

## Differences in xylem development between Dutch and Japanese tomato (*Solanum lycopersicum*) correlate with cytokinin levels in hypocotyls

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- **Background and Aims** Dutch tomato cultivars tend to have a greater yield than Japanese cultivars even if they are grown under the same conditions. Factors contributing to the increased yield of the Dutch cultivars were a greater light use efficiency and greater leaf photosynthetic rate. On the other hand, the relationship between tomato yields and anatomical traits is still unclear. The aim of this study is to identify the anatomical traits related to the difference in yield between Dutch and Japanese cultivars.
- **Methods** Anatomical properties were compared during different growth stages of Dutch and Japanese tomatoes. Hormone profiles and related gene expression in hypocotyls of Dutch and Japanese cultivars were compared in the hypocotyls of 3- and 4-week-old plants.
- **Key results** Dutch cultivars have a more developed secondary xylem than Japanese cultivars, which would allow for greater transport of water, mineral nutrients and phytohormones to the shoots. The areas and ratios of the xylem in the hypocotyls of 3- to 6-week-old plants were larger in the Dutch cultivars. In reciprocal grafts of the Japanese and Dutch cultivars, xylem development at the scion and rootstock depended on the scion cultivar, suggesting that some factors in the scion are responsible for the difference in xylem development. The cytokinin content, especially the level of *N*<sup>6</sup>-( $\Delta^2$ -isopentenyl) adenine (iP)-type cytokinin, was higher in the Dutch cultivars. This result was supported by the greater expression of *Sl-IPT3* (a cytokinin biosynthesis gene) and *Sl-RR16/17* (a cytokinin-responsive gene) in the Dutch cultivars.
- **Conclusions** These results suggest that iP-type cytokinins, which are locally synthesized in the hypocotyl, contribute to xylem development. The greater xylem development in Dutch cultivars might contribute to the high yield of the tomato.

**Key words:** Adenosine phosphate-isopentenyltransferase, cytokinin, tomato, vascular tissues, xylem development, yield.

### INTRODUCTION

Tomatoes have been bred for many different traits, such as yield, fruit size and sugar content. With respect to yield, Higashide and Heuvelink (2009) found that the fresh fruit weights of Dutch cultivars were much greater than those of a Japanese cultivar. Factors contributing to the increased yield of the Dutch cultivars were a greater light use efficiency and greater leaf photosynthetic rate (Higashide and Heuvelink, 2009). The numbers of flowers and fruit, and the fruit set ratio in Japanese tomato were also lower than the values reported in the Dutch cultivars, though there was no significant difference

in truss number (Higashide and Heuvelink, 2009). However, it is unclear whether there is a difference in the internal structures of the Japanese and Dutch tomatoes and if a difference in internal structures can explain the difference in tomato yields.

Vascular tissues consist of phloem, xylem and cambium. Phloem transports photosynthetic products and plant growth regulators (phytohormones) mainly from the shoots to the roots, while xylem transports water, mineral nutrients and phytohormones from the roots to the shoots. Xylem also provides mechanical support and stores water and mineral nutrients (Myburg and Sederoff, 2001; Matsumoto-Kitano *et al.*, 2008; White, 2012). Phloem and xylem differentiate from cambium

cells in the centrifugal and centripetal directions, respectively. On the xylem side of the cambium, the cells first undergo several stages of differentiation that involve cell division, expansion, maturation, lignification, secondary cell wall thickening and programmed cell death (Chaffey, 1999). The rate of cell production in the cambium is the primary determinant of stem and root thickening, which is important for biomass production (Larson, 1994; Jang et al., 2015).

Cytokinins have diverse functions in plant growth and development (Hwang et al., 2012). Generally, cytokinin signalling is considered to be an important regulator of cambium development (Matsumoto-Kitano et al., 2008; Jang et al., 2015). Cytokinins have one of several types of isoprene-derived side chain, such as *N*<sup>6</sup>-( $\Delta^2$ -isopentenyl) adenine (iP), *trans*-zeatin (*tZ*), *cis*-zeatin (*cZ*) and dihydrozeatin (DZ) (Sakakibara, 2006; Kiba et al., 2013). The first step in their biosynthesis is catalysed by adenosine phosphate-isopentenyltransferase (IPT). In *Arabidopsis thaliana*, knocking out four genes encoding cytokinin biosynthetic IPT by T-DNA insertions adversely affected cambium formation and reduced xylem differentiation in the root and shoot. The differentiation was restored by treating the mutant with exogenous cytokinin (Matsumoto-Kitano et al., 2008). Also in *arabidopsis*, cytokinin receptor loss-of-function mutants *ahk2* and *ahk3* (*ARABIOPSIS HISTIDINE KINASE 2* and *3*) and plants with reduced levels of endogenous cytokinins show defects in procambium proliferation and an absence of secondary growth (Hejatko et al., 2009). In poplar (*Populus trichocarpa*), overexpressing the cytokinin catabolic gene *CYTOKININ OXIDASE 2* decreased the cytokinin concentration and reduced secondary growth (Nieminen et al., 2008). These findings strongly indicate that cytokinins are important regulators of vascular development.

To understand the reasons for the differences in yield between Dutch and Japanese tomatoes, we compared anatomical traits of two Dutch tomato cultivars and two Japanese tomato cultivars, all of which were different from those examined by Higashide and Heuvelink (2009). We found that the areas and ratios of the secondary xylem in the hypocotyls and the stems of young (e.g. 5-, 6- or 10-week-old) plants were larger in the Dutch cultivars. We then further compared the cultivars with respect to (1) the development of secondary vascular tissues in the hypocotyls, epicotyls and stems; (2) cytokinin levels in the hypocotyls; and (3) the expression of cytokinin-related genes. Our results suggest that cytokinins are important factors in the difference in xylem development between Dutch and Japanese cultivars.

## MATERIALS AND METHODS

### Plant materials and growth conditions

The tomato (*Solanum lycopersicum* L.) seeds used in this study were from F<sub>1</sub> hybrid lines of the Dutch cultivars ‘Managua’ (MNG; Rijk Zwaan, de Lier, The Netherlands) and ‘Endeavor’ (ENV; Rijk Zwaan), and the Japanese cultivars ‘CF Momotaro York’ (MY; Takii Seeds, Kyoto, Japan) and ‘Reiyo’ (RYO; Sakata seed, Yokohama, Japan). The seeds were soaked in distilled water at 4 °C under dark conditions for 24 h. Water-swollen seeds were germinated in plugs filled with vermiculite. The plugs were covered with a plastic film and black cloth. The seeds were grown at 25 °C under a 14 h light/10 h dark

photoperiod. The plastic film and black cloth were removed when the seeds germinated. A commercial nutrient solution (Otsuka-A; Otsuka AgriTechno, Tokyo, Japan) was diluted 20 times and was added to each plug every day after germination. Two-week-old seedlings were transplanted into rockwool squares (8 × 8 cm; Grodan BV, Roermond, The Netherlands).

### Sectioning

Samples were cut 0.5 cm from the base of the hypocotyl and fixed with FAA fixative solution [5 % (v/v) formalin, 5 % acetic acid and 63 % ethanol]. Transverse sections (120 µm) were cut with a Plant Microtome MTH-1 (Nippon Medical & Chemical Instruments Co., Ltd, Osaka, Japan) according to the manufacturer’s instructions, and stained for xylem with 0.05 % toluidine blue O solution. Images were taken with a light microscope (DM5000 B; Leica Microsystems, Wetzlar, Germany) with a CCD camera (DFC310 FX; Leica Microsystems). The areas of the xylem and hypocotyl (or stem) were measured using ImageJ software (Version 1.49p). The ratio of xylem area was calculated by dividing the xylem area by the whole section area. To count the number of phloem vessels, sections from 4-week-old plants were stained with 0.5 % aniline blue solution, a fluorescent dye that binds to callose, which is present in the sieve plates of phloem vessels. Stained sections were viewed under a fluorescence microscope using UV light (DM5000; UV filter: excitation filter, Ex 360/40; dichroic mirror, DM-400; suppression filter, BP 470/40) with a CCD camera (DFC310 FX; Leica Microsystems).

### Grafting

Using hypocotyls of 17-day-old plants, the rootstock of one cultivar was grafted to the scion of another cultivar. Grafted plants were incubated under high humidity for 7 d and then cultured for another 11 d under normal conditions (a total of 18 d after grafting). An approx. 5 mm section of the stem immediately above the grafting point was used for making sections. The rest of the scion was immediately weighed with a balance, transferred to an oven for 7 d and weighed again to obtain the dry weight.

### Hormone measurement

Hypocotyls were cut 1.5 cm from the base, weighed, frozen in liquid nitrogen and transferred to a freeze drier (Freezone 1; Labconco Corp., Kansas City, MO, USA) for 48 h. Cytokinins, auxin [indole-3-acetic acid (IAA)] and abscisic acid (ABA) were extracted and identified as described previously (Kojima et al., 2009; Shinozaki et al., 2015).

### Exogenous cytokinin application

Tomato seedlings were treated with cytokinins by spraying and immersion. After 2 weeks of growth, MY plants were sprayed with 10 µM 6-benzylaminopurine (6-BA) (Wako Pure Chemical Industries, Ltd, Japan) or 0.1 % dimethylsulfoxide (DMSO) solution (mock) every 2 d until the 3 week stage, and

then used for RNA extraction. Three-week-old seedlings were immersed in a solution of  $3 \mu\text{g mL}^{-1}$  kinetin,  $100 \mu\text{M}$  6-BA or 0.1 % DMSO solution (mock) for 1 h and allowed to stand for another 4 h. The hypocotyl was cut at 1.5 cm from the base and the lower part was used for RNA extraction.

#### RNA extraction and quantitative reverse transcription-PCR (qRT-PCR)

Total RNA was extracted from frozen tissues of hypocotyls at different growing stages using an RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocols. For qRT-PCR, 10 ng of the RNA was used as a template. Transcript levels were measured using a One Step SYBR Prime Script RT-PCR Kit II (Takara Bio Inc., Otsu, Japan) and a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocols. A PCR fragment of each gene was purified and quantified, and then was used to draw standard curves for absolute quantification. Total RNA from four biological replicates was pooled for use as the qRT-PCR template. Primer sequences are shown in Supplementary data Table S1.

## RESULTS

### Difference in xylem development between Dutch and Japanese tomato cultivars

We compared cross-sections of hypocotyls and stems of 10-week-old plants of the Dutch cultivar MNG and the Japanese cultivar MY. The secondary xylem areas and their ratios in the hypocotyl were larger in MNG than in MY (Fig. 1). To confirm this observation, we compared xylem development in the hypocotyls of 3-, 4-, 5- and 6-week-old plants of MNG and another Dutch cultivar ENV, and of MY and another Japanese cultivar RYO. Xylem areas and their ratios were not different between the Dutch and Japanese cultivars 3 weeks after germination (Fig. 2). However, the area and ratio of xylem in MNG were larger than those of other cultivars at 4 weeks after germination. Xylem area was significantly larger in the Dutch cultivars (MNG and ENV) 5 weeks after germination. The xylem ratio was significantly larger in the Dutch cultivars (MNG and ENV) than in Japanese cultivars (MY and RYO) 5 and 6 weeks after germination (Fig. 2). In addition, the number of phloem vessels and the amount of shoot growth were not different between the Dutch and Japanese cultivars (Supplementary data Fig. S1). These findings suggest that secondary xylem development in the hypocotyls is slower and less extensive in the Japanese cultivars.

In 6-week-old plants, the Dutch cultivars also had larger xylem areas (Fig. 3A) and xylem ratios (Fig. 3B) at the epicotyl and first and second internodes than the Japanese cultivars (Fig. 3B). In all cultivars, the xylem ratio decreased with increasing stem position (Fig. 3B).

### Effect of reciprocal grafting on xylem development

In the following, grafts are denoted as S/R, where S is the scion and R is the rootstock. MNG/MNG and MY/MY were used as controls. The shoot growths were similar among the

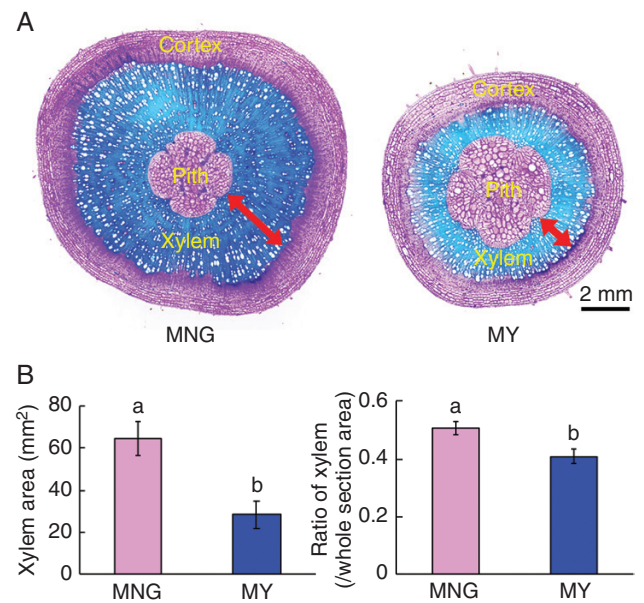


FIG. 1. Comparison of xylem development at the base of 10-week-old Dutch (MNG) and Japanese (MY) tomato hypocotyls. (A) Cross-sections. (B) Xylem areas. (C) Ratio of xylem. Xylem area is indicated by red arrows. Values are means ( $n \geq 5$ )  $\pm$  s.d. Different lower case letters indicate a significant difference among all the conditions ( $P < 0.05$ , one-way ANOVA and then LSD test for multiple comparisons).

grafted plants (Fig. 4A). Xylem areas (Fig. 4B) and xylem ratios (Fig. 4C) in the hypocotyl above the graft union were significantly larger in MNG/MY and MNG/MNG than in MY/MNG and MY/MY. In addition, xylem areas (Fig. 4D) and xylem ratios (Fig. 4E) in the hypocotyl below the graft union were significantly larger in MNG/MY and MNG/MNG than in MY/MNG and MY/MY, suggesting that development of the secondary xylem of both scion and rootstock is affected by the genotype of the scion. Three other grafting combinations between RYO and MNG, RYO and ENV, and MY and ENV also showed that the genotype of the scion affected the secondary xylem development of both scion and rootstock (Supplementary data Figs S2 and S3). Taken together, these results suggest that the shoots determine xylem development in the scion and rootstock during the early stage of tomato development.

### Hypocotyls of Dutch tomato cultivars contain greater levels of *iP*-type cytokinin

In hypocotyls of 3-week-old plants, the total concentration of DZ-type cytokinins (about  $0.1 \text{ pmol g f. wt}^{-1}$  in each cultivar) and the total concentration of cZ-type cytokinins (about  $3 \text{ pmol g f. wt}^{-1}$  in each cultivar) were much lower than those for iP and tZ cytokinins (about 50 and 15  $\text{pmol g f. wt}^{-1}$ , respectively) (Fig. 5). Similar results were obtained in hypocotyls of 4-week-old plants. The most abundant cytokinins in all four cultivars were iP-type cytokinins. In hypocotyls of 3-week-old plants, iP-type cytokinins were significantly more abundant in the Dutch cultivars than in the Japanese cultivars (Fig. 5A), but this was not the case in 4-week-old plants (Fig. 5B). iP7G, which accounted for >86 % of the iP-type cytokinins, was significantly more abundant in the Dutch cultivars than in the



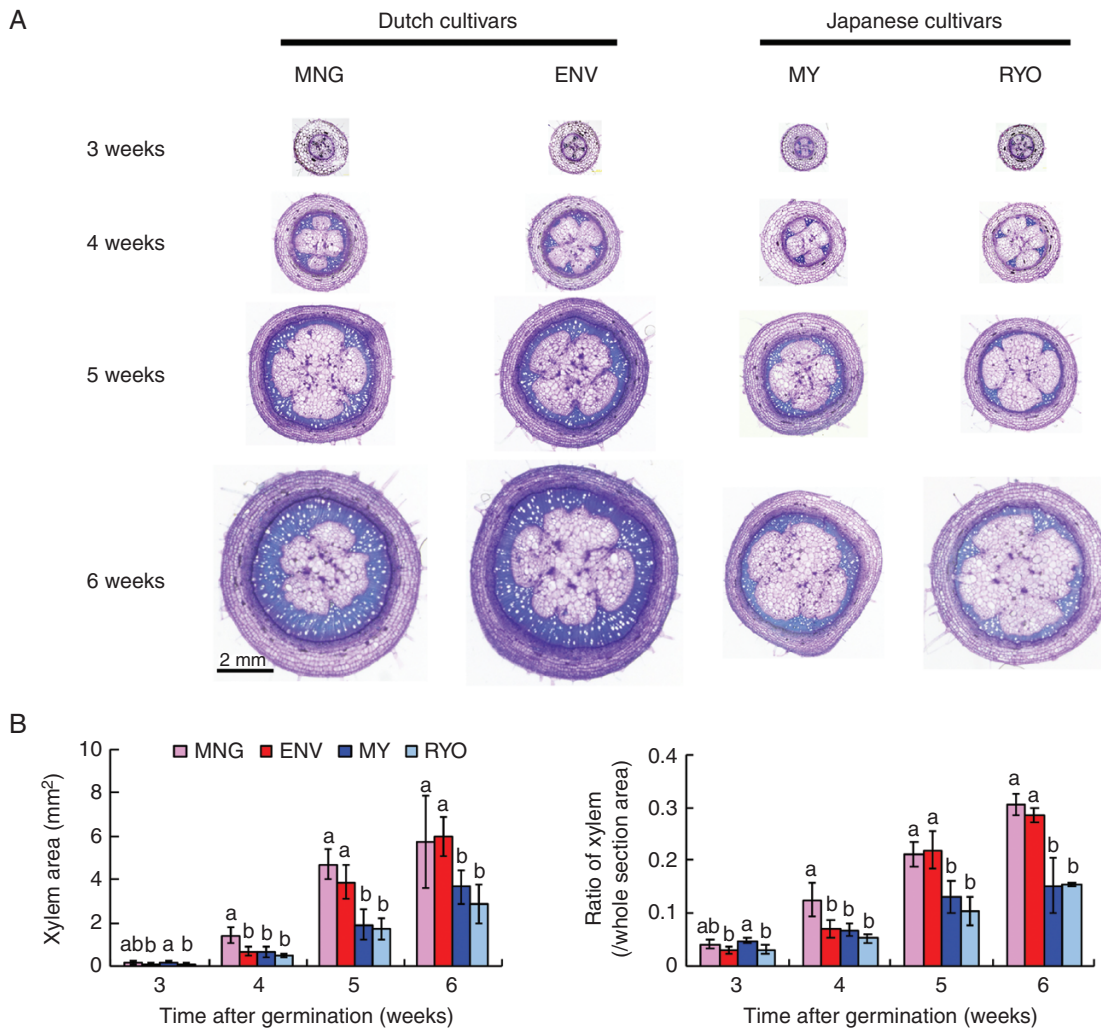


FIG. 2. Xylem formation in hypocotyl of Dutch and Japanese tomato seedlings during 6 weeks. (A) Cross-sections at the base of hypocotyls of Managua (MNG), Endeavor (ENV), CF Momotaro York (MY) and Reiyō (RYO) from the 3- to 6-week-old stage. Scale bar = 2 mm. (B) Xylem area and ratio at the base hypocotyls of 3- to 6-week-old plants. Values are means ( $n \geq 5$ )  $\pm$  s.d. Dutch tomatoes are MNG and ENV; Japanese tomatoes are MY and RYO. Different lower case letters indicate a significant difference among all the conditions ( $P < 0.05$ , one-way ANOVA and then LSD test for multiple comparisons).

Japanese cultivars (Fig. 5A, B; Supplementary data Table S2). However, the concentrations of the other types of cytokinins (*tZ*, *DZ* and *cZ*) were similar between Dutch and Japanese cultivars (Fig. 5C–H; Supplementary data Tables S3–S5).

The concentrations of salicylic acid (SA) and ABA were not significantly different between the Dutch and Japanese cultivars (Supplementary data Fig. S4). IAA concentrations were significantly greater in ENV than in RYO at 4 weeks, but not in other stages or cultivars (Supplementary data Fig. S4). The gibberellins  $GA_{44}$  and  $GA_{53}$  tended to be lower in the Japanese cultivars, while other GAs were not significantly different among the four cultivars (Supplementary data Fig. S4).

#### Expression of cytokinin biosynthesis genes and cytokinin-responsive genes

The first step in isoprenoid (iP) cytokinin biosynthesis is *N*-prenylation of adenosine 5-phosphates (AMP, ADP or ATP) at the *N*<sup>6</sup>-terminus with dimethylallyl diphosphate, which is

catalysed by IPT (Sakakibara, 2006). Because the amounts of total iP and iP7G were greater in the Dutch cultivars (Supplementary data Table S2), we hypothesized that expression of IPT genes would also be greater in the Dutch cultivars. Six tomato *IPT* genes (*Sl-IPT1*–*Sl-IPT6*) were previously identified (Matsuo et al., 2012). Among the six *Sl-IPT* genes, *Sl-IPT3* was expressed the most strongly. *Sl-IPT3* was expressed significantly more strongly in the Dutch cultivars than in the Japanese cultivars both 3 and 4 weeks after germination, while *Sl-IPT1* was expressed significantly more strongly in the Dutch cultivars only 3 weeks after germination (Fig. 6; Supplementary data Fig. S5).

Tomato has five *Responsive Regulator* genes (*Sl-RR3/4*, *Sl-RR7/15*, *Sl-RR8/9a*, *Sl-RR8/9b* and *Sl-RR16/17*) that are markers of cytokinin signalling (Shani et al., 2010; Matsuo et al., 2012). Spraying the hypocotyls with 6-BA (Supplementary data Fig. S6A) or immersing them in 6-BA or kinetin solution (Supplementary data Fig. S6B) upregulated all five *Sl-RR* genes. Among the five *Sl-RR* genes, only *Sl-RR16/17* was expressed more strongly in the Dutch cultivars than in the Japanese cultivars at 3 weeks (Fig. 7).

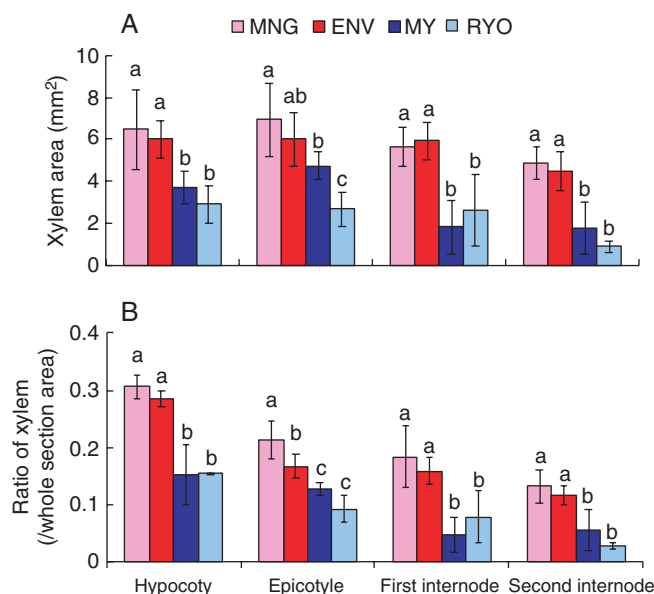


FIG. 3. Xylem development in cross-sections at different positions along the stem of 6-week-old Dutch and Japanese tomato. (A) Xylem area. (B) Xylem ratio. Values are means ( $n \geq 5$ )  $\pm$  s.d. Different lower case letters indicate a significant difference among all the conditions ( $P < 0.05$ , one-way ANOVA and then LSD test for multiple comparisons).

### DISCUSSION

The xylem conducts water and mineral nutrients from the roots to the shoots, and its development is related to the biomass production of plants (Larson, 1994). In tomato, the flow of sap in the xylem was reported to be faster in Dutch cultivars than in Japanese cultivars (Nakano et al., 2013). At first, we hypothesized that xylem development in the Dutch cultivars was faster (Figs 1–3) because of their greater water or nutrient uptake. However, the reciprocal grafting did not affect either shoot growth or xylem development in the scion (Fig. 4A–C; Supplementary data Fig. S2). On the other hand, xylem development in the rootstock was affected by which cultivar was used for the scion (Fig. 4D, E; Supplementary data Fig. S3), suggesting that factors related to the development of secondary xylem in Dutch cultivars were derived from the shoot.

Hormones such as auxin, GAs and cytokinins regulate vascular development (Sachs, 1981; Savidge, 1988; Eriksson et al., 2000; Mähönen et al., 2000). Cytokinins have been shown to be involved in the maintenance of shoot and cambial meristem activities, which determines the shoot or root size, in several plants, including tobacco (*Nicotiana tabacum* L., Werner et al., 2001, 2003), populus (Matsumoto-Kitano et al., 2008), arabidopsis (Nieminen et al., 2008) and radish (*Raphanus sativus* L., Jang et al., 2015). Our findings that the Dutch cultivars have higher concentrations of iP-type cytokinins (Fig. 5) and higher expression levels of *SI-IPT1* and *SI-IPT3* suggest that locally synthesized cytokinins contribute to the difference in the development of secondary xylem between Dutch and Japanese cultivars. So far, four types of active cytokinins have been identified in plants, but two of them (DZ and cZ) play minor roles in the growth of most plants (Matsumoto-Kitano et al., 2008). iP and tZ as well as their sugar conjugates are biologically important cytokinins. tZ-type cytokinins predominate

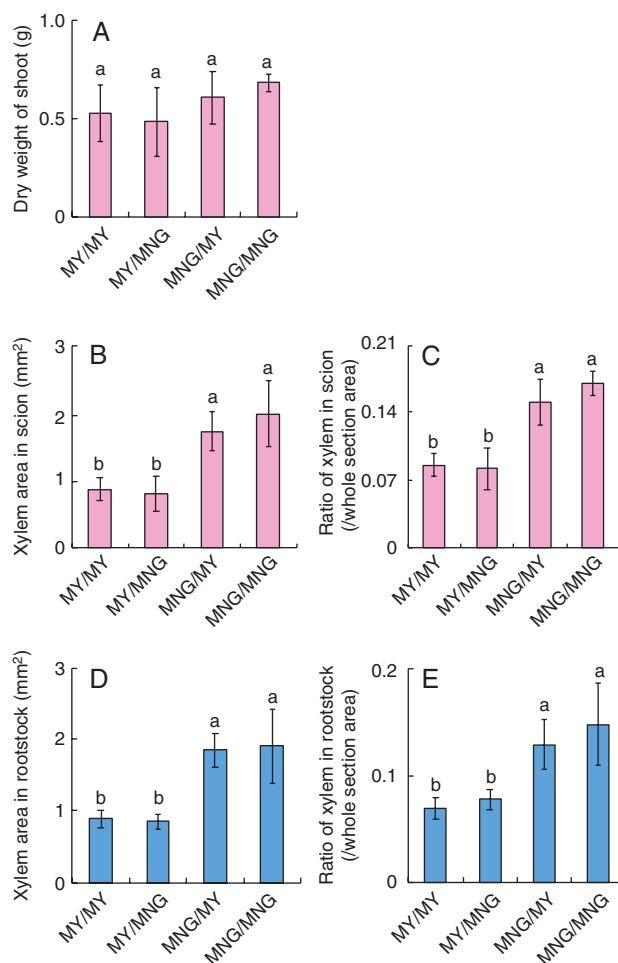


FIG. 4. Effects of grafting between MNG and MY on the xylem development and shoot growth. The cultivars listed before and after the slash correspond to the scion and stock, respectively. Dry weight of shoot (A), xylem areas (B) and ratios of xylem (C) in the scion of grafted plants between MNG and MY. Xylem areas (D) and ratios of xylem (E) in rootstock of grafted plants between MNG and MY. Values are means ( $n \geq 5$ )  $\pm$  s.d. Different lower case letters indicate a significant difference among all the conditions ( $P < 0.05$ , one-way ANOVA and then LSD test for multiple comparisons).

in the xylem sap, whereas iP-type cytokinins predominate in the leaf exudates (Salama and Wareing, 1979; Kakimoto, 2003; Hirose et al., 2008). Recent reciprocal grafting of roots and shoots of cytokinin-deficient mutants revealed that tZ-type cytokinins are translocated from roots to shoots via the xylem to regulate shoot growth. iP-type cytokinins are major components of phloem cytokinins (Hirose et al., 2008). The amounts of iP and iP7G in the Dutch cultivars were greater than those in the Japanese cultivars 3 weeks after germination (Fig. 5A; Supplementary data Table S2). iP7G, which is an inactive form of cytokinin, had the highest concentration (Supplementary data Table S2). However, cytokinin *N*-glucosyltransferase can convert iP to the *N*-glucoside of iP7G. iP7G has been suggested to represent an inactive, but stable storage form of cytokinins (Frébert et al., 2011). Differences in the iP and iP7G levels between the Dutch and Japanese cultivars were consistent with their rates of xylem development. The greater level of iP7G and active iP in the Dutch cultivars might lead to an earlier and

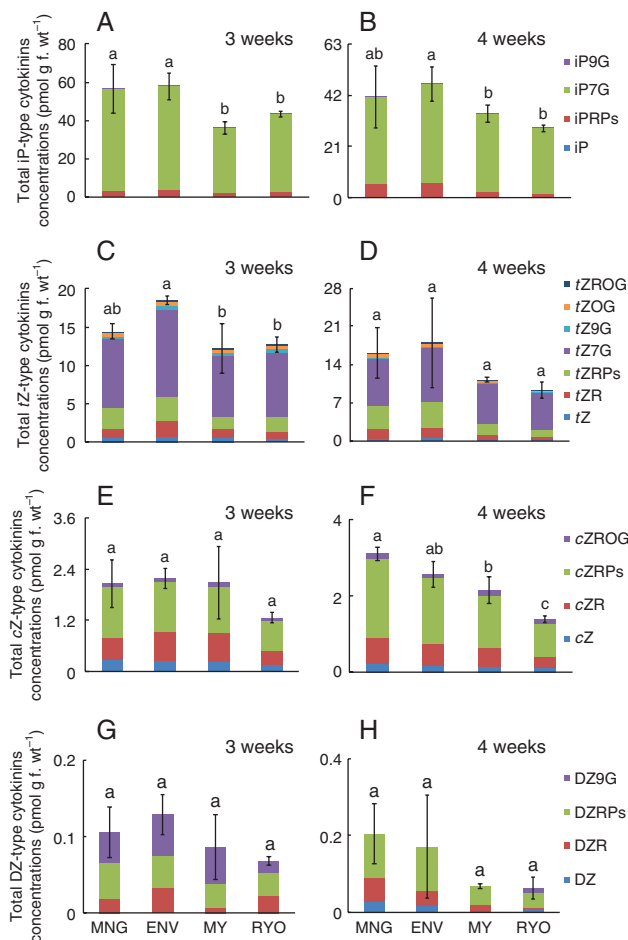


Fig. 5. Cytokinin levels in hypocotyls of 3- and 4-week-old Dutch cultivars MNG and ENV and Japanese cultivars MY and RYO. Dutch and Japanese tomato cultivars were grown for 3–4 weeks, and hypocotyls were harvested for plant hormone analysis. iP-type (A, B), tZ-type (C, D), cZ-type (E, F) and DZ-type cytokinin (G, H) concentrations in 3- and 4-week-old tomatoes, respectively. Values are means ( $n \geq 3$ )  $\pm$  s.d. Different lower case letters indicate a significant difference among all the conditions ( $P < 0.05$ , one-way ANOVA and then LSD test for multiple comparisons).

more rapid development of xylem. The accumulation of iP-type cytokinins (which predominate in the leaves) rather than tZ-type cytokinins (which predominate in the roots) implies that shoot-derived cytokinins rather than root-derived cytokinins are involved in the difference in xylem development between the Dutch and Japanese cultivars. This was supported by the grafting experiments, in which the cultivar used for the rootstock did not affect the xylem size of the scion (Fig. 4B, C; Supplementary data Fig. S2), but the cultivar used for the scion affected the xylem size of the rootstock (Fig. 4D, E; Supplementary data Fig. S3). This indicates that the genotype of the scion has a predominant role in xylem development in the shoot and rootstock. So, iP-type cytokinins might also be the shoot-to-root signal that determines xylem formation in the root. Additionally, the expression of *SI-IPT3* was greater in the hypocotyls of the Dutch cultivars (Fig. 6). The greater expression levels of the cytokinin biosynthesis gene *SI-IPT3* and the cytokinin-responsive gene *SI-RR16/17* (Fig. 7) support the differences in the iP and iP7G levels. These results suggest that

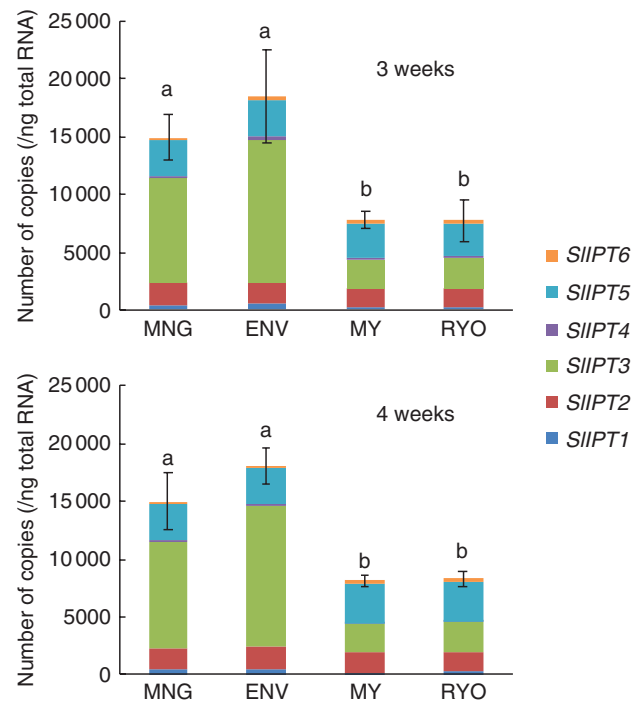


Fig. 6. Expression levels of total *SI-IPT* genes in hypocotyls of 3- and 4-week-old plants of Dutch and Japanese tomato. Values are means ( $n = 4$ )  $\pm$  s.d. Different lower case letters indicate a significant difference among all the conditions ( $P < 0.05$ , one-way ANOVA and then LSD test for multiple comparisons).

local biosynthesis of an iP-type cytokinin contributed to the difference of xylem development between the Dutch and Japanese cultivars.

We did not detect any significant difference in other plant hormones (Supplementary data Fig. S4). Auxin was reported to be involved in the initiation and promotion of vascular development. In the woody plants *Pinus sylvestris* L. (Uggla et al., 1996) and hybrid aspen (*Populus tremula*  $\times$  *Populus tremuloides*, Tuominen et al., 1997), the auxin level peaked in the cambial zone, and steeply decreased toward the mature xylem and phloem. In the Ptt *iaa3m* mutant (IAA3m is a key component in the auxin signalling pathway) of hybrid aspen, the radial and axial dimensions of xylem formation were reduced (Nilsson et al., 2008). However, we did not find a close relationship between IAA concentration and xylem development (Supplementary data Fig. S4). (One exception was the IAA concentration in hypocotyls of 4-week-old plants, which was significantly greater in ENV than in RYO.) GAs have also been reported to be involved in xylem development. Application of GA<sub>3</sub> enhanced xylem development in the shoot of linseed and carrot (McKenzie and Deyholos, 2011; Wang et al., 2015). Overexpression of *GIBBERELLIN 20-OXIDASE1* (*GA20ox*), a GA biosynthetic gene, in populus resulted in increased growth, biomass production and xylem fibre length (Eriksson et al., 2000). *GA20ox* mRNA and bioactive GAs have also been demonstrated to accumulate in the expansion zone of developing xylem (Israelsson et al., 2005). Moreover, decreasing the levels of GA precursor and reduced bioactive GA resulted in reduced secondary growth (Mauriat et al., 2011). The levels

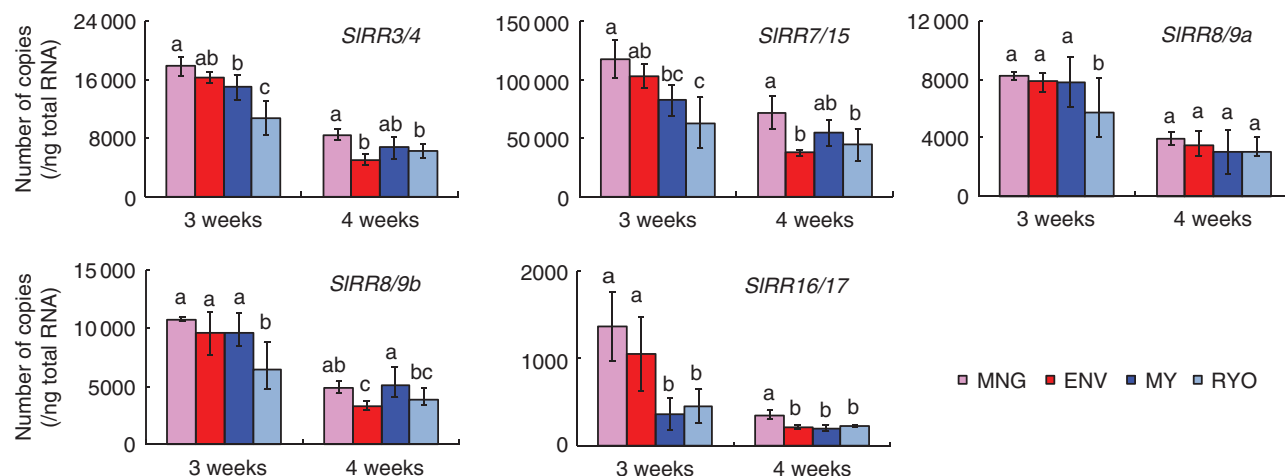


Fig. 7. Expression levels of cytokinin response genes (*SI-RR* genes) in hypocotyls of 3- and 4-week-old plants of Dutch and Japanese tomato. Values are means ( $n = 4$ )  $\pm$  s.d. Different lower case letters indicate a significant difference among all the conditions ( $P < 0.05$ , one-way ANOVA and then LSD test for multiple comparisons).

of GA44 and GA53 tended to be lower in the Japanese cultivars while the levels of the other GAs were not significantly different among the four cultivars (Supplementary data Fig S4), suggesting that other plant hormones did not contribute to the difference of xylem development between the Dutch and Japanese cultivars.

### Conclusions

Our results provide evidence that the xylem in hypocotyls and stems of the Dutch cultivars were more developed than those of the Japanese cultivars at the early and late stages of tomato growth. We attributed this difference to the level of iP-type cytokinins, which are locally synthesized in the phloem by *IPT*. Although we found that xylem development was greater in the Dutch cultivars, further experiments are needed to clarify whether it also contributes to tomato growth and fruit production.

### SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following. Table S1: the primers used for quantitative RT-PCR. Table S2: iP-type cytokinin concentrations in the hypocotyl of MNG, ENV, MY and RYO at the 3 and 4 week stages. Table S3: tZ-type cytokinin concentrations in the hypocotyl of MNG, ENV, MY and RYO at the 3 and 4 week stages. Table S4: cZ-type cytokinin concentrations in the hypocotyl of MNG, ENV, MY and RYO at the 3 and 4 week stages. Table S5: DZ-type cytokinin concentrations in the hypocotyl of MNG, ENV, MY and RYO at the 3 and 4 week stages. Figure S1: comparison of shoot growth and the number of phloem vessels in hypocotyl sections in Japanese and Dutch tomatoes. Figure S2: the effects of grafting on shoot growth and xylem formation of the scion in other combinations of tomato cultivars. Figure S3: the effects of grafting on xylem area and xylem ratio in rootstock under grafting. Figure S4: IAA, GA, ABA and SA levels in

hypocotyls of 3- and 4-week-old plants of MNG, ENV, MY and RYO. Figure S5: the expression levels of six *IPT* genes in 3- and 4-week-old Dutch and Japanese tomato hypocotyls. Figure S6: the effects of cytokinin treatment on expression of *SI-RR* genes in MY.

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