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The effects of K^+-deficiency on H_2O_2 dynamics and sucrose in tomato

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Abstract: Potassium (K^+) deficiency inhibits the transport of photosynthetic products and causes severe crop yield losses. However, the underlying mechanisms are poorly understood. In this study, we used two tomato lines *081018* (K^+-deficiency-sensitive) and *081034* (K^+-deficiency-tolerant), showing tolerance to K^+ deficiency to investigate the relationship between the H_2O_2 and sucrose in the tomato under K^+-deficiency. The H_2O_2 accumulation was increased by the low K^+ condition (0.5 mM) after 8 h in *081018*. The enzymes related to the metabolism of H_2O_2 were decreased, and more malondialdehyde (MDA) was produced. After 24 h, the sucrose content had accumulated significantly in the leaves, however, it was deficient in the roots, and the expression level of the sucrose transporters (*SUT1*) was inhibited. In *081034*, the activity of antioxidant enzymes was increased under K^+-deficiency, and then the H_2O_2 subsequently returned to the control treatment (4 mM) levels and did not produce more MDA. The sucrose content was not significantly different from the control treatment after 24 h. The expression of *SUT1* was not suppressed. These results suggested that the H_2O_2 dynamics played different roles in the two different strains. The transportation of sucrose was suppressed by the H_2O_2 from the leaf (source) to the root (sink) in *081018*, and unrestricted by the advantageous reactive oxygen species dynamics capacity in *081034*.

Keywords: low K^+ stress; oxidation; antioxidant enzyme activity; sucrose transport; *SUT1*

During evolution, plants have developed a series of signal transduction mechanisms to deal with K^+-deficiency stresses (Ho, Tsay 2010). Plants respond to low K^+ stress signals under K^+-deficiency and exhibit appropriate physiological responses to maintain life-sustaining activities (Wang, Wu 2010).

Photosynthetic products assimilated by mature tomato leaves are mainly transported as sucrose from the source to sink tissues, such as the fruits and roots, where they are utilised for growth or storage as a reserve, except for its metabolism (Osorio et al. 2014). The synthesis, transport and distribution of sugars in plants are complex processes. Sucrose transporters (SUTs) are mainly responsible for the process of loading sucrose into the phloem for long-distance transportation (Lemoine 2000; Hackel et al. 2006;

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Schmitt et al. 2008). In recent years, numerous studies have shown that H_2O_2 is not only a toxic substance that damages the cells, but also functions as common signalling molecules at certain concentrations (Neill et al. 2002). H_2O_2 is involved in the cell defence responses, cell death and physiological and biochemical reactions in growth and morphogenesis (Apel, Hirt 2004; Quan et al. 2008), as well as plant stress responses (Suzuki et al. 2012). Under K^+-deficiency stress, the sucrose transport is restricted from leaves to roots, as K^+-deficiency stress is not conducive to the transport and distribution of photosynthetic products from the leaves to the roots (Huber 1984; Cakmak et al. 1994a; Gerardeaux et al. 2010). Some studies have indicated that H_2O_2 plays an important role in K^+-deficiency stress perception by regulating the synthesis and gene expression of the related enzymes (Hafsi et al. 2014; Chérel et al. 2014). It is unknown whether H_2O_2 regulates the synthesis and transport of photosynthesis between the leaves (source) and the roots (sink). In this study, the relationship between H_2O_2 dynamics and sucrose was analysed.

MATERIALS AND METHODS

Plant materials and stress treatments. Two tomato lines, K^+-deficiency-sensitive genotype *081018* and K^+-deficiency-tolerant genotype *081034* (Zhao et al. 2011), were used. The tomato seedlings grew in a greenhouse with day/night temperatures of approximately 26 °/18 °C in a daily average irradiance of 350 μmol/(m^2·s) and at 75% relative humidity (RH). The chosen seedlings that grew well and uniform at the vegetative growing stage (28 days) were cleaned with distilled water three times and transferred to a pot containing 12 L of a nutrient solution. Each pot was filled with a nutrient solution containing 1.5 mM of $Ca(NO_3)_2{\cdot}4H_2O$, 4 mM of KNO_3, 3.5 mM of $NaNO_3$, 0.67 mM of $NH_4H_2PO_4$, 2 mM of $MgSO_4{\cdot}7H_2O$, 0.05 mM of H_3BO_3, 0.009 mM of $MnSO_4{\cdot}4H_2O$, 0.7 mM of $ZnSO_4{\cdot}7H_2O$, 0.32 mM of $CuSO_4{\cdot}5H_2O$, 0.1 mM of $(NH_4)_2MoO_4$, 0.05 mM of $FeSO_4{\cdot}7H_2O$, and 0.04 mM of Na_2-EDTA. At the vegetative growing stage (35 days), the K^+-deficiency treatment was applied by reducing the concentration of KNO_3 from 4 mM to 0.5 mM in the nutrient solution. The concentration of 4 mM of KNO_3 was used as a control. One gram of tissue from the third compound leaf and lateral roots (no taproots) of each seedling was collected at 0 h, 4 h, 8 h, 12 h, 24 h, 2 days, 4 days and 6 days for the K^+-deficiency-treated and control plants. The samples were quickly frozen in liquid nitrogen and stored at –80 °C for the quantitative real-time polymerase chain reaction (qRT-PCR) analysis. Fresh plant samples were used for the determination of the physiological indicators.

Determination of H_2O_2 contents and related protective enzymes. The foliar H_2O_2 concentrations were determined after Patterson et al. (1984).

The superoxide dismutase (SOD) activity was assayed by monitoring the inhibition of a nitro blue tetrazolium (NBT) photochemical reduction. Each sample was homogenised in four volumes of an ice-cold 50 mM phosphate buffer, pH 7.8, containing 0.1 mM of EDTA. Each crude extract was then dialysed for 24 h against a half-strength extraction buffer, and was centrifuged for 20 min at 15 000 × *g*. The supernatant was used for the SOD activity assay. A crude extract (100 µL) was added to a reaction mixture (3 mL) containing 50 mM of the phosphate buffer, pH 7.8, 0.053 mM of NBT, 10 mM of methionine and 0.0053 mM of riboflavin. The reaction was started by exposing to a cool-white fluorescent light (at a photosynthetic photon flux density (PPFD) of 50 µmol/(m^2·s) for 7 minutes. One unit of SOD activity is defined as the amount of enzyme required to inhibit 50% of the reduction in the NBT levels measured at 560 nm.

The catalase (CAT) activity was determined by monitoring the degradation of H_2O_2 for 5 min at 240 nm. Each reaction mixture (3 mL) was based on 25 mM of the phosphate buffer (pH 7.0) containing 0.8 mM of Na-EDTA and 20 mM of H_2O_2. The assay was performed at 25 °C.

The peroxidase (POD). Using guaiacol as the substrate, each reaction mixture consisted of 0.5 mL of a crude leaf or root enzyme extract, 0.5 mL of a 50 mM sodium acetate buffer (pH5.6), 0.5 mL of 20 mM guaiacol, and 0.5 mL of 60 mM H_2O_2. The linear increase in the absorbance at 480 nm, due to the formation of tetraguaiacol, was monitored for 4 min at 30 °C. The POD activity was calculated based on the change in the absorbance and was expressed in mmol tetraguaiacol produced $min^{-1}{\cdot}g^{-1}$·FW of the leaf or root tissue using a molar extinction coefficient of 26.6 mM/cm. A sodium acetate buffer was used as a control.

The ascorbate peroxidase (APX) was extracted from 500 mg of the fresh leaf or root material using 50 mM of the potassium phosphate buffer, containing 1 mM of EDTA, 8% (v/v) glycerol and 1.0 mM

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of ascorbate (pH 7.0). The APX activity was determined in 2 mL of the 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM of ascorbate, 0.1 mM of H_2O_2 and 50 μL of the crude extract. The APX activity was measured by following the linear decrease in the absorbance at 290 nm and expressed in μmol ascorbate $min^{-1} \cdot g^{-1}$ FW using a molar extinction coefficient of 2.8 mM/cm.

Determination of MDA contents. A 200 mg fresh plant sample was homogenised in 0.05 M of the phosphate buffer (pH 7.8), transferred to 15-mL centrifuge tubes containing 1 mL of 0.5% thiobarbituric acid and 20% trichloroacetic acid, and incubated for 30 min in a boiling water bath. After centrifugation at 1,800 × g for 10 min at 4 °C, the supernatant absorbances at 450 nm, 532 nm and 600 nm were measured using a DU-800 spectrophotometer (Beckman-Coulter, Brea, CA, USA). The MDA content was calculated as: MDA (nmol/g FW) = 1 000 × $6.45(OD_{532}–OD_{600}) – 0.56OD_{450}$

Determination of sucrose contents. The sucrose was measured using a Waters 600E high-performance liquid chromatography system (Waters, Milford, MA, USA). A carbohydrate column and a 2410 refractive index monitor were used. The mobile phase comprised 75% acetonitrile and ultrapure water (75 : 25, V/V). The mobile rate was 1.0 mL/min and the temperature of the column was 35 °C. Waters Millennium software was used for the data analysis.

qRT-PCR analysis. The first-strand cDNA was synthesised using a PrimeScript 1st Strand cDNA Synthesis Kit (TIANGEN, Beijing, China). The qRT-PCR was performed in triplicate for each sample using a SYBR Green Real Master Mix. The qRT-PCR amplification was performed using the Bio-Rad CFX Manager 3.1 Real Time PCR System (Applied Biosystems, Foster City, CA, USA) and software (Applied Biosystems). The $2^{-\Delta\Delta Ct}$ method was used to analyse the relative changes in the gene expression levels from three biological replicates. The data were analysed based on the mean values of the triplicates. The specificity of the reactions was verified through a melting-curve analysis.

Statistical Analysis. The data were analysed using a one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) multiple comparison tests in Statistical Productions and Service Solutions 17.0 (SPSS, Chicago, IL, USA). The asterisks (* and **) indicate a significant difference between the controls and the plants under the K^+-deficient conditions at $P < 0.05$ and 0.01, respectively.

RESULTS AND DISCUSSION

H_2O_2 and Sucrose Levels in *081018* and *081034* under K^+-Deficiency Stress. The changing trends in the H_2O_2 concentration and sucrose contents were different between *081018* and *081034* under the K^+-deficiency stress. In *081018*, the H_2O_2 was significantly higher in the leaves and roots than the control after 8 hours. The production of sucrose in the leaves was suppressed during 24 h and significantly lower than the control treatment at 12 hours. After 24 h, the sucrose concentration was significantly higher than the control treatment. The sucrose concentration in the root was always lower than the control treatment from 8 h and reached a highly significant difference at 24 hours. Compared with the control treatment, the sucrose accumulated in the leaves and was scarce in the roots. The transportation of sucrose from the source to the sink was affected. In *081034*, the concentration of H_2O_2 was significantly higher than the control at 8–12 h in the leaves and higher than control at 8 h in the roots, then decreased back to the control level. The concentration of sucrose in the leaves was significantly lower than the control treatment at 8–12 hours. The sucrose concentration in the roots was significantly lower than control just at 12 hours. The concentration of sucrose in the leaves and roots was significantly lower than the control during 24 h, but after 24 h, there was almost no difference. The transportation of sucrose from the source to the sink was normal (Figure 1). Some studies suggest that the soluble sugar increased in the plant leaves and decreased in the sink tissues under K^+-deficiency stress (Cakmak 1994b; Wang et al. 2012; Hu et al. 2015). This phenomenon appeared in 081018, but not in 081034.

Antioxidant enzyme activity in *081018* and *081034* under K^+-deficiency stress. The antioxidant enzyme activities show different trends in the expression for the two tomato lines. In *081018*, the SOD and CAT activity were decreased by the K^+-deficiency treatment, and lower than the control between the leaves and roots. In *081034*, they were higher than the control levels in the leaves and roots. In *081018*, the POD activity was lower than the control in the leaves. The APX activity was lower than the control in the leaves after 12 hours. In *081034*,

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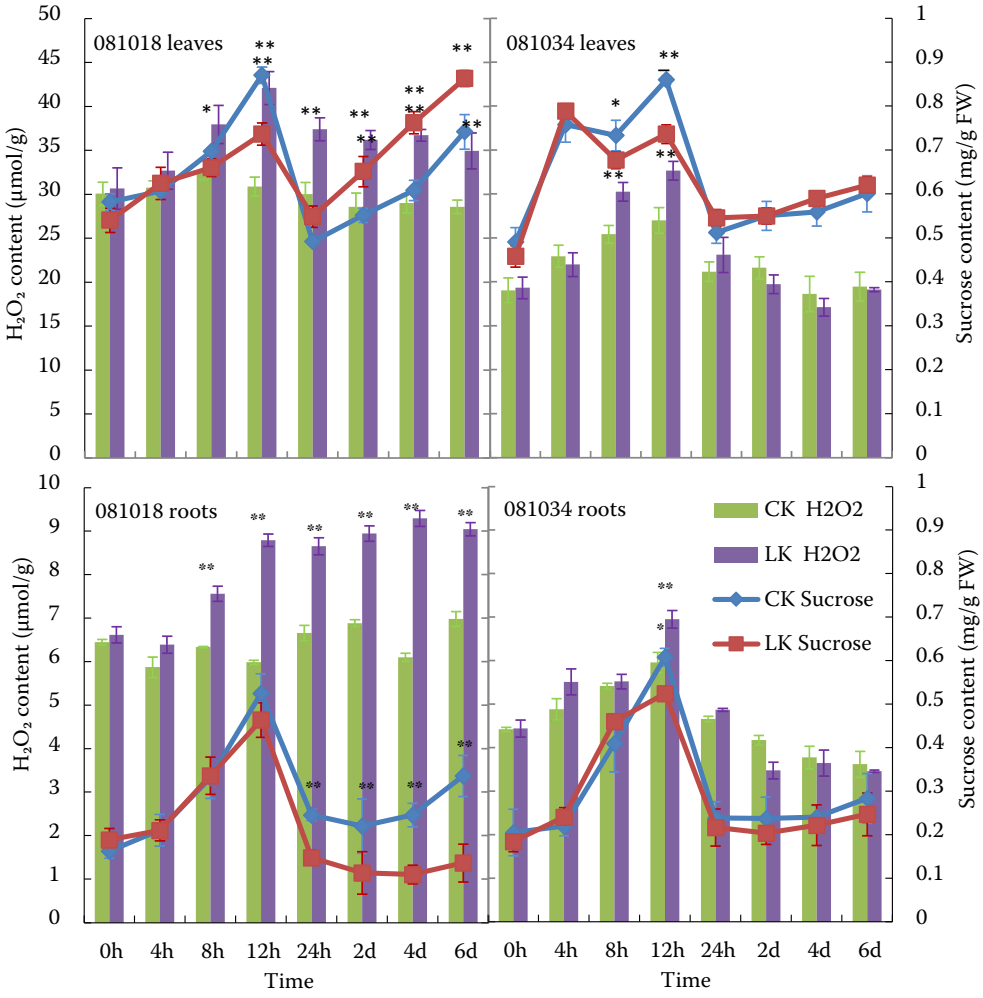

Figure 1. Comparison of the H_2O_2 and sucrose content changes in the leaves and roots of the different tomato genotypes under K^+-deficiency stress

The experiments were repeated three times. CK represents normal K^+ (4 m M); LK represents K^+ deficiency (0.5 m M); *, ** denote significant differences at $P < 0.05$ and $P < 0.01$, respectively

the POD and APX activity were higher than the control levels in the leaves and roots, the POD activity began to increase in the roots at 8 h of treatment and consistently remained higher than the control levels thereafter. The APX activity reached higher levels at 12 h in the leaves and 8 h in the roots (Figure 2). Comparing the content of the enzymes related to the active oxygen metabolism in the two strains, *081034* has the potential for a more optimised active oxygen metabolism.

MDA contents in *081018* and *081034* under K^+-deficiency stress. The MDA content in the two lines showed significantly different expression trends under the K^+-deficiency stress (Figure 3). In *081018*, the MDA content was higher than the control after 4 h of the treatment, while the MDA content in *081034* was not higher than the control. The *081018* tomato has a higher degree of membrane peroxidation, reflecting that its cell damage is more serious.

Expression of *SUT1* in *081018* and *081034* under K^+-deficiency stress. In the *081018* leaves, the expression of *SUT1* was significantly lower than control under the K^+-deficiency stress after 24 h (Figure 4). In the *081034* leaves, there was no significant difference in the expression level between the K^+-deficiency and the control. The expression of *SUT1* was significantly lower than the control at 2, 4, and 6 days in the roots of *081018*, the sucrose transport from the source to the sink was inhibited under the K^+-deficiency, which leads to a carbohydrate accumulation in the leaves and a reduction in the roots (Huber 1982). This was the

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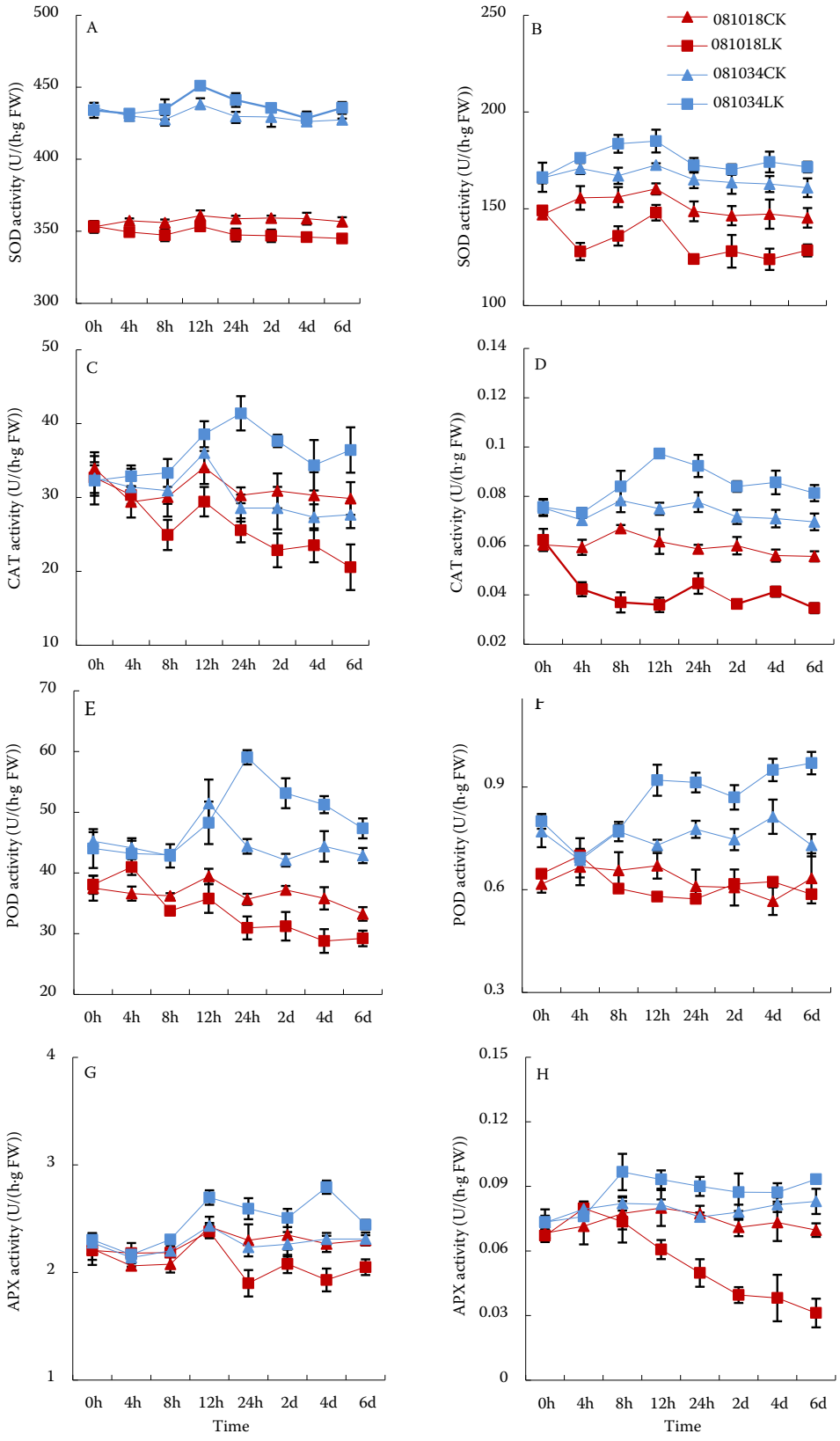

Figure 2. Comparison of the antioxidant enzyme activity changes in the leaves (A, C, E, G) and roots (B, D, F, H) of the different tomato genotypes under K^+-deficiency stress conditions

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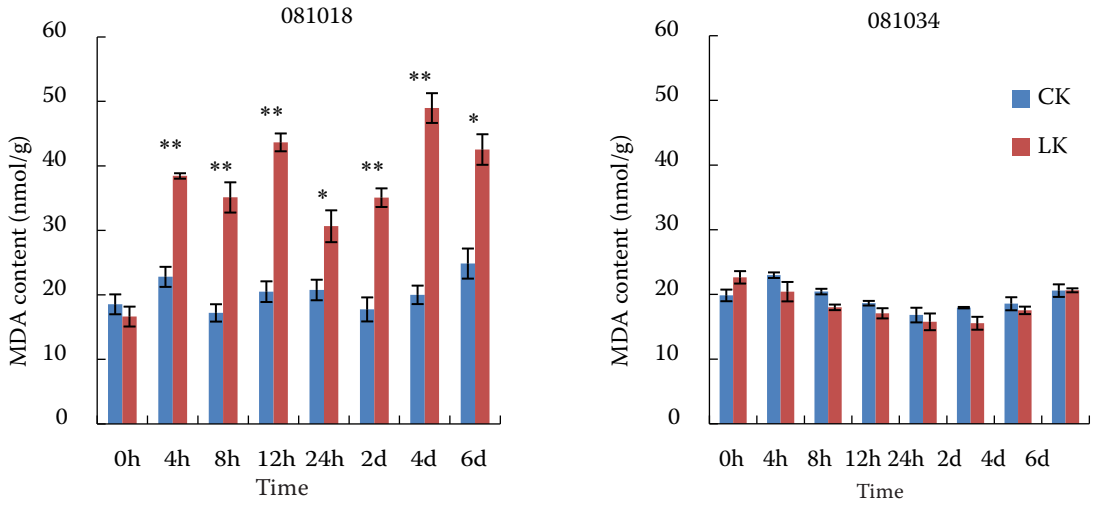

Figure 3. MDA contents in *081018* and *081034* under K^+-deficiency stress conditions

CK – K^+ concentration is 4mM; LK – K^+ concentration is 0.5mM

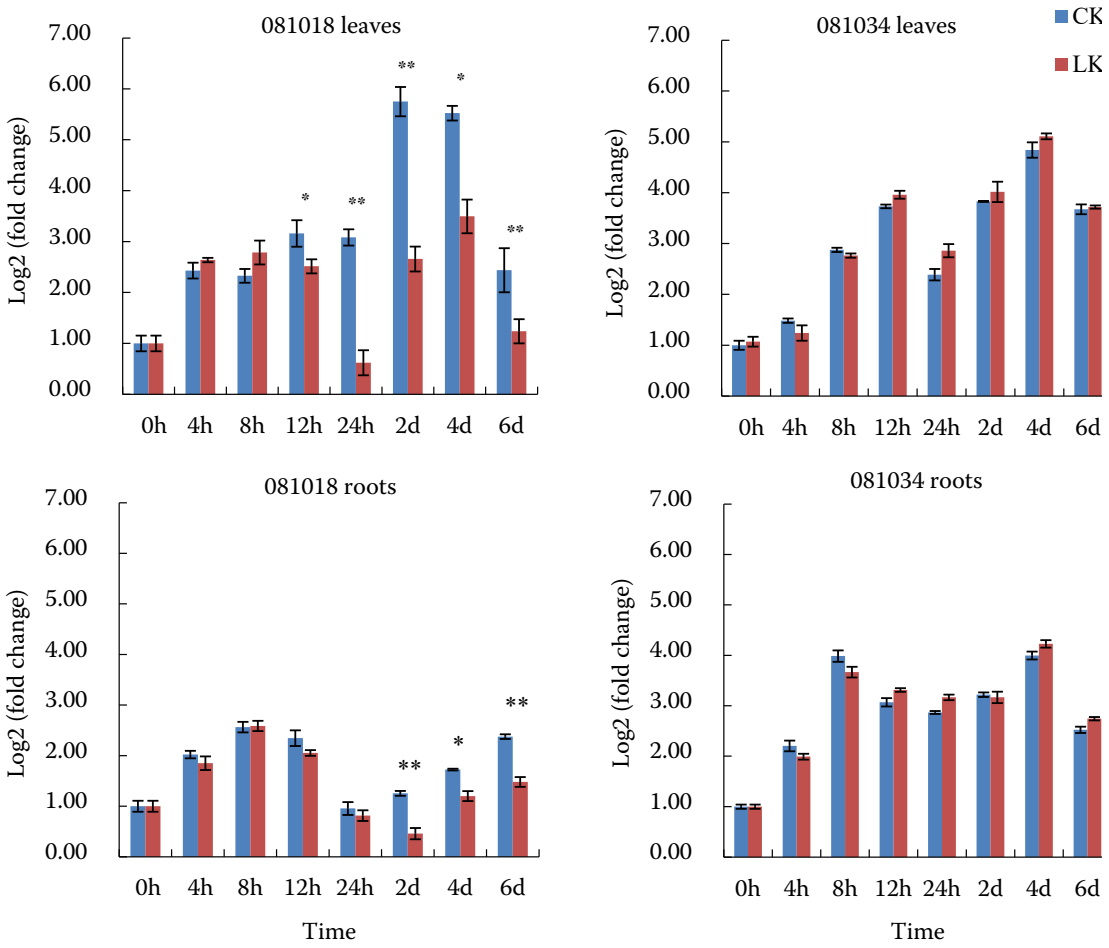

Figure. 4. Comparison of the *SUT1* changes in the leaves and roots of the different tomato genotypes under K^+-deficiency stress conditions

CK – K^+ concentration is 4mM; LK – K^+ concentration is 0.5mM

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same as our experimental results (Figure 1). In the *081034* roots, the expression of *SUT1* was not significantly different from the control. *SUT1* is supposed to be the main phloem loader of sucrose and was immunolocalised in the plasma membranes of the enucleate sieve elements (Schmitt et al. 2008). We believe that the excessive accumulation of H_2O_2 in *081018* has damaged the membrane structure to a certain extent (Figure 3), while the sugar transporters cannot transport sucrose normally. The comparative transcriptome profiling of the 081018 and 081034 tomato lines responded to the K^+-deficiency stress. The number of genes involved in the reactive oxygen metabolism reached 110 during 24 hours. There are only 14 genes related to carbohydrate metabolism (Zhao et al. 2018). In addition, some studies suggested that soluble sugars can act as signal to influence changes in reactive oxygen species (Couée et al. 2006; Moustakas et al. 2011). However, in this research, the changes in the H_2O_2 content and metabolism-related enzymes were more rapid than those of the soluble sugar content after the K^+-deficiency stress.

CONCLUSION

This study shows that the *081034* genotype has a strong H_2O_2 scavenger capacity and no obvious cell damage. The sucrose did not accumulate in the leaves, and the expression of *SUT1* had no significant differences in the plants compared with the control. The H_2O_2 significantly accumulated in *081018*. The membrane peroxidation in the cells was severe. After 24 h, the content of soluble sugars in the leaves was gradually higher than the control, and decreased in the roots, while the expression of *SUT1* was lower than the control. The sucrose accumulation in the leaves was related to the expression of *SUT1* under stress.

Tomatoes with different tolerances to low K^+ have different reaction mechanisms to the accumulation of H_2O_2 in the plants under the K^+-deficiency stress, the metabolism and scavenging ability were two of the key factors for the tomatoes having good tolerance to the K^+-deficiency stress. The poor scavenging ability of H_2O_2 results in the blocked transport of nutrients from the source to the sink, thus affecting the growth. Therefore, H_2O_2 metabolism can be used as a key indicator for screening and cultivating low K^+ tolerant tomatoes.

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