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Identification of Quantitative Trait Loci for Fruit Weight, Soluble Solids Content, and Plant Morphology Using an Introgression Line Population of *Solanum pennellii* in a Fresh Market Tomato Inbred Line

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Abstract

Introgression lines are convenient populations for the identification, fine-mapping, and functional analysis of genes that are responsible for variations in traits, particularly quantitative trait genes. An introgression line population of *Solanum pennellii* LA0716 in a fresh market tomato inbred line 1052 was developed by our group. This population was composed of 214 lines. In the present study, five quantitative trait loci (QTLs) for fruit weight, two QTLs for soluble solids content (SSC), three QTLs for plant height, and one QTL for leaf size were identified using this introgression line population. Among these, *fw3a* and *fw4a* for fruit weight, *ssc7a* for SSC, *h4t2a*, *h4t3a*, and *h4t7a* for plant height, and *lz12a* for leaf size were determined to be novel loci. These results serve as a foundation for fine-mapping and functional analysis of genes underlying these QTLs.

Keywords: tomato; introgression line; quantitative trait locus; fruit weight; soluble solids content; plant morphology

1. Introduction

Solanum pennellii has been used as a tomato model for genetic analysis and improvement. Several loci for abiotic and biotic resistance have been identified in this species; for example, drought resistance (Martin et al., 1989; Xu et al., 2008; Lu et al., 2009; Liu et al., 2011), salt resistance (Zamir and Tal, 1987; Foolad and Jones, 1993; Foolad et al., 1997; Foolad and Chen, 1998; Frary et al., 2010), and pest resistance (Mutschler et al., 1996; Lawson et al., 1997; Blauth et al., 1999). A large number of loci for fruit quality have been identified in the population derived from *S. pennellii*, such as fruit weight and shape (Tanksley et al., 1982; Eshed and Zamir, 1995; Ku et al., 1999; Lippman and Tanksley, 2001; Barrero and Tanksley, 2004; Causse et al., 2004; Baxter et al., 2005; Schauer et al., 2006; Semel et al., 2006), fruit color and pigment content (Liu et al., 2003; Rousseaux et al., 2005; Kachanovsky et al., 2012), and soluble solids content (SSC) (Eshed and Zamir, 1995; Fulton et al., 2002; Causse et al., 2004; Frary et al., 2004; Baxter et al., 2005; Schauer et al., 2006; Semel et al., 2006). Several loci for plant morphology have also been identified in the population derived from *S. pennellii*, such as plant height (de Vicente and Tanksley, 1993; Eshed and Zamir, 1995; Frary et al., 2004; Schauer et al., 2006; Semel et al., 2006) and leaf size (de Vicente and Tanksley, 1993; Holtan and Hake, 2003; Frary et al., 2004).

Introgression lines are nearly isogenic to the recipient genotype, and the phenotypic features that differentiate these are associated with the introgressed segment. Therefore, introgression lines are a convenient population for the identification, fine-mapping, and functional analysis of genes that are responsible for variations in traits, particularly quantitative trait genes. Eshed and Zamir (1994) developed an introgression line population of *S. pennellii* LA0716 in the processing tomato inbred line M82. This population has been extensively used in the identification of QTLs for fruit quality (Eshed and Zamir, 1994, 1995, 1996; Monforte et al., 2001; Gur and Zamir, 2004; Gur et al., 2011),

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harvest index and earliness (Gur et al., 2010), nutrient substance (Schauer et al., 2006; Stevens et al., 2007; Almeida et al., 2011), primary metabolites (Baxter et al., 2005; Gur et al., 2010), and carbon isotope composition (Xu et al., 2008). Among these loci, the genes underlying *fw2.2* for fruit weight (Frary et al., 2000) and *brix9-2-5* for SSC (Fridman et al., 2004) have been positionally cloned. The value of fresh market tomato is generally higher than that of processing tomato. To genetically analyze and improve fresh market tomato, we developed an introgression line population of *S. pennellii* LA0716 in a fresh market tomato inbred line 1052. This population was composed of 214 lines. This population has been used to identify loci for reticulate fruit surface, green stem, dark leaf veins, and potato leaf (Qiu et al., 2011). In the present study, five QTLs for fruit weight, two QTLs for SSC, three QTLs for plant height, and one QTL for leaf size were identified using this introgression line population.

2. Materials and methods

2.1. Plant materials

The fresh market tomato inbred line 1052 was developed by our group. The seeds of *S. pennellii* LA0716 were obtained from the Tomato Genetic Resource Center (TGRC). The BC_5S_2 population, which was composed of 214 lines, was grown in a plastic greenhouse in Shunyi District, Beijing, in the summer of 2013. Three replicates for every line and five plants for every replicate were prepared. Every replicate was randomly planted. The same experiment was repeated using the same conditions in the summer of 2014.

2.2. DNA isolation and marker development

Tomato genomic DNA was isolated using the modified CTAB method (Fulton et al., 1995; Boiteux et al., 1999). The quality of DNA was checked in a 1% agarose gel, and DNA concentration was measured by using a Biospec-nano (Shimadzu Corporation, Japan).

Based on the map Tomato-EXPEN 2000, 219 cleaved amplified polymorphic sequence (CAPS) and simple sequence repeat (SSR) markers were selected to screen for polymorphisms between fresh market tomato inbred line 1052 and *S. pennellii* LA0716. Finally, 168 CAPS and SSR markers were used to genotype the introgression lines. The physical position of these markers was identified based on the SL2.50 version of the tomato reference genome.

The volume of the PCR reaction for the CAPS markers was 15 μL, which included 3 μL of the DNA template (50–100 ng · μL^{-1}), 0.4 μL of the forward primer (10 μmol · L^{-1}), 0.4 μL of the reverse primer (10 μmol · L^{-1}), 7.5 μL of a 2 × GoTaq Green Master Mix, and 3.7 μL of ddH_2O. The PCR conditions were as follows: 94 °C for 4 min, followed by 35 cycles of 94 °C for 40 s, 50–60 °C for 40 s, 72 °C for 1 min; 72 °C for 10 min; and then 16 °C for forever. The PCR products were digested with 0.2 μL of an enzyme (10 U · μL^{-1}), 2.4 μL of a 10 × buffer, and 6.4 μL of ddH_2O for 4–16 h. The digested products were separated in a 1.5%–1.8% agarose gel via electrophoresis.

The volume of the PCR reaction for the SSR markers was 10 μL, which included 2 μL of the DNA template (50–100 ng · μL^{-1}), 0.25 μL of the forward primer (10 μmol · L^{-1}), 0.25 μL of the reverse primer (10 μmol · L^{-1}), 5 μL of a 2 × GoTaq Green Master Mix, and 2.5 μL of ddH_2O. The PCR conditions were as follows: 94 °C for 4 min; followed by 32 cycles of 94 °C for 30 s, 50–60 °C for 30 s, 72 °C for 1 min; 72 °C for 7 min; and then 16 °C for forever. The PCR products were separated via 8% PAGE.

2.3. Phenotyping

Eight to ten red ripe fruits were harvested from the second and third trusses of each plant to measure fruit weight. Then 3–5 fruits were selected for the measurement of SSC using an electronic refractometer (AtagoPAL-1, Tokyo, Japan). Plant height was measured from the boundary between the soil and tomato plant to the fourth truss when the fruits on the fourth truss were at the ripening stage. Leaf size was determined using the fifth to seventh leaf after these had fully expanded. The leaf size was classified by size, namely, large, medium, and small.

2.4. Data analysis

Levene's test of equality of error variance of fruit weight, SSC, and plant height was performed using the SPSS 17.0 software. If no significant equality of error variance was detected, the *t*-test was used to detect the equality of means. The genotype of every introgression line was analyzed and the genotypic map was drawn using the GGT2.0 software.

3. Results

3.1. QTLs for fruit weight

The fruit of the recurrent parent of these introgression lines was 120 g in weight, and the fruit weight of the introgression lines was within the range of 60–136 g. In the present study, five QTLs for fruit weight were detected and designated as *fw1a* (*fruit weight 1a*), *fw2a*, *fw3a*, *fw3b*, and *fw4a*. All small fruit alleles of these QTLs were from *S. pennellii* LA0716. Compared to the recurrent parent, *fw2a* reduced the weight of fruits by 23%–50%. The physical region of *fw2a* contained the *FW2.2* gene, which had been earlier cloned (Frary et al., 2000). *fw3b* reduced the weight of fruits by 24%–27%. Its physical region encompassed the *FW3.2* gene, which had been previously cloned (Chakrabarti et al., 2013). The region of *fw1a* overlapped with *fw1.2*, which has been previously described (Grandillo and Tanksley, 1996; Lippman and Tanksley, 2001). However, *fw3a* and *fw4a* were thus defined as novel loci for tomato fruit weight.

3.1.1. fw1a

The fruit weight of the introgression lines, IL1-3, IL1-4, IL1-5, IL1-6, and IL1-7, were within the range of 79–95 g. These were reduced by 18.8%–32.5% relative to the fruit weight of the recurrent parent. The introgressed region from *S. pennellii* LA0716 that spanned C2_At4g15520 and U223116 was shared among these lines. On the other hand, the introgression lines IL1-2 and IL1-11 contained the introgressed fragment upstream of C2_At4g15520 and downstream of U223116, respectively (Fig. 1). The fruit weight of IL1-2 increased by 14.5%, and that of IL1-11 was reduced by 5.1%. Therefore, we assigned *fw1a* to the