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Effects of salt stress on the expression of key genes related to nitrogen assimilation and transport in the roots of the cultivated tomato and its wild salt-tolerant relative

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ABSTRACT

Inorganic nitrogen is a key element for plant growth under salt stress. A comparative study including physiological responses, ion content, transcript regulation of ammonium/nitrate transporters (*AMTs*/*NRTs*) as well as key enzymes for nitrogen assimilation was undertaken in wild salt-tolerant tomatoes (*Solanum pennellii*) and cultivated tomatoes (*Solanum lycopersicon*) exposed to 100 mM NaCl for 1 and 7 days. In comparison to *S. lycopersicon*, *S. pennellii* was more salt tolerant as evidenced by its higher survival rate, lower biomass reduction, and less salt injury (reduced electrolyte leakage and proline accumulation). In root tissues of both species, salt exposure (7 days) reduced the mRNA expression levels of low affinity nitrate transporters (*NRT1.1* and *NRT1.2*). This was associated with a decline in both nitrate content and expression level of the nitrate reductase gene (*NR*). Salt-stressed root tissues of *S. pennellii* showed relatively higher mRNA expression of the high affinity ammonium transporters (*AMT1.1* and *AMT1.2*) compared to *S. lycopersicon*. The root ammonium content was increased only in *S. lycopersicon* going hand in hand with a reduction in mRNA level of cytosolic glutamine synthetase (*GS1*) after 7 days of salt stress, whereas the expression level of *GS1* was unchanged in *S. pennellii*, suggesting a lower salt-induced inhibition in ammonium assimilation in this species. Our comparative study demonstrated that the salt-tolerant and salt-sensitive tomato species show differential contribution of the nitrogen transporters and key genes associated with nitrogen assimilation under salt stress. While the reduction in the expression of key components of NO_3^- uptake (*NRT1.1, NRT1.2*) and assimilation (*NR* gene) in both species, likely contributed to the reduction in plant growth under salt stress, the observed salt tolerance for *S. pennellii* was associated with relative higher mRNA expression of ammonium uptake and assimilation genes. These results provide crucial knowledge for tomato breeding employing salt-tolerant wild species in salt-induced nitrogen- deficient environments.

1. Introduction

Salinity is one of the most prevalent stresses that limits plant growth and crop productivity. While elevated salinity is common in arid and semiarid regions, agricultural practices and the limitation of fresh water supplies have contributed to increased saline lands in all climatic regions (Niu and Cabrera, 2010). The adverse effects of salinity on plants result primarily from osmotic and/or ionic stresses caused by the salt-induced decrease in soil water potential and the accumulation of ions (mainly Na^+ and Cl^-) in the plant tissues respectively (Hasegawa et al., 2000). In addition, salt-stressed plants often exhibit nutrient imbalances (Hasegawa et al., 2000); for example, uptake, assimilation and nitrogen (N) content in plant cells can be reduced by high salt levels in soils in a number of economically important plant species, including tomato (*Solanum lycopersicon*) (Flores et al., 2000; Dluzniewska et al., 2007). This decrease in N content in plants exposed to salt stress has been attributed to the reduction in NO_3^- uptake by roots due to the competition with NO_3^- and Cl^- (Botella et al., 1994; Yao et al., 2008). In addition, salt-induced changes in membrane integrity (Ca^{2+} displacement and oxidative damage) could further affect NO_3^- uptake (Frechilla et al., 2001). Nitrogen fertilization plays a critical role in the alleviation of salinity stress (Flores et al., 2001). Therefore, maintenance of N homeostasis under saline environment seems to be a key element for salt tolerance (Popova et al., 2003; Ehlting et al., 2007) and N uptake through root transporters may play a key

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role for maintaining N balance in salt-stressed plants (Senadheera et al., 2009 and Zhang et al., 2014).

Nitrate (NO_3^-) and ammonium (NH_4^+) ions, the two main sources of N available in soils for plants (Von Wirén et al., 2000), are absorbed by roots using a variety of transporters. Nitrate transporters (NRTs) mediate NO_3^- uptake across the plasma membrane of the plant root cells by a mechanism of proton-coupled symport (Ullrich and Novacky, 1990) using two kinetically distinct nitrate transporters depending on the nitrate concentration in the soil (Forde, 2000; Hildebrandt et al., 2002; Gojon et al., 2011). The high affinity transport system (HATS) is encoded by the *NRT2* gene family and is used with optimal environmental NO_3^- concentrations below 100 μM. The low affinity transport system (LATS) is encoded by *NRT1* and operates at optimal NO_3^- concentrations higher than 1 mM. In *S. lycopersicon*, two families of nitrate transporter genes, *NRT1* and *NRT2*, have been identified as contributors to the NO_3^- uptake system. Ammonium transporters (AMTs) function as NH_4^+ uniporters and mediate NH_4^+ uptake through high-affinity ammonium uptake system (Ludewig et al., 2007). The proteins responsible for low-affinity system for ammonium have not been identified yet, but it has been suggested that the low-affinity system might be mediated by aquaporins and cation channels (Tsay and Hsu, 2011). In *S. lycopersicon* roots, two ammonium transporters *AMT1.1* and *AMT1.2* are involved in root ammonium uptake, while the expression of *AMT1.3* was detected in leaves, suggesting for this transporter a different role in N metabolism (Von Wirén et al., 2000).

Plant roots are key organs for N uptake but also represent the first defense barrier against salt stress as they are in direct contact with soil solutions (Senadheera et al., 2009). In salt-stressed roots of *Oryza sativa* (rice) and *Populus simonii* (Chinese poplar) changes in mRNA expression levels of NH_4^+ and NO_3^- transporters have been suggested to be involved in salt tolerance through the regulation of the uptake and transport of NH_4^+ and NO_3^- (Senadheera et al., 2009; Zhang et al., 2014). In NO_3^- fed *O. sativa*, NH_4^+ and NO_3^- transporter genes have been reported to be differentially expressed in roots of salt-tolerant *O. sativa* and salt-sensitive plants (Senadheera et al., 2009). These differences in gene expression in salt-stressed root tissues could be beneficial to withstand salt-induced N deficiency in the salt-tolerant cultivar (Senadheera et al., 2009). In their study, the regulation of nitrogen transporters during salt stress was investigated in the presence of only one source of N. Since NO_3^- and NH_4^+ are the two major nitrogen sources for non-legume plants (Tsay and Hsu, 2011), studying the regulation of different N transporters in plants fed with both NH_4^+ and NO_3^- could be key to understand nitrogen uptake mechanisms during salt stress. A recent study in salt-stressed *Populus simonii,* a moderately salt-tolerant species, showed that the mRNA expression of NH_4^+ transporters were up-regulated in salt-stressed roots, whereas the expression of most of the NO_3^- transporters were down-regulated. This suggests a role of NH_4^+ transporters in NH_4^+ flux during acclimation to salinity stress (Zhang et al., 2014). Despite the efforts made to understand the effect of salt stress on nitrogen transporters and their involvement in salt tolerance, most of the research has focussed on glycophytes; while halophytes that have the ability to cope with salinity, have been rather neglected.

The use of comparative studies of closely related species that differ in their salt tolerance is a powerful strategy to understand the mechanisms of tolerance to this particular stress (Sun et al., 2010). *Solanum lycopersicon*, one of most important vegetable crops in the world, is moderately sensitive to salinity (Sun et al., 2010). The availability of wild halophytic *Solanum* species makes this species an ideal model crop for studying salinity tolerance. Thus, the objective of our study was to compare salinity responses of well-characterized salt-sensitive species, *S. lycopersicon* cultivar "Manitoba" and the halophyte species, *S. pennellii* to identify the contribution of different N transporters and N assimilation genes of roots in salt tolerance. Therefore, the physiological responses and the mRNA expression levels of selected key genes encoding N transporters and N assimilation enzymes in salt-stressed roots of both species with NH_4NO_3 supplies have been investigated. We hypothesized that under salt stress, the expression pattern of ammonium and nitrate transporter genes will be different between salt tolerant and salt sensitive tomato species and that these differences could be related to salt tolerance. Investigating the effect of salinity-stress on root mRNA expression levels of N-transporter genes and genes important in N-assimilation will make a valuable contribution to breed tomato cultivars that can better withstand salt stress.

2. Materials and methods

2.1. Plant growth and salt treatment

Seeds of the cultivated tomato, *Solanum lycopersicon* (cv. Manitoba, obtained from T&T seeds, Winnipeg, Canada), and its wild salt-tolerant relative, *S. pennellii* (obtained from Tomato Genetics Resource Center, Davis, CA, USA—accession LA0716), were selected for this study. In general, tomato plants are more sensitive to salinity stress at the seedling stage (Sun et al., 2010); thus, our study was carried out at this stage. The seeds were sown into seedling trays containing a mixture of peat moss: perlite (2: 1, v: v). When the first true leaf emerged (two to three weeks), the seedlings were transferred for one week to an aerated hydroponic solution (half strength modified Hoagland solution containing 2 mM NH_4NO_3; 1 mM KH_2PO_4; 1.5 mM $CaCl_2$; 0.5 mM KCl_2; 1 mM $MgSO_4$; 23 μM H_3BO_3; 5 μM $MnCl_2$; 0.4 μM $ZnSO_4$; 0.2 μM $CuSO_4$; 0.07 μM H_2MoO_4 in addition to 0.007 μM Fe-EDTA) (Sabra et al., 2012) for acclimation (six plants per 10-l containers). The nutrient solution was renewed every 3 days to prevent nutrient deficiency. Plants were grown under the following greenhouse conditions: 24/19 °C day/night temperatures, 18 h/6 h photoperiod with natural light supplemented by sodium lamps (P.L. light systems, Beamsville, ON, Canada). Seedlings from both species were exposed to salinity stress at the stage of three true leaves by adding 100 mM NaCl to the nutrient solution. Three plants from each of the two species were placed in 10-L containers, which were distributed in a completely randomized block design of six replicates.

After 1 day and 7 days of treatment, root tissues were collected and stored in RNAlater® (Ambion) at −80 °C for subsequent mRNA expression analysis. To confirm the difference in salt tolerance of the two species, several key physiological and biochemical parameters (Sun et al., 2010; Sabra et al., 2012) were measured after 7 days of salt treatments, including relative water content (LRWC), electrolyte leakage (EL) and proline content. The remaining plants were harvested, washed three times with distilled water; the area of fully-expanded leaves was measured with a leaf meter (model 3000, LI-COR Inc. Nebraska, USA) and the tissues were lyophilized for determining dry weight and ion analysis.

As all seedlings survived after one week of 100 mmol l^{-1} NaCl, in a second experiment, we exposed *S. lycopersicon* and *S. pennellii* plants to 200 and 300 mmol l^{-1} NaCl for 7 days in parallel experiments (similar set up as the one described above) with four replicates. Survival rates were recorded to further determine the difference in salt tolerance of the two tomato species.

2.2. Leaf relative water content (LRWC)

Leaf relative water content was estimated after 7 days of salt treatment in fully-expanded detached leaflets. After recording the fresh weight (FW), leaf samples were placed in distilled water in a closed Petri dish in the dark at 4 °C for 24 h to determine