論文の内容の要旨

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Effects of seedling raising method on the vegetative and reproductive growth of tomato plants

（トマトの栄養成長と生殖成長に影響を及ぼす育苗方法の影響）

Traditionally, tomato seedlings were raised in small pots with capacities of 200 to 600 cm^3 and transplanted into fields just before or at anthesis. Recently, tomato crops are raised from transplants that are cultivated in separate nurseries, and then shipped to farmers. Thus, use of cell trays (cell volumes ranging from 12 to 50 cm^3) became more popular for easy transportation of transplants. In addition, seedling raising in cell trays makes it possible to mechanize sowing and transplanting. However, the small rooting volume available in cell trays is known to retard shoot growth during the seedling-raising period and stimulate vegetative growth after transplanting, leading to delay of flowering in the tomato seedlings. To control overgrowth, most Japanese farmers transplant cell-raised seedlings into small pots and then into fields. This requires additional labor for the farmers.

Growth retardation observed in plants grown in limited rooting volumes have been associated with several factors such as reduced root respiration in restricted roots, depression of photosynthesis and accumulation of abscisic acid in the xylem sap. Root environment such as nutrient levels, root temperature and water availability are known to affect shoot growth and flowering, suggesting that a root-to-shoot signal plays a role in plant response to root environment. The effect of these root environments, however, differs among cultivars, and little is known about the interaction effect of genotype and limited rooting volumes on vegetative and reproductive growth.

Vegetative traits and flowering time of tomato have been studied using marker-based quantitative trait loci (QTL) analysis. However, no QTL analysis has been done on how these traits are

affected by limited rooting volumes. In this study, the effect of the seedling raising method (in pots or cell trays) on early vegetative and reproductive growth of tomato was studied by QTL analysis using a population derived from *Solanum esculentum* and *S. pimpinellifolium*. The possible role of ethylene in root-to-shoot signaling during root restriction was also examined.

1. Effects of limited rooting volume on early vegetative growth and flower induction in tomato

The limited rooting volume of cell trays is known to cause growth inhibition and flowering delay in tomato transplants. However, little is known about how shoot growth and flower induction proceeds in response to root restriction. Thus, leaf initiation and flower induction was monitored in tomato seedlings raised in 128-cell trays. Leaf initiation and fresh weight accumulation became inhibited in tomato cv. 'Sunroad' seedlings after 2 weeks of growth in the cell trays and the seedlings initiated flowers only after transplanting into larger containers. Response to root restriction was different among cultivars when raised either in small pots or 128-cell trays. The cultivar 'Reika' was the most sensitive to limited rooting volumes; when grown in pots, 'Reika' seedlings were the largest compared with 'Marryroad' and 'First Power'. 'Reika' flowered first when grown in pots, but in cell trays, 'Reika' flowered last and produced a large number of leaves preceding the first inflorescence (LN). Flower induction and leaf initiation were also examined in a BC_1F_6 population (hereafter referred to as recombinant inbred lines, RILs) developed from *Solanum lycopersicum* 'M570018' and *S. pimpinellifolium* (PI124039). At 8 days after cotyledon expansion (before root restriction occurred), approximately 80% of the 110 RILs were completely vegetative and the number of leaves initiated was 6 to 9. Quantitative trait loci (QTL) analysis detected one QTL each, *ri_8d1* and *li_8d1*, which influenced the percentage of reproductive seedlings per line (reproductive index, RI) and the number of leaves initiated (LI), respectively. Co-localization of these QTLs indicates that this region controls meristem identity, which determines both leaf initiation rate and phase change from vegetative to reproductive growth.

2. QTLs controlling vegetative overgrowth in tomato seedlings after transplanting

To explore the interaction effects of the seedling raising method and genotypes on the vegetative and reproductive growth of tomato seedlings, seeds from the 110 RILs were either, 1) directly sown into pots, or 2) raised in 128-cell trays and transplanted. Both seedlings were cultivated until flowering. Two experiments were performed in autumn, 2007 and spring, 2009. Four vegetative traits were evaluated – plant height, length of largest leaf (LL), number of lateral shoots and shoot fresh weight (SFW), along with the flowering time traits, days from sowing to anthesis (DTF) and LN. Transplanted plants grew more vigorously and flowered later than direct-sown plants in both experiments. Among the RILs, environment (direct-sown or transplanted) effects and genotype × environment interaction effects were significant for all traits. Ten additive (main effect) QTLs and three epistatic (QTL × QTL) interactions were detected for the vegetative traits, while six additive QTLs and six epistatic interactions were detected for DTF and LN. One additive QTL (*ll9*) and an

epistatic QTL pair (*sfw1*−*sfw5*) exhibited QTL × environment (QE) interactions, accounting for the observed vigorous vegetative growth in the seedlings after transplanting. No flowering QTL exhibited QE interactions, suggesting that the DTF and LN QTLs detected in this study are independent of the environment. The seedling raising method may affect other genetic factors upstream to the QTLs that directly control DTF and LN. Alternatively, several developmental processes influence DTF and LN, and QE interaction at these processes may not be detected when measuring only DTF or LN.

3. QTL analysis of flowering-time-related traits in tomato

None of the flowering time QTLs previously detected exhibited QE interaction. However, several developmental processes influence DTF and LN, and QTLs controlling these processes may exhibit QE interaction. To understand how these developmental processes are genetically integrated with DTF and LN, days to macroscopic flower bud appearance (DMB), flower development duration (FDD: DTF − DMB), the number of leaves initiated (LI) and reproductive index (RI) were measured, along with DTF and LN. Composite interval mapping detected 12 QTLs for the six traits, which included two QTLs for DTF on chromosomes 1 and 6. The two DTF QTLs explained 43% of the phenotypic variation in this trait. The presence of *S. pimpinellifolium* alleles in the detected QTLs increased the rate of leaf initiation, reduced LN, and hastened flower induction, floral development and anthesis. QTLs for LN, LI, RI, DMB and FDD clustered with the DTF QTLs; *dmb1*, *fdd1*, *li_14d1*, *li_19d1* and *ri_19d1* clustered with *dtf1* on chromosome 1, and *ln6* and *fdd6* with *dtf6* on chromosome 6. These results suggest that the QTLs on chromosomes 1 and 6 form functional “gene clusters” that drive tomato flowering in synergy. Alternatively, the two DTF QTLs may act as “master genes” that control flowering time through pleiotropic effects on multiple developmental processes. Tight clustering of the developmental QTLs with the DTF and LN QTLs suggests that any developmental process influencing tomato flowering time is also independent of the environment.

4. QTL analysis of transplanting time and other root-growth-related traits in tomato

If the repressed vegetative growth and flowering delay of cell-tray-raised tomato seedlings were associated with limited rooting volume, the extent of growth restriction and flowering delay would increase as transplanting time was delayed. Transplanting time is determined by field conditions, the age of the seedlings, size of the container, etc., but little information is available on the effect of genetic background on transplanting time. To identify the QTLs affecting root development and transplanting time, root dry weight (RDW), shoot dry weight, root/shoot ratio (RSR), time at which 50% of the seedlings have expanded cotyledons (CE50), period from cotyledon expansion of the first seedling to the last (emergence span, ES), root ball formation (RBW) and time at which seedlings are easily plucked from the cell trays (transplanting time, TRD) were measured in the RIL population. RSR was significantly correlated to RDW, but not to SDW. RBW, CE50 and TRD were significantly correlated to each other. A total of 8 additive QTLs were detected for the five traits, i.e., RSR, RBW, CE50, ES and TRD. One epistatic interaction each was identified for RSR and TRD. QTLs for RSR,

RBW, CE50, ES and TRD clustered near marker LEOH37 on chromosome 4. This indicates that this region influences both root and shoot growth at the early stage of development of tomato seedlings as well as transplanting time. Several root-growth-related QTLs were mapped to regions where DTF and LN QTLs were previously identified, suggesting that the roots may exert some influence on flowering time in tomato.

5. Possible role of ethylene in growth inhibition and flowering delay of root-restricted tomato seedlings

Shoot growth inhibition and delay in flowering in tomato seedlings raised in the small rooting volumes of cell trays are considered to occur as the result of the action of signals transmitted from root to shoot. One possible signal molecule is the ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC). To clarify the involvement of ethylene with shoot growth inhibition and flowering delay during root restriction, the effect of ethylene-producing ethephon and ethylene inhibitors, aminovinylglycine (AVG) and silver thiosulfate (STS), on DTF and LN was examined. In addition, the expression of the ethylene biosynthesis genes, ACC synthase and ACC oxidase, in pot- and cell-tray-grown tomato seedlings was measured using real-time PCR. Foliar application of ethephon increased both DTF and LN in pot-grown plants, while inhibiting ethylene perception via STS reduced DTF and LN in cell-grown seedlings. In contrast, disruption of ACC synthase activity in the leaves using AVG, did not affect DTF and LN. Repression of leaf initiation and fresh weight accumulation was evident by 14 days after sowing (DAS) in the cell trays, coinciding with root accumulation on the root ball surfaces. Prolonged growth in cell trays delayed flowering after transplanting. Plants transplanted at 23 DAS flowered 19 days later than pot-grown plants. Of the 8 tomato ACC synthase (LeACS) and 5 ACC oxidase (LeACO) gene isoforms, LeACS2, LeACO1 and LeACO4 transcript levels increased in the leaves of cell-grown seedlings compared with pot-grown seedlings. Up-regulation of these genes coincided with the repression of leaf initiation and flower induction, and increase in root density on the root ball surface. Transplanted plants did not exhibit up-regulation of the LeACS2, LeACO1 and LeACO4, which was observed in cell-tray-grown seedlings of the same age. Transient up-regulation of the LeACO genes suggests that ACC may be one of the root-to-shoot signal molecules during early root restriction.

In conclusion, seedling raising in cell trays caused growth restriction and floral initiation during growth in cell trays, but stimulation of vegetative growth after transplanting. Growth inhibition and flowering delay of seedlings during growth in cell trays may be controlled by ethylene. This study also revealed that some QTLs controlling vegetative growth interacts with seedling raising methods, leading to vigorous vegetative growth after transplanting. This information should be useful in developing seedling raising methods using cell trays without vegetative growth stimulation after transplanting and flowering delay.