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Research article

Phenotyping two tomato genotypes with different nitrogen use efficiency

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ABSTRACT

Nitrogen (N) supply usually limits crop production and optimizing N-use efficiency (NUE) to minimize fertilizer loss is important. NUE is a complex trait that can be dissected into crop N uptake from the soil (NUpE) and N utilization (NUtE). We compared NUE in 14 genotypes of three week old tomatoes grown in sand or hydroponic culture supplied with nitrate (NO_3^-). Culture method influenced measured NUE for some cultivars, but Regina Ostuni (RO) and UC82 were consistently identified as high and low NUE genotypes. To identify why these genotypes had contrasting NUE some traits were compared growing under 0.1 and 5 mM NO_3^- supply. UC82 showed greater root $^{15}NO_3^-$ influx at low and high supply, and stronger *SlNRT2.1/NAR2.1* transporter expression under low supply when compared with RO. Conversely, RO showed a higher total root length and thickness compared to UC82. Compared with UC82, RO showed higher shoot *SlNRT2.3* expression and NO_3^- storage at high supply, but similar NO_3^- reductase activity. After N-starvation, root cell electrical potentials of RO were significantly more negative than UC82, but nitrate elicited similar responses in both root types. Overall for UC82 and RO, NUtE may play a greater role than NUpE for improved NUE.

1. Introduction

Nitrogen (N) availability is one of the most important factors limiting plant growth and productivity in both natural and agricultural environments (Marschner, 1995). Plant roots acquire N from the soil mainly as ammonium (NH_4^+), nitrate (NO_3^-) or amino acids (Miller and Cramer, 2005). In temperate climates, NO_3^- is the dominant N supply form as in most agricultural soils microbial conversion of organic N and NH_4^+ to NO_3^- rapidly occurs (Forde and Clarkson, 1999). Nitrate is also an important signal for plant growth and development, regulating N metabolism and assimilatory pathways (Stitt, 1999). Nitrate uptake by roots is an active process with transport systems that operate over different concentration ranges allowing plants to maximize acquisition depending on soil NO_3^- availability (Forde and Clarkson, 1999). A high affinity transport system (HATS) operates at low NO_3^- concentrations (0–0.2 mM) and has two defined parts. The constitutive transport system (cHATS) is always expressed and characterized by a greater NO_3^- affinity, and the inducible transport system (iHATS) generated by an increased NO_3^- supply and with a greater uptake capacity (Forde and Clarkson, 1999; Glass et al., 2002; Glass, 2009). The low affinity transport system (LATS), is also constitutively expressed and mediates NO_3^- uptake at high external concentrations (>1 mM) and displays linear kinetics (Glass, 2009). After NO_3^- is taken up by roots, it can be reduced to nitrite (NO_2^-) and then NH_4^+ and amino acids by N-regulated enzymes or translocated to the shoot where it is assimilated (Miller and Cramer, 2005; Glass, 2009). Within the *Arabidopsis* genome the NO_3^- influx transporters are encoded by two gene families, NPF or NRT1 (Léran et al., 2014) and NRT2 (Williams and Miller, 2001). Some of the NRT2 transporters require a small partner protein called NAR2.1 (or NRT3.1) for their function (Tong et al., 2005; Orsel et al., 2006). The activity of some root uptake transporters is regulated by internal and external N supply and is coordinated with N metabolism (Glass et al., 2002). Several transporters have a particular role in long distance xylem and phloem NO_3^- transport within the plant (Wang and Tsay, 2011; Xia

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et al., 2015). Nitrate uptake by roots requires energy to overcome the negative electrical potential across plasma membrane of root epidermal and cortical cells (Miller et al., 2001) which is provided by activity of the plasma membrane H^+-ATPase (PM H^+-ATPase), a key enzyme in plant nutrition (Palmgren, 2001).

The importance of optimizing N management practices together with genetic improvements to decrease excess fertilizer applications is well known (Good and Beatty, 2011) and much of the N fertilizer routinely applied to crops is leached, causing environmental damage (Good et al., 2004; Sebilo et al., 2013). The physiological and molecular steps involved in NO_3^- uptake and assimilation can be used to identify traits that are important for N Use Efficiency (NUE). This may be because the first step for this type of cultivar comparison requires a consistent definition of NUE. Plant NUE can be defined as the biomass produced per unit of applied N (Moll et al., 1982) or the dry mass production for N unit taken up from the soil (Hirose, 1971). Whatever the crop, root, leaf, fruit or seed the method to measure NUE usually depends on calculating the plant biomass production per unit of applied N (Good et al., 2004; Xu et al., 2011). Clearly, NUE is a complex trait that must be encoded by many different genes and their environmental interactions, but it can be dissected into two components. Firstly, the ability of the plant to take up N from the soil termed "nutrient uptake efficiency" and secondly the ability of the plant to transfer N to plant organs and yield, known as "nutrient utilization efficiency" (Xu et al., 2011). Several studies on model and crop species have highlighted the genetic variability and the complex regulatory mechanisms controlling NUE under growth limiting and non-limiting N supply (Krapp et al., 2011). Given the importance of the topic it is surprising that relatively few papers have compared measures of NUE for the same germplasm growing in different environments.

Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops. Long storage types of tomato are of great interest for their adaptation to abiotic stress conditions and they are often cultivated in Mediterranean regions where both drought and N-limited conditions are frequent. Improving tomato NUE is particularly important as large amounts of N fertilizer are required to obtain the best yield. It follows then that the identification of high and low NUE tomato genotypes, and the subsequent identification of their contrasting physiological and molecular traits, can be used to provide tools for developing marker-assisted breeding strategies. Although both NO_3^- and NH_4^+ are important N sources for tomato, we have focused on NO_3^- as this form is more readily leached from the soil. A model simulating diurnal net uptake rate patterns has been set up in tomato assuming a homeostatic mechanism, i.e. negative feedback regulation by plant NO_3^- content on uptake rates (Càrdenas-Navarro et al., 1998). Several tomato NO_3^- transporter genes, belonging to the NPF and NTR2 families, have been characterized in roots and chiefly in root hairs (Lauter et al., 1996; Ono et al., 2000; Wang et al., 2001). Like *Arabidopsis*, the tomato genes *NTR2.1* and *NTR2.2* appear very similar in their coding regions (95% identity) and their expression was predominantly in roots (Ono et al., 2000), with transcription maximum achieved 4 h after a 200 μM NO_3^- treatment (Ono et al., 2000). The recently completed sequencing of the tomato genome (Tomato Genome Consortium, 2012) now provides access to the sequence for more key candidate genes previously identified in model species like *Arabidopsis* as being important in NO_3^- uptake and assimilation and therefore likely to have a role in NUE.

In the present study, biomass production was used to calculate NUE for a collection of Italian tomato cultivars and one from California supplied with NO_3^- grown in hydroponics and sand to identify contrasting NUE genotypes. Two lines representing consistently high and low NUE ranges were selected for a more detailed analysis comparing their morphological, physiological and molecular traits growing in hydroponic culture. Nitrate transporter activity measurements using NO_3^--elicited changes in root cell membrane potential and ^{15}N influx, tissue NO_3^- reductase (NR) activity, and root morphology were evaluated. Finally, the expression of some assimilatory (NR) and NO_3^- transporter (*SlNPF6.3*, *SlNRT2.1*, *SlNRT2.3* and *SlNAR2.1*) genes was compared. This analysis identified some differences between the two contrasting phenotypes and these traits may be used as potential markers for tomato breeding to select for improved NUE.

2. Materials and methods

2.1. Plant materials

Thirteen recognized tomato landraces from distinct geographic regions of Southern Italy were chosen for this study. Ten landraces, namely *Pizzutello di Paceco, Pizzutello di Nubia, Linosa, Buttighieddu, Piriddu, Sinacori, POP 2, Inverno, Stella, Patataro* came from Sicily (University of Palermo, Italy), one, *Regina Ostuni* from Apulia (University of Bari, Italy) and two, *Vesuviano* and *San Marzano*, from Campania (CRA, Monsampolo del Tronto, Italy). In addition, the North American cultivar UC82, kindly supplied as seed by the Tomato Genetics Resource Center - Department of Plant Sciences, University of California Davis, was included.

2.2. Silver sand experiment

Seeds of each type of tomato were washed with 5% (v/v) NaClO for 15 min to surface sterilize the seed and then were germinated in a Petri dish (diameter 90 mm) on filter paper with 0.1 mM $CaSO_4$. After 7 d of germination, seedlings of uniform size were selected and transferred to pots (diameter 7 cm, 110 cm^3 volume), one plant per pot, filled with silver sand and the surface exposed to light was covered using black plastic film to prevent algal growth. Seedlings were daily watered with 5 mL modified Hoagland nutrient solution, containing 2.5 mM K_2SO_4, 2 mM $MgSO_4$, 1 mM KH_2PO_4, 1 mL L^{-1} Hoagland micronutrients and 2 mL L^{-1} FeEDTA. Nitrate was added as $Ca(NO_3)_2$ to the solution to give the following NO_3^- concentrations: 0, 0.1, 0.3, 0.5, 0.75, 1, 2.5, 5 and 10 mM. Furthermore, $CaSO_4$ in a range 0–5 mM concentration was added to the nutrient solution to adjust the Ca^{2+} concentration to the same value in all the treatments. The pH of the nutrient solution was adjusted to 5.8 with KOH. Tomato seedlings were placed in a growth chamber maintained at 23 °C, 70% RH and 16 h photoperiod with a light intensity of 340 μmol m^{-2} s^{-1} for a further 2 weeks. Five tomato seedlings (21-days old), for each NO_3^- concentration and genotype, were collected and divided into leaves, stem and roots. Finally, the plant material was placed in an oven at 70 °C for two days to determine leaf (LDW, g), stem (StDW, g) and root dry weight (RDW, g). Shoot dry weight (SDW, g) was calculated by adding LDW to the StDW.

2.3. Hydroponic experiments

Four tomato genotypes (UC82, Regina Ostuni, Linosa and Piriddu) were also grown in an aerated hydroponic system. After germination (as described above), seedlings of uniform size were placed into plastic pots (10 × 10 cm), with three plants per unit supported by netting above 700 mL of aerated nutrient solution, as described above containing 0, 0.1, 0.3, 0.5, 0.75, 1, 2.5, 5 and 10 mM NO_3^-. Care was taken to ensure there was the same amount of vigorous aeration in the pots. The nutrient solution was renewed every two days and the pH was adjusted to 5.8 with KOH. The plants were placed in a growth chamber in the same experimental