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# Quantitative trait loci analysis of lateral shoot growth in tomato

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ABSTRACT

Branching is an important agronomic trait to determine plant architecture and fruit yield. In tomato, the regulatory mechanisms of shoot branching patterns have been studied mainly in mutants defective in meristem identity. Little is known about quantitative trait loci (QTLs) and their genetic interaction (epistasis) in lateral shoot development. Here, the genetic control of lateral bud outgrowth initiation and subsequent lateral bud development into branches were investigated. We used a $BC_1F_7$ population developed from *Solanum lycopersicum* (SL) and its close wild relative *Solanum pimpinellifolium* (SP) to measure the number of leaves on primary shoot (LN), the proportion of nodes with lateral buds (LS0) and lateral branches longer than 5 cm (LS5) and 10 cm (LS10) on main axis, the proportion of nodes with secondary lateral branches longer than 1 cm (LSLS1) on primary lateral branches, and the lengths of the longest primary and secondary lateral branches (LLSL1 and LLSL2, respectively). Composite interval mapping detected 17 additive QTLs; those for LS5, LS10, and LLSL1 clustered near C2_At5g49480 or LEATPACb on chromosome 1 and LEOH361 on chromosome 4. One epistatic (QTL × QTL) interaction was identified for LS10 QTLs on chromosomes 1 and 12, where recombinant-type alleles increased lateral bud development into primary lateral branches. We analyzed further the QTLs clustered on chromosome 1 using two $BC_3F_4$ populations containing introgression region from the SP, which revealed the co-location of QTLs for lateral shoot development and days to flowering time. These results suggest that lateral shoot development is regulated by some additive and epistatic QTLs, some of which possibly exhibit a pleiotropic effect on flowering time.



## 1. Introduction

The patterns of axillary/lateral bud formation and their development determine plant architecture (Schmitz and Theres, 1999). Lateral buds have two developmental modes, monopodial and sympodial (Pnueli et al., 1998). The model plant *Arabidopsis* has monopodial shoot architecture, and its shoot development can be separated into three phases, vegetative, inflorescence, and flowering (Schmitz and Theres, 1999). The vegetative shoot consists of a short internode and rosette leaves. The inflorescence shoot is extended by internode expansion after the transition from the vegetative to reproductive stage. At the same time, the axillary buds become visible. Later, these axillary buds develop into lateral inflorescence shoots. The flowering shoots consist of an intermediate-length internode and solitary flowers with or without subtending bracts (Pnueli et al., 1998). By contrast, tomato has sympodial shoot architecture, and its development can be separated into two phases, vegetative and reproductive, which alternate regularly (Pnueli et al., 1998). The vegetative phase is terminated by the development of a cymose inflorescence after the production of 8–12 leaves. When reproductive growth is initiated by the outgrowth of the inflorescence, vegetative growth restarts with the development of lateral buds just below the inflorescence. This generates three more leaves until terminating with the next inflorescence initiation.

In tomato, several mutants defective in meristem identity has been used to study the genetic control of shoot development and branching habit. The bushy (*bu*) mutant stimulates branching, resulting in a characteristic bushy appearance (Young and MacArthur, 1947). Campbell and Nonnecke (1974) reported another unusual branching mutant, lateral promoter (*Lp*), in which buds in the axils of the cotyledons appeared early, causing a bushier appearance. The self-pruning (*sp*) gene in tomato replaces flowers with leaves in the inflorescence and suppresses the transition of the vegetative shoot apex to a reproductive shoot (Pnueli et al., 1998). The lateral suppressor (*ls*) mutant does not form most of the axillary meristem (Schumacher et al., 1999) and is related to two proteins involved in negative regulation of GA signal transduction (GAI, RGA). In the blind (*bl*) mutant, the sympodial meristem is absent, and the number of flowers per inflorescence is reduced

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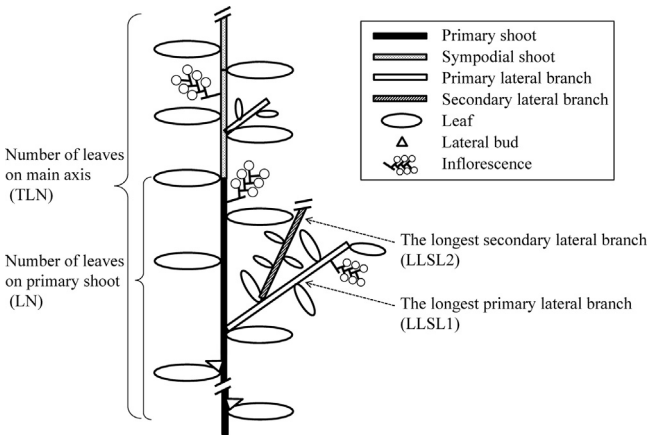


**Fig. 1.** Illustration of inflorescence and lateral branch development in tomato. To evaluate the lateral shoot development in a $BC_1F_7$ and two $BC_3F_4$ populations, the number of leaves on primary shoot (LN), the number of leaves on the main axis (TLN) was measured. The proportion of buds to LN was calculated as an indicator of the proportion of lateral buds released from apical dominance (LS0). The proportions of the number of these primary lateral branches to TLN were designated LS5 and LS10, respectively. The proportion of the number of secondary lateral branches longer than 1 cm to the leaf number of the longest primary branches (LSLS1), the length of the longest primary lateral branches (LLSL1) and the longest secondary lateral branches (LLSL2) were measured. The total number of inflorescences (IN) and the average number of flowers (AFN) on the longest primary lateral branch were measured 62 days after sowing.

(Schmitz et al., 2002). The jointless (*j*) mutant suppresses sympodial meristem identity, which reverts the inflorescence meristem to vegetative growth after forming 1–2 flowers (Mao et al., 2000; Szymkowiak and Irishi, 2006). Although genes regulating branching habit and shoot development in these mutants have been identified, lateral shoot development is also controlled by the cumulative effects of quantitative trait loci (QTLs), like most important agronomic traits such as yield, plant height, and flowering time. QTL analysis using phenotypic values and genetic marker information could enable us to determine the number of QTLs associated with lateral shoot development, their locations and epistatic effects between QTLs.

In cereal crops, many QTLs that affect branching have been identified. Teosinte branched (*tb1*) is a major QTL that controls the reduction in axillary branching from teosinte to maize (Doebley et al., 1995). In rice, the identification of genes that underlie QTLs affecting branching type has been advanced using rice genome information (Doust, 2007). Peng et al. (2014) also reported that QTLs for primary panicle branch number was regulated by a gene for plant height and tiller number. In spray cut chrysanthemum, 16 additive QTLs for branching traits were identified (Peng et al., 2015). By contrast, QTLs controlling lateral shoot development has been little studied in tomato although the number of lateral shoots affects vegetative and reproductive traits, such as leaf mass, canopy structure, the number of flowers and yield (Navarrete and Jeannequin, 2000). In the present study, therefore, we tried to identify QTLs related to the initiation of lateral bud outgrowth and subsequent lateral bud development in tomato using 111 $BC_1F_7$ lines and 100 $BC_3F_4$ lines (Fig. 1). Our results are useful for understanding the genetic basis of lateral shoot development and branching type in tomato.

## 2. Materials and methods

### 2.1. Mapping population

One hundred and eleven $BC_1F_7$ lines (hereafter referred to as BILs) were derived from an initial cross between the commercial cultivar *Solanum lycopersicum* (M570018, normal branched tomato cultivar) and its close wild relative *Solanum pimpinellifolium* (PI124039, highly branched wild tomato) and a backcross of the $F_1$ to the 'M570018' cultivar. The resultant $BC_1F_1$ population was advanced using the single-seed descent method to obtain the $BC_1F_6$ population, which was used for genotyping. The subsequent $BC_1F_7$ generation was used for phenotypic evaluation. To confirm the effects of detected QTLs in chromosome 1 on flowering time and branch development, two $BC_3F_1$ lines containing a 45 cM heterozygous fragment on chromosome 1 were selected and advanced to $BC_3F_4$ populations by selfing. In addition to a fragment on chromosome 1, these two lines contained 13 and 16.2 cM heterozygous fragments near C2_At3g55120 and SSR115 to C2_At3g55120 markers on chromosome 5. A total of 100 plants from two $BC_3F_4$ populations (1762050-7-16 and 1762050-7-34) were used for the QTL analysis.

### 2.2. Phenotyping

For phenotypic evaluation, the 111 $BC_1F_7$ lines, along with their parents, were grown in a greenhouse with natural daylight in The University of Tokyo, Japan. Seven seeds from each of the BILs were sown into 200 mL plastic pots (7 cm diameter) containing mixed commercial growth medium (Engeibaido, Kureha, Tokyo, Japan; Soilmix, Sakata Seed Co., Yokohama, Japan) on 15 July 2010. On 12 August (four weeks later), five out of seven seedlings were selected and transplanted into 1 L plastic pots (14.5 cm diameter). The pots were arranged in two double rows with 130 cm spacing between the centers of the rows. The plants were spaced 30 cm apart within each row and 45 cm apart between rows.

For QTL analysis using $BC_3F_4$ families, two seeds from 50 lines, each of the two $BC_3F_4$ families were sown into 500 mL plastic pots (9 cm diameter), with five pots per line, on 7 October 2010, and thinned to one plant per pot after emergence. On 29 October, the plants were transplanted into 1 L plastic pots (14.5 cm diameter). The plants were spaced 27 cm apart within rows and 45 cm apart between rows. The plants were watered daily throughout the experiment. A randomized complete block design was used in both experiments. No lateral branches were pruned in either experiments.

To evaluate the number of axillary buds (lateral buds) in the 111 $BC_1F_7$ lines, the number of leaves before the inflorescence (LN), i.e., the number of leaves on the primary shoot was measured 46 days after sowing the seeds, at which time the macroscopic appearance of the first inflorescence had occurred. At the same time, the number of visible lateral buds was counted, and the proportion of buds to LN was calculated as an indicator of the proportion of lateral buds released from apical dominance (LS0). The numbers of primary lateral branches longer than 5 cm and 10 cm and the number of leaves on main axis (monopodial and sympodial shoots), TLN, were measured 62 days after sowing. The proportions of the number of these primary lateral branches to TLN were designated LS5 and LS10, respectively. The proportion of the number of secondary lateral branches longer than 1 cm to the leaf number of the longest primary branches (LSLS1), the length of the longest primary lateral branches (LLSL1), and the longest secondary lateral branches (LLSL2) were measured 64 days after sowing. The total number of inflorescences (IN) and the average number of flowers (AFN) on the longest primary lateral branch were measured 62 days after sowing. In QTL analysis using the two $BC_3F_4$ families, the traits for which QTLs were detected in the experiment using $BC_1F_7$ BILs were measured; LS5 and LS10 were measured 72 days after sowing, while LSLS1, LSLS2, LLSL1, and LLSL2 were measured 74 days after sowing. The number of leaves (LN) before inflorescence was measured 46 days after sowing, and days to flowering (DTF) were measured as well. However, LS0 was not measured in this experiment because