to Huang (Huang 2011).

Fas gene expression was detected by Fas-rt-F 5'-TCTTCACTCTTCCCCTTTG-3' and Fas-rt-R 5'-GCTGCATGGAACACTAACC-3' by taken actin as a quantitative internal control, Forward 5'-GGAATGGGACAGAAGGAT-3', Reverse 5'- CAGTCAGGAGAACAGGGT-3', with the following profile: 94°C 2 min; different cycles (*actin* 21 cycles, Fas-rt 34 cycles) of 30 s at 94°C, 30 s at 52°C, and 1 min at 72°C; 72°C 5 min.

The 3' RACE cDNA was synthesized using a traditional reverse transcription procedure, but with a special oligo(dT) primer Adaptor. According to 3' end known sequence of *fas*, specific primer AP 5'- CTGATCTAGAGGTACCGGATCC -3' was designed and synthesized for 3'-RACE according to the kit manual (SMARTTM RACE cDNA synthesis Kit, Clontech from USA). Both nested specific primers GSP1 and GSP2 were from the kit. Used primers AP and GSP1 for the first reaction, then taken the first PCR product as template in the second nested reaction with primers AP and GSP 2. The amplified fragment was cloned into the pGEM-T (Tiangen) and then sequenced.

The 5' end sequence was determined by 5' RACE following TaKaRa code D315 instruction. Both nested PCR primers GSP3 and GSP4 were from the kit. Used 5' RACE outer primer provided by the kit and GSP3 for the first reaction, then taken the first PCR product as template in the second nested reaction with primers 5' RACE inner primer also from the kit and GSP4. The amplified fragment was cloned into the pGEM-T (Tiangen) and then sequenced.

Semi-quantitative RT-PCR primers mfas-RT-F and mfas-RT-R were designed to detect novel transcripts expression. To avoid the interference of *fas* gene, mfas primers particularly spanned the first intron and the second intron of novel transcripts, with the following profile: 94°C 2 min; different cycles (*actin* 21 cycles, *mfas* 34 cycles) of 30 s at 94°C, 30 s at 54°C, and 1 min at 72°C; 72°C 5 min. The primers are mfas-RT-F 5'- CACATTTACATGCGGAGCAG-3', mfas-RT-R 5'-ACTGTTGCCTTTGCAGCTTT-3'.

Results and Discussion

Genome analysis results indicated that FL1 has a wild-type *fas*, while MLK1 *fas* was inversional mutation, and F1 (MLK1×FL1) represented both FL1 and MLK1 type (Fig. 1). Meanwhile, *fas* expression were detected in vegetative organ (Fig. 2a) and reproductive organ (Fig. 2b) in FL1. The results showed that *fas* strongly expressed in stems, leaves, apical meristems and

fruits, but not in roots (Fig. 2a). In contrast, there was no expression of *fas* in each tissues of MLK1 (Fig. 2a, b).

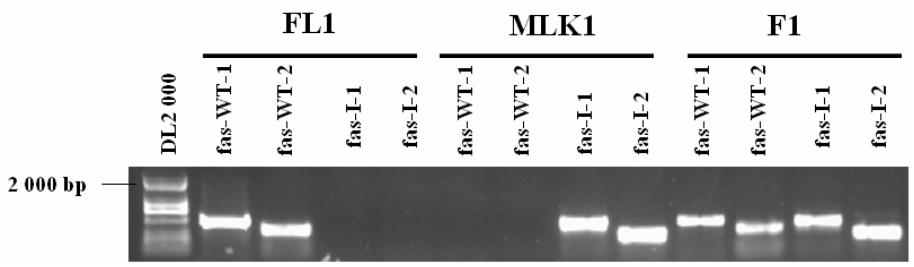

Fig.1. Genome type analysis at the *fas* locus in tomato.

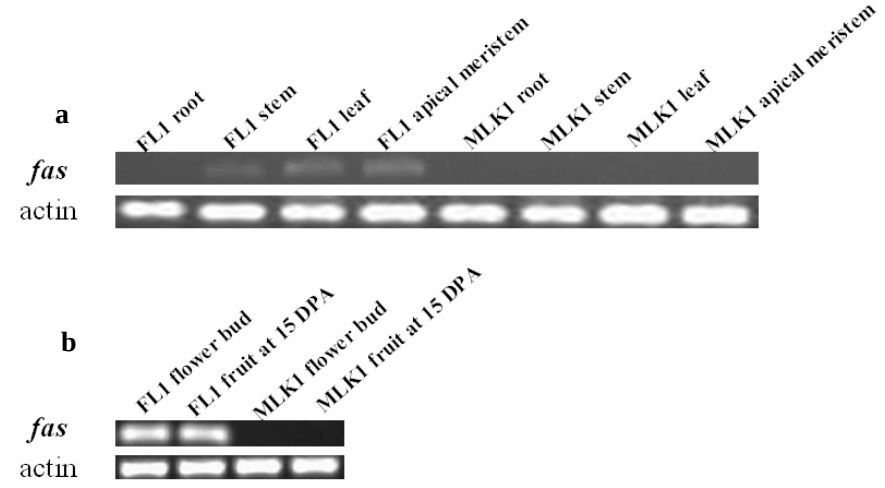

Fig. 2. Expression analysis of *fas* gene using RT-PCR.

These results confirmed that MLK1 tomato (*Solanum lycopersicum*) was *fas* mutant type, and it was inversion. According to this research, the inversional mutation was same to previous results that cultivars with larger fruits harbored a 249 kb inversion at *fas* locus by Huang (Huang 2011). Meanwhile, no expression of *fas* gene in MLK1 was due to the mutation altering its chromosomes structure. Even though low expression of *fas* gene was still detected in larger cultivars reported by Huang, it might be novel transcripts which located at the same locus in tomato (*Solanum lycopersicum*). The special primer should be designed for avoiding this interference.

In wild type tomato (*Solanum lycopersicum*), *fas* gene was 534 bp in length containing six exons (Fig. 3a). Two novel transcripts at *fas* locus, 2085 bp and 1930 bp, were obtained in MLK1. Analysis of novel transcripts showed that 2085 bp long was composed of the first exon (1478 bp), the second exon (120 bp), the third exon (148 bp), the fourth exon (49 bp), the fifth exon (76 bp) and the sixth exon (54 bp) (Fig. 3b). The other was composed of the first exon (1478 bp), the second exon (120 bp), the third exon (148 bp), and the fourth exon (184 bp) (Fig. 3c). Comparison results showed that only the first exon was obvious different between *fas* gene and novel transcripts (Fig. 3a, b, c).

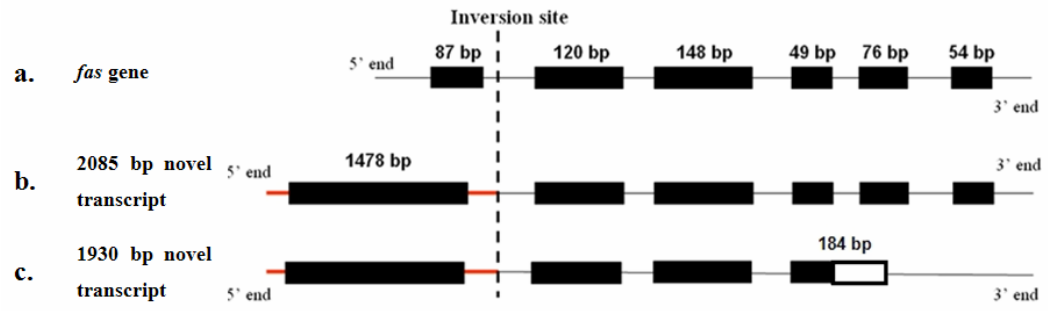

Fig. 3. The structure of *fas* gene and novel transcripts in tomato.

Based on sequence information of *fas* and novel transcripts, it provided us an opportunity to design special primers to distinguish *fas* gene and novel transcripts. The results showed that novel transcripts were expressed in MLK1, but not in FL1 (Fig. 4).

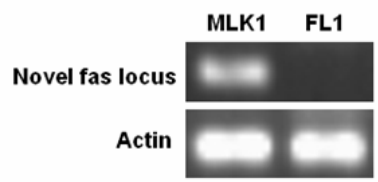

Fig. 4. Expression analysis of novel transcript at *fas* locus.

There were alternative splicing in most genes, which were related to splicing, transcription, flower, disease resistance, enzyme activity and grain quality of physiological processes and so on (Wang 2005). Due to alternative splicing, two novel transcripts at *fas* locus were got in MLK1 tomato (*Solanum lycopersicum*). While, the 5' end of *fas* gene and novel transcripts was obviously different, but similar at the 3' end. That's the reason why low expression of *fas* was still detected in larger fruit by Huang if primers located at the similar 3' end.

The function of *fas* gene was related to control fruit weight in tomato (*Solanum lycopersicum*). However, evolution lead to mutation in *fas* and created novel transcript which led to dramatically increased fruit weight. Using *fas* wild-type FL1, and *fas* mutant-type MLK1, two pairs of primers were designed to detect *fas* and novel transcripts with great specificity and efficiency, respectively. According to the test of genome and expression of *fas*, it provided an efficient method to identify *fas* type to predict fruit weight without waiting long time for fruit formation in tomato.

Acknowledgements

The authors thank Qingbo Zhang for his help with the analysis of the novel transcripts at the *fas* locus technical assistance. They thank Tao Xu for his help with the writing skill. They aslo thank all members of the Protected Horticulture laboratory, in particular Yanling Zhang for their help with fruit and image collections. The research reported herein was supported by grants from Doctoral Program of Higher Education of Special Research Foundation (200801570001).

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(Manuscript received on 14 August, 2015; revised on 29 November, 2015)