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ORIGINAL PAPER

The effectiveness of grafting to improve drought tolerance in tomato

Zhihuan Zhang[1,2,3] · Yu Liu[1,2,3] · Bili Cao[1,2,3] · Zijing Chen[1,2,3] · Kun Xu[1,2,3]

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Abstract

Drought is a common stress in crop growth that limits plant growth, development and yield. Therefore, we explored the feasibility of bio-saving water for tomato production by grafting with drought-tolerant seedlings. In this experiment, we carried out grafting on different drought-tolerant tomato seedlings to study the effects of grafting on root growth and plant nutrient uptake under drought stress. We measured the effects of grafting on root growth, nutrient uptake, carbohydrate content and organic acids in tomato leaves and roots under drought stress. The growth and vigor of roots as well as the concentration of N, P, K, Ca and Mg of the plant were significantly inhibited by drought. The nutrient content was significantly decreased in leaves, and the content of carbohydrates and organic acids was increased in both leaves and roots under drought conditions. Treatment with drought-tolerant tomato seedlings significantly enhanced root growth and increased the element content under water deficit conditions compared to those grafted with drought-susceptible seedlings. In the plants grafted with drought-tolerant seedlings, the contents of carbohydrates and organic acids increased. Our results indicated that the grafted tomato seedlings showed beneficial root growth because drought-tolerant seedlings increased in inorganic and organic osmotic adjustment substances that helped the plants absorb more water and promoted plant growth.

Keywords Tomato · Grafting · Drought · Major elements · Carbohydrate · Organic acids

Introduction

Drought is one of the most prevalent limiting factors that influence plant growth and development and has become the primary cause for reductions in crop yields, inflicting economic as well as nutritional insecurity (Boyer 1982; Luo 2010; Liu et al. 2014). The subject of plant response to drought stress has been extensively studied and comprehensively reviewed (Todaka et al. 2015; Thatcher et al. 2016; Blum 2017; Kalaji et al. 2018). Roots perceive a water deficit in soil and communicate this environmental constraint as a stress signal toward the shoot, most likely through the xylem. Moreover, root exudation has also been suggested to play a primary role in some nutrient acquisition mechanisms operating in water deficit soils. Among these exudations, the K-concentration and organic acids play a crucial role in improving osmoregulation (Sánchez-Rodríguez et al. 2014; Altunlu and Gul 2012). In particular, the release of organic acids from roots may undergo complexation reactions with target metals or nontarget metals to complex metals in solution (Colla et al. 2010) and induce the dissolution of unavailable, insoluble nutrients such as ferric oxyhydroxides (Jones et al. 1996).

One way to avoid or minimize yield losses in production caused by drought conditions would be to graft plants onto rootstocks capable of increasing the water use efficiency (WUE) of crops (Kumar et al. 2017; Pagliarani et al. 2017). Because appropriate and compatible rootstocks can enhance plant performance by improving both nutrient acquisition and utilization efficiency, grafting is widely used as an alternative to breeding in horticultural crops (Albacete et al. 2015; Nawaz et al. 2016; Huang et al. 2016a; Rouphael et al. 2018).Owing to the vigor of rootstocks, compared with self-rooted plants, grafted plants usually show an increased

✉ Kun Xu
xukun@sdau.edu.cn

1 College of Horticulture Science and Engineering, Shandong Agricultural University, Tai'an 271018, People's Republic of China

2 Collaborative Innovation Center of Fruit & Vegetable Quality and Efficient Production in Shandong, Tai'an, People's Republic of China

3 State Key Laboratory of Crop Biology, Key Laboratory of Biology and Genetic Improvement of Horticultural Crops in Huanghuai Region, Ministry of Agriculture and Rural Affairs, Tai'an, People's Republic of China

uptake of water and minerals under favorable growth conditions (Martínez-Ballesta et al. 2010). For example, Colla et al. (2010) reported that watermelon plants grafted onto bottle gourd rootstocks and ungrafted plants in a high pH (8.1) nutrient solution showed a significant decrease in leaf macronutrient concentration, especially for P and Mg, compared to plants grafted onto pumpkin rootstocks. Moreover, Sánchez-Rodríguez et al. (2014) indicated that the susceptible tomato scion "Josefina" when grafted onto drought-tolerant "Zarina" rootstocks, increased the accumulation of macro and micronutrients (N, P, K, Fe and Cu). In addition, working with similar plants, Sánchez-Rodríguez et al. (2013) concluded that drought-tolerant seedlings could stimulate nitrate reductase (NR) activity and NO_3^- assimilation.

Given that tomato is one of the most important vegetable crops cultivated worldwide, its large growth volume requires significant water resources (Ma et al. 2017; Jiang et al. 2017). However, primary production of tomatoes is concentrated in semiarid regions, where water stress is frequent, so it is of great interest to ascertain the effective method to improve water-stress tolerance. Many studies have enhanced the drought resistance of tomato plants by overexpressing certain genes or proteins in the plant (Wang et al. 2014; Zhao et al. 2018; Wang et al. 2018a, b), but this method is costly, time-consuming, and difficult to operate. So in this experiment, we first selected the corresponding rootstocks and try to achieve biological water saving through tomato grafting cultivation. In previous experiments, we observed that tomato plants grafted onto drought-tolerant rootstocks showed higher water utilization efficiency and higher yield compared with those grafted onto drought-sensitive rootstocks under water stress (Zhang et al. 2017, 2019a, b). The goal of biological water saving was achieved by selecting the corresponding rootstock for graft cultivation. From this perspective, the aim of the present work is to determine the response of reciprocal grafts to moderate water stress between a drought-tolerant genotypes 'T' and a more sensitive cultivar 'S' examining the uptake and concentration of nutrients in different treatments.

Materials and methods

Plant material and experimental design

The experiment was carried out in a solar greenhouse located in Shandong Agricultural University in Tai'an (36° 09′ N, 117° 09′ E), eastern China, in Spring and Autumn of 2018. Two different tomato genotypes, drought-tolerant ('606', T) and drought-sensitive ('112', S), were used as plant materials (Zhang et al. 2019a, b). The experimental design was split-plot, the main plot was grafting composed of the self-rooted grafting tomato ('T/T' (scion/rootstock) and 'S/S') and reciprocal grafting ('T/S' and 'S/T'), and the subplot was soil water content composed of 40% (drought) and 80% (watered) treatments. The tomatoes were grown in plastic pots (diameter 110 mm, height 120 mm; one seedling per pot) containing 0.5 kg of substrate composed of sandy loam-soil/peat mixture (1:1, v/v). The plants were watered twice daily at 9:00 and 17:00, using the pot weighing method to estimate soil moisture. Each treatment was replicated three times, and each replicate included ten seedlings. After 15 days of water treatment, every five plants were randomly selected from each treatment to collect root and leaf samples for the measurement of related indicators.

Soil moisture and root activity

Diurnal variation of soil moisture under different water treatments was measured using an integrated meteorological instrument (SP350, LSI LASTEM S.r.l., Italy). Following 15 days of drought treatment, the root tips were dyed using Evans blue according to the method of Baker and Mock (1994). The tomato root tips were removed from treatments cleanly and submerged in a 0.025% Evans blue solution for 30 min. The roots were washed several times to wash off the excess dye using deionized water and then observed and photographed under an optical microscope (SZX16, Olympus Corporation, Tokyo, Japan).

For the root vigor measurements, 5 strains of the root were selected from each treatment using the TTC (2,3,5-triphenyltetrazolium chloride) method to determine root vigor (Zou 1993). First, 0.5 g of apical roots were weighed and cut into 2 cm long segments and then placed into graduated test tubes, followed by the addition of 5 mL of 0.4% TTC solution and 5 mL of M/15 phosphate buffer. Later, the root was fully immersed in the liquid and maintained for 4 h at 37 °C. Then, 2 mL of 1 mol L^{-1} sulfate was added for 15 min to terminate the reaction. Afterwards, the roots were removed, wiped dry and placed into the original test tube; 10 mL of 95% ethyl alcohol was added, followed by extraction for 24 h for root whitening. The colorimetric method was used at a wavelength of 485 nm using an ultraviolet spectrophotometer (UV-2450, SHIMADZU, Japan) to obtain the absorbance values.

Analysis of root growth

The roots were washed free of soil to scan the entire root and then dried to measure the root dry weight. Root scanning can measure the root length, surface area, volume, average diameter rand root tip number. Roots were stained with pure water and scanned with a digital scanner (STD4800, RegentInc., Canada) to generate high-definition digital images. Images were analyzed using WinRHIZO™ Basic software (Regent Instruments Inc., Quebec, QC, Canada) for root length.

Mineral analysis

Plant tissues (leaf, and root) were dried for 48 h at 75 °C and ground separately in a Wiley mill to pass through a 20-mesh screen. Then, 0.5 g of the dried plant tissues were analyzed for the following major and minor elements: N, P, K, Ca and Mg. The nitrogen concentration in the plant tissues was determined after mineralization with sulfuric acid by the "Kjeldahlmethod" (Bremner 1965). Phosphorus concentrations were determined by titration with molybdenum antimony reagent in the presence of dinitrophenol (Su-Cheng et al. 1990). K, Ca and Mg concentrations were determined by dry ashing at 400 °C for 24 h, dissolving the ash in 1:20 HNO_3, and assaying the solution obtained using an inductively coupled plasma emission spectrometer (iCAP7000 SERIES; Thermo SCIENTIFIC).

Measurement of carbohydrate and organic acids

The carbohydrates sucrose, fructose and glucose and the main organic acids in tomato leaves and roots were measured by capillary zone electrophoresis (Pharmaceutical Analysis System, PA 800 plus, BECKMAN COULTER, USA). Fresh samples of different treatments were ground and centrifuged at 4 °C and 10,000 rpm for 15 min, and the supernatant was diluted appropriately. Before injection in the capillary, all solutions were degassed in an ultrasonic bath and forced through a 0.22 µm membrane filter. To measure carbohydrate and organic acids, hydrodynamic injection at 0.5 psi for 3 s and 18 s was used, and the detection wavelengths were 254 nm and 200 nm, respectively. The separation was performed at − 8.1 kV and − 9 kV at 25 °C. Between injections, the capillary was rinsed with separation buffer for 2 min.

Statistical analysis

All data were statistically analyzed by a two-way ANOVA using the DPS software package (DPS for Windows, 2009). The differences between the means were carried out by Duncan's multiple range test at $P < 0.05$.

Results

Soil moisture conditions and plant growth

The dynamic change in the soil water content under different water treatments in one day is shown in Fig. 1. Under drought conditions, the grafting of different genotypes of tomato seedlings affected the decrease of root vigor significantly (Fig. 2). Drought stress severely decreased the plant root dry weight and root vigor of all cultivars after treatment

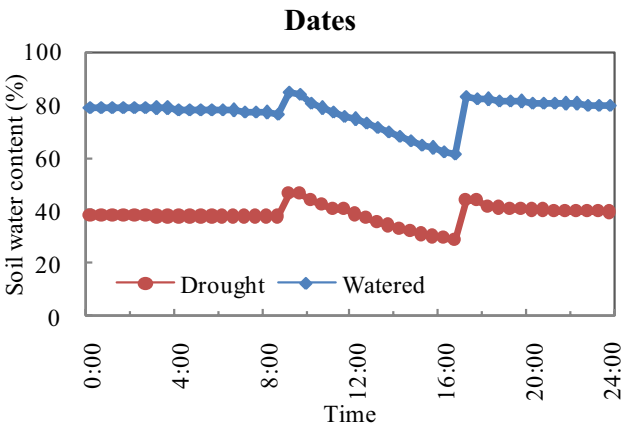

Fig. 1 Daily variation of soil moisture under different water treatments

for 15 days compared with the watered treatment. Grafting with 'T' seedlings as rootstock, on the other hand, significantly alleviated the deleterious effect of drought stress on the root dry weight and root vigor of both 'T/T' and 'S/T'. The plant root dry weight of different grafting combinations was significantly different from those of the 'T/S' and 'S/S' treatments under drought conditions. The tomato plant root vigor of 'T/S' and 'S/T' grafting combinations was decreased by 5.65% and 1.65% compared with T/T treatments and increased by 5.80% and 10.28% compared with S/S, respectively, under drought conditions (Fig. 2b).

Root growth

In Table 1, the length, volume of root and surface area of root were highly influenced by the grafting combination, soil water content and their interaction. However, the average diameter of the root was highly influenced by the soil water content but was not significant in the grafting combination and the grafting × water interaction, whereas the root tip number was significantly affected by the grafting combination and soil water content, with no significant grafting × water interaction. In grafted plants, the length, surface area, volume, average diameter of the root and root tip number decreased in response to drought stress. Moreover, under drought stress, the growth reduction of roots in comparison to the watered treatment were clearly lower in the plants with the grafting combinations of 'S/T' and 'T/T' compared to those plants with the combinations 'T/S' and 'S/S', while grafting combination 'S/S' showed the highest percentage reduction of root growth among treatments. For example, under drought condition the root surface area in 'T/T' and 'S/T' treatments were 512.81 cm^2 and 463.93 cm^2, respectively, while in the grafting combination 'T/S' and 'S/S' the root surface area were 445.23 cm^2 and 364.03 cm^2,

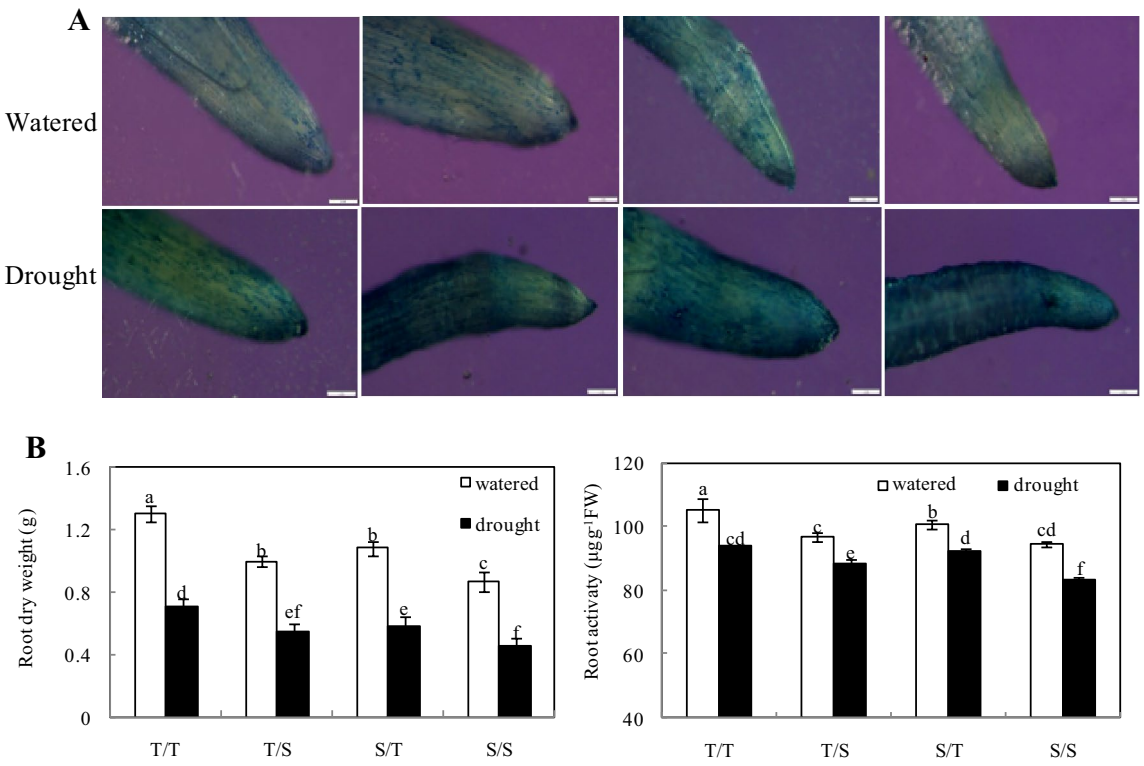

Fig. 2 Effect on roots of tomato grafting onto different drought-tolerant seedlings under water stress. **a** Evans blue dyeing pictures in root tips of tomato under different stresses. **b** Root dry weight and root vigor. All data were determined 15 days after drought treatment. The data are mean ± SD and the different letters indicate a significant difference at $P < 0.05$ according to Duncan's test. (Color figure online)

Table 1 Effects of grafting combination and drought stress on root growth of tomato plants

Grafting combination	Soil water content (%)	Length (cm)	Surface area (cm^2)	Volume (cm^3)	Average diameter (mm)	Root tips number
T/T	40	1793.04 ± 16.55	512.81 ± 5.46	34.27 ± 0.99	0.70 ± 0.01	3099.5 ± 27.57
	80	2505.80 ± 45.97	847.16 ± 4.73	51.76 ± 0.92	0.79 ± 0.01	4193 ± 67.88
	Mean	2149.43a	679.99a	43.02a	0.75a	3646.25a
T/S	40	1377.33 ± 29.67	445.23 ± 7.45	26.74 ± 0.52	0.71 ± 0.03	2737 ± 135.76
	80	2314.94 ± 35.34	813.95 ± 5.49	48.05 ± 0.10	0.77 ± 0.01	3901 ± 24.04
	Mean	1846.14c	646.01c	37.40c	0.75a	3319.00bc
S/T	40	1617.55 ± 28.97	463.93 ± 9.47	30.75 ± 1.57	0.76 ± 0.01	2935 ± 149.9
	80	2410.98 ± 16.12	828.08 ± 5.91	49.04 ± 0.57	0.77 ± 0.02	3990 ± 33.94
	Mean	2014.27b	629.60b	39.90b	0.77a	3462.50b
S/S	40	1297.71 ± 22.04	364.03 ± 12.58	24.23 ± 0.19	0.73 ± 0.02	2483.5 ± 146.37
	80	2210.69 ± 19.79	802.71 ± 7.38	46.99 ± 0.32	0.77 ± 0.01	3819 ± 104.65
	Mean	1754.20d	583.37d	35.61d	0.76a	3151.25c
Variance analysis		P value				
Grafting combination		0.0003	0	0.0026	0.2868	0.0015
Soil water content		0	0	0	0.0006	0
Grafting × water		0.0120	0.0002	0.0237	0.0790	0.3154

Values are means ± SD of three replicates. The different letters indicate a significant difference at $P < 0.05$ according to Duncan's test. The P value is obtained by multiple comparison and representing significant or nonsignificant, respectively

respectively. In addition, plants grafted onto 'T' rootstocks showed higher root length, surface area and volume of the root when treated with drought stress.

Mineral composition and partitioning

The concentration and distribution of major elements in tomato plants as a function of the grafting combination and soil water content are displayed in Table 2. There were significant differences in the content of major elements in the leaves between different grafting treatments. Drought stress significantly decreased the N, P and K concentrations in leaves and increased the K concentration in roots. The concentrations of N, P and K in leaves and roots were significantly affected by the grafting combination, the soil water content and the grafting × water interaction. Under different soil water contents, the N content in T/T and S/T treatments were higher at 16.36 mg g^{-1} and 15.97 mg g^{-1}, respectively. While The concentration of N in roots was not significantly affected in the 'T/T' and 'S/T' treatments, and a significant decrease in N concentration was recorded in the 'T/S' and 'S/S' treatments. In addition, the content of P and K was significantly affected in the four grafted plants, and both contents were highest in roots of the 'T/T' treatment at 1.84 mg g^{-1} and 47.80 mg g^{-1} (Table 2).

The concentrations of Ca and Mg in both plant tissues were significantly affected by the grafting combination, the soil water content and the grafting × water interaction. In leaves, the Ca and Mg concentrations decreased as the soil water content decreased from 80 to 40%, while they increased in roots. In addition, the Ca and Mg concentrations in the leaves and roots were significantly higher in the 'T/T' and 'S/T' treatments than in the 'T/S' and 'S/S' treatments (Table 2).

The carbohydrate content

For the concentration of carbohydrates (sucrose, fructose, glucose and total carbohydrates), the effect of the water stress treatment was highly significant (Table 3). The concentrations of sucrose, fructose, glucose and total carbohydrates increased in both leaves and roots, followed by soil water content reduction; all showed higher levels in the leaves. Except for the concentration of sucrose in leaves, glucose in roots and fructose both in leaves and roots, which were all highly influenced by the soil water content and the grafting combination but were not significant in their interaction, the other carbohydrates were significantly affected by the grafting combination, soil water content and the grafting × water interaction. Regardless of the leaves or roots, the concentration of carbohydrates in plants grafted onto 'T' rootstocks was higher than that in plants grafted onto 'S' rootstocks. The total carbohydrate content in the leaves of 'T/T' treatment with the highest performance at 102.34 mg g^{-1}, was higher by 15.35%, 45.93% and 72.26% compared with S/T, T/S and S/S, respectively.

Organic acids in leaves and roots

Six types of organic acids, namely, oxalic, succinic, citric, malic, tartaric acid and proline, were detected in the tomato plant tissues, as shown in Table 4. Drought stress condition induced a larger increase in organic acids. The organic acids in leaves and roots were significantly influenced by the grafting combination and soil water content but not by the grafting × water interaction, while the proline content was affected by the grafting combination, soil water content and the grafting × water interaction.

Moreover, when averaged over the water content, the oxalic and succinic acid concentrations in leaves were significantly higher in 'T/T'(avg. 0.31 and 0.66 mg g^{-1}), followed by 'S/T' (avg. 0.27 and 0.61 mg g^{-1}), 'T/S' (avg. 0.22 and 0.57 mg g^{-1}), and finally 'S/S' (avg. 0.19 and 0.55 mg g^{-1}). The concentration of citric acid was significantly higher in plants grafted onto 'T' rootstock seedlings, with the highest values recorded on 'T/T' and 'S/T' grown under ordinary watering conditions. Furthermore, the concentrations of malic, tartaric acid and proline were significantly higher in 'T/T', with the highest average values recorded: 2.93, 0.31 and 0.25 mg g^{-1}.

In addition, compared with leaves, the concentrations of oxalic and tartaric acid were higher in roots, while the concentrations of succinic, citric, and malic acid and proline decreased in roots. When averaged over the grafting combination, decreasing the soil water content significantly increased the root concentrations of xalic, succinic, citric, malic, and tartaric acid sand proline. When averaged over the water content, the organic acid in roots was significantly higher in 'T/T', especially for malic acid (avg. 1.48 mg g^{-1}), while the lowest values for the organic acids were recorded with plants grafted onto 'S' rootstock, particularly the 'S/S' treatment.

Discussion

Researchers have demonstrated that plants respond to the reduction in soil water content with decreased shoot and root growth (Alexieva et al. 2010; Clauw et al. 2016; Andrade et al. 2017; Moles et al. 2018). In the present experiment, significant depression of root vigor and root growth under drought stress-treated tomato plants was observed, and that effect varied with different grafting combinations. Under drought stress conditions, root vigor and root growth reductions in comparison to the control (watered treatment) were clearly lower in 'T/T' and 'S/T' than in 'T/S' and 'S/S'.

Table 2 Effects of grafting combination and drought stress on the concentration of major element in leaves and roots of tomato plants

Grafting combination	Soil water content (%)	Leaves					Roots				
		N (mg g^{-1})	P (mg g^{-1})	K (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})	N (mg g^{-1})	P (mg g^{-1})	K (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})
T/T	40	15.87 ± 0.08	1.54 ± 0.003	34.00 ± 0.34	43.25 ± 0.89	7.85 ± 0.07	11.28 ± 0.04	1.72 ± 0.006	51.23 ± 1.75	20.00 ± 0.21	17.01 ± 0.03
	80	16.84 ± 0.15	1.81 ± 0.013	35.40 ± 0.06	51.82 ± 0.40	8.92 ± 0.02	12.19 ± 0.12	1.95 ± 0.009	44.36 ± 0.67	18.02 ± 0.10	14.27 ± 0.22
	Mean	16.36a	1.68a	34.71a	47.54a	8.39a	11.74a	1.84a	47.80a	19.02a	15.69a
T/S	40	15.02 ± 0.06	1.47 ± 0.007	30.28 ± 0.08	38.12 ± 0.65	7.58 ± 0.10	10.61 ± 0.08	1.63 ± 0.007	49.16 ± 0.59	18.61 ± 0.04	14.73 ± 0.62
	80	16.21 ± 0.12	1.70 ± 0.009	35.34 ± 0.06	41.90 ± 1.20	8.03 ± 0.16	11.62 ± 0.07	1.89 ± 0.006	40.81 ± 1.07	17.50 ± 0.09	13.76 ± 0.15
	Mean	15.62c	1.59c	32.81c	40.02c	7.81c	11.12b	1.76c	44.99bc	18.06c	14.50c
S/T	40	15.38 ± 0.03	1.51 ± 0.008	32.04 ± 0.76	41.03 ± 1.08	7.78 ± 0.11	11.34 ± 0.19	1.68 ± 0.003	50.13 ± 0.24	19.25 ± 0.04	15.90 ± 0.28
	80	16.56 ± 0.06	1.77 ± 0.008	34.39 ± 0.18	49.78 ± 0.78	8.53 ± 0.08	11.80 ± 0.04	1.92 ± 0.01	42.04 ± 0.31	17.82 ± 0.06	14.16 ± 0.131
	Mean	15.97b	1.65b	33.21b	45.41b	8.16b	11.57a	1.80b	46.09b	18.54b	15.06b
S/S	40	12.61 ± 0.48	1.42 ± 0.008	28.96 ± 0.43	36.02 ± 0.89	7.00 ± 0.083	10.02 ± 0.17	1.59 ± 0.004	48.8 ± 1.81	18.21 ± 0.07	14.33 ± 0.13
	80	15.69 ± 0.19	1.60 ± 0.020	32.06 ± 0.16	40.71 ± 0.50	7.78 ± 0.03	11.45 ± 0.06	1.87 ± 0.002	39.71 ± 1.16	17.33 ± 0.06	13.19 ± 0.31
	Mean	14.15d	1.52d	30.52d	38.37d	7.39d	10.74c	1.74d	44.26c	17.78d	13.79d
Variance analysis		P value									
Grafting combination		0	0.0001	0.0001	0.0007	0.0025	0	0.0001	0.0009	0.0003	0.0012
Soil water content		0	0	0.0001	0.0002	0.0001	0	0	0	0	0.0007
Grafting × water		0.0006	0.0319	0.0084	0.0423	0.0419	0.0052	0.0414	0.0007	0.0135	0.0014

Table 3 Effects of grafting combination and drought stress on the carbohydrate contents in leaves and roots of tomato plants

Grafting combination	Soil water content (%)	Leaves				Roots			
		Sucrose (mg g^{-1})	Fructose (mg g^{-1})	Glucose (mg g^{-1})	Total carbohydrate (mg g^{-1})	Sucrose (mg g^{-1})	Fructose (mg g^{-1})	Glucose (mg g^{-1})	Total carbohydrate (mg g^{-1})
T/T	40	41.64 ± 0.29	7.55 ± 0.10	20.55 ± 1.95	149.63 ± 12.74	27.81 ± 1.80	4.91 ± 0.19	12.20 ± 0.29	73.16 ± 0.65
	80	22.66 ± 0.61	4.29 ± 0.25	7.05 ± 0.39	55.05 ± 3.46	13.72 ± 1.19	2.66 ± 0.22	4.32 ± 0.27	45.67 ± 0.22
	Mean	32.15a	5.92a	13.81a	102.34a	20.77a	3.79a	8.26a	59.42a
T/S	40	30.52 ± 2.07	5.51 ± 0.26	14.65 ± 0.46	97.49 ± 3.39	17.96 ± 0.89	3.39 ± 0.06	10.22 ± 0.42	59.92 ± 0.43
	80	18.38 ± 0.18	3.45 ± 0.36	5.65 ± 0.21	42.76 ± 2.87	9.69 ± 0.78	1.80 ± 0.04	3.22 ± 0.10	37.47 ± 0.91
	Mean	24.46c	4.48c	10.15bc	70.13c	13.83bc	2.60c	6.73c	48.70c
S/T	40	38.96 ± 2.63	6.50 ± 0.11	16.92 ± 0.43	130.15 ± 0.51	20.09 ± 0.53	4.13 ± 0.22	10.98 ± 0.50	66.85 ± 0.97
	80	20.64 ± 0.33	3.94 ± 0.19	6.15 ± 0.10	47.29 ± 2.06	11.97 ± 0.27	2.07 ± 0.20	3.57 ± 0.07	42.50 ± 1.91
	Mean	29.81ab	5.23b	11.54b	88.72b	16.03b	3.10b	7.28b	54.68b
S/S	40	35.35 ± 1.78	4.99 ± 0.27	12.92 ± 0.23	85.00 ± 4.27	15.87 ± 1.19	3.12 ± 0.04	8.38 ± 0.44	56.25 ± 1.53
	80	19.42 ± 0.09	2.89 ± 0.06	4.62 ± 0.09	33.81 ± 4.95	8.22 ± 0.11	1.56 ± 0.07	2.46 ± 0.26	32.55 ± 2.14
	Mean	27.39bc	3.94d	8.77c	59.41d	12.05c	2.34c	5.43d	44.40d
Variance analysis		*P* value							
Grafting combination		0.0198	0.0007	0.0157	0.0007	0.0076	0.0038	0.0008	0.0037
Soil water content		0	0.0001	0	0	0	0	0	0
Grafting × Water		0.0617	0.1137	0.0137	0.0283	0.0151	0.0712	0.075	0.0103

Table 4 Effects of grafting combination and drought stress on the content of organic acid in leaves and roots of tomato

Grafting combination	Soil water content (%)	Leaves						Roots					
		Oxalic Acids (mg g^{-1})	Succinic Acids (mg g^{-1})	Citric Acids (mg g^{-1})	Malic Acids (mg g^{-1})	Tartaric Acids (mg g^{-1})	Proline (mg g^{-1})	Oxalic Acids (mg g^{-1})	Succinic Acids (mg g^{-1})	Citric Acids (mg g^{-1})	Malic Acids (mg g^{-1})	Tartaric Acids (mg g^{-1})	Proline (mg g^{-1})
T/T	40	0.40 ± 0.01	0.75 ± 0.01	2.73 ± 0.13	3.94 ± 0.10	0.40 ± 0.01	0.32 ± 0.01	0.89 ± 0.1	0.57 ± 0.01	0.92 ± 0.16	2.18 ± 0.06	0.60 ± 0.01	0.25 ± 0.01
	80	0.22 ± 0.01	0.57 ± 0.01	1.89 ± 0.12	1.91 ± 0.13	0.21 ± 0.01	0.18 ± 0.00	0.59 ± 0.2	0.36 ± 0.01	0.34 ± 0.04	0.77 ± 0.11	0.22 ± 0.01	0.12 ± 0.01
	Mean	0.31a	0.66a	2.31a	2.93a	0.31a	0.25a	0.74a	0.47a	0.63a	1.48a	0.42a	0.19a
T/S	40	0.28 ± 0.02	0.64 ± 0.01	2.18 ± 0.02	3.31 ± 0.1	0.31 ± 0.01	0.26 ± 0.01	0.77 ± 0.00	0.42 ± 0.06	0.55 ± 0.03	1.67 ± 0.07	0.50 ± 0.02	0.21 ± 0.01
	80	0.16 ± 0.01	0.48 ± 0.01	1.46 ± 0.07	1.03 ± 0.17	0.18 ± 0.01	0.16 ± 0.00	0.51 ± 0.01	0.33 ± 0.01	0.29 ± 0.04	0.60 ± 0.04	0.18 ± 0.01	0.10 ± 0.00
	Mean	0.22c	0.57bc	1.83b	2.17c	0.25b	0.21c	0.64bc	0.38bc	0.43bc	1.14b	0.35c	0.15bc
S/T	40	0.34 ± 0.01	0.70 ± 0.04	2.49 ± 0.12	3.49 ± 0.02	0.33 ± 0.01	0.29 ± 0.01	0.80 ± 0.03	0.47 ± 0.02	0.78 ± 0.11	1.98 ± 0.08	0.55 ± 0.01	0.228 ± 0.00
	80	0.20 ± 0.04	0.51 ± 0.02	1.78 ± 0.02	1.50 ± 0.01	0.19 ± 0.01	0.17 ± 0.01	0.54 ± 0.01	0.34 ± 0.00	0.3 ± 0.04	0.64 ± 0.09	0.19 ± 0.01	0.10 ± 0.00
	Mean	0.27b	0.61b	2.14a	2.50b	0.27b	0.23b	0.67b	0.41ab	0.54ab	1.32ab	0.38b	0.17b
S/S	40	0.24 ± 0.01	0.63 ± 0.01	2.17 ± 0.03	3.13 ± 0.06	0.31 ± 0.02	0.24 ± 0.00	0.70 ± 0.02	0.37 ± 0.03	0.45 ± 0.02	1.81 ± 0.09	0.48 ± 0.00	0.18 ± 0.01
	80	0.14 ± 0.01	0.45 ± 0.02	1.31 ± 0.01	0.90 ± 0.23	0.17 ± 0.01	0.15 ± 0.00	0.49 ± 0.02	0.31 ± 0.01	0.19 ± 0.02	0.62 ± 0.05	0.16 ± 0.00	0.08 ± 0.01
	Mean	0.19d	0.55c	1.75b	2.02c	0.24b	0.19d	0.60c	0.34c	0.33c	1.22b	0.32c	0.13c
Variance analysis		P value											
Grafting combination		0.0025	0.0098	0.0208	0.0050	0.0150	0.0015	0.0114	0.0239	0.0428	0.0324	0.0048	0.0043
Soil water content		0.0002	0	0	0	0	0	0	0.0009	0.0004	0	0	0
Grafting × water		0.1527	0.5067	0.1161	0.2788	0.0635	0.0040	0.0985	0.0805	0.0805	0.0626	0.0895	0.0011

Underground stresses allow the plant to have a greater root surface area and longer root length for the absorption of water and nutrients (Xiong et al. 2002); an increase in the root growth rate then leads to increasing the root:shoot ratio (Xu et al. 2015; Wang et al. 2018a, b).

It is a common phenomenon that plant growth decreased slowly by scion-rootstock grafting in vegetable crops in response to abiotic stress (Schwarz et al. 2010; Singh and Agrawal 2016; Marsic et al. 2018), particularly in plants grafted onto drought-tolerant rootstock. For instance, Martínez-Ballesta et al. (2010) suggested that in vegetable plants, the enhancement of growth and plant yield by rootstock is mainly due to their strong access to soil nutrients as a consequence of the vigorous root system used as rootstock. Koevoets et al. (2016) demonstrated that a deep root system has shown beneficial effects on plant growth by acquiring water stored in deeper soil layers, thus leading to more drought tolerance. In the present study, we observed that root growth was significantly decreased under drought stress; in particular, the length, surface area and volume of the roots changed greatly in response to different grafting combinations, with the highest value for 'T/T', followed by 'S/T'. The results demonstrated that drought-tolerant rootstocks contributed to greater root length, surface area and volume for the absorption of water and nutrients (Colla et al. 2010; Huang et al. 2016b; Pompeiano and Patton 2017).

In general, under water stress, the restricted transpiration rate and increased membrane permeability and active transport depressed the nutrient absorption and transport in the plant (Sánchez-Rodríguez et al. 2013, 2014; Kumar et al. 2017). However, grafted plants are capable of increasing the uptake and translocation of nutrients as a result of the enhancement of vigor by the rootstock's root system and its effects on plant growth (Savvas et al. 2010). Even though the concentrations of N, P, K, Ca and Mg in leaves were significantly reduced under drought conditions, when averaged, the water content of the plants grafted onto 'T' rootstocks ('T/T and 'S/T') had a higher nutrient concentration than those grafted onto 'S' rootstocks('S/S and 'T/S'). This suggests that plants grafted onto 'T' rootstocks enhance the uptake and translocation of nutrients toward the shoot.

According to our research, the results showed that the content of K, Ca and Mg in leaves significantly decreased under drought conditions but increased in the root system (Table 2). This result indicated that the root system would enhance the absorption of mineral elements under a certain degree of drought conditions. Higher concentrations of K, Ca and Mg can increase the content of inorganic osmotic adjustment substances, maintain the stability of the cell membrane structure, reduce the osmotic potential of root xylem and promote root water absorption. In addition, the plants grafted onto 'T' rootstocks exhibited higher uptake and accumulation of K, Ca and Mg than those grafted onto 'S' rootstocks. Sánchez-Rodríguez et al. (2014) had similar results with plants of susceptible tomato grafted onto drought-tolerant rootstocks, observing higher accumulation of macro and micronutrients (N, P, K, Fe, and Cu) in grafted plants.

Carbohydrates are the material basis for plant metabolism. From the perspective of energy metabolism, an important reason for the decrease in plant dry weight under stress was the reduction of carbon assimilation products caused by the decrease in photosynthesis (Nebauer et al. 2011; Jover et al. 2012). Among them, sucrose is one of the main products of plant photosynthesis (Choudhury et al. 2010; Tauzin and Giardina 2014). Maintaining a dynamic balance between sucrose synthesis, transport, distribution, and use is important for normally growing plants. However, under environmental stress, soluble sugar and starch tend to accumulate in large amounts, and negative feedback inhibition of photosynthesis leads to slowing of plant growth (Nafziger and Koller 1976; Rook et al. 2010; Richter et al. 2015). In our previous study we fund that under drought stress, grafted plants with drought-tolerant tomato seedlings enhanced photosynthetic capacity of plants through a series of active oxygen metabolism regulation (Zhang et al. 2019a). Higher photosynthetic capacity is conducive to the accumulation of photosynthetic products, leading to an increase in the content of carbon compounds in the plants. In this study, drought stress increased the soluble sugar and the accumulation of sucrose in the leaves. These soluble sugars can participate in the osmotic adjustment of plants and facilitate the absorption of water by plants under stress conditions (Farhangiabriz and Torabian 2017; Abdellaoui et al. 2018).

Working with watermelon, Colla et al. (2010) concluded that the plants grafted onto pumpkins under high pH levels, facilitating higher nutrient uptake (higher P and Mg), were associated with the considerable exudation of organic acids by roots into the soil. Jones (1998) reported that organic acids in the rhizosphere promoted the mobilization and uptake of nutrients by plants and microorganisms. Our results showed that as soil moisture decreased, the concentration of organic acids, such as oxalic, succinic, citric, malic, tartaric acids and proline, in tomato leaves and roots increased. Our results are consistent with the findings of Venekamp et al. (1989), who reported that in field bean plants, organic acids as sources for drought-induced proline synthesis cause the concentrations to increase due to water loss. In the current study, the plants grafted onto 'T' rootstocks contained higher concentrations of organic acids than those grafted onto 'S' rootstocks. These results support that the higher concentration of organic acids in tomato plants grafted onto 'T' rootstocks accumulated more osmotic adjustment substances and increased root osmotic potential, which was conducive to absorbing water in the soil. If the organic acid content was too high, the Ca element absorbed

by the plant can neutralize excess intermediate metabolites accumulated in the plant to form insoluble calcium salts such as calcium oxalate, calcium citrate and calcium malate, thereby resulting in regulating pH, eliminating acid toxicity, and maintaining the intracellular environment (Sagoe et al. 1998).

Conclusion

Our results demonstrate that drought stress caused a reduction in root vigor and a decrease in nutrient concentrations in the leaves, which then inhibited root growth and consequently reduced the growth rate of tomato plants. However, the plants grafted onto 'T' rootstocks ('T/T and 'S/T') improved the growth of tomato plants under drought stress by regulating nutrient transport and distribution. 'T' rootstock-grafted plants under drought conditions enhanced the absorption of mineral elements and increased the accumulation of carbohydrates and organic acids, thereby achieving higher osmotic potential of the plant roots and maintaining cell structure, thus contributing to improving plant growth and stress resistance.

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