

# Grafting tomato (*Solanum lycopersicum*) onto the rootstock of a high-altitude accession of *Solanum habrochaites* improves suboptimal-temperature tolerance

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## Abstract

Grafting is regarded as a promising tool to broaden the temperature optimum of elite tomato cultivars. However, suitable low-temperature tolerant tomato rootstocks are not yet available and its breeding is hampered by a lack of variation in low-temperature tolerance within the cultivated tomato. In this study, therefore, the impact of grafting tomato (*Solanum lycopersicum* Mill. cv. Moneymaker, *Sl*) onto the rootstock of a cold-tolerant high-altitude accession of a related wild species (*Solanum habrochaites* LA 1777 Humb. & Bonpl., *Sh*) was examined at different combinations of optimal (25 °C) and/or suboptimal (15 °C) air/root-zone temperatures (RZT), i.e. 25/25, 25/15, 15/25 and 15/15 °C. Self-grafted tomato plants were used as controls. Both scion/rootstock combinations, *Sl/Sl* and *Sl/Sh*, were grown hydroponically and compared for biomass production and partitioning, plant morphology, carbohydrate partitioning and leaf C and N status. Grafting tomato onto *Sh* increased the relative growth rate of shoots with 26 and 11% at 25/15 and 15/15 °C, respectively. This increase could be attributed to stimulation of the leaf expansion rate. Graft combinations with *Sh* rootstocks were characterized by higher root mass ratios, particularly at 15 °C RZT. Suboptimal RZT strongly reduced the relative growth rate of *Sl* roots but not of *Sh*. This was correlated to differences in inhibition of root elongation. In contrast to tomato grafted onto *Sh*, leaf total C and total N concentrations increased in self-grafted tomato plants in response to 15 °C RZT. The increase in leaf total C concentration of *Sl/Sl* graft combinations at 15 °C RZT could be ascribed largely to starch accumulation. This study illustrates that growth of vegetative tomato plants at suboptimal temperature is for a significant part inhibited by its poor root development. Grafting tomato onto a low-temperature rootstock provides an alternative tool to reduce, in part, the grow-limiting effects of suboptimal RZ temperature on the shoot. To improve the low-temperature tolerance of existing commercial tomato rootstocks, *S. habrochaites* LA 1777 appeared to be a valuable germplasm pool.

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**Keywords:** Grafting; Rootstock; Root-zone heating; *Solanum habrochaites*; *Solanum lycopersicum*; Suboptimal temperature; Tomato

## 1. Introduction

Throughout its ontogeny, growth and development of the cultivated tomato (*Solanum lycopersicum*, synonym *Lycopersicon esculentum*; Spooner et al., 2005) are severely inhibited by suboptimal (Venema et al., 1999a; van der Ploeg et al., 2007)

and chilling temperatures (Venema et al., 2005). Because of its low-temperature sensitivity, which includes numerous physiological disorders under suboptimal temperatures, tomatoes are either cultivated year-round indoors in greenhouses or as a typical summer crop outdoors in the temperate climate zone. The production of tomatoes in greenhouses requires particularly during the cold and dark winter months high-energy inputs for heating. Due to increasing energy prices and public concern about environmental problems related with CO<sub>2</sub> emissions from the combustion of fossil fuel, the horticultural sector is forced to improve its energy-efficiency. Although traditional breeding over the last 25 years has led to the generation of tomato cultivars with a twofold improved energy-efficiency (kg tomatoes produced MJ<sup>-1</sup> energy input), which was almost exclusively the result of higher production levels (van der Knijff et al., 2004),

**Abbreviations:** a.s.l., above sea level; DMC, dry matter content; LER, leaf expansion rate; LMR, leaf mass ratio; N<sub>r</sub>, reduced nitrogen; PI, platychron index; PMR, petiole mass ratio; RMR, root mass ratio; RZ(T), root-zone (temperature); RGR, relative growth rate; SLM, specific leaf mass; *Sh*, *Solanum habrochaites*; *Sl*, *Solanum lycopersicum*; SMR, stem mass ratio; SSM, specific stem mass; TNC, total non-structural carbohydrates.

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there is now also need to reduce the absolute amount of energy input (van der Ploeg and Heuvelink, 2005). A simple option is to decrease the greenhouse temperature, which requires the breeding of new cultivars that are better adapted to low temperatures. As a fast alternative for the relatively slow breeding process aimed to lower the energy demand of tomato, grafting of existing elite commercial cultivars onto selected rootstocks is regarded as a promising tool (Heuvelink and Kierkels, 2005). In the Netherlands, approximately 75% of the commercially grown greenhouse tomatoes are already grafted onto rootstocks that are selected for high vigour. The main general objective of using rootstocks is to increase scion growth and development rate, yield and fruit quality. This technique, which can also be applied to other thermophilic vegetables like pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.) and cucumber (*Cucumis sativus* L.), is now being expanded greatly in order to reduce infections caused by soil-born pathogens and nematodes (Lee, 1994), to enhance nutrient uptake (Ruiz-Sifre et al., 1997; Rivero et al., 2004), and to increase the resistance to abiotic stresses like drought (White and Castillo, 1989; Weng, 2000; Bhatt et al., 2002), salinity (Chung and Choi, 2002; Santa Cruz et al., 2002; Estañ et al., 2005), flooding (Yetisir et al., 2006), heat (Abdelhafeez et al., 1975; Rivero et al., 2003a,b) and low (soil) temperatures (den Nijs, 1980, 1984; Tachibana, 1982; Zijlstra and den Nijs, 1987; Bulder et al., 1991; Rivero et al., 2003b).

The use of rootstocks to improve fruit yield is already a common practice for successful production of Cucurbitaceae (cucumbers, squash, and melon) at the open-ground cultivation in unheated polythene greenhouses during the cold seasons in Japan and Korea, when low soil temperatures may seriously affect the performance of seedlings or may even kill them (Lee, 1994; Ahn et al., 1999; Lee et al., 2005b). Because of their resistance to low temperature, figleaf gourd (*Curcubita ficifolia* Bouché) and bur cucumber (*Sicos angulatus* L.) are used as rootstock for cucumber. Several studies demonstrated that these rootstocks improve vegetative growth and early yield at suboptimal temperatures (den Nijs, 1980, 1984; Tachibana, 1982; Bulder et al., 1991) and under conditions where only the roots are subjected to chilling (8 °C) temperatures (Ahn et al., 1999). The physiological basis of the higher level of low-temperature tolerance of figleaf gourd rootstocks is supposed to be associated with the maintenance of (i) a higher absorption rate of water and nutrients (Tachibana, 1987) and (ii) a higher cytokinin synthesis which stimulates root meristem activity and translocation of photosynthate to the roots (Tachibana, 1988). The former coincides with a better adaptability of the plasmamembrane H<sup>+</sup>-ATPase to low temperature (Choi et al., 1995; Ahn et al., 1999) and increase in cytochrome respiration (Tachibana, 1989). Recently, it was reported that the differences in root hydraulics in response to chilling between cucumber and figleaf gourd are related both to changes in the activity of aquaporins and in root anatomy (Lee et al., 2005a). The ability of the figleaf gourd root system to maintain a low level of H<sub>2</sub>O<sub>2</sub> in the cytoplasm and to detoxify reactive oxygen species appeared to be one of the mechanisms facilitating the maintenance of water transport activity at low temperatures (Rhee et al., 2007).

The few grafting experiments performed with genotypes of the cultivated tomato illustrated that a vigorous rootstock might increase the resistance to thermal fluctuations as reflected in greater biomass production (Rivero et al., 2003b). Rootstock genotype significantly affected the vegetative growth rate, earliness and yield of different scion genotypes under low-energy conditions without significant interactions between rootstock and scion (Zijlstra and den Nijs, 1987). For tomato it was found that the intraspecific variation in adaptability to low air (Nieuwenhof et al., 1993, 1997) and root temperatures (Nieuwenhof et al., 1993, 1999) is small, which leaves very little room for improvement by selective breeding. Due to their wide range of altitudinal distribution, related wild tomato species (e.g. *Solanum habrochaites* synonym *Lycopersicon hirsutum*; Spooner et al., 2005) are of particular interest as potential germplasm sources to widen the genetic variation for low-temperature tolerance of the cultivated tomato (Venema et al., 2005). Many physiological studies illustrated the superior low-temperature tolerance of the above-ground parts of high-altitude accessions of *S. habrochaites*, which thrive at temperatures that are detrimental to the cultivated tomato (Miltau et al., 1986; Yakir et al., 1986; Vallejos and Pearcy, 1987; Venema et al., 1999a,b). In addition to the shoot also root traits like (i) elongation rate (Zamir and Gadish, 1987), (ii) the volumes of secreted xylem sap and quantities of K<sup>+</sup> transport (Brunet et al., 1990) and (iii) NH<sub>4</sub><sup>+</sup> absorption (Smart and Bloom, 1991; Bloom et al., 1998) appeared to be less susceptible to chilling in these high-altitude accessions than in the cultivated tomato. In addition, shoots of tomato start to wilt if their roots are exposed to temperatures of around 5 °C in contrast to a high-altitude accession of *S. habrochaites* (Bloom et al., 2004). This difference was associated with the propensity of this wild tomato species to close stomata during chilling, whereas the stomata of tomato remained open. Reciprocal grafting experiments with wilted or non-wilted phenotypes of the backcross generations BC<sub>1</sub> and BC<sub>2</sub> of a cross between this wild and cultivated tomato species (Truco et al., 2000) indicated that the differential stomatal behaviour during root chilling has distinct shoot and root components (Bloom et al., 2004).

Given that root performance of the high-altitude accession LA 1777 of *S. habrochaites* (*Sh*) is more tolerant to low-temperature than of tomato, the use of this high-altitude wild tomato accession as rootstock could improve tomato shoot (scion) growth at suboptimal temperatures. In order to test this hypothesis, the impact of grafting tomato (*Solanum lycopersicum* Mill. cv. Moneymaker, *Sl*) either onto the root of *Sh* or onto its own root was compared on biomass accumulation and partitioning, plant morphology, carbohydrate partitioning and leaf C and N status at four combinations of optimal (25 °C) and/or suboptimal (15 °C) air/root-zone temperatures (RZT).

## 2. Materials and methods

### 2.1. Plant material

Seeds of *S. lycopersicum* Mill. cv. Moneymaker (*Sl*) and *S. habrochaites* LA 1777 (*Sh*) were kindly provided by the lab-

oratory of Plant Breeding of the Wageningen University and by the C.M. Rick Tomato Genetics Resource Center of the University of California, Davis (<http://www.tgrc.ucdavis.edu>), respectively. Seeds of *S. habrochaites* LA 1777 originate from Rio Casma, Province Ancash in Peru at an altitude of 3150 m a.s.l.

## 2.2. Grafting procedure and growth conditions

Seeds of both species were germinated on a moist rock wool plug with vermiculite on top. Due to uneven emergence and seedling development, seeds of *S. habrochaites* were germinated 10 days earlier than the relative large seeds of *S. lycopersicum*. Germination was performed at a temperature of 25 °C, a relative humidity of around 78%, a 17 h photoperiod with 120  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of photosynthetically active radiation (PAR). After 18 (*Sl*) or 28 days (*Sh*) when the plants started to initiate their third true leaf, plants were grafted using the Japanese top grafting method. The scion and the rootstock were cut off with a razor blade at an angle of 45° just above the cotyledons at a maximum of 2 cm above the rock wool plug.

As scion we used tomato cv. Moneymaker and grafted it either onto its own root, as control, or onto the root of line accession (LA) 1777 of the wild tomato *S. habrochaites*. Scion (*S. lycopersicum* cv. Moneymaker, *Sl*) and rootstock (either *S. lycopersicum* cv. Moneymaker; *Sl*, or *S. habrochaites* LA 1777; *Sh*) were connected using a silicon-grafting clip ( $\emptyset$  2 mm). This procedure resulted in two scion/rootstock combinations: *Sl/Sl* and *Sl/Sh*. After grafting, both graft combinations were put immediately into a clear, closed plastic tunnel of 30 cm height with inside a relative humidity of nearly 100% and a temperature of 20–21 °C. The exposure to direct sunlight was prevented. From day 5, the tunnel was ventilated daily till the morning of day 7 when the plastic was removed. The seedlings were transferred to a greenhouse at 25  $\pm$  1 °C with a minimum of 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of PAR at plant level during a 12-h photoperiod. Grafts were treated for another 5 days to ensure full recovery. The grafts were then transferred to aerated hydroponic culture tanks holding 30 L of a 25%-strength Hoagland's nutrient solution. After another week of acclimation to the hydroponic culture plants in comparable vegetative stages (i.e. those who started to initiate their fifth true leaf) were selected and randomly distributed over two identical climate rooms with an optimal (25 °C) or suboptimal (15 °C) day/night temperature. In the 25 °C climate room, half of the plants were subjected to 15  $\pm$  2 °C root-zone temperature (RZT), whereas half of the plants in the 15 °C climate room were subjected to 25  $\pm$  2 °C RZT. The RZT was controlled thermostatically by water baths (Julabo Labortechnik GmbH, Seelbach, Germany) that circulated the nutrient solution. In this way, four combinations of air/RZ temperatures, i.e. 25/25, 25/15, 15/25 and 15/15 °C, were obtained. In both climate rooms light was supplied by Philips TLD reflex 58 W/830 and 840 tubes in a ratio of 1:1. At plant level PAR was 250  $\pm$  25  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and the relative humidity was 50  $\pm$  10%. During these treatments, which lasted for 14 days, the plants were grown on a 50%-strength Hoagland's nutrient solution of pH 5.8, which was refreshed after 7 days.

## 2.3. Plant harvest and growth analysis

Eight plants per graft combination were harvested at the start (day 0) and after 14 days of treatment at the four air/RZ temperature regimes. After thoroughly washing the roots in de-ionised water, plants were divided into leaves, petioles, stems and roots, and fresh masses were recorded. Leaf area was measured with a leaf area meter (model 3100, LI-Cor Inc., Lincoln, NE, USA). Subsequently, the plant material was dried during 72 h at 70 °C and dry masses were determined. The partitioning of biomass to leaves (LMR), petioles (PMR), stem (SMR) and root (RMR) was calculated by dividing the FM of the respective tissues by the total plant FM. Dry matter content (DMC) was calculated as the percentage DM of FM. The specific stem mass (SSM), a parameter indicative for stem thickness, was calculated from the ratio between stem mass and length.

The mean relative growth rate (RGR) of the whole-plant, shoot (RGR<sub>shoot</sub>) and root (RGR<sub>root</sub>), and leaf expansion rate (LER) during the four treatments was calculated according to Hunt (1982) using the ln-transformed mean values for total, shoot and root fresh masses, and total leaf area, respectively, for the time interval of 14 days during which the vegetative tomato plants were in the exponential growth phase.

## 2.4. Qualification of vegetative development

To quantify the effect of rootstock genotype on vegetative development at the four air/RZ temperature treatments, the plastochron index (PI) was measured twice a week on eight plants of each graft combination at each treatment. The plastochron duration is defined as the time interval between the initiation of consecutive leaves on a plant. The PI was determined according to the method of Erickson and Michelini (1957) and calculated as

$$\text{PI} = n + \frac{\log L_n - \log R}{\log L_n - \log L_{n+1}}$$

where  $n$  is the serial number of the youngest leaf, counted from the cotyledons, exceeding the reference length  $R$  of 10 mm,  $L_n$  the length in mm of leaf  $n$ , which by definition is longer or equal to  $R$ , and  $L_{n+1}$  is the length in mm of leaf  $n+1$ , which by definition is shorter than  $R$ . The daily change in PI over the 14 days (plastochron day<sup>-1</sup>) was obtained by calculating the regression coefficient between PI and time.

## 2.5. Soluble-sugar and starch analysis

Soluble-sugar and starch concentrations were determined in both shoots and roots of plants that had been treated for 14 days at the four air/RZ temperatures. Soluble-sugars were extracted from 50 mg oven-dried material with 80% ethanol in five extraction steps. The remaining pellet was boiled in 3% (v/v) HCl for 3 h in order to hydrolyse starch. After addition of a 10% Al(OH)<sub>3</sub> (w/v) suspension the extract was centrifuged again. For both extracts, soluble sugar was determined colorimetrically at 620 nm using the anthrone method (Fales, 1951).

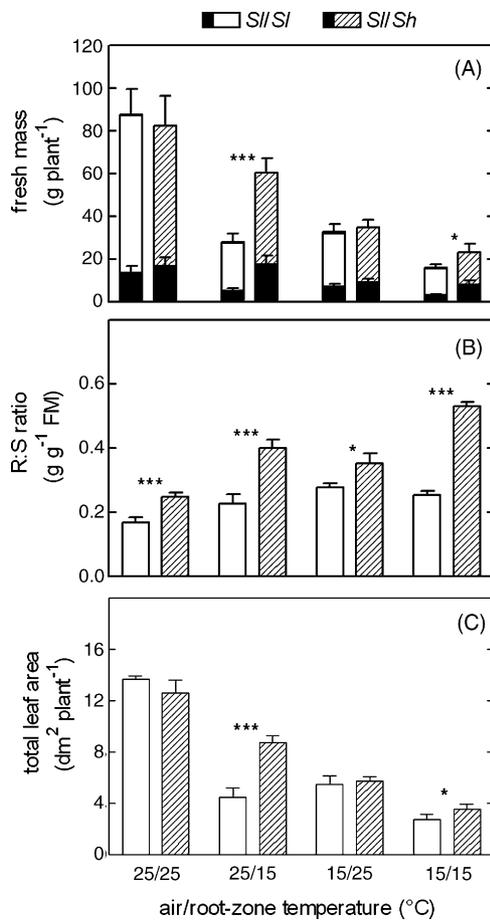


Fig. 1. Total fresh mass (A) divided into root (black) and shoot mass (upper part), root:shoot ratio (B) and total leaf area (C) of tomato (*Solanum lycopersicum* cv. Moneymaker; *Sl*) scions grafted onto their own rootstock (*Sl/Sl*; open bars) or onto the rootstock of *S. habrochaites* LA 1777 (*Sl/Sh*; arced bars) after growth for 14 days at four combinations of air/RZ temperatures. Data show the mean of eight plants ( $\pm$ S.D.). Significant differences between graft combinations per treatment are denoted by \* $P \leq 0.05$  and \*\*\* $P \leq 0.001$ . Asterisks shown in panel (A) present significant differences in total FM.

## 2.6. Leaf $\text{NO}_3^-$ , total N and total C analysis

Powdered oven-dried leaf material was extracted for  $\text{NO}_3^-$  determination by incubating the samples overnight in demineralised water (20 mg in 5 ml). Homogenates were filtered through one layer of Miracloth and the filtrate was centrifuged at  $30,000 \times g$  for 15 min ( $0^\circ\text{C}$ ).  $\text{NO}_3^-$  was separated from the other anions by an HPLC apparatus as described by Durenkamp and De Kok (2002). Total N and total C analysis were carried out on powdered oven-dried leaf material by an automated CHNS-element analyser (Interscience model EA 1110, Breda, The Netherlands). Reduced-N concentration ( $N_r$ ) was calculated from the difference between total N and  $\text{NO}_3^-$ -N concentration.

## 2.7. Data presentation and statistical analysis

As proposed by Equiza et al. (2001), all data were presented on a FM basis in order to minimise weight interference by the accumulation of TNC. Within every graft combination the effects of the four temperature treatments were tested by a one-

way ANOVA, and where appropriate, a Tukey post-test. Within each temperature treatment the effect of the rootstock on performance of the graft was analysed using an unpaired Student's *t*-test. For both tests the GraphPad Prism software package version 3.0 (GraphPad Software Inc., San Diego, CA, USA) was used.

## 3. Results

### 3.1. Growth, biomass partitioning and plant morphology

Air and root-zone (RZ) temperature both significantly affected biomass accumulation of the two graft combinations (Fig. 1A). Significant effects of rootstock genotype on total fresh mass (FM) were only observed in treatments with  $15^\circ\text{C}$  RZT. Tomato (*Sl*) scions grafted onto *S. habrochaites* (*Sh*) rootstocks, denoted as *Sl/Sh*, showed a 115 and 43% higher total FM at 25/15 and 15/15  $^\circ\text{C}$ , respectively, than when they were grafted onto their own rootstock (*Sl/Sl*). Shoot and root FM separately were 87% and 236% higher, respectively, in *Sl/Sh* graft combinations than in *Sl/Sl* grafts at 25/15  $^\circ\text{C}$ . At 15/15  $^\circ\text{C}$ , only root FM was significantly higher in grafts with *Sh* as rootstock. Grafting tomato onto *Sh* instead of onto its own root increased the relative growth rate (RGR) at 25/15 and 15/15  $^\circ\text{C}$  with 27 and 15%, respectively (Table 1). At 25/15  $^\circ\text{C}$ , shoot and root similarly contributed to the stimulation of RGR at the whole-plant level, whereas the increase in RGR at 15/15  $^\circ\text{C}$  could be attributed mainly to a higher  $\text{RGR}_{\text{root}}$  of the *Sh* rootstock.  $\text{RGR}_{\text{root}}$  of *Sl/Sh* graft combinations were only affected by air temperature, whereas *Sl/Sl* grafts revealed intermediate  $\text{RGR}_{\text{root}}$  when either the shoot or the root was subjected to  $15^\circ\text{C}$ .

Graft combinations with *Sh* rootstocks showed higher root-to-shoot (R:S) ratios than those with *Sl* rootstocks under all four air/RZ temperature treatments, particularly those with  $15^\circ\text{C}$  RZT (Fig. 1B). R:S ratios of *Sl/Sl* grafts were only slightly affected by air/RZ temperature, whereas in *Sl/Sh* grafts R:S ratios of 15/15  $^\circ\text{C}$ -grown plants were 3.1-times higher than in 25/25  $^\circ\text{C}$ -grown plants with intermediate R:S ratios at 25/15 and 15/25  $^\circ\text{C}$ . Suboptimal air temperature resulted generally in tomato plants with higher leaf mass ratio (LMR) and lower peti-

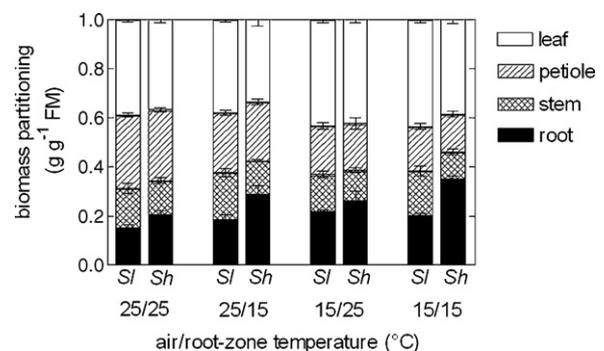


Fig. 2. Partitioning of biomass between root (RMR), stem (SMR), petiole (PMR) and leaves (LMR) of tomato (*Solanum lycopersicum* cv. Moneymaker; *Sl*) scions grafted onto their own rootstock (*Sl/Sl*) or onto the rootstock of *S. habrochaites* LA 1777 (*Sl/Sh*) after growth for 14 days at four combinations of air/RZ temperatures. Data show the mean of eight plants  $\pm$  S.D.

Table 1

Growth and morphologic characteristics of tomato (*Solanum lycopersicum* cv. Moneymaker, *Sl*) scions grafted onto their own rootstock (*Sl/Sl*) or onto the rootstock of *S. habrochaites* LA 1777 (*Sl/Sh*) after growth for 14 days at four combinations of air/RZ temperatures

Parameter	Graft	Air/root-zone temperature (°C)			
		25/25	25/15	15/25	15/15
RGR (mg g <sup>-1</sup> FM day <sup>-1</sup> )	<i>Sl/Sl</i>	276	191	202	151
	<i>Sl/Sh</i>	266	243	203	173
RGR <sub>shoot</sub> (mg g <sup>-1</sup> FM day <sup>-1</sup> )	<i>Sl/Sl</i>	276	187	195	145
	<i>Sl/Sh</i>	266	235	198	159
RGR <sub>root</sub> (mg g <sup>-1</sup> FM day <sup>-1</sup> )	<i>Sl/Sl</i>	279	212	234	178
	<i>Sl/Sh</i>	264	268	220	211
LER (mm <sup>2</sup> cm <sup>-2</sup> day <sup>-1</sup> )	<i>Sl/Sl</i>	25	17	18	14
	<i>Sl/Sh</i>	25	22	19	16
Plastochron (d <sup>-1</sup> )	<i>Sl/Sl</i>	0.51 ± 0.04 c	0.37 ± 0.06 b	0.37 ± 0.05 b	0.29 ± 0.04 a
	<i>Sl/Sh</i>	0.49 ± 0.04 d	0.42 ± 0.04 c	0.32 ± 0.05 b	0.26 ± 0.03 a
SLM-FM (m <sup>2</sup> kg <sup>-1</sup> FM)	<i>Sl/Sl</i>	4.00 ± 0.26 ab	4.23 ± 0.13 b	3.87 ± 0.08 a	3.96 ± 0.11 a
	<i>Sl/Sh</i>	4.16 ± 0.19 ab	4.36 ± 0.16 b	3.89 ± 0.04 a	4.04 ± 0.10 a
SLM-DM (m <sup>2</sup> kg <sup>-1</sup> DM)	<i>Sl/Sl</i>	35.15 ± 0.17 b	26.03 ± 3.63 a	32.06 ± 1.29 b	26.25 ± 1.27 a
	<i>Sl/Sh</i>	32.57 ± 2.39 b*	32.97 ± 4.68 b*	25.95 ± 3.73 a**	27.62 ± 1.75 ab
DMC leaves	<i>Sl/Sl</i>	11.4 ± 0.5 a	16.4 ± 1.9 b	12.1 ± 0.5 a	15.1 ± 0.7 b
	<i>Sl/Sh</i>	12.8 ± 0.4 a***	13.4 ± 1.5 ab*	15.3 ± 2.4 b**	14.7 ± 0.7 ab
DMC root	<i>Sl/Sl</i>	5.9 ± 0.4 a	6.9 ± 0.7 b	5.5 ± 0.4 a	7.6 ± 0.6 b
	<i>Sl/Sh</i>	5.4 ± 0.7	5.1 ± 0.4***	5.7 ± 0.4	5.6 ± 0.4***
Stem length (mm)	<i>Sl/Sl</i>	200 ± 8 d	147 ± 10 c	111 ± 8 b	94 ± 10 a
	<i>Sl/Sh</i>	179 ± 14 b**	169 ± 16 b*	104 ± 10 a	87 ± 13 a
SSM (g FM cm <sup>-1</sup> )	<i>Sl/Sl</i>	0.68 ± 0.03 d	0.36 ± 0.03 b	0.46 ± 0.03 c	0.31 ± 0.02 a
	<i>Sl/Sh</i>	0.63 ± 0.07 c	0.48 ± 0.05 b***	0.41 ± 0.04 b	0.32 ± 0.03 a

Data show the mean of eight plants (±S.D.). Values within a graft combination with different letters are significantly different ( $P \leq 0.05$ ). Significant differences between graft combinations at each treatment are \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ .

ole mass ratio (PMR) (Fig. 2). Graft combinations with *Sh* as rootstock were characterized by a higher root mass ratio (RMR) and a lower stem mass ratio (SMR) at all temperature treatments than those with *Sl* as rootstock. RZT affected biomass partition-

ing in both graft combinations differently. In response to 15 °C RZT, SMR increased only slightly in self-grafted tomato plants (*Sl/Sl*), whereas a pronounced increase in RMR was observed in tomato grafted onto *Sh* at the expense of the partitioning

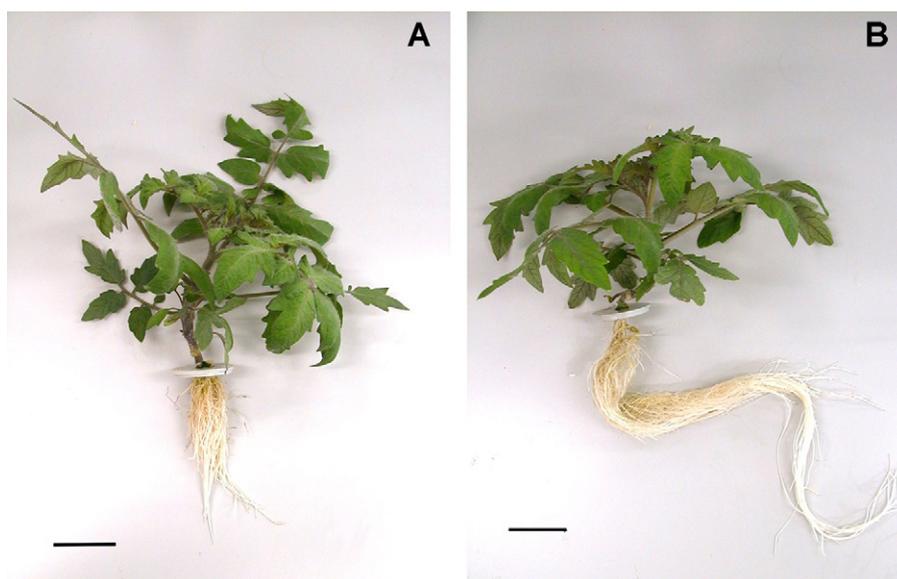


Fig. 3. Tomato (*Solanum lycopersicum* cv. Moneymaker) scions grafted onto their own rootstock (*Sl/Sl*; A) or onto the rootstock of *S. habrochaites* LA 1777 (*Sl/Sh*; B) after growth for 14 days at 15/15 °C air/RZ temperature. Bar = 5 cm.

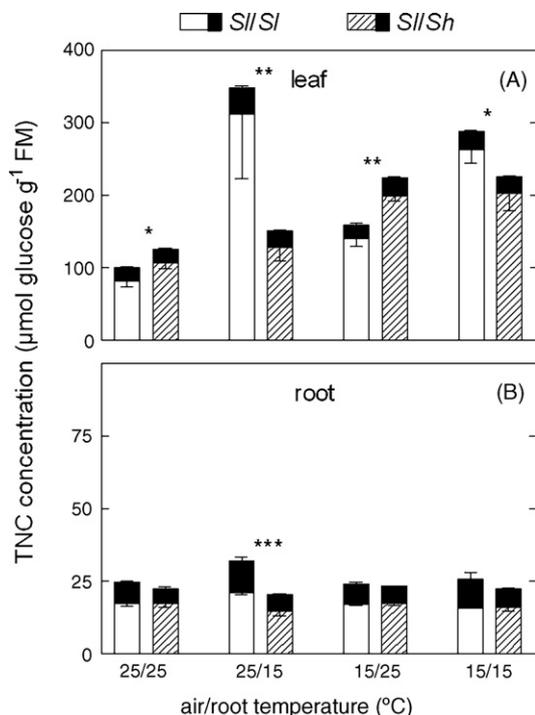


Fig. 4. Total non-structural carbohydrate (TNC) concentration in leaf (A) and root (B), consisting of soluble sugars (upper black part) and starch (lower white/arced part), of tomato (*Solanum lycopersicum* cv. MoneyMaker; *SI*) scions grafted onto their own rootstock (*SI/SI*; open bars) or onto the rootstock of *S. habrochaites* LA 1777 (*SI/Sh*; arced bars) after growth for 14 days at four combinations of air/RZ temperatures. Data show the mean of four plants  $\pm$  S.D. Significant differences between graft combinations per treatment are denoted by \* $P \leq 0.05$ ; \*\* $P \leq 0.01$  and \*\*\* $P \leq 0.001$ .

of biomass to petiole and leaves. Root morphology of *SI* rootstocks particularly differed from *Sh* rootstocks in the extent root elongation was inhibited at 15 °C RZT (Fig. 3).

Grafting tomato onto *Sh* increased the total leaf area considerably with 95% at 25/15 °C (Fig. 1C). Also at 15/15 °C leaf area was significantly ( $P=0.035$ ) higher in grafts with *Sh* as rootstock. In *SI/Sh* grafts the leaf expansion rate (LER) was 29 and 14% higher at 25/15 and 15/15 °C, respectively, than in *SI/SI* grafts (Table 1). The leaf initiation rate presented by the plastochron day<sup>-1</sup> parameter, however, was not significantly affected by the rootstock genotype at any of the temperature treatments. Although no differences were found between both graft combinations in the way air and/or RZ temperature affected the specific leaf mass (SLM) expressed on a FM basis, there were differences in SLM when calculated on a DM basis. *SI/SI* grafts showed a decrease in SLM-DM in response to 15 °C RZT, whereas in *SI/Sh* grafts SLM-DM was significantly decreased when the shoot was subjected to 15 °C. These differences in SLM-DM strongly correlated with those found in leaf dry matter content (DMC) and leaf carbohydrate accumulation (Fig. 4A). Suboptimal RZT increased root DMC of *SI/SI* grafts in contrast to *SI/Sh* grafts. Suboptimal air temperature severely reduced stem length in both graft combinations. Under fully optimal-temperature conditions (25/25 °C) tomato scions grafted onto *Sh* were characterized by 10% shorter stems. Suboptimal RZT decreased stem length with 26% in *SI/SI* grafts but not signifi-

cantly in *SI/Sh* grafts. The impact of air/RZ temperature on the specific stem mass (SSM), a parameter indicative for stem thickness, correlated with differences found in shoot FM and total leaf area (Fig. 1A and C). Differences in SSM between both graft combinations were observed only at 25/15 °C, the treatment at which grafts with *Sh* rootstocks were characterized with a higher shoot biomass and total leaf area than their counterparts with *SI* rootstocks.

### 3.2. Carbohydrate concentration

The way air/RZ temperatures affected leaf total non-structural carbohydrate (TNC) concentrations depended strongly on the rootstock genotype (Fig. 4A). In *SI/SI* grafts leaf TNC concentrations were significantly ( $P \leq 0.05$ ) higher particularly at 15 °C RZT. In leaves of *SI/Sh* grafts, on the contrary, carbohydrates accumulated at 15 °C air temperature irrespective of the RZT. Leaf TNC concentrations of *SI/Sh* grafts were higher at 25 °C RZT and lower at 15 °C RZT in comparison with *SI/SI* grafts. The higher leaf TNC concentrations in both graft combinations at 15 °C air and/or RZ temperature could mainly be attributed to an increase in starch concentration. In *SI/SI* grafts the leaf starch:soluble-sugar ratio was 2.2 times higher at 15/15 °C than at 25/25 °C (Table 2). Intermediate ratios were found when either the scion or rootstock was subjected to 15 °C. In leaves of *SI/Sh* grafts leaf starch:soluble-sugar ratios were 1.5 times higher in treatments with 15 °C air temperature. TNC concentrations in the root were much lower than in the leaf, which was mainly due to lower starch concentrations (Fig. 4B). Root soluble-sugar concentrations of *SI/SI* grafts were 1.5 times higher at 15 °C RZT, whereas in grafts with *Sh* rootstocks these concentrations were unaffected by air/RZ temperature. Root TNC concentration was significantly higher only in *SI/SI* grafts at 25/15 °C due to a significant ( $P \leq 0.001$ ) increase of both soluble-sugar and starch concentration.

### 3.3. Leaf C and N status

In contrast to tomato grafted onto *Sh*, exposure of the RZ to 15 °C significantly increased leaf total C and total N concentrations in self-grafted tomato plants (Table 2). Leaf total C concentrations of *SI/SI* grafts were 52 and 34% higher at 25/15 and 15/15 °C, respectively, than at 25/25 °C. These higher total C concentrations could for a great part, but not fully, be ascribed to accumulation of non-structural carbohydrates in the leaves. Leaf total N concentrations of *SI/SI* grafts were 18 and 6% higher at 25/15 and 15/15 °C, respectively, than at 25/25 °C. Relatively to the increase in total C concentration the increase in total N was smaller in leaves. As a consequence leaf C:N ratios of *SI/SI* grafts were 28 and 26% higher at 25/15 and 15/15 °C, respectively, than at 25/25 °C. Leaves of *SI/SI* grafts were characterized by lower NO<sub>3</sub><sup>-</sup> concentrations and increased N<sub>r</sub>:NO<sub>3</sub><sup>-</sup> ratios when the roots were subjected to 15 °C. The higher leaf total N concentrations in *SI/SI* grafts observed at 15 °C RZT could therefore fully be ascribed to higher reduced-N (N<sub>r</sub>) concentrations. As mentioned, the concentrations of total C and total N, as well as those of NO<sub>3</sub><sup>-</sup> and N<sub>r</sub>, were not significantly affected

Table 2

Leaf concentrations of total C, total C corrected for total non-structural carbohydrates (TNC), total N,  $\text{NO}_3^-$  and reduced nitrogen ( $\text{N}_r$ ), and leaf ratios of starch:soluble-sugars, C:N and  $\text{N}_r:\text{NO}_3^-$  in tomato (*Solanum lycopersicum* cv. Moneymaker, *Sl*) scions grafted onto their own rootstock (*Sl/Sl*) or onto the rootstock of *S. habrochaites* LA 1777 (*Sl/Sh*) after growth for 14 days at four combinations of air/RZ temperatures

Parameter	Graft	air/root-zone temperature ( $^{\circ}\text{C}$ )			
		25/25	25/15	15/25	15/15
Total C	<i>Sl/Sl</i>	4009 $\pm$ 142 a	6079 $\pm$ 334 c	4109 $\pm$ 119 a	5363 $\pm$ 198 b
	<i>Sl/Sh</i>	4431 $\pm$ 165**	4549 $\pm$ 497**	5085 $\pm$ 693*	4949 $\pm$ 169*
Total C-TNC	<i>Sl/Sl</i>	3406 $\pm$ 88 ab	3992 $\pm$ 269 c	3155 $\pm$ 162 a	3634 $\pm$ 72 b
	<i>Sl/Sh</i>	3677 $\pm$ 114*	3646 $\pm$ 605	3731 $\pm$ 423*	3594 $\pm$ 108
Starch:soluble-sugars ratio	<i>Sl/Sl</i>	5.0 $\pm$ 0.7 a	9.0 $\pm$ 1.5 b	8.5 $\pm$ 1.8 b	11.2 $\pm$ 1.2 b
	<i>Sl/Sh</i>	6.2 $\pm$ 0.7 a*	6.4 $\pm$ 1.0 a*	9.3 $\pm$ 1.1 b	9.5 $\pm$ 0.8 b*
Total N	<i>Sl/Sl</i>	446 $\pm$ 12 a	528 $\pm$ 16 c	438 $\pm$ 12 a	473 $\pm$ 5 b
	<i>Sl/Sh</i>	467 $\pm$ 9*	453 $\pm$ 61	437 $\pm$ 8	459 $\pm$ 4*
$\text{NO}_3^-$	<i>Sl/Sl</i>	42 $\pm$ 4 b	27 $\pm$ 6 a	46 $\pm$ 5 b	34 $\pm$ 5 ab
	<i>Sl/Sh</i>	39 $\pm$ 6	32 $\pm$ 3	38 $\pm$ 2*	38 $\pm$ 2
$\text{N}_r$	<i>Sl/Sl</i>	404 $\pm$ 9 a	501 $\pm$ 10 c	392 $\pm$ 17 a	440 $\pm$ 4 b
	<i>Sl/Sh</i>	428 $\pm$ 13*	421 $\pm$ 59*	399 $\pm$ 8	420 $\pm$ 4***
C:N ratio	<i>Sl/Sl</i>	9.0 $\pm$ 0.1 a	11.5 $\pm$ 1.0 b	9.4 $\pm$ 0.2 a	11.3 $\pm$ 0.5 b
	<i>Sl/Sh</i>	9.5 $\pm$ 0.3 a*	10.0 $\pm$ 0.7 ab	11.6 $\pm$ 0.4 c***	10.8 $\pm$ 0.3 bc
$\text{N}_r:\text{NO}_3^-$ ratio	<i>Sl/Sl</i>	9.8 $\pm$ 1.0 a	19.2 $\pm$ 4.7 b	8.5 $\pm$ 1.2 a	13.4 $\pm$ 2.0 a
	<i>Sl/Sh</i>	11.1 $\pm$ 1.8*	13.0 $\pm$ 1.4*	10.5 $\pm$ 0.5*	11.0 $\pm$ 0.7

Concentrations are given in  $\mu\text{mol g}^{-1}$  FM and ratios in  $\text{mol mol}^{-1}$ . Data represent the mean of four individual plants ( $\pm$ S.D.). Values within a graft combination with different letters are significantly different ( $P \leq 0.05$ ). Significant differences between graft combinations per treatment are \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ .

by air and/or RZ temperatures in leaves of tomato scions grafted onto *Sh*.

#### 4. Discussion

In contrast to the findings of Bloom et al. (2004), who reported that the cultivated tomato (*S. lycopersicum*, *Sl*) and its wild relative *S. habrochaites* (*Sh*) were graft-incompatible, our grafting technique and subsequent plant handling procedure was successful in grafting *Sl* scions onto rootstocks of *Sh*. However, we did not succeed in making the reciprocal graft combination, i.e. grafting *Sh* scions onto rootstocks of *Sl*, due to the fact that *Sh* scions started to wilt dramatically without the capacity to recover.

In accordance with comparative physiological studies on the impact of low temperature on root performance of tomato and high-altitude accessions of related wild species (Zamir and Gadish, 1987; Brunet et al., 1990; Smart and Bloom, 1991; Bloom et al., 1998, 2004), the root system of *Sh* showed little dependence on RZT (15 or 25  $^{\circ}\text{C}$ ) in comparison with *Sl* (Table 1). The most promising result from this study is that grafting tomato onto a *Sh* rootstock is able to improve  $\text{RGR}_{\text{shoot}}$  at suboptimal (15  $^{\circ}\text{C}$ ) RZT by stimulating the expansion rate of leaves (LER). In addition, *Sh* rootstocks did not adversely affect shoot growth and LER at optimal (25  $^{\circ}\text{C}$ ) RZT. That the poor performance of *Sl* rootstocks at suboptimal temperature contributed considerably to the low  $\text{RGR}_{\text{shoot}}$  by inhibiting the LER was also demonstrated by the 15/25  $^{\circ}\text{C}$  air/RZ temperature treatment. Root-zone heating increased  $\text{RGR}_{\text{shoot}}$  and LER of *Sl/Sl* grafts both with about 30% with respect to 15/15  $^{\circ}\text{C}$ . The

higher LER of tomato grafted onto *Sh* rootstocks could not be attributed to a faster initiation rate of new leaves since the rootstock genotype did not significantly affect plastochron duration (Table 2). Although the leaf initiation rate of intact (ungrafted) *Sh* plants is faster than in tomato at high- and low-temperature regimes (Miltau et al., 1986; Venema et al., 1999b), this result also implicates that this shoot character is not affected by traits residing in the root. Leaf total N and carbohydrate concentrations showed that neither the uptake of N nor the availability of carbohydrates limited shoot growth at 15  $^{\circ}\text{C}$  RZT (Table 2). The results demonstrated that the better development and/or functioning of *Sh* rootstocks compared to *Sl* rootstocks at 15  $^{\circ}\text{C}$  RZT apparently was the dominant feature contributing to the higher LER. The factors by which *Sh* rootstocks increased LER of tomato scions at 15  $^{\circ}\text{C}$  RZT may include (i) a lower reduction of the absorption and upward transport capacity of water and nutrients and/or (ii) a less impaired balance and/or supply rate of root-derived phytohormones to the scion (Jones, 1986; Tachibana, 1987, 1988; Brunet et al., 1990; Kamboj et al., 1999; Sorce et al., 2002; Clearwater et al., 2004; Lee, 1994; Lee et al., 2005a,b). In addition to these factors, the smaller reduction in root sink strength at 15  $^{\circ}\text{C}$  RZT resulted in a less pronounced accumulation of TNC in the leaves of tomatoes grafted onto *Sh* (Fig. 4A), which might prevent source leaves from a direct negative effect on photosynthesis at the metabolic level and through sugar modulated down-regulation of photosynthetic genes (Hurewitz and Janes, 1983; Paul and Foyer, 2001).

The differences in R:S ratios (Fig. 1B) and root biomass partitioning (Fig. 2) among both graft combinations at the four

temperature treatments showed a striking good similarity with those found in a similar experiment with intact (ungrafted) *Sl* and *Sh* plants (J.H. Venema, B. Dijk, M. Durenkamp, P.R. van Hasselt, T.L. Pons, unpublished results). It can be concluded that *Sl* plants have a low capacity to adjust their R:S ratio to suboptimal temperatures in comparison with *Sh*. The mechanisms that control allocation of biomass and its modulation by developmental and environmental signals are not well understood (Stitt and Scheible, 1998). This grafting experiment illustrates that the partitioning of biomass in tomato is strongly affected by the sink strength of the root genotype. Increase of the R:S ratio at 15 °C RZT may adjust the root system to overcome restrictions to water absorption which might be related to increased water viscosity and a decrease of the root hydraulic conductance at lower RZT (Equiza et al., 2001). Bloom et al. (2004) demonstrated with two independent methods that the root hydraulic conductance in *Sl* and *Sh* declined equally with temperature emphasizing the importance of increasing the partitioning of biomass to the roots as an adaptive mechanism to compensate for the decrease in water movement through the roots when these are subjected to suboptimal temperatures.

Although a more detailed comparative root morphological study is required, it appeared from the present study that the variation in root growth between both rootstock genotypes at 15 °C RZT was associated with the extent in which root elongation was inhibited (Fig. 3). In chilling-sensitive maize plants the inhibition in root elongation rate at suboptimal temperature was in the short term related to a reduction in the *in vivo* wall extensibility of the tissue and not to a decrease in turgor pressure and/or change in yield stress threshold (Pritchard et al., 1990). Among *Arabidopsis* ecotypes, variation in root elongation rate was positively correlated with cyclin-dependent kinase (CDK) activity, suggesting that cell cycle activity (production rate of new cells) is an important determinant of root elongation rate within and among closely related species (Beemster et al., 2002). Low-temperature inhibits CDK activity in maize leaves (Granier et al., 2000), however, there is still little knowledge how low-temperature affects the regulation of the cell cycle in roots (West et al., 2004). We are presently characterizing the physiology of the difference in inhibition of root elongation rate among both tomato species at suboptimal temperature.

In conclusion, the vegetative growth rate of tomato at suboptimal temperature is for a significant part limited by its poor root development. Root-zone heating and grafting onto a low-temperature tolerant rootstock appeared to be useful tools for tomato to increase shoot growth at suboptimal cultivation temperatures by stimulation of the leaf expansion rate. The genetic variability in low-temperature tolerance of the root system among tomato cultivars and high-altitude wild tomato accessions (e.g. *S. habrochaites* LA 1777) provides a valuable germplasm pool for plant breeders to improve the low-temperature tolerance of existing commercial vigorous rootstocks. Such new rootstocks may help to broaden the temperature optimum of elite tomato cultivars and so to increase the energy efficiency of tomato greenhouse cultivation in the temperate climate zone. Investigations are in progress to elu-

cidate the crucial physiological and molecular mechanisms that underlie the positive impact of *Sh* rootstocks on tomato scion performance at suboptimal (RZ) temperatures. With this knowledge appropriate criteria for the selection and breeding of vigorous 'energy-efficient' tomato rootstocks can be defined.

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