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TFT6 and *TFT7*, two different members of tomato 14-3-3 gene family, play distinct roles in plant adaption to low phosphorus stress

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ABSTRACT

14-3-3 proteins are a large family of proteins but exact roles of their members in plant response to abiotic stresses are not clear, especially under nutrient deficiency. We investigated the expressions of all the tomato 14-3-3 gene family members (*TFT1–TFT12*) under low phosphorus stress (LP) and found that *TFT6* belongs to the later responsive gene while *TFT7* belongs to the early responsive gene. When the two genes were separately introduced into *Arabidopsis* and overexpressed, their plant growth under LP was much enhanced compared with wild-type plant. *TFT6* overexpressing plants showed reduced starch synthase activity, reduced starch content but enhanced sucrose loading into phloem in the shoot under LP. *TFT7* overexpressing plants had much enhanced H^+ flux along their root tip and activity of plasma membrane H^+-ATPase in the roots under LP. Our results suggest that *TFT6* and *TFT7* play different roles in plant adaption to LP. *TFT6* acts mainly in leaves and is involved in the systemic response to LP by regulating leaf carbon allocation and increasing phloem sucrose transport to promote root growth, while *TFT7* directly functions in root by activating root plasma membrane H^+-ATPase to release more protons under LP.

Key-words: 14-3-3 proteins; carbon allocation; proton; root; starch; sucrose.

INTRODUCTION

14-3-3 proteins are a large family of proteins found in virtually every eukaryotic organism and tissue (Moore & Perez 1967; Comparot, Lingiah & Martin 2003). Functionally, 14-3-3 proteins are phosphoserine-binding proteins that regulate the activities of a wide array of targets *via* direct protein–protein interactions (Lancien & Roberts 2006; Elmayan *et al*. 2007). In higher plants, 14-3-3 proteins have been shown to play important roles in regulating plant development and stress responses (Roberts, Salinas & Collinge 2002). Most higher plants have more than one 14-3-3 isoform (Roberts 2003; Paul, Sehnke & Ferl 2005). Although 14-3-3 proteins possess a highly conserved target-binding domain, some researchers suggest that individual 14-3-3 isoforms may have specific functions (Sehnke *et al*. 2002; Paul *et al*. 2005). Our previous gene-specific expression results also showed that the isoform specificity may exist in the tomato 14-3-3 gene family under abiotic stress. However, at present there are few genetic evidences for isoform specificity of plant 14-3-3 proteins under abiotic stress, especially under nutrient deficiency.

Phosphorus deficiency is one of the major limiting factors for plant growth and development. Evolution must have led plants to have developed flexible strategies to cope with phosphorus deficiency through a complex of biological processes (Vance, Uhde-Stone & Allan 2003; Lambers *et al*. 2006). Some recent studies have suggested that in higher plants, 14-3-3 proteins may play an important role in response to phosphorus deficiency. For instance, the activities of phosphorus transporters are modulated by signalling proteins under phosphorus deficiency. Signalling proteins known to interact with phosphorus deficiency response factors include protein kinases, phosphatases, and 14-3-3 proteins (Cao *et al*. 2007; Baldwin *et al*. 2008). Apart from their well-established roles in regulating plasma membrane H^+-ATPase, which plays an important role in phosphorus acquisition (Yan *et al*. 2002; Shen *et al*. 2006), 14-3-3 proteins are also regulatory partners of ion channels (Véry & Sentenac 2003). In addition, 14-3-3 proteins are thought to be involved in carbohydrate metabolism and transport, which play important roles in phosphorus-deficient responses (Comparot *et al*. 2003; Schoonheim, Pereira & Boer 2009).

Tomato (*Solanum lycopersicum*) is becoming a model plant of vegetable for studying physiological and molecular mechanisms (Fei *et al*. 2006). In tomato plants, 14-3-3 proteins are encoded by a multi-gene family. To date, at least 12 genes predicted to encode 14-3-3 proteins, named in sequence of *TFT1–TFT12*, have been identified in tomatoes (Xu & Shi 2006). In this work, to explore the isoform specificity of tomato 14-3-3 proteins under phosphorus

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deficiency and their roles in this process, we first investigated the expression of tomato 14-3-3 gene family under low-phosphorus-stress (LP). We found that *TFT6* (one member of non-ε group in tomato 14-3-3 family) may belong to the later responsive gene, while *TFT7* (one member of ε-like group in tomato 14-3-3 family) may belong to the early responsive gene. In an effort to further study their physiological functions, we introduced *TFT6* and *TFT7* into *Arabidopsis* plants and studied whether overexpressions of *TFT6* or *TFT7* could enhance the response of plants to LP under the hydroponic systems. Our results indicate that *TFT6* and *TFT7*, two different members of tomato 14-3-3 gene family, play distinct roles in the adaption of plants to LP. The obtained results can extend our understanding of the isoform specificity of plant 14-3-3 proteins in response to abiotic stress, especially under nutrient deficiency such as LP.

MATERIALS AND METHODS

Plant materials, growth conditions and stress treatment

Tomato (*Solanum lycopersicum* L. 'Hezuo903') plants were grown hydroponically in black pots containing the modified Hoagland's solution (control: CK), which consists of the following macronutrients: KNO_3, 0.5 mM; Ca $(NO_3)_2$, 1.0 mM; KH_2PO_4, 1.0 mM; and $MgSO_4$, 0.3 mM; and the following micronutrients: H_3BO_3, 13.3 μM; $MnCl_2$, 3.0 μM; $CuSO_4$, 0.5 μM; $ZnSO_4$, 1.0 μM; Na_2MoO_4, 0.1 μM; NaCl, 2 μM; $CoCl_2$, 0.01 μM; and $NiSO_4$, 0.1 μM. The solutions were supplemented with 20 μM Fe-EDDHA. For LP, 2 μM phosphorus was kept in the nutrient solution. The pH of the solution was adjusted to 6.0 every day, and the solution was renewed every 2 d. The samples were harvested at 0, 1, 2, 4, 6 and 8 d after the plants were exposed to CK or LP. The roots and leaves of tomato plants were then separated, frozen and stored at −80 °C. In addition, the *Arabidopsis* seeds were grown hydroponically using a sugar-free agar medium solution culture system as described by Xu & Shi (2008). Fifteen-day-old *Arabidopsis* plants were treated with CK (1 mM phosphorus) and LP (2 μM phosphorus) for 15 d under hydroponic system. Then, 30-day-old *Arabidopsis* plants were frozen immediately into liquid nitrogen, and stored at −80 °C. Each independent experiment was arranged with three replicates and each replicate contained 10 *Arabidopsis* plants. Furthermore, each replicate was harvested separately and analysed separately. In addition, the independent experiment was repeated twice at different time with same growth conditions.

DNA and protein sequence database analysis

Database searches were performed at the National Center of Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov) and The Institute for Genomic Research (TIGR, http://www.tigr.org) web sites. The phylogenetic tree was generated using DNAMAN version 4.0 (Lynnon Biosoft Company, Foster City, CA, USA).

Real-time RT-PCR

Real-time RT-PCR was assayed according to the method of Xu & Shi (2006). Gene-specific primers were designed using Primer 5 software (Supporting Information Table S1). Because α-tubulin is a strongly and constitutively expressed housekeeping gene in tomato plants (Wang, Garvin & Kochian 2002; Coker & 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 & Davies 2003), were monitored. α-Tubulin mRNA, which was defined as 100 relative expression units (REU), was used as an internal standard in the gene expression of tomato plants. *At-ACT2* is a strongly and constitutively expressed 'house-keeping' gene in *Arabidopsis* plants (Panchuk, Zentgraf & Volkov 2005), and then quantification of mRNA levels of *Arabidopsis* genes was based on comparison with the level of mRNA for *At-ACT2*, which was also defined as 100 REU. As an additional control in *Arabidopsis* plants, mRNA levels were monitored for a moderately expressed 'house-keeping' gene *At-L23a* coding for the ribosomal protein L23a (Panchuk *et al*. 2005). The expression level of a gene in tomato or *Arabidopsis* plants was defined as the ratio of the copy number of the studied gene to the copy number of α-tubulin (tomato) or *At-ACT2* (*Arabidopsis*) multiplied by 100 REU. All transcripts of these studied genes were confirmed by DNA sequencing.

Generation of transgenic *Arabidopsis* plants

The full-length coding sequence of *TTF6* or *TFT7* was first amplified from the tomato cDNA library and was ligated into the pMD18-T vector (TaKaRa, Kyoto, Japan). The vector was then digested using the BamHI/SacI double digestion, and the resulting DNA was subcloned into the pBI121 linearized by the double digestion with the same restriction enzymes. The coding region of *TTF6* or *TFT7* was confirmed by DNA sequencing. Transformation of wild-type (WT) *Arabidopsis* plants (Columbia) was done according to the floral dip method using *Agrobacterium tumefaciens* C58 (Clough & Bent 1998), and then T_3 homozygous lines for kanamycin resistance were used for further studies.

Measurement of dry weight and phosphorus concentration

When the *Arabidopsis* plants were 15 d, they were treated with CK and LP for 15 d. After that, plant shoots were collected and dried at 70 °C for 3 d, then weighed. The phosphorus concentrations of *Arabidopsis* roots were determined colourimetrically 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).

Measurement of root growth

When the *Arabidopsis* plants were 15 d, they were treated with CK and LP for 15 d. After that, the total root length was measured using a root analysis instrument (WinRHIZO; Regent Instruments Inc., Quebec, ON, Canada) according to the method of Xu & Shi (2007).

Analysis of proton extrusion

The proton extrusion of *Arabidopsis* roots was analysed following the method of Jin *et al*. (2009). When the *Arabidopsis* plants were 15 d, they were treated with CK and LP for 15 d. After that, plants were transferred to 100 mL pots filled with 1 mM phosphorus solution or 2 μM phosphorus solution. After 12 h, the solution was titrated with 1.00 mM NaOH to pH 6.0. The amounts of protons excreted by roots were calculated from the amount of NaOH used.

Carbohydrate determination

Arabidopsis plant samples (roots and leaves) were extracted in 0.5 mL of 80% ethanol (v/v) for 20 min at 70 °C, and soluble sugar, glucose and sucrose were determined (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). The extract was incubated for 36 h at 37 °C to convert the starch to glucose, which was determined as above.

Enzyme measurements

Arabidopsis leaves were weighed and homogenized in a chilled mortar and pestle and 5 mL ice-cold extraction buffer containing 50 mM Mes-NaOH (pH 6.9), 5 mM MgCl2, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.1% (v/v) 2-mercaptoethanol and 2% (w/v) insoluble Polyvinylpyrrolidone (PVP). Extracts were filtered through two layers of miracloth and were centrifuged at 27 000 *g* for 20 min at 4 °C. Supernatants were applied directly to a 2×30 cm Sephadex G-25 column (Sigma, St Louis, MO, USA) pre-equilibrated with extraction buffer (less PVP) at 4 °C. The activity of sucrose phosphorus synthase was determined as described by Sicher & Kremer (1984). Starch synthase (SS) activity was assayed by the method of Zuk, Weber & Szopa (2005). The protein concentration was determined with bovine serum albumin (BSA) as the standard according to the paper of Bradford (1976).

Collection and analysis of phloem exudates

Phloem exudates were collected from excised *Arabidopsis* leaves according to the method of Fan *et al*. (2009). After *Arabidopsis* leaves were cut, the tip of the petiole was recut in EDTA buffer (5 mM Na2EDTA, pH 7.5, osmotically adjusted to 270 mosmol with sorbitol) with fresh razor blades without wounding. The leaves were washed with a large volume of sterile EDTA buffer to remove contaminants and then placed in 200 μL new EDTA buffer. During phloem sap exudation, the leaves were illuminated and incubated in a chamber. After 1 h of bleeding, the buffer solution containing phloem exudates was analysed for sucrose content.

Assay of plasma membrane H^+-ATPase activity

Plasma membrane vesicles of *Arabidopsis* root were prepared according to the method of Shen *et al*. (2006). The plasma membrane was stored at –80 °C until analysis. The protein concentration was determined by the method of Bradford (1976). Root plasma membrane H^+-ATPase activity was determined by the method of Shen *et al*. (2006). In a reaction volume of 0.5 mL that contained 30 mM BTP/MES, pH 6.5, 5 mM MgSO4, 50 mM KCl and 4 mM TRIS-ATP, plasma membrane H^+-ATPase activity was measured. Brij 58 (0.02% w/v) was applied to obtain membrane vesicles of uniform sideness. Reactions were initiated by adding 3–5 μg of membrane protein. Reactions proceeded for 30 min at 30 °C and were stopped with 1 mL of stopping solution containing 2% (v/v) concentrated H_2SO_4, 5% (w/v) sodium dodecyl sulphate 0.7% (w/v) sodium molybdate, followed by 50 μL of 10% (w/v) ascorbic acid. Colour development of the phosphomolybdate complex proceeded for 30 min. Absorbance at 700 nm was measured with a spectrophotometer. Plasma membrane H^+-ATPase activity was calculated as the phosphorus liberated in the excess of boiled-membrane controls. To assess the purification of the H^+-ATPase activity, its activity was expressed as the difference in activity in the presence and absence of 0.1 mM vanadate. As the oxidation state of vanadate varies greatly, after calculation and weighing, solid vanadate was directly added to the reaction mixture. A standard curve of phosphorus in the reaction mixture was included in each assay.

Measurement of net H^+ flux with the SIET system

Net fluxes of H^+ were measured noninvasively using scanning ion-selective electrode technique (SIET; SIET system BIO-003A; Younger USA Science and Technology Corporation; Applicable Electronics Inc.; Science Wares Inc., Falmouth, MA, USA). The principle of this method and instrument are detailed in Li *et al*. (2010). *Arabidopsis* plants were equilibrated in measuring solution for 20–30 min; these equilibrated *Arabidopsis* plants were transferred to the measuring chamber, a small plastic dish (3 cm diameter) containing 2 to 3 mL of fresh measuring solution. When the root became immobilized at the bottom of the dish, the microelectrode was vibrated in the measuring solution between two positions, 5 and 35 μm from the root surface, along an axis perpendicular to the root. The

background was recorded by vibrating the electrode in measuring solution not containing roots. After backfilling, electrode tip was filled with a commercially available ionophore H^+ cocktail (95297 from Fluka, Milwaukee, WI, USA) All measurements of H^+ fluxes were carried out at Xuyue Science and Technology Co., Ltd (Beijing, China).

Grafting of *Arabidopsis* plants

Grafting is a technique whereby tissues or organs from one plant are inserted into another plant so that the two sets of vascular tissues can grow and join together. Grafting of 6-day-old *Arabidopsis* plants grown on sterile vertical agar plates was performed as described previously (Turnbull, Booker & Leyser 2002) with minor modifications. Seeds of WT *Arabidopsis* plants and transgenic *Arabidopsis* plants (L6-3 and L7-5) were surface sterilized and stratified at 4 °C for 3 d. The seeds were then laid onto sterile half-strength MS agar (0.8%) plates. The plates were kept vertically in constant light (approximately 120 μE) at 21 °C for 3 d in an *Arabidopsis* growth chamber, and then kept at 25 °C under 8 h photoperiod (60 μE) for another 3 d. The seedlings were grafted using silicon tubing collars, and grafted plants were kept on the identical agar plates and under the same growth conditions for another 6 d until the graft junction had healed. Successfully grafted plants (12 d old) were transferred to the hydroponic system, and grown for another 3 d. After that, 15-day-old grafted *Arabidopsis* plants were treated with CK and LP for 15 d under hydroponic system. Then, 30-day-old *Arabidopsis* plants were used for experimental analysis.

RESULTS

Expression of tomato 14-3-3 gene family

Using α-tubulin as the internal control gene (= 100 REU), no significant change was found in phosphoglycerate kinase mRNA levels in tomato root or leaf under phosphorus-sufficient conditions (CK; 1 mM phosphorus) or LP (2 μM phosphorus) (Supporting Information Fig. S1). So, these results suggest that selection of the housekeeping genes for real-time RT-PCR in tomato plants is reliable and accurate. Subsequently, the expression patterns of all 12 tomato 14-3-3 genes were analysed under CK or LP. (Fig 1). The expression level of all 14-3-3 genes was kept stable in tomato leaf or root under CK over the entire treatment. Under LP, 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 tomato leaf or root. The phylogenetic tree (Supporting Information Fig. S2) shows that the 14-3-3 gene family comprises two major groups, a non-ε group and an ε-like group: the *TFT1*, *TFT4* and *TFT6* genes form the non-ε group, and the *TFT7* gene form the ε-like group. Among these phosphorus-responsive tomato 14-3-3 genes which belong to non-ε group, the expression of *TFT1* or *TFT4* was down-regulated in tomato leaf under LP throughout the entire treatment. After LP for 8 d, the expression level of *TFT1* or *TFT4* in leaves was about 50% of that of phosphorus-sufficient plants. However, the expression level of *TFT6* was stable in tomato leaf under LP over the first 2 d of treatment. After LP for 8 d, the expression level of *TFT6* in leaves was about 25% of that of phosphorus-sufficient plants. Among these members of ε-like group in tomato 14-3-3 family, *TFT7* is the only phosphorus- responsive gene. After LP for 1 d, the expression level of *TFT7* in roots was about 2.5-fold of that of phosphorus-sufficient plants. The genes identified as responding to phosphorus deficiency can be grouped into the 'early' genes that respond rapidly and may play a role in phosphorus-deficient sensing and signalling pathways, and the 'late' genes that respond slowly and may alter the morphology or metabolism of plants upon prolonged phosphorus deficiency (Lambers *et al*. 2006; Richardson 2009). Thus, under LP, *TFT6* (one member of non-ε group in tomato 14-3-3 family) may belong to the later responsive gene, while *TFT7* (one member of ε-like group in tomato 14-3-3 family) may belong to the early responsive gene.

Identification and selection of transgenic *Arabidopsis* plants

To investigate the role of *TFT6* or *TFT7* in the adaption of plants to LP, we separately overexpressed *TFT6* or *TFT7* in *Arabidopsis* plants under control of a CaMV 35S promoter. *At-ACT2*, our choice of a housekeeping gene in *Arabidopsis* plants, is a strongly and constitutively expressed housekeeping gene in *Arabidopsis* (Panchuk *et al*. 2005). To avoid bias, real-time RT-PCR is referenced to another housekeeping gene (*At-L23a*) as the control gene. Our results showed that no significant change was found on the expression level of *At-L23a* in the WT plants, *TFT6*-overexpressing plants or *TFT7*-overexpressing plants (Supporting Information Fig. S1). Thus, it is very clear that our experiment systems are reliable and accurate. According to the results (Supporting Information Fig. S3), six transgenic lines (from L6-1 to L6-6) were confirmed to contain the *TFT6* transcript. The expression level of *TFT6* in L6-6 line was the highest, and the *TFT6* expression levels of L6-1 and L6-2 lines were lower. The other lines (L6-3, L6-4 and L6-5) showed this gene expression with the medium levels. Also, six transgenic lines (from L7-1 to L7-6) were confirmed to contain the *TFT7* transcript. The expression level of *TFT7* in L7-6 line was the highest, and the *TFT7* expression levels of L7-1, L7-2 and L7-3 lines were lower. The other lines (L7-4 and L7-5) showed this gene expression with the medium levels. Also, no significant change was found on the expression level of *Arabidopsis* endogenous 14-3-3 genes between the WT plants and transgenic plants under CK or LP (Supporting Information Table S2). Thus, three *TFT6*-overexpressing lines (L6-1, L6-3 and L6-6) and three *TFT7*-overexpressing lines (L7-2, L7-5 and L7-6) were selected for further studies.

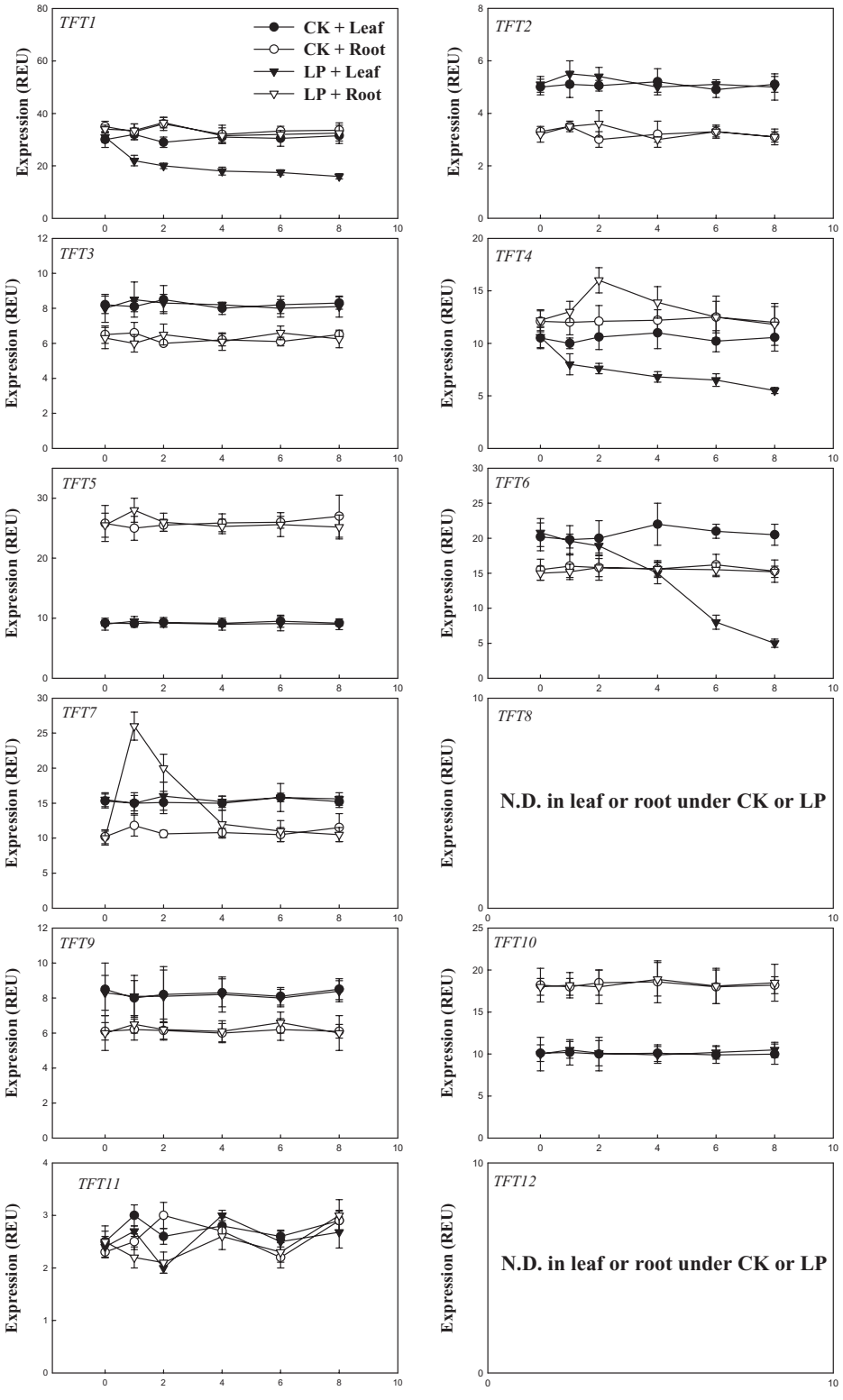

Figure 1. Expression of 14-3-3 gene family members in tomato plants (leaf and root) under phosphorus-sufficient conditions (Control: CK; 1 mM) or low phosphorus stress (LP; 2 μM) over 0, 1, 2, 4, 6 and 8 d. Relative expression levels were calculated and normalized with respect to α-tubulin mRNA [= 100 relative expression units (REU)]. Changes in the REU of gene mRNA were checked for statistical significance according to Student's *t*-test ($P < 0.05$). The values are the means and SD of six replicates from two independent experiments. N.D. indicated that expression is not detectable.

Enhanced tolerance to LP in transgenic *Arabidopsis* plants

According to the result of Fig 2a, no significant difference in the shoot dry weight was observed between transgenic plants (*TFT6*-overexpressing *Arabidopsis* lines and *TFT7*-overexpressing *Arabidopsis* lines) and WT plants under CK. Under LP, both transgenic plants and WT plants had their growth inhibited (Fig. 2b), but transgenic plants were less affected than the WT ($P < 0.05$). Furthermore, *TFT6*-overexpressing line (L6-3) or *TFT7*-overexpressing line (L7-5) grew as well as the WT plants under CK (Fig. 2b). Whereas, under LP, compared with WT, transgenic plants (L6-3 and L7-5) showed the larger leaves (Fig. 2b). These results suggest that both *TFT6*- and *TFT7*-overexpressing plants were more tolerant to LP than WT plants.

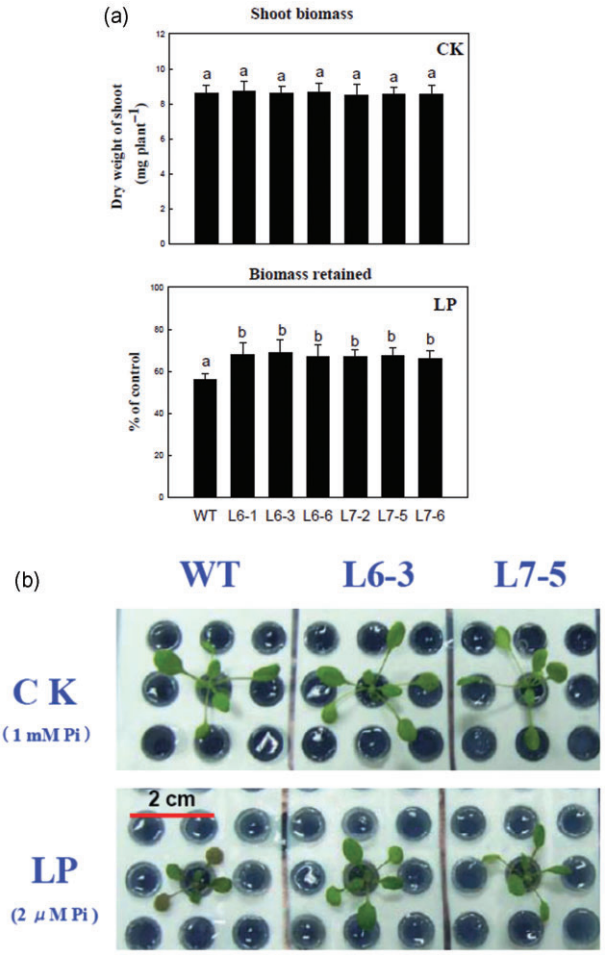

Figure 2. The shoot biomass and phenotype of 30-day-old wild-type (WT) *Arabidopsis* plant, *TFT6*-overexpressing *Arabidopsis* plant and *TFT7*-overexpressing *Arabidopsis* plant under normal growth condition (Control: CK) or low phosphorus stress (LP). (A) 15-day-old *Arabidopsis* plants (WT and transgenic lines) were treated with 1 mM phosphorus (CK) and 2 μM phosphorus (LP) for 15 d under hydroponic system. Then, 30-day-old *Arabidopsis* plants were used for analysis of shoot dry weight. Bars with the same letter (a or b) are not significantly different at $P < 0.05$ level. (B) In the same time, visual symptoms of 30-day-old WT, *TFT6*-overexpressing *Arabidopsis* line (L6-3) and *TFT7*-overexpressing *Arabidopsis* line (L7-5) were recorded using camera. The data were subjected to analysis of variance and *post hoc* comparisons were done with Duncan's multiple range test at $P < 0.05$ level. The statistical software program used was SPSS version 13.0. The values are the means and SD of six replicates from two independent experiments.

Roots play important role not only in the nutrient uptake but also in the adaption to environment stress (Desnos 2008). So, under CK or LP, phosphorus content, total root growth, soluble sugar content and proton extrusion were analysed in the roots of WT plant, *TFT6*-overexpressing plants (L6-1, L6-3, L6-6) and *TFT7*-overexpressing plants (L7-2, L7-5, L7-6) (Table 1). There was no significant difference in phosphorus content between transgenic plants and WT plants under CK. Under LP, the phosphorus content of transgenic plants was higher than that of WT plants ($P < 0.05$). Clearly, under CK or LP, the total root growth or root soluble sugar content in *TFT6*-overexpressing plants was significantly higher than WT plants ($P < 0.05$), while root proton extrusion in *TFT7*-overexpressing plants was significantly higher than WT plants ($P < 0.05$). These results suggest that *TFT6* is involved in the adaptation of tomato to LP by improving root growth and root soluble sugar content, and *TFT7* is involved in LP response by modulating root to release more protons.

Activities of SS and sucrose phosphorus synthase (SPS)

Some researches have shown that 14-3-3 proteins may play important role in the modulation of carbohydrate metabolism under stresses (Yan *et al*. 2002; Comparot *et al*. 2003; Shen *et al*. 2006; Schoonheim *et al*. 2009). Thus, in order to explore the involvement of *TFT6* or *TFT7* in this adaptation response to LP, we analyzed some parameters of carbohydrate metabolism in WT and transgenic *Arabidopsis* plants. The activities of SS and SPS were determined in leaves of WT plant, *TFT6*-overexpressing lines (L6-1, L6-3 and L6-6) and *TFT7*-overexpressing lines (L7-2, L7-5, L7-6) (Fig 3). Under CK or LP, no significant difference in the SPS activity was observed between transgenic plants and WT plants. However, the activity of SS in *TFT6*-overexpressing plants (L6-1, L6-3 and L6-6) was significantly lower than that of WT plants ($P < 0.05$) under CK or LP.

Carbohydrate content

There was no significant difference in the carbohydrate content or the ratio of sucrose/starch between WT plants and *TFT7*-overexpressing plants under CK or LP (Fig 4). Also, no significant difference in the soluble sugar content or sucrose was observed between *TFT6*-overexpressing plants and WT plants under CK. Nevertheless, the content of leaf starch in *TFT6*-overexpressing plants was significantly lower than WT plants ($P < 0.05$) under CK. Under LP, the content of soluble sugar, sucrose and starch in the leaves of *TFT6*-overexpressing plants was significantly

Table 1. Phosphorus content, total root growth, soluble sugar content and proton extrusion in the roots of wild-type (WT *Arabidopsis* plant), *TFT6*-overexpressing *Arabidopsis* plants (L6-1, L6-3, L6-6) and *TFT7*-overexpressing *Arabidopsis* plants (L7-2, L7-5, L7-6) under normal growth control (Control: CK) or low phosphorus stress (LP)

	WT	L6-1	L6-3	L6-6	L7-2	L7-5	L7-6
Root phosphorus content (μg plant^{-1})							
CK	9.2 ± 0.4 a	9.5 ± 0.4a	9.3 ± 0.5a	9.2 ± 0.5a	9.2 ± 0.4a	9.3 ± 0.3a	9.2 ± 0.3a
LP	5.0 ± 0.5 a	6.0 ± 0.1b	6.9 ± 0.3b	7.0 ± 0.5b	6.8 ± 0.5b	6.6 ± 0.6b	6.7 ± 0.3b
Total root growth (cm plant^{-1})							
CK	61 ± 2.1 a	68 ± 1.5b	69 ± 1.8b	65 ± 0.9b	61 ± 1.1a	60 ± 2.8a	61 ± 2.5a
LP	45 ± 5.0 a	65 ± 1.2b	62 ± 3.2b	60 ± 3.3b	48 ± 3.5a	51 ± 2.5a	50 ± 2.0a
Root soluble sugar content (mg glucose equivalents g^{-1} DW)							
CK	29 ± 0.5 a	32 ± 0.5b	33 ± 0.6b	35 ± 1.1b	28 ± 0.7a	30 ± 0.8a	29 ± 0.9a
LP	20 ± 2.0 a	25 ± 0.3b	26 ± 0.5b	25 ± 0.9b	20 ± 1.5a	22 ± 0.9a	21 ± 1.3a
Root proton extrusion (μmol h^{-1} 10g^{-1} FW)							
CK	1.5 ± 0.2 a	1.5 ± 0.1a	1.6 ± 0.2a	1.5 ± 0.1a	2.1 ± 0.2b	2.0 ± 0.1b	2.2 ± 0.3b
LP	2.5 ± 0.2a	2.5 ± 0.3a	2.9 ± 0.3a	2.8 ± 0.5a	4.1 ± 0.5b	3.9 ± 0.1b	3.8 ± 0.2b

Note: Fifteen-day-old *Arabidopsis* plants (WT and transgenic lines) were treated with 1 mM phosphorus (Control: CK) and 2 μM phosphorus (LP) for 15 d under hydroponic system. Then, 30-day-old *Arabidopsis* plants were used for analysis. The data were subjected to analysis of variance and *post hoc* comparisons were done with Duncan's multiple range test at $P < 0.05$ level. The statistical software program used was SPSS version 13.0. The values are the means and SD of six replicates from two independent experiments. The total root length was measured using a root analysis instrument (WinRHIZO; Regent Instruments Inc., Quebec, ON, Canada).
DW, dry weight; FW, fresh weight.
Values with the same letter (a or b) are not significantly different at $P < 0.05$ level.

lower than that of WT plants ($P < 0.05$). Furthermore, the ratio of sucrose/starch in the leaves of *TFT6*-overexpressing plants was significantly higher than that of WT plants ($P < 0.05$) under CK or LP.

Sucrose transport in transgenic *Arabidopsis* plants or WT plants

The expression of SUC2 (sucrose transporter 2) gene and exudate sucrose in phloem were analysed in shoot of WT plants, *TFT6*-overexpressing plants (L6-1, L6-3 and L6-6) and *TFT7*-overexpressing plants (L7-2, L7-5, L7-6) (Fig 5). Under CK or LP, no significant difference in the SUC2 expression or phloem exudate sucrose was observed between *TFT7*-overexpressing plants and WT plant, while the SUC2 expression or phloem exudate sucrose in the shoot of *TFT6*-overexpressing plants was significantly higher than that of WT plants ($P < 0.05$)

Activity of plasma membrane H$^+$-ATPase

Firstly, in order to evaluate the purity of plasma membrane, the activity of various inhibitor-sensitive ATPases in both the microsome and membrane fractions was analyzed (Supporting Information Table S3). In the microsome, molybdate-sensitive ATPase was the major enzyme, which indicates the presence of unspecific acid phosphatases (Yan *et al*. 2002). However, in the plasma membrane, vanadate-sensitive ATPase occupied about 90% of the total activity, and the activity of other inhibitor-sensitive enzyme was negligible. These results suggest that the isolation techniques used for the plasma membrane of root were practicable. After that, activities of plasma membrane H$^+$-ATPase were analysed in the whole root of WT plant, *TFT6*-overexpressing plants (L6-1, L6-3 and L6-6) and *TFT7*-overexpressing plants (L7-2, L7-5, L7-6) (Fig 6). Under CK or LP, no significant difference in the activity of plasma membrane H$^+$-ATPase was observed between *TFT6*-overexpressing plants and WT plants. However, the activity of plasma membrane H$^+$-ATPase in *TFT7*-overexpressing plants was significantly higher than WT plants ($P < 0.05$) under CK or LP.

Plot of H$^+$ flux along the root tip of *Arabidopsis* plants

Under CK or LP, proton (H$^+$) fluxes were measured along the root tip of WT plant, *TFT6*-overexpressing plants (L6-3) and *TFT7*-overexpressing plants (L7-5), concentrating on the following zones: 50, 100, 150, 200, 250, 300, 400, 520, 600, 700, 850, 900, 1000, 1100, 1200 and 1500 μm from the root cap junction (Fig. 7). At the meristem zone (0–200 μm from the root cap junction), either in transgenic *Arabidopsis* plants or in WT plants, H$^+$ influxes were increased along the root tip and the highest H$^+$ influx occurred at a distance of 200 μm from the root cap junction under CK or LP. Besides, no significant difference in the H$^+$ influx was observed between transgenic *Arabidopsis* plants and WT plants at the meristem zone under CK or LP. At the transition zone (200–520 μm from the root cap junction), either in transgenic *Arabidopsis* plants or in WT plants, H$^+$ influxes were decreased along the root tip and the lowest H$^+$ influx occurred at a distance of 520 μm from the root cap

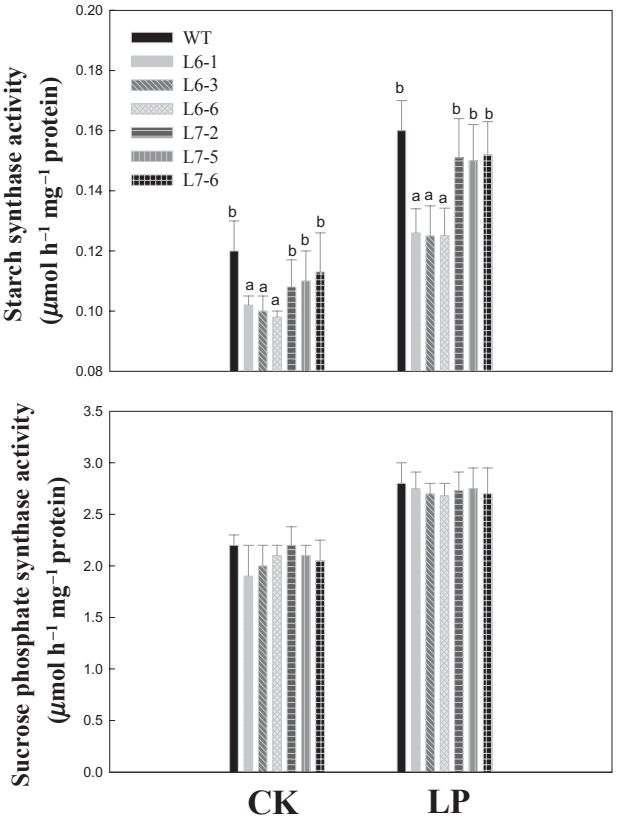

Figure 3. The activities of starch synthase (SS) and sucrose phosphorus synthase (SPS) in the leaves of wild-type (WT) *Arabidopsis* plant, *TFT6*-overexpressing *Arabidopsis* lines (L6-1, L6-3 and L6-6) and *TFT7*-overexpressing *Arabidopsis* lines (L7-2, L7-5, L7-6) under normal growth condition (Control: CK) or low phosphorus stress (LP). Fifteen-day-old *Arabidopsis* plants (WT and transgenic lines) were treated with 1 mM phosphorus (CK) and 2 μM phosphorus (LP) for 15 d under hydroponic system. Then, 30-day-old *Arabidopsis* plants were used for experimental analysis. The data were subjected to analysis of variance and *post hoc* comparisons were done with Duncan's multiple range test at $P < 0.05$ level. The statistical software program used was SPSS version 13.0. The values are the means and SD of six replicates from two independent experiments. Bars with the same letter (a or b) are not significantly different at $P < 0.05$ level.

junction under CK or LP. However, the H^+ influx in *TFT7*-overexpressing plants was significantly lower than WT plants or *TFT6*-overexpressing plants ($P < 0.05$) at the transition zone under CK or LP. At the elongation zone (520–850 μm from the root cap junction), the H^+ influx switched to sustained H^+ efflux in transgenic plants or WT plants under CK or LP. Furthermore, the H^+ efflux in *TFT7*-overexpressing plants was significantly higher than those of WT or *TFT6*-overexpressing plants ($P < 0.05$) at the elongation zone under CK or LP. Also, at the root hair zone (850–1500 μm from the root cap junction), under CK or LP, the H^+ efflux in *TFT7*-overexpressing plants was significantly higher than those of WT or *TFT6*-overexpressing plants ($P < 0.05$)

Physiological parameter in grafted *Arabidopsis* plants

We performed reciprocal grafting using WT *Arabidopsis* plants and transgenic *Arabidopsis* plants (L6-3: *TFT6*-overexpressing *Arabidopsis* plants; L7-5: *TFT7*-overexpressing *Arabidopsis* plants). Nine combinations of grafted plants were generated, including three self-grafted controls: WT leaf to WT root (designated as WTL/WTR), L6-3 leaf to L6-3 root (T6L/T6R) and L7-5 leaf to L7-5 root (T7L/T7R); six reciprocal grafts: L6-3 leaf to WT root (T6L/WTR), WT leaf to L6-3 root (WTL/T6R), L7-5 leaf to WT root (T7L/WTR), WT leaf to L7-5 root (WTL/T7R), L6-3 leaf to L7-5 root (T6L/T7R) and L7-5 leaf to L6-3 root (T7L/T6R). Under CK or LP, dry weight and phosphorus content were measured in these grafted plants. There was no significant difference in the dry weight or phosphorus content among these grafted plants under CK (data not shown). Under LP, according to the results of Fig 8, no significant difference in the dry weight or phosphorus content was observed among the four grafted plants (WTL/WTR, WTL/T6R, T7L/WTR, T7L/T6R). However, under LP, compared with these grafts (WTL/WTR, WTL/T6R, T7L/WTR, T7L/T6R), the dry weights or phosphorus contents in the five grafted plants (T6L/T6R, T7L/T7R, T6L/WTR, WTL/T7R, T6L/T7R) were higher (Fig 8). Further, the dry weight or phosphorus content in graft T6L/T7R was the highest among all grafted plants under LP (Fig 8). Further, the sucrose content and proton extrusion of root were measured in these grafted plants. There was no significant difference in the root sucrose content or root proton extrusion among these grafted plants under CK (data not shown). However, under LP, the root sucrose content in the three grafted plants (T6L/T6R, T6L/WTR, T6L/T7R) were higher (Fig 8). Also, the root proton extrusion in the three grafted plants (T7L/T7R, WTL/T7R, T6L/T7R) were higher under LP.

DISCUSSION

Response of *TFT6* to LP

Long-term phosphorus deficiency results in the accumulation of starch in leaf. If the starch accumulation occurs too much in leaf, the transport and synthesis of sucrose will be inhibited. This phenomenon eventually leads to an inhibition of plant growth and development, and then the reduction of starch accumulation will contribute to the adaption of plants to LP (Pieters, Paul & Lawlor 2001; Wissuwa, Gamat & Ismail 2005). Moreover, the elevated ratio of sucrose/starch can reduce the starch accumulation by improving the sucrose translocation from source leaf to root (Tamoi *et al.* 2011). Thus, the increased loading of sucrose to the phloem functions to relocate carbon resource to the roots, which increases the root growth to acquire phosphorus efficiently from soil under phosphorus deficiency (Hammond & White 2008; Lei *et al.* 2011). Our results suggested that overexpression of *TFT6* decreased the leaf starch accumulation (Fig 4), and the phloem

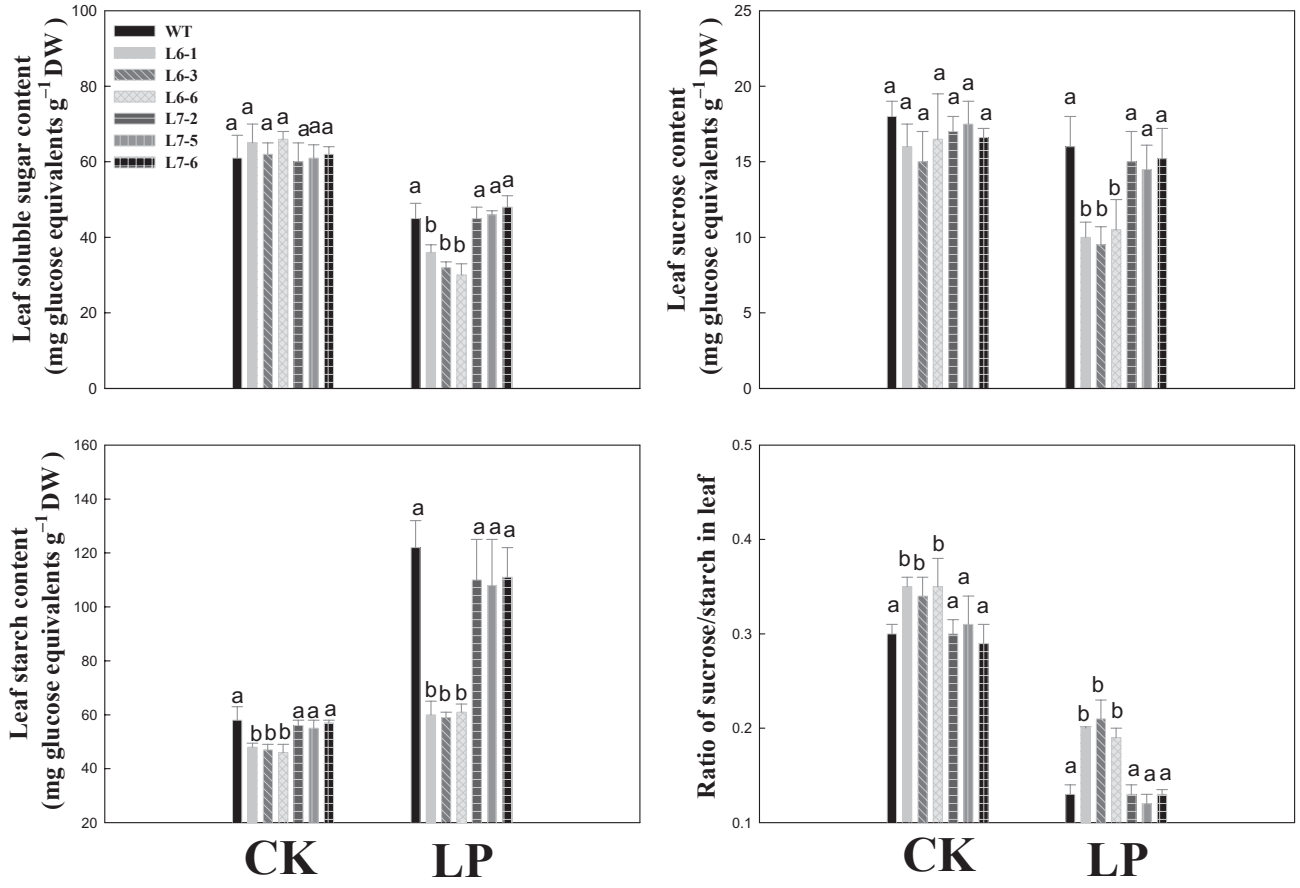

Figure 4. The soluble sugar content, sucrose content, starch content and the ratio of sucrose/starch in the leaves of wild-type (WT *Arabidopsis* plants), *TFT6*-overexpressing *Arabidopsis* plants (L6-1, L6-3, L6-6) and *TFT7*-overexpressing *Arabidopsis* plants (L7-2, L7-5, L7-6) under normal growth condition (Control: CK) or low phosphorus stress (LP). Fifteen-day-old *Arabidopsis* plants (WT and transgenic lines) were treated with 1 mM phosphorus (CK) and 2 μM phosphorus (LP) for 15 d under hydroponic system. Then, 30-day-old *Arabidopsis* plants were used for experimental analysis. The data were subjected to analysis of variance and *post hoc* comparisons were done with Duncan's multiple range test at $P < 0.05$ level. The statistical software program used was SPSS version 13.0. The values are the means and SD of six replicates from two independent experiments. DW, dry weight. Bars with the same letter (a or b) are not significantly different at $P < 0.05$ level.

exudate sucrose in *TFT6*-overexpressing plants was also significantly higher than WT plants under LP (Fig 5). In addition, there is growing evidence that sucrose is transported from shoot to root due to the action of SUC2 (Lloyd & Zakhleniuk 2004; Lei *et al*. 2011). So, the SUC2 expression in the shoot of *TFT6*-overexpressing plants was significantly higher than WT plants under LP (Fig 5). Taken together, our results suggest that *TFT6* is involved in the response of plants to LP by regulating leaf carbon allocation and phloem sucrose transport to promote root growth.

Response of *TFT7* to LP

The plant adaptations to low phosphorus include not only the increase in root length for phosphorus uptake in greater soil volume, but also the rhizosphere acidification to acquire phosphorus efficiently (Lambers *et al*. 2006; Richardson 2009). In higher plants, 14-3-3 proteins are known to activate plasma membrane H^+-ATPase which plays the key role in proton exudation (Kanczewska *et al*. 2005). Compared with WT plants, root proton extrusion or the activity of plasma membrane H^+-ATPase of *TFT7*-overexpressing plants was higher under CK or LP (Table 1 & Fig 6). So, these results suggest that *TFT7* is involved in the response of plants to LP by activating root plasma membrane H^+-ATPase to release proton. In addition, our previous results showed that *TFT7* was also up-regulated in tomato root under iron deficiency (Xu & Shi 2006). Just like under LP, plants also release proton to acquire iron efficiently under iron deficiency (Santi & Schmidt 2009). Furthermore, iron is accumulated to a higher extent in plants under LP (Rouached, Arpat & Poirier 2010). Thus, by activating root plasma membrane H^+-ATPase to release proton, *TFT7* may also play a role in the adaptation to iron deficiency.

Some researches have shown that in higher plants, root tip may be considered to be a 'brain-like' organ consisted of four distinct zones of growth activity (meristematic zone, transition zone, elongation zone and root hair zone)

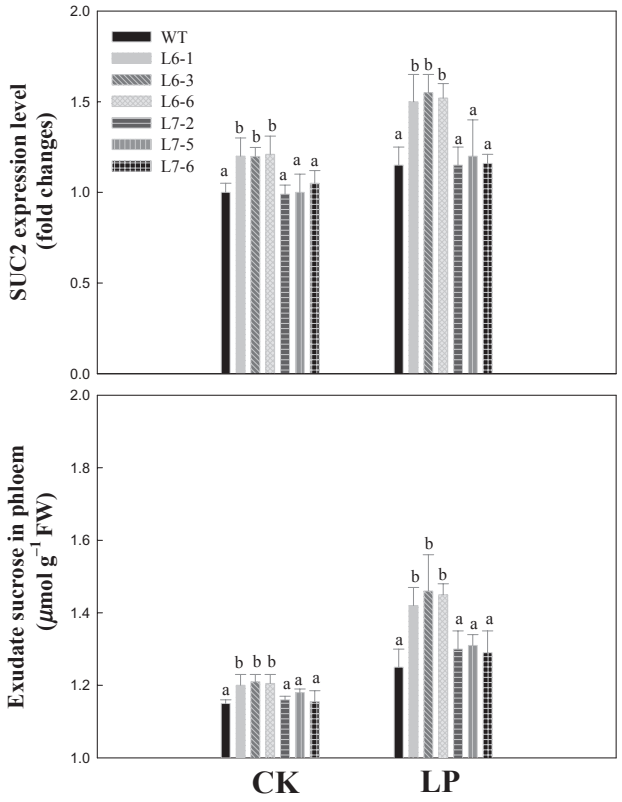

Figure 5. The shoot SUC2 gene expression and phloem exudate sucrose in the wild-type (WT) *Arabidopsis* plant, *TFT6*-overexpressing *Arabidopsis* lines (L6-1, L6-3 and L6-5) and *TFT7*-overexpressing *Arabidopsis* lines (L7-2, L7-5, L7-6) under normal growth condition (Control: CK) or low phosphorus stress (LP). Fifteen-day-old *Arabidopsis* plants (WT and transgenic lines) were treated with 1 mM phosphorus (CK) and 2 μM phosphorus (LP) for 15 d under hydroponic system. Then, 30-day-old *Arabidopsis* plants were used for experimental analysis. The data were subjected to analysis of variance and *post hoc* comparisons were done with Duncan's multiple range test at $P < 0.05$ level. The statistical software program used was SPSS version 13.0. The values are the means and SD of six replicates from two independent experiments. The expression of SUC2 in WT under CK was taken as onefold. FW, fresh weight. Bars with the same letter (a or b) are not significantly different at $P < 0.05$ level.

(Darwin 1880; Baluska *et al*. 2010). Among the four distinct zones, the transition zone is the most active zone in root tip. Under CK, the pattern of H^+ flux along the root tip of WT plants or transgenic plants was consistent with the paper of Staal *et al*. 2011, which support the acid growth theory. Under LP, compared with WT plants, H^+ efflux in root hairs zone of *TFT7*-overexpressing plants is higher. In fact, root hairs are very important for phosphorus acquisition (Wu & Wang 2008), and then *TFT7* plays the important role in response to LP by enhancing the proton release of root hairs. Moreover, the shift between H^+ influx and H^+ efflux in transition zone of *TFT7*-overexpressing plants was faster than that of WT plants (Fig 7). Besides, some researches showed that proton play the important role in the sensing and signalling of root tip in response to environment stimuli (Ramos *et al*. 2009; Monshausen *et al*. 2011). Furthermore, root tip may constitute a site to sense local phosphorus (Chiou & Lin 2011). So, these results suggest that *TFT7* may also play a role in the sensing and signalling of LP by regulating the proton release in the transition zone of root tip.

TFT6 and *TFT7* play the distinct roles in the adaption to LP

Plants have developed diverse responses to cope with phosphorus deficiency. These responses are achieved by the coordination of the adaptive network comprising local and systemic response (Chiou & Lin 2011; Peret *et al*. 2011). The localized responses mainly happen in roots and are partially involved in root proton exudation, while the systemic responses mainly occur in shoot and are partially associated with carbohydrate metabolic pathways. We used grafting to generate chimeric *Arabidopsis* plants that overexpress *TFT6* or *TFT7* either in shoots or in roots (Fig 8). Our results showed that as long as *TFT6* is overexpressed in shoots, plants show higher tolerance to LP with higher sucrose in roots. Similarly, as long as *TFT7* is over-expressed in roots, plants show higher proton extrusion and better tolerance to LP. When both genes are separately expressed in shoots and roots (the T6L/T7R graft), plants showed the highest tolerance to LP. Thus, our results suggest that *TFT6*

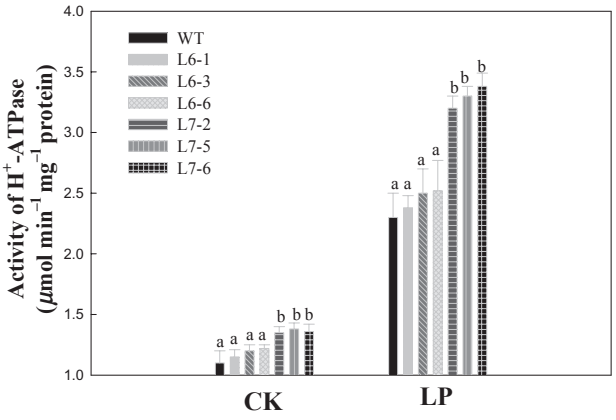

Figure 6. Activity of plasma membrane H^+-ATPase in the whole root of wild-type (WT) *Arabidopsis* plant, *TFT6*-overexpressing *Arabidopsis* lines (L6-1, L6-3 and L6-5) and *TFT7*-overexpressing *Arabidopsis* lines (L7-2, L7-5, L7-6) under normal growth condition (Control: CK) or low phosphorus stress (LP). Fifteen-day-old *Arabidopsis* plants (WT and transgenic lines) were treated with 1 mM phosphorus (CK) and 2 μM phosphorus (LP) for 15 d under hydroponic system. Then, 30-day-old *Arabidopsis* plants were used for experimental analysis. The data were subjected to analysis of variance and *post hoc* comparisons were done with Duncan's multiple range test at $P < 0.05$ level. The statistical software program used was SPSS version 13.0. The values are the means and SD of six replicates from two independent experiments. Bars with the same letter (a or b) are not significantly different at $P < 0.05$ level.

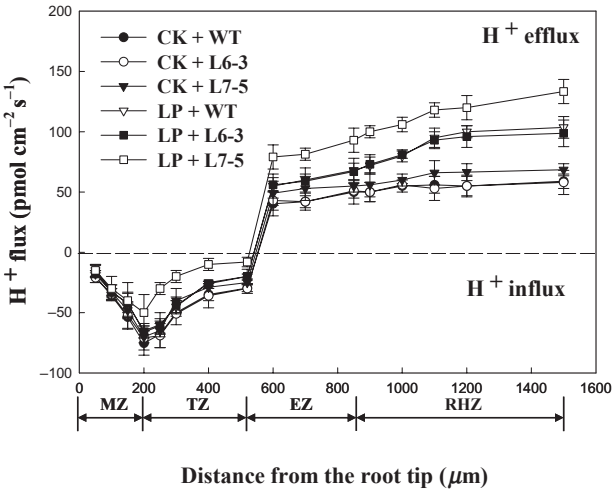

Figure 7. Plot of H^+ flux along the root tip of wild-type (WT) *Arabidopsis* plant, *TFT6*-overexpressing *Arabidopsis* lines (L6-3) and *TFT7*-overexpressing *Arabidopsis* lines (L7-5) under normal growth condition (Control: CK) or low phosphorus stress (LP). Fifteen-day-old *Arabidopsis* plants (WT and transgenic lines) were treated with 1 mM phosphorus (CK) and 2 μM phosphorus (LP) for 15 d under hydroponic system. Then, 30-day-old *Arabidopsis* plants were used for experimental analysis. The data were subjected to analysis of variance and *post hoc* comparisons were done with Duncan's multiple range test at $P < 0.05$ level. The statistical software program used was SPSS version 13.0. The values are the means and SD of six replicates from two independent experiments. The flux of was measured using the SIET system. Note (distance from the root cap junction): MZ (meristem zone, 0–200 μm); TZ (transition zone, 200–520 μm); EZ (elongation zone, 520–850 μm); RHZ (root hair zone, 850–1500 μm).

acts mainly in leaf, and *TFT7* acts mainly in root under LP. Furthermore, due to the fact that *At-SIZ1* (*Arabidopsis* SUMO E3 ligase), *At-PHF1* (an ER-located SEC-related protein in *Arabidopsis*) and *At-SPX1* (SPX domain-containing proteins in *Arabidopsis*) play important roles in phosphorus-signalling pathway (Rouached *et al*. 2010), we also studied the expressions of the three genes in WT plants, *TFT6*-overexpressing plants and *TFT7*-overexpressing plants under CK or LP. However, no significant changes were found on the expression levels of the three genes between the WT plants and transgenic plants under CK or LP (Supporting Information Table S2). Therefore, we conclude that possibly, *TFT6* and *TFT7* are independently regulated from the phosphorus-signalling pathway.

In conclusion, our work demonstrates that *TFT6* and *TFT7*, two different members of tomato 14-3-3 gene family, play the distinct roles in the adaption of plants to LP. *TFT6* is involved in the systemic adaption of plants to low phosphorus stress by regulating leaf carbon allocation and phloem sucrose transport to promote root growth (mainly acting in shoot), while *TFT7* is involved in the local adaption of plants to low phosphorus stress by activating root plasma membrane H^+-ATPase to release proton (mainly acting in root). This finding will help to further elucidate the regulative mechanism that controls plant responses to LP. However, comprehensive analyses including the patterns of gene expressions and proteomic profiling are needed to provide information for the accurate roles and regulations of *TFT6* and *TFT7* in plants.

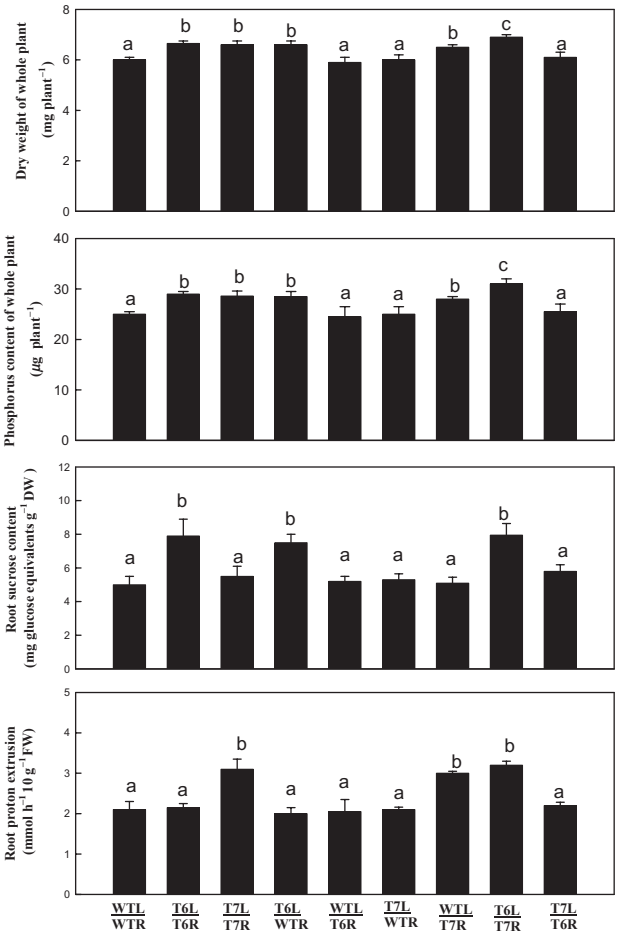

Figure 8. Dry weight, phosphorus content, sucrose content and root proton extrusion of grafted plants between wild-type *Arabidopsis* plants and transgenic *Arabidopsis* plants (L6-3 and L7-5) under low phosphorus stress (LP). Fifteen-day-old grafted *Arabidopsis* plants were treated with 1 mM phosphorus (Control: CK) and 2 μM phosphorus (LP) for 15 d under hydroponic system. Then, 30-day-old *Arabidopsis* plants were used for experimental analysis. The data were subjected to analysis of variance and *post hoc* comparisons were done with Duncan's multiple range test at $P < 0.05$ level. The statistical software program used was SPSS version 13.0. The values are the means and SD of six replicates from two independent experiments. Note: WTL means 'wild type leaf'; WTR means 'wild type root'; T6L means 'Leaf of *TFT6*-overexpressing *Arabidopsis* plants (L6-3)'; T6R means 'Root of *TFT6*-overexpressing *Arabidopsis* plants (L6-3)'; T7L means 'leaf of *TFT7*-overexpressing *Arabidopsis* plants (L7-5)'; T7R means 'root of *TFT7*-overexpressing *Arabidopsis* lines (L7-5)'. DW, dry weight; FW, fresh weight. Bars with the same letter (a or b) are not significantly different at $P < 0.05$ level.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Expression of the phosphoglycerate kinase gene in tomato plants (root or leaf) and *At-L23a* gene (*Arabidopsis thaliana* ribosomal protein L23a) in the whole plant of wild-type (WT) *Arabidopsis*, *TFT6*-overexpressing *Arabidopsis* plant (L6-1) and *TFT7*-overexpressing *Arabidopsis* plant (L7-1) under phosphorus-sufficient conditions (Control: CK; 1 mM phosphorus) or low phosphorus stress (LP; 2 μM phosphorus). Total RNA was extracted from tomato plants or *Arabidopsis* plants. Relative expression levels were calculated and normalized with respect to tomato α-tubulin mRNA [= 100 relative expression units (REU)] and *Arabidopsis At-ACT2* mRNA (= 100 REU). α-Tubulin or *At-ACT2* is a strongly and constitutively expressed 'house-keeping' gene in tomato plants or *Arabidopsis* plants. Changes in the REU of gene mRNA were checked for statistical significance according to Student's *t*-test ($P < 0.05$). Bars show the mean ± SD ($n = 6$).

Figure S2. Phylogenetic position of *TFT6* or *TFT7* in the cladogram illustrating relationships between protein sequences encoded by genes in the 14-3-3 gene family of tomato (*TFT1–TFT12*) and *Arabidopsis* plants (*GRF1–GRF12*).

Figure S3. Expression of *TFT6* and *TFT7* (tomato 14-3-3 protein) in wild-type (WT) *Arabidopsis* plants, *TFT6*-overexpressing *Arabidopsis* lines (from L6-1 to L6-6) and *TFT7*-overexpressing *Arabidopsis* lines (from L7-1 to L7-6) by real-time RT-PCR. Total RNA was extracted from 15-day-old *Arabidopsis* plants. Relative expression units (REU) were calculated and normalized with respect to *At-ACT2* mRNA (= 100 REU). *At-ACT2* is a strongly and constitutively expressed 'house-keeping' gene in *Arabidopsis* plants. The values are the means and SD of six replicates from two independent experiments.

Table S1. Genes and gene-specific primers used for real-time RT-PCR.

Table S2. Expression of *Arabidopsis* endogenous 14-3-3 genes and three *Arabidopsis* phosphorus-signalling pathway genes in the wild-type (WT) plants and transgenic plants under normal growth condition (Control: CK) or low phosphorus stress (LP). No significant changes were found on the expression level of *Arabidopsis* endogenous 14-3-3 genes (GRF1–GRF11) and three *Arabidopsis* phosphorus-signalling pathway genes (*At-SIZ1*, *At-PHF1* and *At-SPX1*) between the WT plants and transgenic plants under CK (data not shown). So, the expression of these studied genes (GRF1–GRF11; *At-SIZ1*, *At-PHF1* and *At-SPX1*) in WT

plants under CK was taken as onefold, and then the expression level of these studied genes in WT plants or *TFT6*-overexpressing *Arabidopsis* lines (L6-1, L6-3, L6-6) or *TFT7*-overexpressing *Arabidopsis* lines (from L7-1, L7-5, L7-6) under LP was shown in this table. The values are the means and SD of six replicates from two independent experiments.

Table S3. Activities of membrane-bound proteins (μmol min^{-1} mg^{-1} proteins) in microsomes and plasma membrane vesicles isolated from the whole root of wild-type (WT) *Arabidopsis* plants. The inhibitor-sensitive was calculated by subtracting the ATP-hydrolytic activity in the presence of inhibitor from the activity of control. The data were subjected to analysis of variance and *post hoc* comparisons were done with Duncan's multiple range test at $P < 0.05$ level. The statistical software program used was SPSS version 13.0. The values are the means and SD of six replicates from two independent experiments. N.D. indicated that expression is not detectable.