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Temporal and Tissue-Specific Expression of Tomato 14-3-3 Gene Family in Response to Phosphorus Deficiency*[1]

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ABSTRACT

Plants adapt to phosphorus (P) deficiency through a complex of biological processes and many genes are involved. Tomato (*Solanum lycopersicum* L. 'Hezuo906') plants were selected to grown hydroponically to study the temporal and spatial gene expression patterns of the 14-3-3 gene family and their roles in response to P deficiency in tomato plants. Using real-time reverse-transcriptase polymerase chain reaction (RT-PCR), we investigated the expression profiles in different tissues (root, stem and leaf) at short-term and long-term P-deficient stress phases. Results revealed that i) four members of 14-3-3 gene family (*TFT1*, *TFT4*, *TFT6* and *TFT7*) were involved in the adaptation of tomato plants to P deficiency, ii) *TFT7* responded quickly to P deficiency in the root, while *TFT6* responded slowly to P deficiency in the leaf, iii) expression response of *TFT4* to P-deficient stress was widely distributed in different tissues (root, stem and leaf) while *TFT8* only displayed stem-specific expression, and iv) temporal and tissues-specific expression patterns to P deficiency suggested that isoform specificity existed in tomato 14-3-3 gene family. We propose that *TFT7* (one member of ε-like group in tomato 14-3-3 family) is the early responsive gene and may play a role in the adaptation of tomato plants to short-term P deficiency, while *TFT6* (one member of non-ε group in tomato 14-3-3 family) is the later responsive gene and may play a role in the adaptation of tomato plants to long-term P deficiency.

Key Words: isoform specificity, mRNA, P starvation, primer pairs, real-time RT-PCR

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INTRODUCTION

Phosphorus (P) deficiency is one of the major factors limiting plant growth in many ecosystems and a major soil constraint for agricultural productivity (Devaiah *et al.*, 2007b; Schachtman and Shin, 2007). In order to survive, plants have developed flexible strategies to cope with soil P deficiency, and thus improve P mobilization and uptake from the soil (Vance *et al.*, 2003; Wu *et al.*, 2003; Hoffland *et al.*, 2006). These strategies include the production of enzymes, such as phosphatases and nucleases; symbiotic relationship with mycorrhizal fungi; increased synthesis and secretion of organic acids; enhanced expression of high-affinity P transporters; plasma membrane H^+-ATPase-dependent proton extrusion; a larger root system capable of exploring greater soil volume to ensure a sufficient uptake of P; and a differential distribution of photosynthates between shoots and roots, which results in increased root-shoot ratios due to the prolific growth of lateral roots. Researches in this area in recent decades have led to the identification of structural genes of primary importance for P acquisition and utilization (Desnos, 2008). Further, it is apparent that P acquisition and utilization are a highly regulated and complex set of processes relying not only on P transporters and enzymes involved in P assimilation and utilization, but also on powerful regulatory mechanisms controlling the abundance and activity of transporters and enzymes (Sánchez-Calderón *et al.*, 2006; Devaiah *et al.*, 2007a).

14-3-3 proteins (a family of regulatory proteins) are phosphoserine-binding proteins that regulate the activities of a wide array of targets *via* direct protein-protein interactions (Chevalier *et al.*, 2009). Plant 14-3-3 proteins bind to a range of transcription factors and other signaling proteins, and play roles in regulating plant development and stress responses (Roberts,

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2003; Xu *et al.*, 2008). Some recent studies have suggested that in higher plants, 14-3-3 proteins may play an important role in response to P deficiency. For example, the activities of P transporters are modulated by signaling proteins in response to P starvation in higher plants. Signaling proteins are known to interact with P-deficiency-response factors such as protein kinases, phosphatases, and 14-3-3 proteins (Baldwin *et al.*, 2008). Apart from their well-established roles in regulating the activity of the plasma membrane H^+-ATPase, which plays an important role in P acquisition (Yan *et al.*, 2002), 14-3-3 proteins are also regulatory partners of ion channels (Véry and Sentenac, 2003). In addition, in higher plants, 14-3-3 proteins are thought to be involved in carbohydrate metabolism and phytohormone signaling, which play important roles in P-deficient responses (Comparot *et al.*, 2003).

Tomato is becoming the model plant for studying the physiological, biochemical, and molecular biological mechanisms of vegetables (Fei *et al.*, 2006). In tomato plants, 14-3-3 proteins are encoded by a multigene family. To date, at least 12 genes, predicted to encode 14-3-3 proteins, named *TFT1–TFT12*, have been identified (see http://www.lancs.ac.uk/staff/robertmr/tft_ests.htm). Although the 14-3-3 gene family in tomato is highly conserved, tomato 14-3-3 gene family comprises two major groups, a non-ε group and a ε-like group: the *TFT1*/*TFT10*, *TFT5*/*TFT6*, *TFT2*/*TFT3*, and *TFT4*/*TFT11* genes form the non-ε group, and the *TFT7*/*TFT12* and *TFT8*/*TFT9* genes form the ε-like group (Xu and Shi, 2006). Northern blot analysis of the expression of the 14-3-3 gene family in response to fusicoccin stress in tomato leaves revealed that 14-3-3 genes have different expression patterns in leaves after challenge (Roberts and Bowles, 1999). In addition, the expression of *TFT7* (X95905), one of the 14-3-3 gene family members, was induced by phosphorus, potassium and iron deficiencies in tomato roots (Wang *et al.*, 2002; Xu and Shi, 2006). Furthermore, recent results showed that *TFT7* positively regulates immunity-associated programmed cell death by enhancing protein abundance and signaling ability of mitogen-activated protein kinase kinase α (Oh *et al.*, 2010). Although the expression of genes encoding 14-3-3 proteins under biotic and abiotic stresses has been investigated (Roberts *et al.*, 2002), expression profiling of the entire 14-3-3 gene family in response to P deficiency in tomato plants (roots, stems, and leaves) has not been reported.

Previously, Cao *et al.* (2007) reported the gene expression of 14-3-3 gene family in the whole-plant tissue under a single P-deficient treatment point (P-deficient stress for 7 d) using northern blotting; however, little is known about their temporal and spatial expression patterns in response to P deficiency in higher plants. Elucidation of temporal (time-dynamic) and spatial (tissue-specific or cell-specific) expression patterns would lead to a better understanding of the plant 14-3-3 proteins response to P-deficient stress. Therefore, the primary objective of this study was to investigate the expression profiles of 14-3-3 gene family in response in different tomato tissues (root, stem, leaf) to different P-deficient stress phases (short-term or long-term phase) using real-time reverse-transcriptase polymerase chain reaction (RT-PCR).

MATERIALS AND METHODS

Experimental

Tomato (*Solanum lycopersicum* L. 'Hezuo906') plants were grown hydroponically in black pots containing 3 L of modified 20% Hoagland's solution (control). Plants were grown in a controlled environmental growth chamber with the light period of 250 mol m^{-2} s^{-1} photosynthetic photon flux at 25 °C, 70% relative humidity for 16 h (from 6 a.m. to 10 p.m.); and a dark period at 21 °C and 70% relative humidity for 8 h (from 10 p.m. to 6 a.m.). The pH of the solution was adjusted to 6.0 every day, and the solution was renewed every 3 d. After three weeks of growth, nutrient solution to the treatments was deprived of P. Plants were harvested at 0, 0.5, 1, 3, 6, and 9 d after exposed to P deficiency. Control plants grown under nutrient-sufficient conditions were also harvested at the same time points. The root, stem, and leaf of the plants were then separated, frozen, and stored at −80 °C until later analysis.

Measurements

When the above experimental treatments were finished, tomato plants (roots, stems and leaves) were separated and roots were washed, and then placed in an oven at 105 °C for 0.5 h to inactivate enzymes. To obtain the dry weight, harvested plant parts were dried to a constant weight at 70 °C. The P concentrations of plant tissues were determined colorimetrically by the phosphovanadate method (Hanson, 1950) after digestion in a mixture of HNO_3, $HClO_4$, and H_2SO_4 (3:1:1, v/v/v).

Net photosynthesis rates were measured on the youngest fully expanded leaf using a portable photosynthesis system (Li-6400, Li-COR Inc., Lincoln, NE, USA). For the carbohydrate determination, plant samples were extracted with 0.5 mL of 80% ethanol (v/v) for 20 min at 70 °C, and glucose, fructose, and sucrose

were determined as described by Stitt *et al.* (1989). Starch was extracted from the residue by grinding in 0.5 mL of 50 mol m^{-3} sodium acetate (pH 4.8) containing α-amylase (10 U) and amyloglucosidase (6 U).

After harvesting, the primary root length, lateral root length, root surface area, and root volume were measured using a root analysis instrument (WinRHIZO; Regent Instruments Inc., Quebec, ON, Canada). The proton extrusion rate of tomato roots was analyzed following the method of Jin *et al.* (2009).

Real-time RT-PCR was performed in 25 μL reaction mixture composed of cDNA by using the DNA Engine Opticon 2 system (MJ Research, USA) for continuous fluorescence detection (Xu and Shi, 2006). RNA extraction, cDNA preparation and primer pairs for real-time RT-PCR followed the method of Xu and Shi (2006). Since α-tubulin is a strong and constitutively expressed housekeeping gene in tomato plants (Wang *et al.*, 2001; Coker and Davies, 2003), quantification of the mRNA levels was based on comparison with the level of α-tubulin mRNA. As an additional control, the mRNA levels of a moderately expressed housekeeping gene, phosphoglycerate kinase (Coker and Davies, 2003), were monitored. α-Tubulin mRNA, which was defined as 100 REU (relative expression units), was used as an internal standard in all experiments. The expression level of a gene was defined as the ratio of the copy number of the studied gene to the copy number of α-tubulin multiplied by 100 REU.

Statistical analysis

For each treatment, three replicates were included in the analysis. In addition, the complete experiment was repeated twice at different time under the same growth conditions. Statistical analysis was conducted using SigmaPlot V8.0. Changes in the relative expression levels of gene mRNA and other physiological data were analyzed for statistical significance according to Student's *t*-test ($P < 0.05$).

RESULTS

Tomato growth under P-sufficient and P-deficient conditions

In this study, the dry weight, P concentration and content in tomato roots, stems, and leaves were analyzed under P-sufficient and P-deficient conditions at different days after treatment (0, 0.5, 1, 3, 6, and 9 d) (Table I). During the first 3 d, no significant differences in dry weight, P concentration and P content were observed between P-sufficient and P-deficient conditions. However, the stem weight, leaf weight, P concentration and content were significantly lower under P-deficient than under P-sufficient conditions during the experiment time period from 6 to 9 d of P starvation.

TABLE I

Dry weights, P concentrations, and P contents of tomato roots, stems, and leaves under P-sufficient (+P) or P-deficient (−P) conditions at different days after treatment (DAT)

Treatment	DAT	Dry weight	P concentration	P content
	d	mg $plant^{-1}$	mg P g^{-1}	mg P $plant^{-1}$
Root (+P)	0	50.1±1.0[a)]	5.50±0.19	0.27±0.02
	0.5	50.3±2.1	5.51±0.24	0.28±0.01
	1	51.6±1.6	5.62±0.27	0.29±0.02
	3	55.0±2.5	5.59±0.28	0.31±0.02
	6	59.1±1.0	5.56±0.21	0.33±0.02
	9	61.2±1.1	5.58±0.22	0.34±0.01
Root (−P)	0	50.1±1.9	5.51± 0.21	0.27±0.02
	0.5	50.2±2.1	5.50± 0.19	0.28±0.01
	1	51.9±1.4	5.45±0.20	0.28±0.02
	3	55.2±1.2	5.08±0.26	0.28±0.01
	6	58.5±1.3	4.50±0.22*	0.26±0.01*
	9	60.3±1.8	4.01±0.23*	0.24±0.02*
Stem (+P)	0	126.5±2.1	6.03±0.22	0.76±0.03
	0.5	126.6±1.9	6.01±0.29	0.76±0.04
	1	127.2±1.8	6.12±0.26	0.78±0.05
	3	128.3±1.4	6.08±0.20	0.78±0.03
	6	133.8±1.1	6.06±0.18	0.81±0.05
	9	139.6±1.5	6.10±0.21	0.85±0.06
Stem (−P)	0	126.5±2.0	6.02±0.29	0.76±0.04
	0.5	126.5±1.3	6.00±0.27	0.76±0.05
	1	127.0±1.2	5.98±0.25	0.76±0.03
	3	128.1±1.4	5.69±0.28	0.73±0.06
	6	129.2±1.0*	5.38±0.23*	0.69±0.03*
	9	130.1±1.3*	5.02±0.24*	0.65±0.05*
Leaf (+P)	0	209.8±1.9	6.50±0.26	1.36±0.16
	0.5	209.8±2.1	6.52±0.23	1.37±0.10
	1	210.5±1.5	6.61±0.27	1.39±0.11
	3	213.2±1.4	6.58±0.28	1.40±0.13
	6	219.1±1.3	6.56±0.20	1.44±0.12
	9	226.9±1.1	6.59±0.22	1.50±0.15
Leaf (−P)	0	209.8±1.8	6.52±0.25	1.36±0.15
	0.5	209.9±2.0	6.50±0.19	1.36±0.16
	1	210.5±1.3	6.39±0.24	1.35±0.12
	3	211.9±1.4	6.08±0.30	1.29±0.14
	6	212.8±1.1*	5.52±0.19*	1.17±0.10*
	9	213.9±1.0*	5.01±0.21*	1.07±0.13*

*Significant at $P < 0.05$ between P-sufficient and P-deficient treatments for a given test day.
[a)] Means±standard deviations ($n = 6$).

The net photosynthesis rate (Pn) and various carbohydrate (non-structural carbohydrate, glucose, fructose, sucrose, and starch) contents in tomato leaves were also analyzed under P-sufficient and P-deficient conditions for 0, 0.5, 1, 3, 6, and 9 d (Fig. 1). There were no significant differences in Pn and carbohydrate content between the two treatments for the first 3 d. However, after 6 and 9 d of P starvation, the Pn was

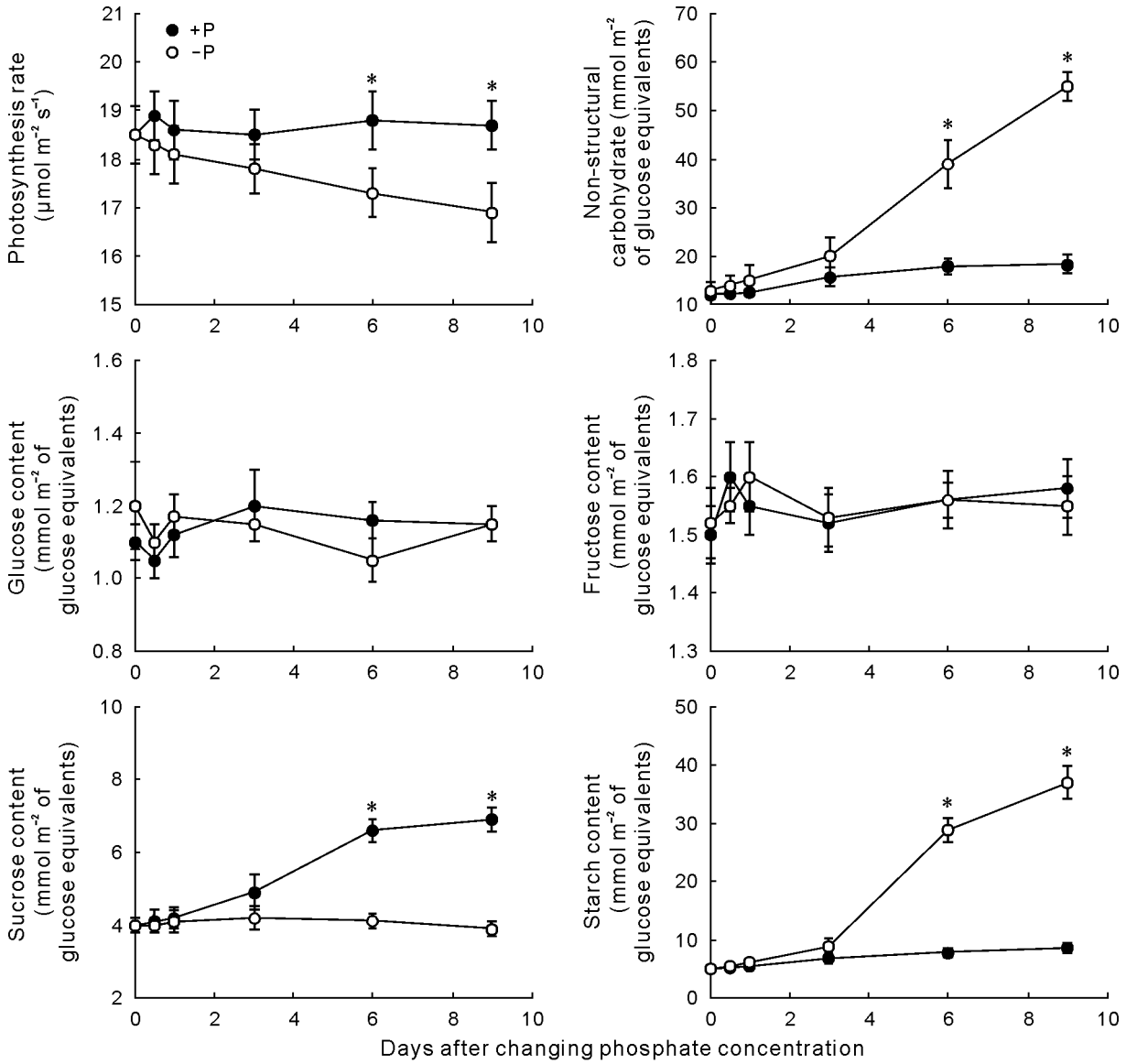

Fig. 1 Net photosynthesis rate and various carbohydrate (non-structural carbohydrate, glucose, fructose, sucrose, and starch) contents in tomato leaves under P-sufficient (+P) and P-deficient (−P) conditions over 0, 0.5, 1, 3, 6, and 9 d. One asterisk (*) indicates significant difference at $P < 0.05$ between P-sufficient and P-deficient treatments for a given test day. Error bars represent the standard deviations of the means ($n = 6$).

significantly lower in P-deficient than in P-sufficient conditions. In addition, in 6 and 9 d treatments, the contents of non-structural carbohydrate and starch in tomato leaves were significantly higher in P-deficient than in P-sufficient conditions; whereas, the sucrose content of tomato leaves was significantly lower in P-deficient than in P-sufficient conditions.

As presented in Fig. 2, during the first 3 d, no significant differences in root volume, primary root length, lateral root length, root surface area and root sucrose content were observed between P-sufficient and P-deficient conditions. However, in 6 and 9 d treatments, root sucrose content, the lateral root length and the root surface area were significantly higher in P-deficient than in P-sufficient conditions, and the primary root length was significantly lower under P-deficient conditions than under P-sufficient conditions. The rate of root proton extrusion was significantly higher under P-deficient than under P-sufficient conditions.

Real-time RT-PCR analysis of 14-3-3 gene expression in tomato plants

To avoid bias, real-time RT-PCR is typically referenced to a housekeeping gene as the internal control gene. Using α-tubulin as the internal control gene (= 100 REU), no significant change was found in phosphoglycerate kinase mRNA levels in tomato plants under P-sufficient and P-deficient conditions over the entire treatment (Fig. 3). These results suggested that the se-

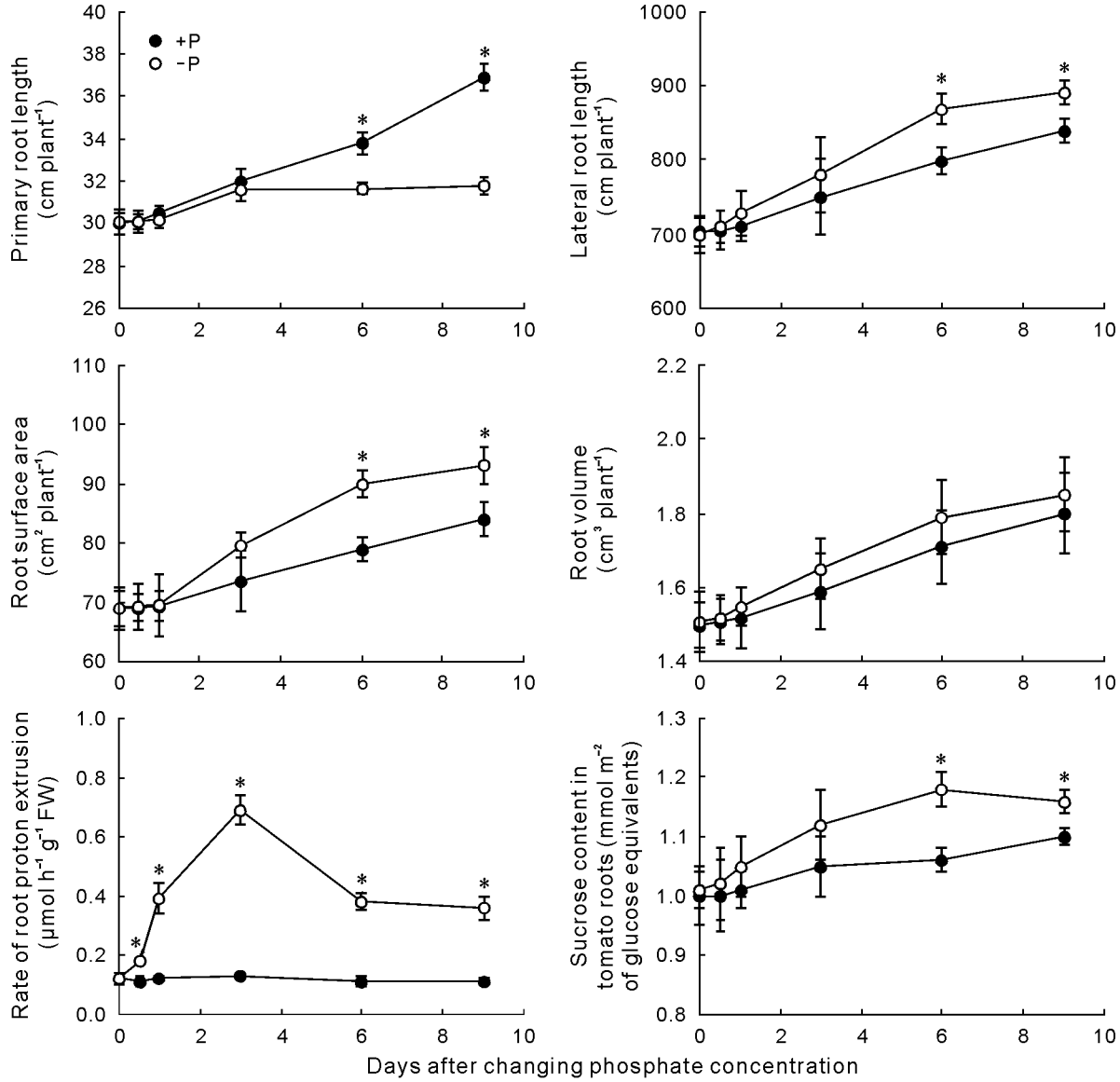

Fig. 2 Morphological response, proton extrusion, and sucrose content in tomato roots under P-sufficient (+P) or P-deficient (−P) conditions over 0, 0.5, 1, 3, 6, and 9 d. One asterisk (*) indicates significant difference at $P < 0.05$ between P-sufficient and P-deficient treatments for a given test day. Error bars represent the standard deviations of the means ($n = 6$).

lection of the housekeeping genes for real-time RT-PCR in our experimental systems was reliable and accurate.

Subsequently, the expression patterns of all twelve 14-3-3 genes were analyzed under P-sufficient or P-deficient conditions using real-time RT-PCR (Figs. 4 and 5, Table II). Irrespective of P-nutritional status of the plant, no *TFT12* mRNA was detected in tomato plants using two different primer pairs (Figs. 4 and 5). Further, although no *TFT8* expression was detected in tomato roots or leaves using two different primer pairs, it was detected in the stems; however, no significant change was found under P-sufficient and P-deficient conditions throughout the treatment period. In addition, except for *TFT8* and *TFT12*, there was no significant difference in the expression of 14-3-3 genes in the roots, stems, or leaves under P-sufficient conditions over the entire treatment.

Under P-deficient stress, the steady-state transcript levels of most of the 14-3-3 gene family members appeared relatively unchanged, but significant differences in four genes, *TFT1*, *TFT4*, *TFT6*, and *TFT7*, were observed in roots, stems, and leaves. The expression of *TFT1* was up-regulated in stems under P-deficient over the first 3 d of treatment, and down-regulated in leaves under P deficiency throughout the entire treatment. Under P deficient conditions, the mRNA level of *TFT4* was up-regulated in roots over the first 3 d of treatment and up-regulated in stems over the entire treatment; whereas, down-regulated in

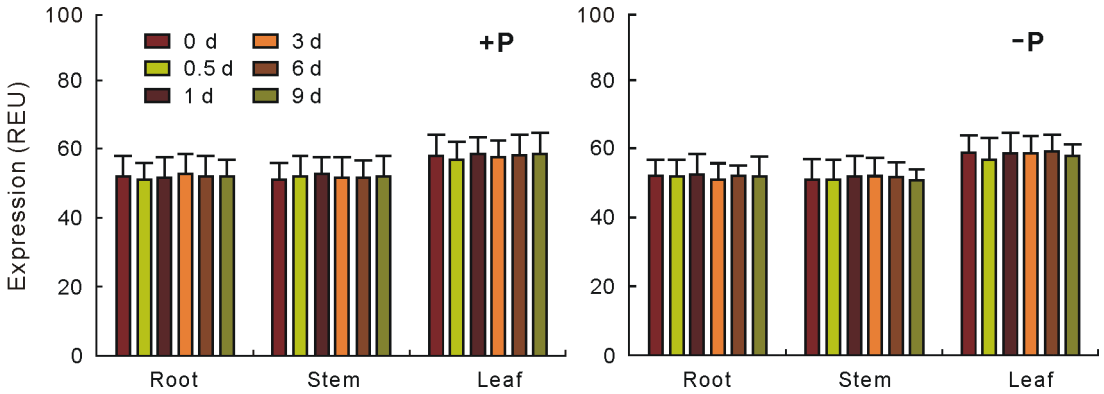

Fig. 3 Expression of the phosphoglycerate kinase gene in roots, stems, and leaves of tomato plants under P-sufficient (+P) and P-deficient (−P) conditions over 0, 0.5, 1, 3, 6, and 9 d. Relative expression levels were calculated and normalized with respect to α-tubulin mRNA (= 100 REU). Error bars represent the standard deviations of the means ($n = 6$).

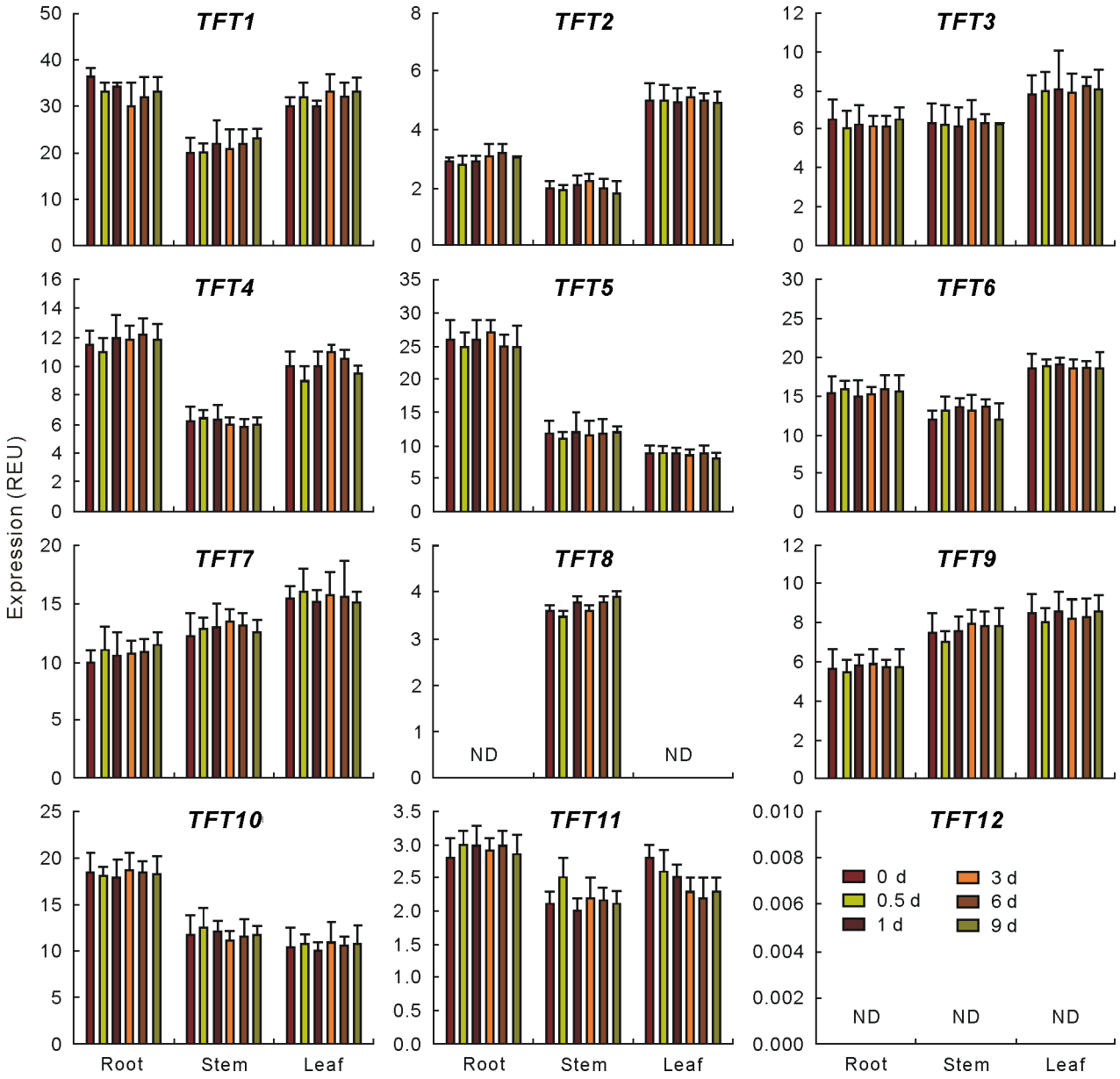

Fig. 4 Expression of 14-3-3 gene family members in the roots, stems, and leaves of tomato plants under P-sufficient conditions over 0, 0.5, 1, 3, 6, and 9 d. Relative expression levels were calculated and normalized with respect to α-tubulin mRNA (= 100 REU). Error bars represent the standard deviations of the means ($n = 6$). ND = not detectable.

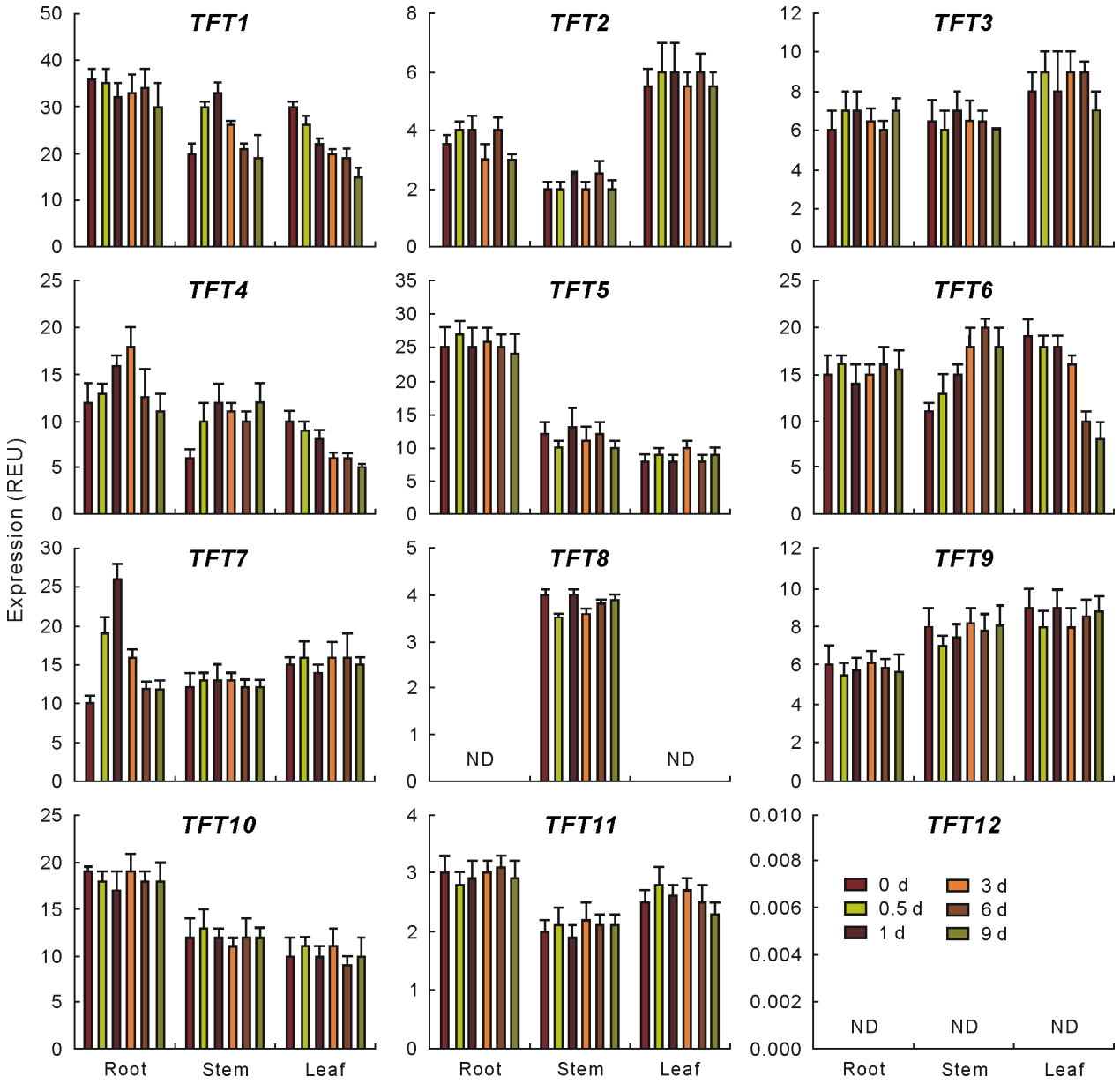

Fig. 5 Expression of 14-3-3 gene family members in the roots, stems, and leaves of tomato plants under P-deficient conditions over 0, 0.5, 1, 3, 6, and 9 d. Relative expression levels were calculated and normalized with respect to α-tubulin mRNA (= 100 REU). Error bars represent the standard deviations of the means ($n = 6$). ND = not detectable.

leaves during the entire treatment. The mRNA level of *TFT6* was up-regulated in stems and down-regulated in leaves under P deficiency over the entire treatment. Finally, the mRNA level of *TFT7* was up-regulated in roots under P deficiency in the first 3 d of treatment only.

DISCUSSION

Response of tomato to P deficiency

Our results (Table I, Figs. 1 and 2) suggested that the response of tomato plant to P deficiency was a progressive process and could be divided into two different phases which are characterized by different morphological and physiological adaptations. The first phase (short-term response phase) was from the beginning to 3 d of P starvation. During this time, the P concentration and content of all studied plant tissues remained relatively constant (Table I). Correspondingly, plant did not show any morphological differences in the dry weight of root, stem and leaf or in root length and root surface area (Table I, Fig. 2). Also, there was no difference in carbohydrate metabolism such as photosynthesis rate, sugar content or starch content of lea-

TABLE II

Summary of expression profile of 14-3-3 gene family of tomato plant in response to short-term and long-term[a)] P deficiency

Gene	mRNA level in root, stem and leaf under short- or long-term P-sufficient condition	mRNA level[b)] in response to P deficiency					
		Root		Stem		Leaf	
		Short-term	Long-term	Short-term	Long-term	Short-term	Long-term
TFT1	→	→	→	↑	→	↓	↓
TFT2	→	→	→	→	→	→	→
TFT3	→	→	→	→	→	→	→
TFT4	→	↑	→	↑	↑	↓	↓
TFT5	→	→	→	→	→	→	→
TFT6	→	→	→	↑	↑	↓	↓
TFT7	→	↑	→	→	→	→	→
TFT8	→	ND	ND	→	→	ND	ND
TFT9	→	→	→	→	→	→	→
TFT10	→	→	→	→	→	→	→
TFT11	→	→	→	→	→	→	→
TFT12	ND	ND	ND	ND	ND	ND	ND

[a)]Short-term means P deficiency over 0, 0.5, 1, or 3 d, and long-term means P deficiency over 6 or 9 d.
[b)]↑ indicates up-regulation of mRNA level; ↓ indicates down-regulation of mRNA level; → indicates no change in mRNA level; ND indicates that mRNA level is not detectable.

ves as well as sucrose content of roots between P-deficient and P-sufficient plants (Figs. 1 and 2). However, after P starvation for 12 h, proton extrusion rate of roots was significantly increased in comparison with that of roots supplied with P (Fig. 2). Thus, the main adaption of tomato plants to short-term P deficiency was to increase the root proton extrusion. The second phase (long-term response phase) occurred after 6 d of P deficiency stress (from 6 to 9 d). This phase was characterized by significant decrease in P concentration and content in root, stem and leaf (Table I). In addition, the dry weight of stem and leaf was significantly decreased after 6 d P starvation (Table I). Further, the length of primary root was significantly reduced (Fig. 2), while the lateral root length and the total root surface area were increased during P deficiency (Fig. 2). For carbohydrate metabolism in leaves, there was significant decrease in photosynthesis rate and sucrose content, while the starch and non-structural carbohydrate contents were increased due to P deficiency (Fig. 1). The main adaption of tomato plants to long-term P starvation was to increase the root growth and adjust leaf carbohydrate metabolism.

Members of tomato 14-3-3 gene family in response to P deficiency

The genes responding to P deficiency can be grouped into 'early' genes that respond rapidly and may play a role in P-deficient sensing and signaling pathways, and the 'late' genes that respond slowly and may alter the morphology, physiology or metabolism of plants upon prolonged P deficiency (Hammond *et al.*, 2004; Lambers *et al.*, 2006; Richardson, 2009). In this study, the response of all members of 14-3-3 gene family to P-deficiency was significantly different. Four members of the 14-3-3 gene family (*TFT1*, *TFT4*, *TFT6*, and *TFT7*) showed differential expression, while the others members did not show any changes in transcriptional expression during the entire experiment period of 9 d (Fig. 5, Table II). The expression of *TFT4* showed a short-term up-regulation response in the roots, and this gene was always up-regulated in stems and down-regulated in leaves under either short-term or long-term P-deficiency stress. Thus, expression response of *TFT4* to P-deficient stress is widely found in different tomato tissues. These results indicated that *TFT4* might mediate a cross-talk between P-deficient adaptations among tomato root, stem or leaf.

Accumulation of starch in leaves has been shown to be a typical response to P deficiency, and 14-3-3 protein may play an important role in this process (Comparot *et al.*, 2003; Cao *et al.*, 2007). In leaves, *TFT1*, *TFT4* and *TFT6* expression level continuously decreased in response to P deficiency. This trend correlated not only with the continuously decreased photosynthesis activity but also with the starch accumulation in the leaves of P-deficient tomato plants (Fig. 1). In particular, starch accumulation showed a close inverse correlation to *TFT6* transcription after P starvation for 6 or 9 d (Figs. 1 and 5). According to the two-phase mode for the P-deficient response of tomato plant (Table I, Figs. 1 and 2) and the definition of 'early' or 'late' genes to P deficiency, our results suggested that *TFT6*

(one member of non-ε group in tomato 14-3-3 family) belonged to the 'late' responsive member of 14-3-3 gene family to P deficiency and might play a role in the adaption of tomato plants to long-term P-deficiency stress. In addition, these results provide some evidence towards the hypothesis that *TFT6* might be involved in preventing the long-term P-deficient responses.

14-3-3 proteins play a key role in the activation of plasma membrane H^+-ATPase (Chevalier *et al.*, 2009); in turn plasma membrane H^+-ATPase is involved in the release of proton and possibly plays a central role in the adaption of plants to P deficiency (Yan *et al.*, 2002). In roots, the transcriptional up-regulation was observed for both *TFT4* and *TFT7*, in particular, for *TFT7* (Fig. 5). After the beginning of P starvation for 12 h, the expression intensity of *TFT7* was almost doubled and reached 2.5 times higher level after 24 h P starvation. During this time there was no change in P concentration, morphology, carbohydrate metabolism in roots and shoots (Table I, Figs. 1 and 2). In contrast, root proton extrusion responded quickly to P starvation treatment. A marked increase in proton extrusion was recorded for P-deficient tomato roots after 12 h and reached the maximum after 3 d P starvation (Fig. 2). Proton extrusion showed a close positive correlation to *TFT7* transcription after short-term P starvation (Figs. 2 and 5). According to the two-phase mode for the P-deficient response of tomato plant (Table I, Figs. 1 and 2) and the definition of 'early' or 'late' genes to P deficiency, our results suggested that *TFT7* (one member of ε-like group in tomato 14-3-3 family) belonged to the 'early' responsive member of 14-3-3 gene family to P deficiency and might play a role in the adaptation of tomato plants to short-term P deficiency. Furthermore, these results provide some evidence towards the hypothesis that *TFT7* might play a role in P-deficient sensing and signaling pathways by activating plasma membrane H^+-ATPase.

Isoform specificity in tomato 14-3-3 gene family

14-3-3 proteins possess a highly conserved target-binding domain, which is able to recognize several short consensus amino acid sequence motifs containing phosphoserine or phosphothreonine (Fu *et al.*, 2000). However, data suggesting that individual 14-3-3 isoforms do have specific functions in higher plants are accumulating in the literature (Sehnke *et al.*, 2002; Alsterfjord *et al.*, 2004; Paul *et al.*, 2005). Our results also support the notion that isoform specificity existed in the 14-3-3 gene family in tomato (Figs. 4 and 5, Table II). In this study, we found that the 14-3-3 proteins exhibited diverse patterns of gene expression in response to P deficiency in tomato plants (roots, stems, and leaves). In tomato roots, short-term P deficiency greatly increased the expression of *TFT4* and *TFT7*. In tomato stems, short-term P deficiency caused a significant up-regulation of three genes (*TFT1*, *TFT4*, and *TFT6*). The up-regulation of two genes, *TFT4* and *TFT6*, was also observed in tomato stems under long-term P deficiency. In tomato leaves, significant down-regulation of three genes, *TFT1*, *TFT4*, and *TFT6*, was observed under both short-term and long-term P deficiency. So, our results suggested specific functions for particular 14-3-3 genes in the adaption of tomato plants to P deficiency.

There are several potential reasons for isoform specificity existing in the 14-3-3 gene family in tomato. First, although the 14-3-3 protein sequences are highly conserved, variation does exist within the N- and C-terminal domains. It was suggested that the N and C termini have functions in isoform specificity (Börnke, 2005). Second, there is increasing evidence that the promoter is associated with gene-specific expression patterns in higher plants. In potato, the specificity of 14-3-3 gene expression in response to stress is promoter-dependent (Aksamit *et al.*, 2005). Third, in many published studies on plants, tissue- and cell-specific expression was observed in the 14-3-3 gene family. For example, in *Arabidopsis thaliana*, 14-3-3 gene expression exhibited cell- and tissue-specific localization (Paul *et al.*, 2005). Our results also showed that tissue-specific expression occurred in the 14-3-3 gene family. For example, no *TFT8* expression was detected in tomato roots or leaves, but it was detected in tomato stems.

In conclusion, using real-time RT-PCR, we performed a detailed analysis of the temporal and tissue-specific expression patterns of the entire set of the 14-3-3 gene family in response to P deficiency in tomato plants. Our results suggested that some members of the tomato 14-3-3 gene family (*TFT1*, *TFT4*, *TFT6*, and *TFT7*) might play important roles in the adaptation of tomato to P deficiency. In particular, *TFT7* responded quickly to P deficiency in tomato root tissue and belonged to the 'early' responsive 14-3-3 gene to P-deficient stress. Moreover, *TFT6* responded slowly to P deficiency in tomato leaf tissue and belonged to the 'late' responsive 14-3-3 gene to P-deficient stress. The expression response of *TFT4* to P-deficient stress was widely found in different tissues (root, stem, leaf), while the expression of *TFT8* was only detected in stem irrespective of P-sufficiency or deficiency. Thus, temporal and tissue-specific expression patterns to P deficiency also indicated that isoform specificity existed

in the 14-3-3 gene family in tomato plants. However, the actual roles and complexity of interactions of the 14-3-3 proteins in the manipulation of P availability in tomato plants are challenging goals for future research. To our knowledge, this is the first comprehensive study of temporal and tissue-specific expression profiles of 14-3-3 gene family response to P deficiency in higher plants. Therefore, it would make a substantial contribution to the research of the adaption of higher plant to P deficiency.

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