A Graft-transmissible RNA from Tomato Rootstock Changes Leaf Morphology of Potato Scion

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Abstract. Heterografting experiments were conducted using potato (*Solanum tuberosum*) as scion and tomato (*Lycopersicon esculentum*) as stock to test whether an RNA molecule responsible for changing leaf shape can be transported and function across the grafting junction. Results showed that a graft-transmissible RNA from the tomato rootstock without any leaves can indeed change leaf morphology of the potato scion. Therefore, if a transgenic stock plant with acquired RNA transport competence could be constructed, it would provide an effective system for transformation of scion plants. This combination of grafting and RNA transport could provide a novel technique for cultivar improvement in horticultural crops.

In higher plants, in addition to carbohydrates, amino acids, and other nutrients, specific RNA molecules and some proteins are transported in the phloem (Wu et al., 2006). Ruiz-Medrano et al. (1999) identified several mRNA molecules in the phloem sap through the characterization of the cDNA clones obtained from pumpkin (*Cucurbita maxima* Duchesne) sap. Yoo et al. (2004) demonstrated the presence of an endogenous population of small RNA species, ranging from 18 to 25 nucleotides in size, in the phloem sap of pumpkin, cucumber (*Cucumis sativus* L.), white lupin (*Lupinus albus* L.), castor bean (*Ricinus communis* L.), and yucca (*Yucca filamentosa* L.). Because the sequences of these small RNAs correspond to several putative target genes, it was proposed that phloem sap contains bona fide small regulatory RNA (Eckardt, 2004; Yoo et al., 2004).

By using heterografting techniques with cucumber as scion and pumpkin as stock, Lucas's group at the University of California at Davis showed evidence of a selective system for the delivery of specific RNA molecules into developing tissues of scions through the sieve element (Haywood et al., 2002; Ruiz-Medrano et al., 1999; Xoconostle-Cázares et al., 1999). Gómez et al. (2005) revealed that several phloem proteins having a wide RNA-binding activity are able to move through intergeneric grafts from melon (*Cucumis melo* L.) to pumpkin. It has long been believed that a long-distance signal, named florigen, is transported from leaves to the shoot apex (Zeevaart, 1976). Recently,

it was suggested that *Flowering Locus T* (*FT*) mRNAs transcribed in leaves moves through the phloem to the shoot apex, where the *FT* protein is produced and interacts with another transcription factor (Abe et al., 2005; Blázquez, 2005; Wigge et al., 2005).

Therefore, the long-distance RNA translocation system appears to regulate the whole plant growth in response to specific environmental conditions. Kim et al. (2001) reported that a homeobox fusion transcript (Chen et al., 1997) in Mouse ear (*Me*) tomato (*Lycopersicon esculentum* Mill.) can alter the morphology of wild-type tomato grafted on *Me*. We are intrigued about the consequences of this phenomenon in horticultural crops, because most fruit trees and many vegetables are grafted (Lee, 1994). So we grafted potato (*Solanum tuberosum* L.) scions onto *Me* tomato rootstock to test whether the RNA molecule can move from *Me* tomato and whether it alters the potato leaf morphology. We present evidence for the existence and function of this supracellular information signal between scion and rootstock.

Materials and Methods

Plant materials. Seed of *Me* tomato (LA324) was obtained from C.M. Rick (University of California, Davis). Potato (cv. Kitaakari) and wild-type tomato (cv. Momotaro) were purchased from the market at Hirosaki in Japan. They were grown in an insect- and pathogen-free greenhouse at Hirosaki University.

Grafting experiments. Tomato and potato plants ≈8 weeks old were used for grafting. Cleft grafting was carried out as follows: rootstocks from *Me* tomato were prepared by cutting off the shoot at ≈7 cm (below the cotyledons) leaving a stem with no leaves or lateral buds. The diameter of the cut surface was ≈10 mm. A vertical cut, 1 to 2 cm long, was then made in the center of the stem. For

the scions, apical shoots longer than 2 cm were prepared from potato plants, the apex was cut off, all leaves were removed at the base of the petiole to leave lateral buds, and the base of the scion was trimmed to a wedge. The wedge was inserted into the slit, and the scion/stock junction was secured with parafilm. During the first week after grafting, plants were covered with a plastic bag to avoid dehydration. Grafted plants were grown for another 3 weeks in the greenhouse at Hirosaki University before samples were taken for analysis.

Reverse transcription–polymerase chain reaction and DNA gel blot analysis. Total RNA from the sample tissues was extracted with guanidium thiocyanate followed by ultracentrifugation on a CsCl gradient (Wakasa et al., 2006). The RNA was amplified with a BD SMART mRNA Amplification Kit (Clontech PR37036, Mountain View, Calif.). For reverse transcription–polymerase chain reaction (RT-PCR) of *PFP-T6* fusion RNA, primers described by Kim et al. (2001) were used. First-strand cDNA was synthesized with oligo-dT primer. PCR amplification with primers PFP2 and LeT6–2 was performed under the following standard conditions: 94 °C for 3 min followed by 40 cycles of 94 °C for 1 min, 52 °C for 2 min, and 72 °C for 3 min. To identify the PCR products, aliquots of the PCR products were electrophoresed on agarose gel (2.0%), and then the separated bands were blotted on a Hybond N-Plus nylon membrane (Amersham Pharmacia Biotech, Arlington Heights, Ill.). The DNA fragment amplified from *Me* tomato genomic DNA by using LeT6–1 and LeT6–2 primers (Kim et al., 2001) was used as the probe. The fragment was labeled with ³²P-dCTP using a Prime-It II (Stratagene, La Jolla, Calif.) random primer labeling kit.

Isolation and RNA extraction from phloem sap. Phloem sap was collected as follows. Stems of 4-week-old *Me* tomato were cut with a razor blade at ≈7 cm above the ground. The first sap exudate was collected in a sterile micropipette and discarded. Subsequent exudates were collected in microtips and immediately frozen at –80 °C. The RNA fraction was isolated using TRI Reagent LS (Molecular Research Center, Cincinnati). Phloem sap (250 μL) and TRI Reagent LS (750 μL) were mixed and left for 5 min at room temperature, then chloroform (200 μL) was added, and the mixture was shaken for 15 s. The mixture was left at room temperature for 15 min and then centrifuged at 12 000 rpm for 15 min. The aqueous phase was recovered, 2-propanol (500 μL) was added, and then the mixture was left for 10 min at room temperature. The RNA was then precipitated and resuspended in RNase-free water.

Results and Discussion

Thirteen of the 20 grafted potato scions on the *Me* tomato rootstock produced leaves (Table 1, Fig. 1). All the leaves had rounded lobes, which closely resembled those of *Me*

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Table 1. Phenotypic changes seen in potato scions after grafting.

Tomato stock	Total number of scions	Number of scions with new leaf growth	Number of scions with leaf morphology resembling	
			<i>Me</i> -type	Potato type
<i>Me</i>	20	13	13	0
Wild-type	2	2	0	2

Me = Mouse ear.

Fig. 1. Leaf morphology. (A) Leaves that developed from lateral buds of potato scion grafted on *Me* tomato. Arrow indicates the scion/stock junction. (B) Comparison of leaf morphology. Top row, *Me* tomato; middle row, potato scion on *Me* stock; last row, potato. Leaves are of similar ages. Scale bars = 10 mm. *Me* = Mouse ear.

tomato (Fig. 1B). The potato leaves also showed reduced trichome development, which is another character of *Me* (Chen et al., 1997; Kim et al., 2001). Because potato scions grafted onto wild-type tomato stock showed no changes (Table 1), the *Me* root system on its own therefore can alter the scion leaves.

RT-PCR and DNA gel blot analysis revealed the presence of the responsible chimeric transcript (Chen et al., 1997; Kim et al., 2001; Parnis et al., 1997)—pyrophosphate-dependent phosphofructokinase (PFP)-*LeT6* (KNOTTED-1-like homeobox)—in the potato scion as well as in the wild-type tomato scion (Fig. 2). Therefore, the *Me*-like potato leaves appeared to have been induced by the transcript transported through the graft junction from the *Me* stock. Kim et al. (2001) reported that PFP mRNA moved singularly through the phloem. We also verified the presence of PFP mRNA in the sap from the *Me* stock by RT-PCR (data not shown).

The results indicate that the rootstock without leaves supplied enough of the chimeric transcript to alter the scion leaves. Although the PFP enzyme functions in young leaves and actively growing areas of leaves, PFP activity is ubiquitously distributed in plants (Nielsen and Stitt, 2001). The detection of PFP transcript in the sap from the cut surface also demonstrated the action of PFP promoter activity in the lower parts of the plant, mainly the root system.

Since the time of ancient Greece, grafting has been used to propagate superior clones of fruit trees. This technique has now been

Fig. 2. Detection of fusion gene transcript. Reverse transcription–polymerase chain reaction experiments amplified the *PFP-LeT6* fusion gene. The products were electrophoresed and hybridized to an *LeT6* cDNA probe. Lane 1, *Me* tomato; 2, wild-type tomato on *Me*; 3, potato on *Me*; 4, tomato; 5, potato. *Me* = Mouse ear.

expanded to vegetable crop production to reduce infections incited by pathogens, to increase resistance to drought, and to enhance nutrient uptake. There are reports highlighting changes in characters of the scion by grafting (Ohata, 1991; Taller et al., 1999). Some of these changes might be responsible for the transport of the gene transcript (Liu, 2006). If rootstocks can be made by gene engineering to produce a desirable RNA that can alter the scion phenotype, this would create a novel system for cultivar improvement in vegetable and tree crops.

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