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Quantitative trait loci controlling flowering time and related traits in a *Solanum lycopersicum* × *S. pimpinellifolium* cross

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Abstract

Flowering time is an important factor determining early yield in tomato. However, the quantitative trait loci (QTLs) controlling flowering time and their relation to other QTLs for morphological and physiological traits have not been well studied. The aim of this study was to map the chromosomal regions controlling days to flowering (DTF) concurrently with other traits, such as the number of leaves preceding the first inflorescence (LN), length of the largest leaf (LL), number of lateral shoots (LS), fresh weight (FRW) and plant height (PH). This was undertaken using an inbred backcross population derived from a cross between the commercial cultivar *Solanum lycopersicum* cv. 'M570018' and its close wild relative *S. pimpinellifolium* (PI124039). *S. pimpinellifolium* flowers earlier than the cultivated tomato. Plants were grown in spring and summer. Composite interval mapping detected 16 QTLs for the six traits evaluated. These QTLs explained 10–42% of the individual phenotypic variation. QTLs detected in spring generally did not differ from those detected in summer. In chromosome 1, the DTF QTL was co-located with the QTLs for LL, LS and FRW, while in chromosome 3 it was co-located with the QTLs for LN, FRW (summer) and PH. One DTF QTL that was detected in chromosome 3 and conferred by the *S. pimpinellifolium* allele hastens flowering. The co-location of the DTF QTL with the LN QTL suggested that the DTF QTL in chromosome 3 controls the period from the vegetative to reproductive phase. Co-locations of DTF QTLs with the other traits might be pleiotropic effects of a single gene or cluster of genes via physiological relationships among traits because they were found to be highly significantly correlated.

Keywords: First inflorescence; Lateral shoot development; Number of leaves; Tomato

1. Introduction

Tomato (*Solanum lycopersicum*) is the second most important vegetable crop in the world, after potato. Consequently, it has been the subject of numerous studies for genetic improvement. Tomato is one of the first plant species in which researchers began to map quantitative trait loci (QTLs) of agronomic importance using molecular markers (Atherton and Harris, 1986).

Most QTL studies in tomato have used crosses of cultivated tomato with wild relative species, such as *S. chmielewskii* (Paterson et al., 1988), *S. cheesmaniae* (Paterson et al., 1991; Goldman et al., 1995; Paran et al., 1997), *S. habrochaites* (Bernacchi et al., 1998), *S. parviflorum* (Fulton et al., 2000), *S. pennellii* (de Vicente and Tanksley, 1993; Eshed and Zamir, 1995), *S. peruvianum* (Fulton et al., 1997) and *S. pimpinellifolium* (de Vicente and Tanksley, 1993; Grandillo and Tanksley, 1996; Tanksley et al., 1996; Chen et al., 1999; Doganlar et al., 2002). Introgressions of chromosomal segments from wild species have been shown to improve the cultivated tomato, not only for qualitative traits, but also for quantitative traits (Eshed and Zamir, 1994). *S. pimpinellifolium* is very close to the cultivated tomato and is the only wild species for which natural introgression has been demonstrated with *S. lycopersicum* (Grandillo and Tanksley, 1996). Despite their close relationship, they differ in many morphological aspects, many of which are polygenically inherited (Luckwill, 1943; Grandillo and Tanksley, 1996). Furthermore, *S. pimpinellifolium* flowers earlier than the cultivated species.

Although QTL studies conducted for tomato have revealed 50 traits, most are focused on fruit-related traits (Eshed and Zamir, 1996; Grandillo and Tanksley, 1996; Tanksley et al., 1996; Fulton et al., 1997, 2000; Bernacchi et al., 1998; Chen et al., 1999; Grandillo et al., 1999; Ku et al., 2000; Doganlar et al., 2002; Lecomte et al., 2004; Chaib et al., 2006). QTLs controlling flowering time have not been well studied. In tomato, flowering time is a key character for high yield; shortening the vegetative phase leads to an increase in early yield, and lengthening it may sustain high yield for a long period by the formation of a large

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number of leaves (Atherton and Harris, 1986). Flowering of tomato is complex and no single environmental factor can be regarded as critical for the regulation of flowering time. Flower initiation is directly or indirectly influenced by light, temperature, carbon dioxide, nutrition, moisture and growth regulators (Heuvelink, 2005). Earliness of flowering can be evaluated by the number of leaves preceding the first inflorescence (NLPI), as well as the days required from sowing to anthesis (Honma et al., 1963). The NLPI is highly correlated with flowering time (Honma et al., 1963).

There is evidence to suggest that intraplant competition for assimilates may affect the phase change from vegetative growth to reproductive development in tomato (Sachs and Hackett, 1969; Atherton and Harris, 1986; Heuvelink, 2005). If the competitive potential of the apex is increased compared with other parts of the plant, the NLPI will decrease because flowering time is hastened (Heuvelink, 2005). Removal of young axillary shoots was also reported to promote floral development (Hartmann, 1978; Nourai and Harris, 1983).

A study based on an F_2 population derived from a cross between *S. lycopersicum* 'Vendor Tm2a' and *S. pennellii* (LA716) revealed QTLs for flowering time in chromosomes 1, 2, 3, 5, 10–12 (de Vicente and Tanksley, 1993). Grandillo and Tanksley (1996) reported QTLs for flowering time in chromosomes 1 and 2 using a BC_1 population derived from a cross between *S. lycopersicum* cv. 'M82-2-7' and *S. pimpinellifolium* (LA1589). Using a BC_2F_6 population derived from a cross between *S. lycopersicum* 'E6203' and *S. pimpinellifolium* (LA1589), Doganlar et al. (2002) reported flowering time QTLs in chromosomes 3 and 4. The QTL detected in chromosome 3 was found to correspond to the same region to which a major flowering time QTL was mapped using the interspecific cross 'Vendor Tm2a' and *S. pennellii* (LA716). However, little study has been undertaken on how flowering time QTLs exert pleiotropic effects on other morphological and physiological traits in tomato.

In this study, we mapped QTLs controlling flowering time and other related traits using a cross between *S. lycopersicum* and *S. pimpinellifolium*. This is the first study in which the relationship between flowering time and vegetative growth (number of leaves preceding the first inflorescence, length of the largest leaf, number of lateral shoots, fresh weight, and plant height) has been analyzed at the QTL level.

2. Materials and methods

2.1. Mapping population

One hundred and fourteen BC_1F_3 plants were developed from a cross between a commercial cultivar, *S. lycopersicum* cv. 'M570018' (kindly provided by Dr. Y. Shintaku), and a wild accession, *S. pimpinellifolium* (PI124039), at the University of Tokyo, Japan. In January 2004, (PI124039) (male) was crossed to 'M570018' (female). The F_1 was backcrossed to 'M570018' in May 2004, the latter being used as a male parent. The resultant BC_1F_1 plants were advanced by the single seed descent (SSD) method until BC_1F_3 seeds were obtained in June 2005. A linkage map was constructed using the BC_1F_3 population, and a phenotypic evaluation was made using the resultant BC_1F_4 families.

2.2. Phenotypic evaluation

The 114 BC_1F_4 families, along with their parents, were grown in 1 l pots (15 cm in diameter) filled with commercial compost (Soilmix; Sakata Seed Co., Yokohama, Japan) under greenhouse conditions. The experimental design was a randomized complete block, consisting of 14 replications. During the spring (April–June) and summer (July–September) of 2006, individual plants were scored for six traits at anthesis. Days to flowering (DTF) was counted as the number of days from sowing to anthesis. The number of leaves (LN) was measured as the number of leaves preceding the first inflorescence. The number of lateral shoots (LS) was the number of lateral shoots (larger than 0.5 cm in length) below the first inflorescence. Leaf length (LL), in centimeters, was the length of the largest leaf. Fresh weight (FRW), in grams, was the fresh weight of the aerial portion of the plant. Plant height (PH), in centimeters, indicates the distance from the ground level to the tip of the shoot.

2.3. DNA extraction and polymerase chain reaction (PCR)

The DNA of the 114 BC_1F_3 and parental lines was extracted from 0.1 g of fresh leaf tissue using the Nucleon PhytoPure plant DNA extraction kit (Amersham Biosciences, Buckinghamshire, UK). Thinly cut leaf samples were homogenized with reagents 1 and 2 of the extraction kit using a multibead shocker (Yasui Kikai, Tokyo, Japan) at 2000 rpm for 60 s. Succeeding steps were done following the protocol of the manufacturer. PCR consisted of 1.6 μl of 10 × PCR buffer (ABgene, Epson, UK), 0.8 μl of 2.5 mM dNTP mixture (ABgene), 0.05 μl of 5 U/μl SMART *Taq* DNA polymerase (ABgene), 0.2 μl of 100 μM forward and reverse primers, 5 μl of 40 ng/μl template DNA and 2.35 μl of milliQ H_2O. PCR conditions were (i) 94 °C for 5 min followed by 35 cycles at 94 °C for denaturation for 30 s, (ii) 50–55 °C for annealing for 45 s and (iii) 72 °C for 45 s for extension and one last cycle at 72 °C for final extension for 5 min. Parents were screened for a total of 231 PCR-based molecular markers (93 SSR, 52 COSII and 86 CAPS) obtained from the SOL Genomics Network (http://sgn.cornell.edu/). Polymorphism of COSII and CAPS markers was surveyed using 12 restriction enzymes, i.e., *Afa*I, *Alu*I, *Bam*HI, *Bgl*II, *Dra*I, *Dpn*II, *Eco*RI, *Eco*RV, *Hinc*II, *Hind*III, *Hinf*I and *Kpn*I at 37 °C for 12 h. Eighty polymorphic markers (41 SSR, 13 COSII and 26 CAPS) were used to construct a linkage map.

2.4. DNA gel electrophoresis

SSR and COSII markers were separated on 3% agarose gels in 0.5× TBE (44.5 mM Tris, 44.5 mM boric acid, 1 mM EDTA and pH 8.0) buffer at 160 V for 110 min. Patterns were visualized under UV using ethidium bromide. SSR and COSII markers that did not show polymorphism on agarose gel and all CAPS markers were separated by polyacrylamide gel electro-