Plant Science 263 (2017) 94–106

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Genetic analysis of rootstock-mediated nitrogen (N) uptake and root-to-shoot signalling at contrasting N availabilities in tomato

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ARTICLE INFO

Keywords:
QTL analysis
SNP
Candidate genes
Salicylic acid
Cytokinins
Gene interactions

ABSTRACT

Selecting rootstocks for high nitrogen acquisition ability may allow decreased N fertilizer application without reducing tomato yields, minimizing environmental nitrate pollution. A commercial hybrid tomato variety was grafted on a genotyped population of 130 recombinant inbred lines (RILs) derived from *Solanum pimpinellifolium*, and compared with self- and non-grafted controls under contrasting nitrate availabilities (13.8 *vs* 1.0 mM) in the nutrient solution.

Grafting itself altered xylem sap composition under N-sufficient conditions, particularly Na^+ (8.75-fold increase) concentration. N deprivation decreased shoot dry weight by 72.7% across the grafted RIL population, and one RIL rootstock allowed higher total leaf N content than the best of controls, suggesting more effective N uptake.

Sixty-two significant QTLs were detected by multiple QTL mapping procedure for leaf N concentration (LNC), vegetative growth, and the xylem sap concentrations of Mn and four phytohormone groups (cytokinins, gibberellins, salicylic acid and jasmonic acid). Only three LNC QTLs could be common between nitrogen treatments. Clustering of rootstock QTLs controlling LNC, leaf dry weight and xylem sap salicylic acid concentration in chromosome 9 suggests a genetic relationship between this rootstock phytohormone and N uptake efficiency. Some functional candidate genes found within 2 Mbp intervals of LNC and hormone QTLs are discussed.

1. Introduction

Nitrogen is quantitatively the most important mineral nutrient taken up from the soil by plants [1] and one of the major factors limiting crop productivity and yield [2]. Consequently, nitrogen fertilization has successfully increased crop yield during the last 60 years, although there are differences between countries in the magnitude of N-mediated yield limitation [3]. Concern continues to grow about the effects of nitrate on both environmental and human health, because nitrate can accumulate in high concentrations in the leaves of edible plants and in the drinking water [4]. While the environmental effects of nitrate pollution of water courses are well known [5,6], there is some evidence that excessive nitrate consumption has been linked to infant mortality [7,8], thus farmers are legislatively bound to diminish nitrate effluent from their holdings and crop nitrate levels (eg. Council Directive 91/676/EEC; https://www.epa.gov/ground-water-and-drinking-water/table-regulated-drinking-water-contaminants). Decreasing both environmental pollution and input costs of N fertilizer application is an important goal of modern agriculture. Therefore, developing crops that need less mineral N fertilizer and with a better N use efficiency (NUE) is required.

Tomato is one of the most important horticultural crops. In terms of human health, tomato fruit is a major component of daily meals in many countries and constitutes an important source of minerals, vitamins, and antioxidant compounds. Breeding for NUE or tolerance to N deficiency could take advantage of the genetic diversity of wild *Solanum* species adapted to marginal environments. Since past tomato breeding programs grew plants under optimal (N-sufficient) conditions, this genetic variability has been likely lost in the domestication process. Thus, N-deficiency severely decreases chlorophyll content, leaf photosynthesis, biomass accumulation, and growth of current tomato cultivars [9,10].

Grafting is a biotechnological tool used since ancient times to improve the amount and uniformity of crop yield, and currently most fruit

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http://dx.doi.org/10.1016/j.plantsci.2017.06.012
Received 23 March 2017; Received in revised form 16 June 2017; Accepted 27 June 2017
Available online 14 July 2017
0168-9452/

crops and many horticultural species (including tomato) are grown as scion-rootstock combinations. Although this strategy triples the work required by breeders (selection for rootstock, scion and their combination), rootstock breeding employing wild genetic resources can confer resistance to biotic and abiotic stresses [11–13]. Since rootstocks can affect nutrient absorption, yield and fruit quality under stress conditions [14–17], those traits are usually targeted in rootstock breeding programs. Besides, genetic studies of rootstock effects are a valuable strategy to understand root functions (particularly nutrient uptake and transport) since they are shoot-regulated by root-shoot communication [18]. Following Gallais and Hirel [19], NUE can be considered as the product of N uptake efficiency (N-uptake/soil N reserves) and N utilization efficiency (yield/N-uptake). At high N-inputs, NUE was mainly related to variation in N-uptake, while at low N-input, both components could play a role and were difficult to distinguish one from each other [20]. Since the root is the main organ involved in N uptake, improving rootstock N acquisition under N-deprivation might allow less N fertilizer application, without decreasing tomato yields, but to our knowledge this approach has not been tried yet.

Plants store considerable quantities of nitrate in the vacuoles of root cells, which can be mobilised to the shoot. Following N deprivation of tomato, vegetative growth may be limited before any change in foliar N concentration is detected [21], suggesting that root-to-shoot signalling regulates growth. N deprivation decreases root hydraulic conductance [22] which may decrease leaf turgor thereby limiting growth, but maintaining N-deprived plants at full turgor (by pressurising the roots) was unable to maintain growth of N-deprived plants [23]. Alternatively, N-deprivation may alter root phytohormone delivery to the shoot. N deprivation decreases root export of growth-promoting cytokinins, while increasing export of ABA [24]. Nevertheless, pea mutants with lower root cytokinin and ABA export showed a similar relative growth response to N deprivation [25,26], suggesting that other phytohormones may be involved in growth regulation following N deprivation. The advent of multi-analyte physico-chemical techniques for phytohormone quantification offers substantial opportunities to more comprehensively evaluate changes in xylem sap composition following N deprivation [12].

Currently, QTL analyses can bridge the gap between agronomic performance and the DNA sequences involved. With the advent of the complete tomato genome sequence by the Tomato Genome Consortium [27], and the availability of a large panel of SNPs (SolCAP panel, http://solgenomics.net/), genome assembly allows the rapid identification of candidate genes around the physical position of the SNP (Single Nucleotide Polymorphism) with observed maximum LOD (Logarithm of the Odds) score. Using this approach, several transporter-coding genes within 2Mbp QTL intervals controlling leaf concentration of several nutrients (Na, B, K, Mg and Mo) were identified [28,15] in trying to gain biological information from the QTL analysis.

Using a commercial variety grafted on a *S. pimpinellifollium* RIL population grown under N-sufficient and N-deficit conditions, this study aimed to (1) estimate the heritability of the rootstock effect on vegetative growth, leaf N concentration and xylem sap composition (2) detect the QTLs involved and study their interactions, (3) disentangle the rootstock-dependent root-to-shoot communication and N acquisition pathways (4) investigate the genetic relationship of potential physiological components of rootstock-mediated N acquisition, and (5) infer possible candidate genes for LNC and hormone QTLs.

2. Materials and methods

2.1. Plant material, growth conditions and trait evaluation

This study used 130 F10 lines (P population) derived by single seed descent from the hybrid between a salt sensitive genotype of *Solanum lycopersicum* var. Cerasiforme (formerly *L. esculentum*) and a salt tolerant line from *S. pimpinellifolium* L. (formely *L. pimpinellifolium*) [29].

The commercial tomato hybrid *Solanum lycopersicum* cv. Boludo (Bol) was the scion, and plants from 130 lines of the P population were evaluated as rootstocks. Non-grafted (Bol) and self-grafted (Bol/Bol) plants were used as controls. Self-grafting placed a scion onto the roots of a different plant of the same genotype, and these controls were included to evaluate any physiological change caused by the grafting process *per se*.

Grafted plants having approximately 6 leaves were obtained from the seed company UNIGENIA Bioscience SLV (Murcia, Spain). Grafting was performed using the splicing method when seedlings had developed 3–4 true leaves [30]. Seedlings were cut at the cotyledonary node, using the shoot as scion and the remainder as rootstock. Grafts were made immediately after cutting the plants and grafting clips were used to adhere the graft union. Two experiments were conducted in two adjacent Venlo-type glasshouses of 144 m^2 at Wageningen UR Greenhouse Horticulture in Bleiswijk, The Netherlands. Each glasshouse consisted of 5 benches of double rows of 9.6 m length at a distance of 1.5 m. Plants were grown in 5 L pots filled with perlite using a drip irrigation system, at a density of 2.5 plants m^{-2}. Within each glasshouse, rows were split in two halves, where one half received the N-sufficient nutrient solution (control) and the other half were irrigated with a N-deficient nutrient solution from the beginning of experiment. The plants on the outsides of the rows were considered as borders. The basic experimental design within the glasshouse was an incomplete block design repeated on two planting dates (4 repetitions per rootstock and treatment). Some graft combinations were repeated within the benches to complete the 150 experimental units per N treatment, glasshouse and planting date. Plants were placed in the glasshouses on August 8th (experiment 1) and October 25th, 2012 (experiment 2) and terminated on September 11th and November 12th, 2012, respectively. Both N treatments were maintained during the entire time: 35 and 18 days, respectively.

The N-sufficient nutrient solution contained the following concentrations of macro-nutrients (in mM): Ca^{2+} 5.7; NH^{4+} 1.2; K^+ 8.2; Na^+ 0.3; Mg^{2+} 2.8; NO_3^- 13.8; Cl^- 0; SO_4^{2-} 5.5; $H_2PO_4^-$ 1.5. The N deficient nutrient solution contained Ca^{2+} 5.8; NH_4^+ 0.1; K^+ 8.4; Na^+ 0.3; Mg^{2+} 3.1; NO_3^- 1.0; Cl^- 10.7; SO_4^{2-} 6.5; $H_2PO_4^-$ 1.5 mM. The composition of the micronutrients was Fe^{2+} 15; Mn^{2+} 11.5; Zn^{2+} 5.5; B^{+3} 30; Cu^{2+} 0.8; Mo^{4+} 0.5 μM. Both nutrient solutions had a pH of 5.3 and an EC of $2.5.10^{-4}$ S m^{-1}, which was monitored biweekly. Throughout the experiment, glasshouse climate data were registered every 5 min. Average temperature was 18.5 °C (16 °C night, 23 °C daytime) and average humidity level was 73% (varying between 55% during the daytime and 85% at night). The experiments were terminated when the first truss had just set fruits (experiment 1) or started flowering (experiment 2). Then, xylem sap was obtained by decapitating the plants below the graft union, washing the stump with demineralized water, applying a silicon tube over the stem and collecting the sap using a pipette. The sap was immediately frozen with liquid nitrogen and stored at −80 °C until analysis. The aboveground plant parts were divided into stems and leaves. Shoot fresh weight (ShFW) comprised all leaves (LFW) and stems (SFW). Whole plant leaf area (LA) was determined (LI-3100C Area Meter, LI-COR, Lincoln, Nebraska, USA) along with dry weights (ShDW, LDW and SDW, respectively) after drying until constant weight. All leaves of the plant were used for evaluations. Vegetative and xylem sap components were evaluated in experiments 1 and 2 while leaf N content (LNC) was evaluated in the ground leaf material (comprising all leaves of the plant) from experiment 2. LNC was analysed using an elemental analysis instrument TRUSPEC CN628 (LECO Corporation, MI, USA). Traits were evaluated in plants grown under normal and low N inputs, noted as _C and _N, respectively.

Xylem sap ionomic analysis determined Al, As, Be, Bi, B, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, P, Sb, Se, S, Sr, Ti, Tl, V, Zn concentrations (mg/L) using inductively coupled plasma spectrometry (ICP-OES, Thermo ICAP 6000 Series).