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

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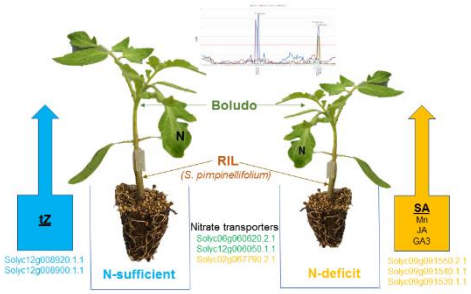
Highlights



Highlights

- Grafting itself increased [Na^+] in the xylem sap under N-sufficient conditions

- Three QTLs governing LNC were common under contrasting N availabilities
- The wild allele increased LNC for most rootstock QTLs under N-sufficient conditions
- QTLs for rootstock-mediated N uptake efficiency were clustered in chromosome 9
- Three coding genes for nitrate transporters were found within LNC QTL intervals

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.

Keywords: QTL analysis; SNP; candidate genes; Salicylic acid; Cytokinins; gene interactions

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 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, 12, 13]. Since rootstocks can affect nutrient absorption, yield and fruit quality under stress conditions [14, 15, 16, 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 18.5ºC (16ºC night, 23ºC 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)

The following phytohormones: cytokinins (*trans*-Zeatin –tZ, *trans*-Zeatin Riboside –ZR and isopentenyladenine - iP), 1-aminocyclopropane-1-carboxylic acid (ACC), abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA), gibberellins (gibberellin A_1 –GA1, gibberellin A_3 -GA3 and gibberellin A_4-GA4) and indole-acetic acid (IAA) were extracted and analysed in the xylem sap as previously described [12] with some modifications. Briefly, the xylem sap was centrifuged and injected in a ultra-high-pressure liquid chromatography coupled with high resolution/high accuracy Orbitrap® mass spectrometry system (EXACTIVE, ThermoFisher Scientific) where the different hormones were quantified by constructing calibration curves of each compound.

2.2 Statistical analysis

A linear model with nitrogen treatments and rootstocks (fixed), benches, rows within benches and replicates (random) was used to assess the significance of each source of variation following the above described design. The Bayesian Information Criteria (BIC) was used to select the best model. Since the model with rootstocks and benches was the most parsimonious with the lowest BIC for most traits, it was used to estimate the adjusted mean traits per rootstock genotype within each N treatment for the QTL analysis and to study the grafting effects by comparing Bol vs. Bol/Bol adjusted means.

Pearson correlation analysis was used to study associations between the different traits.

Broad sense heritability (H^2) was calculated for traits measured in both populations assuming that the individuals from the F_9 were nearly homozygous for all loci. Heritability was calculated as reported previously [31], using the formula: $H^2=V_g/(V_g+V_e)$ where V_g and V_e are the estimates of genotype and environmental variance, respectively, by REML (Restricted Maximum Likelihood). These estimates were obtained by a model with the same sources of variation as above but considering rootstocks as random effects.

2.3 Molecular markers and QTL Analysis

One hundred and thirty P-RILs at F_{10} were genotyped for 7720 SNPs from the SolCAP tomato panel (Illumina BeadXhip WG-401-1004) and a linkage map based on 1899 non-redundant SolCAP SNPs, covering 1326.37 cM of genetic length was used for QTL analysis [15].

QTL analyses of traits whose heritabilities were above 0.01 at least under one N level were carried out using Interval Mapping (IM) and Multiple QTL Mapping (MQM) procedures in MapQTL ® 6 [32]. Kruskal-Wallis (KW) procedure was also used for QTL

analysis of complex traits showing epistasis (LNC and GA3). A 5% experiment-wise significance level was assessed by permutation tests. These LOD critical values ranged from 2.1 to 2.3 depending on the trait and chromosome. Significant QTLs were named by trait, the treatment, the chromosome and a number from 1 to 3 if more than one QTL was detected on the same chromosome for the trait and treatment concerned.

A two-way ANOVA was used to study the interaction (epistasis) between markers corresponding to QTLs controlling LNC and phytohormone trait variation.

Genes covering approximately 2 Mbp around the SNP(s) showing maximum LOD score at each QTL governing LNC and phytohormone traits were downloaded from the Sol Genomics Network (https://solgenomics.net/) and selected for function, root expression in the Heinz cultivar using the tomato eFP Browser (http://bar.utoronto.ca/efp_tomato/cgi-bin/efpWeb.cgi?dataSource=Rose_Lab_Atlas_Renormalized) and for the presence of frameshift InDels in the parental genomes using data reported by Kevei et al. [33].

3. Results

Grafting *per se* (comparing Bol *versus* Bol/Bol in Table 1) affected very few vegetative traits, but increased SFW by 12% (averaged across both N treatments). While grafting increased LA and LFW of N-sufficient plants by 7% and 12% respectively, it decreased SDW of N-deprived plants by 10%. Likewise, grafting had minimal impacts on xylem sap composition, but increased xylem Na^+ concentration and decreased xylem IAA concentration of N-sufficient plants, while tending to increase xylem ABA concentration of N-deprived plants.

Across all graft combinations in the RIL population, the low N treatment decreased vegetative growth by 72.7% and 74.4% for ShDW and LDW, respectively. In fact, the distributions of ShDW and LDW were completely separate under both N levels (Figure 1), as also with leaf N concentration (LNC). Very few rootstocks alleviated the effects of the low N treatment on vegetative growth in comparison to the controls Bol and Bol/Bol (Figure 2). However, when total leaf N content is considered (LTN=LNC x LDW), all rootstocks, including the self-grafted control, mediated larger LTN values than the non-grafted control (Figure 3A). Six rootstocks RILs surpassed the LTN values of the self-grafted control under N-sufficient conditions (but only one under N-deficit), suggesting they increased N uptake efficiency depending on N availability. In general,

the reduction in LTN from N-sufficient to N-deficit conditions (dLTN in Figure 3B) was less than the controls in 28.5% of the RILs.

Correlation analysis (Table 2) showed that LNC was directly related to plant growth (ShFW, ShDW, and LDW), and inversely related to xylem sap tZ concentration under N sufficient conditions; while under N-deficit, LNC was inversely related to xylem sap SA concentration and directly to LA and LFW. Noteworthy, under N deficit, xylem sap SA concentration was also inversely related to both ShFW and xylem sap Mn concentration. When comparing vegetative traits between N levels, stem fresh and dry weights (SFW and SDW) were the only ones that were not significantly correlated between treatments.

The rootstock genotype showed significant effects on LNC and vegetative traits of the scion, in addition to the xylem sap Mn, tZ, JA and SA concentrations (Table 3). Estimated trait heritabilities were low under N-sufficient conditions and generally increased under N-deficit except for ShDW and xylem sap tZ concentration (Table 3). The low N supply significantly affected all traits, including GA3, but GxE interaction was only detected for xylem sap SA concentration (Table 3).

In total, 62 significant QTLs were detected by MQM procedure (Table 4). All of them contributed little to the total variance (PEV) and only two could be considered constitutive (detected under both N treatments, in bold in Table 4): for LDW in chromosome 1 and for LNC in chromosome 6. Surprisingly, the graph of genotypic means for some LNC and phytohormone QTLs suggested the presence of over- and under-dominance gene effects: LNC_C_6, LNC_C_10, LNC_N_6.1, LNC_6.2, SA_N_9 and GA3_N_10.2 (supplementary figure S1). Significant epistasis between QTLs were studied for LNC and xylem phytohormones. They were detected between LNC_N_12.1 and LNC_N_12.2 ($p \leq 0.0198$), between JA_N_5 and JA_N_11 ($p \leq 0.0456$), and particularly for GA3 under low N (Figure 4). Two epistatic interactions involved an overdominant QTL (GA3_N_10.2). Given the complexity of the genetic architecture of LNC and GA3, Kruskal-Wallis procedure for QTL detection was used to analyze them and some QTLs were detected by both methods (asterisks in Tables 4 and 5). The complete list of LNC QTLs detected by this procedure and mRNA coding for nitrate transporters within their 2 Mbp intervals are presented in Table 5. Some LNC QTLs in chromosomes 6, 9 and 12 could be common between N treatments (QTLs in bold in Table

5). The wild allele of the rootstock was beneficial at most LNC QTLs under N sufficient conditions, and for 40% of those under N deficit. The lycopersicum allele was beneficial at LNC QTLs in chromosome 9, with LNC_N_9.2 overdominant (Supplementary Figure S1). Interestingly, this chromosome presented two clusters of QTLs (Figure 5) including both vegetative and LNC traits where the lycopersicum allele increased all of them indicating two regions involved in rootstock mediated N uptake efficiency under N deficit. One of those regions included SA_N_9 where the lycopersicum allele was associated with less SA in the xylem sap. Other clusters of QTLs appeared at three genomic positions (Table 4) in chromosomes 1 (LFW_N_1 and LA_N_1), 3 (LDW_N_3 and LFW_N_3) and 10 (LDW_N_10, ShDW_N_10 and SDW_N_10). Linkage between SDW and LNC QTLs was observed in chromosomes 2, 6 and 10.

Genes covering approximately 2 Mbp around the SNP(s) showing maximum LOD score at each QTL governing LNC and phytohormone traits were downloaded and studied regarding root expression and frameshift InDels to indicate the most likely functional candidates summarized in Table 6. Three nitrate transporters (NTR1 and NTR2) were included in the case of QTLs for LNC_C_6, LNC_C_12 and LNC_N_2 although with low expression in the root of Heinz cultivar. Additionally, a voltage-gated chloride channel was found within LNC_N_2. Regarding plant hormone QTLs, two Cytokinin oxidase/dehydrogenase genes were within tZ_C_12; a Hydroxycinnamoyl CoA shikimate/quinate hydroxycinnamoyltransferase, a Cinnamoyl-CoA reductase and a 4-coumarate CoA ligase were within SA_N_3; three Salicylic acid carboxyl methyltransferase genes were within SA_N_9 and a Gibberellin 2-beta-dioxygenase 7 within GA3_N_6.

4. Discussion

In general, candidate genes for stress tolerance have been identified by the reverse genetics strategy through expression studies in mutants and transgenic genotypes [34]. The present study follows the scarcely used forward genetics strategy, and evaluated the hypothesis that genetic variability in rootstocks derived from wild relatives of tomato can improve N uptake. To our knowledge, this is the first study utilising genetic variability in a wild species (*S. pimpinellifolium)* to identify rootstock-mediated QTLs related to vegetative growth and N content under contrasting N availabilities.

4.1 Can the rootstock improve N uptake efficiency?

To understand the genetics of rootstock-mediated plant N nutrition, it is first necessary to know whether grafting itself affected the analysed traits. Grafting increased plant growth under N-sufficient condition, as in a previous experiment on rootstock-mediated salt tolerance [15]. Moreover, grafting altered xylem sap composition by increasing Na^+ and decreasing IAA concentrations under N-sufficient conditions. Higher xylem Na^+ concentrations could increase osmotically driven water uptake by the roots, and might explain increased LFW and SFW in Bol/Bol compared to Bol plants, as a consequence of higher leaf and stem water content [35]. Interestingly, grafting decreased SDW under N deficit suggesting that low N availability might limit biomass allocation to the stem [36], especially after grafting.

Heritability estimates of rootstock-mediated N-uptake, measured as LNC under each N regime, were larger than those corresponding to vegetative traits, and ranged from 0.33 to 0.39 (Table 3), supporting the hypothesis that selecting specific rootstock genotypes can improve N uptake. Thus, the reduction in total leaf N content, when comparing N sufficient to N-deficit conditions, was smaller than controls in 28.5% of RILs used as rootstocks (dLTN in Figure 3), although very few RILs surpassed Bol/Bol control for LTN. Therefore, the development of selection tools to improve rootstock-mediated N uptake efficiency needs to decipher the genetic control of its components (LNC and LDW) under N-sufficient and -deficit conditions.

4.2 Complex genetic control of rootstock N-uptake under N-deficit differs from that under N-sufficient conditions.

Under N-deficit no LNC_N, no significant QTL was detected by the interval mapping procedure in the population of RILs but, after co-factor adjustment, 6 QTLs controlling LNC_N were found using MQM, a composite interval mapping procedure (Table 4). In our experiment, two of those QTLs, LNC_N_12.1 and LNC_N_12.2 (Figure 4), were involved in an epistatic interaction, and overdominance gene effects were observed at 2 of them, at least (LNC_N_6.1 and LNC_N_6.2; supplementary figure S1). Given this complexity under N deficit, and the distribution of LNC under N-sufficient conditions (Figure 1), a non-parametric QTL detection procedure (KW) could be more reliable than MQM since the former is based on fewer assumptions than the latter. Four QTLs were detected by both procedures.

Since LNC_C and LNC_N were significantly correlated (r=0.37, Table 2), it seems likely that common genes are involved. Accordingly, three LNC QTLs detected by KW in chromosomes 6, 9 and 12, could be considered common between N levels; and

the beneficial allele was from the wild species at two of them (Table 5). The remaining LNC QTLs are N-level specific, consistent with reports that genetic variability is expressed differently under high and low N input in non-grafted plants [19].

Two LNC QTLs co-located with tolerance QTLs for N-deficit (QTLs for vegetative growth) and xylem sap SA concentration in chromosome 9 (Figure 5), and another QTL (LNC_N/C_12 in Table 5) with tZ_C_12, a QTL for the xylem sap tZ concentration (Table 4) in chromosome 12. Additionally, close linkage between QTLs was observed between LNC_C_6 and SDW_C_6, and between LNC_C_4 (Table 5) and Mn_N_4 (Table 4), with the wild allele beneficial for all traits. Therefore, QTL linkage could explain the correlation between LNC and vegetative growth under both N levels, and between LNC and SA or tZ under low or high N inputs, respectively. However, linkage between QTLs cannot explain the correlation found between LNC_N and LA_N (Table 2), which agrees with the observation that foliar nitrate level influences leaf expansion [37].

For N uptake, plants utilize both low- and high-affinity transport systems [38], with the latter further divided into a constitutive component expressed in the absence of nitrate and a nitrate- inducible component. Three nitrate transporters (Solyc06g060620.2.1, Solyc02g067790.2.1, Solyc12006050.1.1), a voltage-gated chloride channel (Solyc02g068080.2.1) and a Porin/voltage-dependent anion-selective channel (Solyc02g067460.2.1) were included in the list of candidate genes of LNC QTLs (Table 6). In Arabidopsis, tonoplastic AtCLCa acts as a $2NO_3^-/1H^+$ antiporter regulating vacuolar nitrate accumulation [39, 40]. Among the candidate genes within LNC_N_2, a high affinity (nitrate-inducible) nitrate transporter (NRT2) was found. The other candidate gene within LNC_N_2 codes for a voltage-gated chloride channel, which shows maximal expression in the root of Heinz cultivar. Within LNC_C_6 and LNC_C_12, low affinity nitrate transporter coding genes were found (Solyc06g060620.2.1 and Solyc12006050.1.1), consistent with observations that low affinity transport systems are expressed under high NO_3^- conditions [41]. Within LNC_C_6, a highly root-expressed (in the Heinz cultivar) protein-P-II uridylyltransferase coding gene, with a frameshift InDel in the wild allele [33], was detected. The combined action of both genes could explain the (pseudo)-overdominance observed at this QTL. Bacterial PII protein performs a role linked to a central processing unit to sense and coordinate cellular responses to changes in key C and N metabolite levels [42]. Plant PII

may precisely sense fluctuations in C signalling molecules that coordinate cellular energy status with N assimilation [43].

A peptide transporter, an amino acid transporter, an aquaporin and several purine permeases could be responsible, at least in part, for the following QTLs: LNC_N_6.1, LNC_N_6.2, LNC_N_9.2, LNC_N_12.1, LNC_N_12.2 and LNC_N_12.3, respectively (Table 6). The use of ureides as N transport molecules (a particular economical form of N transport, i.e. high N/C ratio in the molecule) from root to the N sinks has been shown for some non-leguminous families such as Aceraceae and Boraginaceae [44].

In addition to being a nutrient source, nitrate is also a signalling molecule coordinating multiple cellular processes such as root development and pathogen defence [37]. Hundreds of genes are nitrate responsive, including N and C metabolizing enzymes, redox enzymes and multiple signalling proteins and transcription factors [2]. The complex genetic control of rootstock mediated N uptake and translocation to the leaf under N-deficit might mean that not only transporter-coding genes but also genes involved in the nitrate signalling (i.e transcription factors, lipid signalling, phytohormone signalling, phosphorylation, ubquitination...) could underlie LNC_N QTLs, making it difficult to select candidate genes. Interestingly, genes coding for an ubiquitin-conjugating enzyme E2 and a transmembrane protein within LNC_N_12.3 and an ethylene receptor within LNC_N_12.1 (data not shown) were reported as candidate genes for NUE QTLs in maize [45]. Therefore, more than one candidate gene may underlie each QTL, particularly for QTLs whose intervals are rich in root expressed genes such as LNC_N_6.1 and LNC_N_9.2 (Table 6). Since tight linkage of genes with additive x additive epistasis might explain apparent overdominance gene interaction at a QTL, as demonstrated by simulation studies [46, 47], the epistatic involvement of more than one candidate gene at these QTLs might explain their overdominance (Supplementary Figure S1).

To further understand the signalling pathways modulating N acquisition and root to shoot translocation, genetic analysis of the inheritable xylem components was also performed.

4.3 N-dependent rootstock mediation of xylem sap composition

The rootstock genotype significantly affected the xylem sap concentration of Mn, tZ, and mainly, JA and SA. Besides, N deficit significantly altered xylem sap composition, including GA3, and in the case of SA, this change was rootstock-dependent (Table 3).

There was genetic variability in the xylem sap for Mn concentration only, particularly under N deprivation (Table 3). Here, one QTL was detected in chromosome 4 (Mn_N_4); which was different from those detected for leaf [Mn] under moderate salinity [15], but co-located with a QTL controlling LNC under N-sufficient conditions (LNC_C_4 in Table 5). Two likely candidate genes (Solyc04g015020.2 and Solyc04g015030.2) coding for heavy metal transport/detoxification proteins were found 394620 bp apart from the SNP at the LOD peak of Mn_N_4. Therefore, the N-deficit specific expression of those genes, could explain the detection of Mn_N_4 and consequently, the different xylem sap [Mn] between N availabilities (0.95 ± 0.02 and 2.24 ± 0.05 mg L^{-1} under N-sufficient and N-deficit, respectively). Close linkage between Mn_N_4 and SDW_N_4 (Table 4) might explain the positive correlation detected between SDW and xylem sap Mn concentration under N deficit (Table 2).

Some hormones are root-synthesized and can act as signalling molecules transported via the xylem. Heritable, rootstock mediated effects were detected for the cytokinin (tZ) under N sufficient conditions, and for salicylic acid (SA), jasmonic acid (JA) and gibberellin (GA3) under N deficit.

Lower gibberellin levels can limit plant growth restriction on exposure to several stresses including cold, salt and osmotic stress [48]. Xylem gibberellin (GA3) was only detected in xylem sap of 44 rootstocks under N deficit and its concentration did not correlate to any vegetative trait. Therefore, it does not seem to play a major role as a root to shoot signal of N deficit. Nevertheless, four out of six QTLs detected by MQM were also found by using the KW procedure (asterisks in Table 4). Despite not being polymorphic for frameshift mutations [33] and being quite distant from the LOD peak, a gibberellin 2-beta-dioxygenase could underlie GA3_N_6 (Table 6). GA3_N_12 was clearly epistatic (Figure 4D-F) which could explain its lack of detection by the KW procedure. It appears in the central position of several epistatic interactions (with GA3_N10.2, GA3_N_10.3 and GA3_N_6 in Figure 4D-F) where only the pimpinellifolium allele increases GA3 under N deficit. Three genes were found with frameshift InDels in the parent alleles (Table 6), one of them coding for an ATP synthase subunit H family protein (Solyc12g098720) affected the lycopersicum allele. Genes related to other phytohormones were found within the intervals of some GA3_N QTLs: Solyc06g084070 to auxin within GA3_N_6; Solyc1010g076410 and Solyc10g079210 to ABA within GA3_N_10.1 and GA3_N_10.2, respectively; and Solyc10g0839730 to ethylene within GA3_N_10.3.

In *Arabidopsis*, JA was related to root nitrate assimilation [49]. In our study, five QTLs were detected controlling xylem sap JA concentration under N deficit (Table 4), two of them epistatic (Figure 4). All candidate genes listed in Table 6 showed frameshift Indels in the pimpinellifolium allele. Since this allele at both JA_N_4 and JA_N_5 increased xylem JA, their candidate genes are more likely involved in JA metabolism rather than JA synthesis. Thus, two cytochrome P450 coding genes within JA_N_4 and another within JA_N_5 might be candidates as well as two sulfotransferase coding genes within JA_N_5. Regarding other stresses, several disease resistance proteins and a heat shock protein binding protein related to ABA were also included in the interval of JA_N_5. Two polymorphic, hydroxycinnamoyl transferase (HCT) coding genes were found within the intervals of JA_N_4 and JA_N_9 (Solyc04g078660 and Solyc09g014280). HCT participates in the biosynthesis of secondary metabolites [50]. *HCT1* was up- regulated locally in the bark of Norway spruce (*Picea abies*) trees in response to MeJA [51]. Thus, tight linkage of candidate genes related to different signalling pathways might have an evolutionary role for their integration in potential nodes and cross-talk [52].

Under N-deficit conditions, SA was apparently the most important signalling hormone since its xylem concentration was inversely related to both leaf N accumulation and shoot growth (Table 2). Co-location of SA_N_9 and LDW_N_9 QTLs at the same position in chromosome 9 (Table 4, Figure 5) where the pimpinellifolium allele increases xylem [SA] and decreases LFW and LDW, could contribute to the negative relationship between SA_N and ShFW_N (Table 2). Moreover, since LNC_N_9.2 (Table 5) also co-located to SA_N_9, this would explain the negative correlation between SA and LTN (Table 2). Therefore, this clustering of QTLs appears relevant to improving rootstock-mediated tolerance to low N and N uptake efficiency. The main candidate genes for SA_N_9 encode salicylic acid carboxyl methyltransferases. The presence of underdominance at SA_N_9, i. e. the heterozygote showed less SA_N than any homozygote (Supplementary Figure S1) might be interpreted as the presence of more active Salicylic acid carboxyl methyltransferases alleles in the heterozygote converting more SA into MeSA than in any of the homozygotes (i.e. pseudo-underdominance). This genomic region includes also the gene Solyc09g090360 (Xenotropic and polytropic retrovirus receptor) related to G-protein signalling [2]. Therefore, an alternative explanation for the underdominance in heterozygote could be the competition between wild and mutant subunits to reach the three-dimensional functional conformation. Three

ACC oxidase coding genes were also found within SA_N_9. These were highly root-expressed in the Heinz cultivar (data not shown), and present frameshift InDels in the pimpinellifolium allele [33]. Since ACC oxidase catalyzes the rate-limiting step in ethylene biosynthesis [53], its presence within SA_N_9 suggests a genetic link at SA_N_9 between both ethylene and SA signalling pathways in the root.

Plant cytokinin levels increase when plants are N-supplied and decrease when N-deprived [54], thereby modulating N assimilation and C metabolism [55, 56]. Accordingly, xylem sap tZ concentration was the only hormone that was found associated with LNC under N sufficient conditions (Table 2). This correlation was supported by the linkage between tZ_N_12 (Table 4) and LNC_C/N_12 (Table 5). Likely candidate genes underlying xylem [tZ] under N sufficient condition (Table 6) encode a cytokinin oxidase/dehydrogenase, particularly Solyc12g008920 with a frameshift InDel at the lycopersicum allele, and two chaperone Dna J3 (Solyc05g050810 and Solyc05g050820), with frameshift InDels in both genes [33]. These polymorphisms are consistent with the direction of gene effects at both tZ_C_5 and tZ_C_12. In addition to their function as chaperon proteins, DnaJ proteins are also involved in transcriptional activation by directly binding transcription factors [57], endosome formation [58], carotenoid accumulation [59] and regulation of plasma membrane H^+-ATPase activity via inactivation of the PKS5 kinase [60]. Our results suggest that xylem tZ is the main signalling hormone contributed by the rootstock under N sufficient conditions.

In conclusion, rootstock-mediated increments of cytokinin (tZ) and SA concentrations (under N-sufficient and N-deficit conditions, respectively) were related to decreased leaf N concentration, and also vegetative growth in the case of SA. Co-location and close linkage of QTLs controlling those traits in chromosomes 9 and 12 could explain their correlation. The pimpinellifolium allele increased leaf N concentration at most detected QTLs under N sufficient conditions, where six RILs could improve N uptake efficiency when used as rootstocks of a hybrid tomato. The genetic contribution of the rootstock mediating N leaf nutrition under N deficit is complex, involving gene interactions. The (pseudo-) overdominance detected at some N and hormone QTLs could be explained by tight linkage (even in the same QTL interval) of genes involved, suggesting heterosis could be exploited to improve tomato rootstocks for N uptake efficiency. A list of likely candidate genes, including three nitrate transporter coding genes, is provided that opens the possibilities of molecular breeding for N uptake efficiency in the future.

Author Contribution Statement Conceived: MJA, ICD, FPA; Experimental design: DJA, EAC, MJA; Performed experiment: DJA; Plant phenotyping: DJA, AA, CMA; Analyzed data: MJA, EAC; Wrote paper: MJA, ICD, EAC

Ethical standards: The authors declare that the experiment comply with the current laws.

Conflict of interest: The authors declare that they have no conflict of interest.

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Figure legends

Figure 1- Distributions of (A) LDW (leaf dry weight), (B) ShDW (shoot dry weight) and (C) LNC (leaf nitrogen concentration) under N-sufficient (grey) and N-deficit (white) conditions.

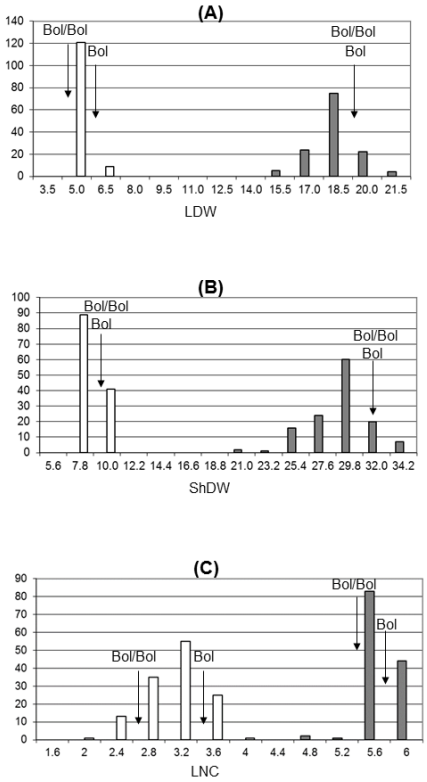

Figure 2- Rootstocks (X axis) ordered by their adjusted means (in grams, at Y axis) for (A) LDW_N, (B) ShDW_N, (C) SDW_N and (D) SFW_N (leaf dry weight, shoot dry weight, stem dry weight and stem fresh weight under N-deficit, respectively). The position of controls Bol and Bol/Bol is indicated.

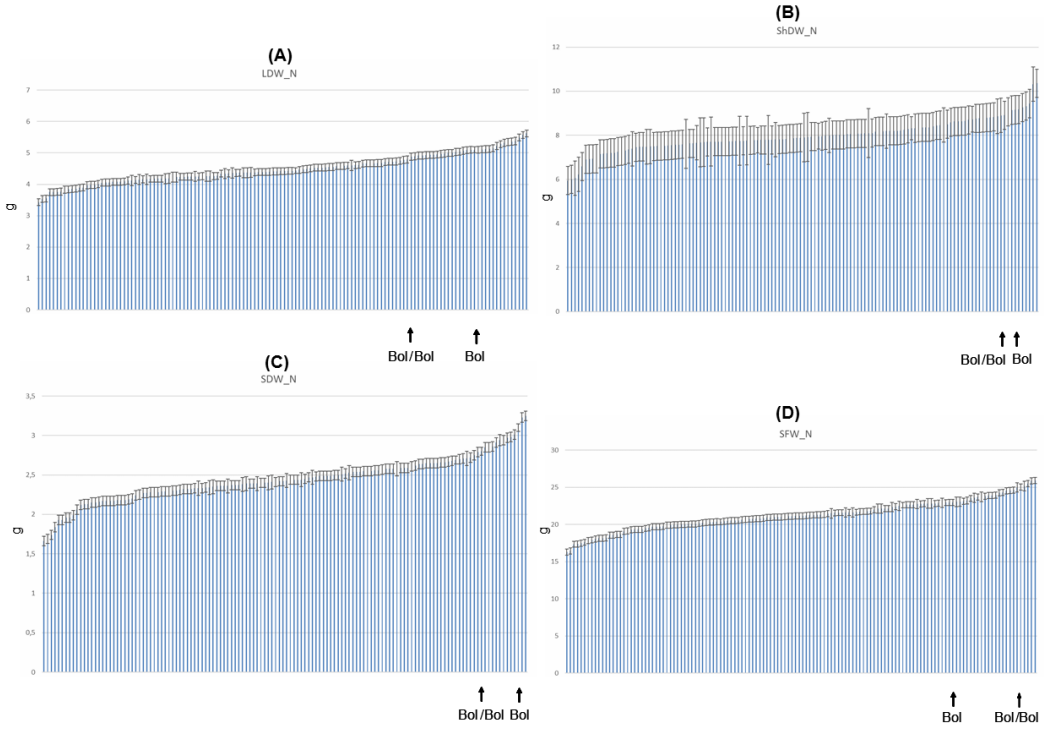

Figure 3- Distributions of (A) total leaf N content (LTN=LNCxLDW) and (B) its reduction from N sufficient to N-deficit conditions (dLTN in %).

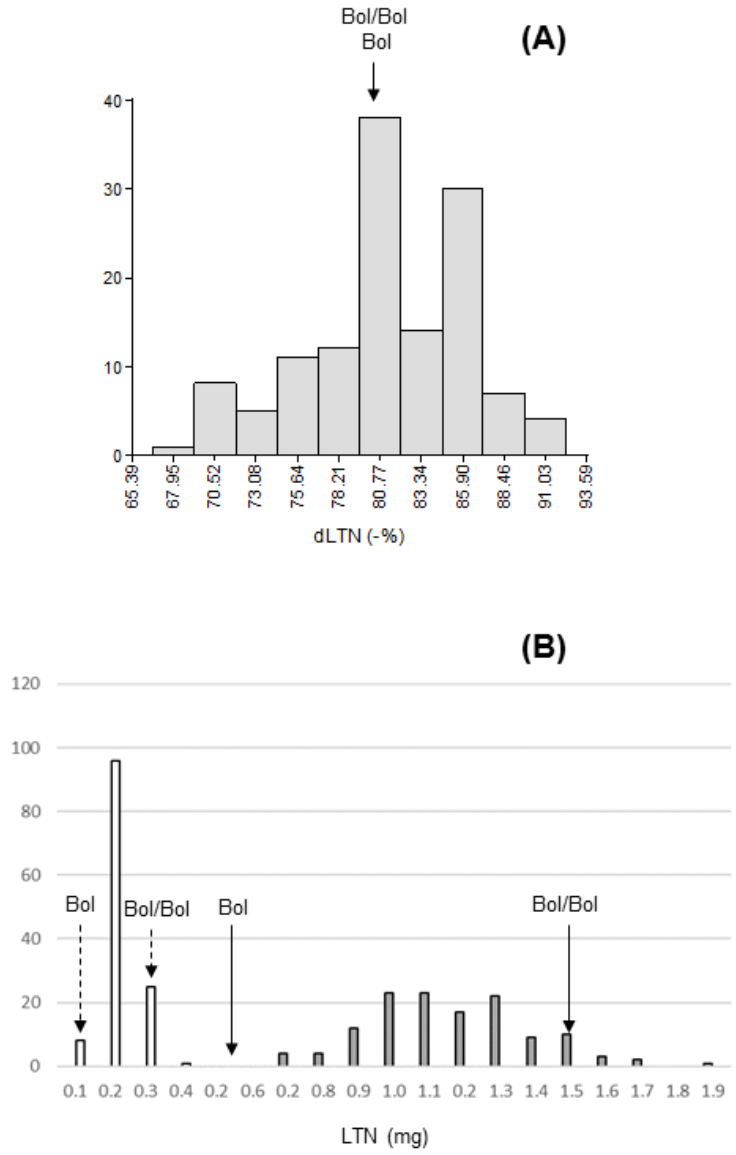

Figure 4- Means and standard errors for significant epistatic interactions between QTLs governing (A) LNC (leaf N concentration), (B) JA (jasmonic acid concentration in the xylem sap) and (C to F) GA3 (gibberellin concentration in the xylem sap). Homozygotes for the lycopersicum or the pimpinellifolium allele are coded as LL or PP, respectively.

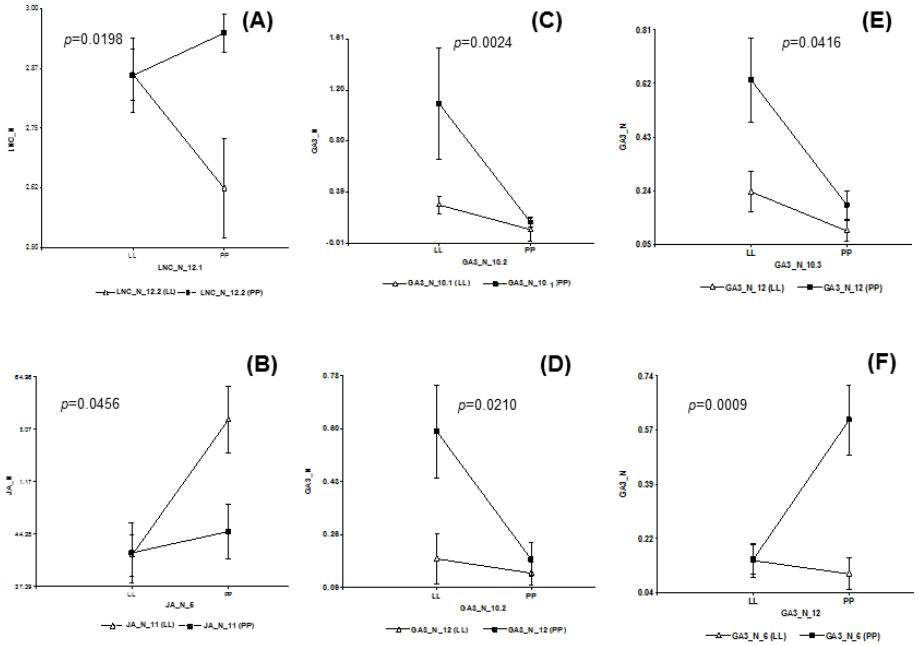

Figure 5- LOD profiles of QTLs (ShDW_N_9, LFW_N_9.1, LFW_N_9.2, LDW_N_9 and SA_9) detected in chromosome 9 forming two clusters. LNC (leaf N concentration) QTLs detected by Kruskal-Wallis (QTLs with asterisks) are also included. Distribution of markers along the chromosome 9 is shown under the X axis and LOD critical value is indicated by the discontinuous line.

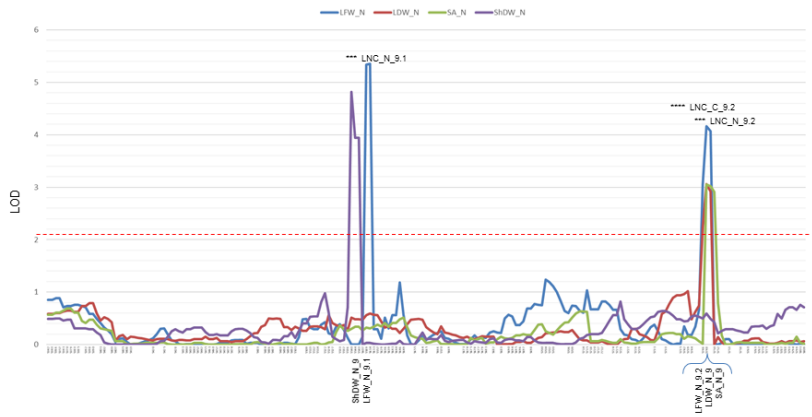

Table 1- *P* values and adjusted means for traits showing significant differences ($p \leq 0.05$) between non-grafted (Bol)- and self-grafted (Bol/Bol)-Boludo variety under N-sufficient (_C) and N-deficit (_N) conditions.

Trait (units)	*p*-value_C	Bol_C	Bol/Bol_C	*p*-value_ N	Bol_N	Bol/Bol_N
LA (cm^2)	0.0354	3798.530	**4072.850**	0.0641	.	.
LFW (g)	0.0001	158.830	**178.660**	0.7026	.	.
SFW (g)	<0.0001	80.070	**91.740**	0.0078	22.960	**24.980**
SDW (g)	0.1567	.	.	<0.0001	**3.100**	2.800
Na (mg/L)	0.0305	9.784	**85.598**	0.6423	.	.
IAA (mg/L)	0.0478	**4.740**	0.000	>0.9999	.	.
ABA (mg/L)	0.3179	.	.	0.0543	6.477	**13.598**

Table 2- Pearson coefficients between significantly correlated traits ($p \leq 0.05$) under N-sufficient (_C) and N-deficit (_N) conditions, and between treatments for a given trait. LTN is total leaf N content (LNC x LDW) and dLTN, its change between treatments.

Trait 1_C	**Trait 2_C**	**Pearson**	***p-value***		**Trait 1_N**	**Trait 2_N**	**Pearson**	***p-value***
dLTN	LA_C	0.27	0.0019		dLTN	LA_N	-0.20	0.0232
dLTN	LTN_C	0.48	<0.0001		dLTN	LFW_N	-0.24	0.0052
LDW_C	LA_C	0.38	<0.0001		dLTN	LNC_N	-0.35	<0.0001
LDW_C	LFW_C	0.75	<0.0001		dLTN	LTN_N	-0.70	<0.0001
LDW_C	SFW_C	0.59	<0.0001		dLTN	SFW_N	-0.18	0.0432
LDW_C	ShFW_C	0.45	<0.0001		LDW_N	LA_N	0.36	<0.0001
LFW_C	LA_C	0.63	<0.0001		LDW_N	LFW_N	0.59	<0.0001
LNC_C	LDW_C	0.18	0.0428		LDW_N	SFW_N	0.53	<0.0001
LNC_C	ShDW_C	0.22	0.0137		LDW_N	ShFW_N	0.40	<0.0001
LNC_C	ShFW_C	0.20	0.0219		LFW_N	LA_N	0.75	<0.0001
LTN_C	LA_C	0.49	<0.0001		LNC_N	LA_N	0.36	<0.0001
LTN_C	LDW_C	0.35	<0.0001		LNC_N	LFW_N	0.28	0.0015
LTN_C	LFW_C	0.48	<0.0001		LNC_N	SA_N	-0.35	0.0001
LTN_C	LNC_C	0.19	0.0316		LTN_N	LA_N	0.45	<0.0001
LTN_C	SDW_C	0.19	0.0339		LTN_N	LDW_N	0.25	0.0034
LTN_C	SFW_C	0.39	<0.0001		LTN_N	LFW_N	0.50	<0.0001
LTN_C	ShFW_C	0.18	0.0446		LTN_N	LNC_N	0.40	<0.0001
SDW_C	LA_C	0.20	0.0219		LTN_N	SA_N	-0.19	0.0309
SDW_C	LDW_C	0.77	<0.0001		LTN_N	SFW_N	0.34	0.0001
SDW_C	LFW_C	0.43	<0.0001		LTN_N	ShFW_N	0.24	0.0059
SDW_C	SFW_C	0.64	<0.0001		Mn_N	LDW_N	0.19	0.0314
SDW_C	ShFW_C	0.44	<0.0001		Mn_N	SA_N	-0.18	0.0399
SFW_C	LA_C	0.45	<0.0001		Mn_N	SDW_N	0.26	0.0034
SFW_C	LFW_C	0.71	<0.0001		Mn_N	SFW_N	0.19	0.0361
ShDW_C	LDW_C	0.58	<0.0001		Mn_N	ShDW_N	0.19	0.0309
ShDW_C	LFW_C	0.37	<0.0001		SA_N	ShFW_N	-0.19	0.0326
ShDW_C	SDW_C	0.64	<0.0001		SDW_N	LA_N	0.22	0.0104
ShDW_C	SFW_C	0.33	0.0001		SDW_N	LDW_N	0.64	<0.0001
ShDW_C	ShFW_C	0.82	<0.0001		SDW_N	LFW_N	0.30	0.0005
ShFW_C	LA_C	0.20	0.0213		SDW_N	SFW_N	0.78	<0.0001
ShFW_C	LFW_C	0.54	<0.0001		SDW_N	ShFW_N	0.38	<0.0001
ShFW_C	SFW_C	0.43	<0.0001		SFW_N	LA_N	0.59	<0.0001
tZ_C	LNC_C	-0.20	0.0230		SFW_N	LFW_N	0.56	<0.0001
					ShDW_N	LDW_N	0.59	<0.0001
Trait_C	**Trait_N**	**Pearson**	***p-value***		ShDW_N	LFW_N	0.30	0.0006
LA_C	LA_N	0.17	0.0487		ShDW_N	SDW_N	0.56	<0.0001
LDW_C	LDW_N	0.20	0.0216		ShDW_N	SFW_N	0.34	0.0001
LFW_C	LFW_N	0.41	<0.0001		ShDW_N	ShFW_N	0.82	<0.0001
LNC_C	LNC_N	0.37	<0.0001		ShFW_N	LA_N	0.31	0.0004
LTN_C	LTN_N	0.26	0.0030		ShFW_N	LFW_N	0.51	<0.0001
ShDW_C	ShDW_N	0.22	0.0105		ShFW_N	SFW_N	0.34	0.0001

ShFW_C	ShFW_N	0.28	0.0014					

Table 3- *P*-values for the significant effects in the mixed model analysis and heritability estimates (H^2_C and H^2_N, for N-sufficient and N-deprivation conditions, respectively). Only traits showing higher heritabilities than 0.01 in at least one treatment are presented. Higher heritabilities than 0.10 are in bold.

Trait	**Rootstock RIL (G)**	**N treatment (E)**	**GxE**	**H^2_C**	**H^2_N**
LNC	<0.0001	<0.0001	0.063	**0.3269**	**0.3915**
LA	0.0023	<0.0001	0.3968	0.0488	**0.1130**
LFW	<0.0001	<0.0001	0.0562	0.0703	**0.2257**
SFW	0.0001	<0.0001	0.4873	0.0000	0.0838
ShFW	<0.0001	<0.0001	0.1467	0.0000	0.0199
LDW	0.0001	<0.0001	0.0714	0.0000	0.0579
SDW	<0.0001	<0.0001	0.7499	0.0000	0.0305
ShDW	<0.0001	<0.0001	0.1548	0.0112	0.0000
Mn	0.0195	<0.0001	0.4525	0.0243	**0.1283**
tZ	0.0439	<0.0001	0.2554	**0.1081**	0.0344
GA3	0.3087	0.0006	0.4558	0.0000	**0.1336**
JA	<0.0001	<0.0001	0.0538	0.0103	**0.1967**
SA	<0.0001	<0.0001	<0.0001	0.0916	**0.1182**

Table 4- List of QTLs (named by the trait, treatment (_C or _N for N-sufficient or N-deficit, respectively) that were detected by using MQM procedure (5% overall significance level) and corresponding SNPs (mostly SolCAP SNPs named by the number) at the LOD peak. The map position (cM) of QTL peaks in the tomato chromosomes (Chr) and the means for both homozygous genotypes, *LL* and *PP*, are indicated. The estimated additive value is **a**, and the percentage of explained variance, PEV. NSML is the number of SNPs associated with the maximum LOD score. LNC or GA3 QTLs with an asterisk were also detected by the Kruskal-Wallis procedure.

QTL	Chr	cM	SNP	LOD	*LL*	*PP*	PEV	a	NSML
GA3_N_6*	6	100.411	31671	2.8	0.17	0.39	5.6	- 0.109	1
GA3_N_9*	9	52.212	43102	3.19	0.39	0.16	6.4	0.116	2
GA3_N_10.1	10	43.446	28675	2.93	0.12	0.44	5.8	- 0.159	2
GA3_N_10.2*	10	49.612	33007	5.42	0.55	0.02	11	0.262	7
GA3_N_10.3*	10	71.483-70.995	33797	3.09	0.74	-0.18	6.2	0.457	3
GA3_N_12	12	100.061	31585	2.7	0.18	0.38	5.3	- 0.103	2
JA_N_11	11	48.442	14367	2.19	50.02	40.74	4.3	4.642	2
JA_N_4	4	84.789	47056	2.77	40.02	50.74	5.5	- 5.358	1
JA_N_5	5	51.538	50722	2.12	40.80	49.88	4.2	- 4.542	2
JA_N_8	8	46.551	4374	2.42	51.04	39.72	4.8	5.658	4
JA_N_9	9	44.817	57020	2.81	50.91	39.85	5.6	5.532	1
LA_C_1	1	49.836	50504	3.67	2924.35	3079.55	8.1	- 77.597	1
LA_C_3	3	17.584	63402	3.64	2929.76	3072.67	8	- 71.456	3
LA_C_7	7	59.313	5863	2.29	2946.81	3057.08	4.9	- 55.136	2
LA_C_8	8	96.781	34911	3.21	3068.29	2935.61	7	66.342	1
LA_C_9	9	65.872	46701	2.59	3059.96	2943.94	5.6	58.012	3
LA_N_1	1	35.609	59944	4.01	626.02	662.03	9.1	- 18.000	1
LA_N_3	3	95.71	62120	3.98	626.13	661.92	9	- 17.899	1
LDW_C_1	1	85.928	15339	3.17	18.00	17.06	8	0.467	2
LDW_C_4	4	17.213	64119	3.11	17.18	17.80	7.1	- 0.314	1
LDW_C_8	8	82.825	65114	2.37	17.78	17.20	5.3	0.293	2
LDW_N_1	1	90.932	SL10945_281	3.55	4.37	4.57	8.6	- 0.096	1
LDW_N_10	10	5.395	46305	2.41	4.39	4.55	5.8	- 0.082	1
LDW_N_11	11	0.000	66678	2.23	4.54	4.40	5.3	0.075	3
LDW_N_3	3	30.046	19508	2.53	4.55	4.39	6.1	0.079	1
LDW_N_9	9	100.475	69640	3.06	4.56	4.38	7.4	0.090	1
LFW_C_3	3	97.904	62037	2.76	165.58	171.09	8.6	- 2.750	1
LFW_N_1	1	34.125	59944	2.34	32.56	33.60	4.7	- 0.520	1
LFW_N_12	12	71.032	55550	2.4	33.60	32.53	4.8	0.530	2
LFW_N_3	3	30.046	19508	2.31	33.52	32.56	4.3	0.480	1
LFW_N_9.1	9	47.117	67830	5.35	33.87	32.24	11.4	0.820	1
LFW_N_9.2	9	100.475	69640	4.16	33.75	32.36	8.7	0.690	1
LNC_C_10*	10	51.917	33113	4.54	5.41	5.56	13.4	- 0.075	5
LNC_C_6.1*	6	37.829	55874	2.41	5.43	5.54	6.8	- 0.051	1
LNC_N_12.1	12	46.682	27059	2.26	3.05	2.73	5.3	0.158	2
LNC_N_12.2	12	60.667	53992	2.84	2.76	3.02	6.9	- 0.128	2

LNC_N_12.3	12	79.758	31966	4.97	3.04	2.73	12.2	0.153	3
LNC_N_2*	2	11.615	20344	2.49	2.98	2.80	6	0.093	3
LNC_N_6.1*	6	48.006	1321	3.7	2.70	3.08	9.1	- 0.191	7
LNC_N_6.2	6	53.531	41942	3.67	3.08	2.69	9	0.199	2
Mn_N_4	4	37.897	41552	2.31	2.08	2.37	7.4	- 0.148	2
SA_N_11	11	39.245	36157	2.6	12.05	8.43	6.9	1.807	1
SA_N_3	3	71.289	35638	2.57	8.44	12.04	6.8	- 1.795	1
SA_N_9	9	100.475	69640	3.05	8.22	12.19	8.1	- 1.985	1
SDW_C_10	10	60.255	SL10386_455	2.95	7.59	7.88	6.3	- 0.150	1
SDW_C_11	11	99.137	44926	2.01	7.85	7.62	4.2	0.116	3
SDW_C_2	2	49.655	49613	3.58	7.90	7.57	7.7	0.163	1
SDW_C_4.1	4	95.113	47385	5.79	7.46	8.01	13	- 0.275	3
SDW_C_4.2	4	105.963	47782	2.25	7.90	7.57	4.8	0.165	1
SDW_C_5	5	62.289-62.315	50963	2.02	7.85	7.62	4.3	0.117	3
SDW_C_6	6	31.355	68943	4.9	7.54	7.93	10.8	- 0.195	1
SDW_N_10	10	2.206	46104	3.98	2.35	2.48	9.6	- 0.063	1
SDW_N_3	3	64.624	35459	3.57	2.48	2.36	8.6	0.059	2
SDW_N_4	4	29.720-29.834	9921	2.35	2.37	2.47	5.5	- 0.048	2
ShDW_C_1	1	112.82	44213	3.01	28.68	27.20	8.8	0.742	1
ShDW_C_4	4	86.962	43517	2.53	27.25	28.64	7.3	- 0.694	2
ShDW_N_10	10	5.395	46305	2.38	7.37	7.70	6	- 0.165	1
ShDW_N_3	3	83.046	58518	2.31	7.69	7.37	5.7	0.159	2
ShDW_N_4	4	30.788	64358	2.33	7.37	7.70	5.8	- 0.164	2
ShDW_N_9	9	46.409	45095	4.82	8.86	6.21	12.6	1.326	1
tZ_C_12	12	19.723	40924	2.72	10.24	8.39	6.5	0.923	2
tZ_C_5	5	70.861-70.880	22599	2.61	6.78	11.57	5.7	- 2.394	24

Table 5- List of QTLs that were detected for LNC using the Kruskal-Wallis procedure and corresponding SNPs at the K peak. Their map position (cM) at the tomato chromosomes (Chr), the means of homozygotes LL and PP, and the mRNA of the nitrate transporter coding gene found (2 Mbp intervals), if any, as candidate gene are also indicated. QTLs with an asterisk were also detected by MQM procedure, and QTLs that could be considered the same under both treatments are in bold.

QTL	Chr	cM	SNP	K*	Signif.	LL	PP	Nitrate transporter
LNC_C_4	4	42.478	41683	9.222	0.010	5.48	**5.54**	
LNC_C_6.1*	6	37.829	55874	9.510	0.010	5.46	**5.56**	Solyc06g060620.2.1
LNC_C_9.2	9	91.838	36845	11.301	0.005	**5.55**	5.46	
LNC_C_10*	10	52.087	33113	9.633	0.010	5.43	**5.55**	
LNC_C_11.1	11	30.915	21019	8.512	0.005	5.47	**5.55**	
LNC_C_11.2	11	41.224	9539	6.675	0.010	5.47	**5.54**	
LNC_C_11.3	11	62.737	53040	11.797	0.001	5.47	**5.56**	
LNC_C_12	12	29.424	41068	6.911	0.010	5.46	**5.56**	Solyc12g006050.1.1
LNC_N_2*	2	11.615	20344	7.672	0.050	**2.98**	2.80	Solyc02g067790.2.1
LNC_N_6.1*	6	47.523	1350	10.058	0.010	2.82	**2.91**	Solyc06g060620.2.1
LNC_N_9.1	9	47.117	67830	9.623	0.010	**2.95**	2.80	
LNC_N_9.2	9	101.937	69669	9.310	0.010	**2.93**	2.77	
LNC_N_12	12	19.952	40952	7.626	0.010	2.79	**2.95**	Solyc12g006050.1.1

Table 6- Summary list of candidate genes for LNC and xylem hormone QTLs mined by function, presence of frameshift Indels (from Kevei et al. 2015) in parental genomes, E (E9) and P (L5), and relative root expression (Root) in Heinz cultivar (Max: maximum, H: high, Med: medium, VL: very low, L: low and N: no data). The start physical position of the gene (start), its mRNA code, and the number of genes counted from the QTL peak (G.Ord) are also indicated.

QTL	InDel	Root	Start	G.Ord	Annotation	mRNA
LNC_C_12		VL	645791	64	Nitrate transporter 1.3 like	Solyc12g006050.1.1
LNC_C_12	P	Max	2943402	329	Plant synaptotagmin	Solyc12g009700.1.1
LNC_C_12		L	4203735	496	Amino acid transporter	Solyc12g011370.1.1
LNC_C_12		N	4473107	523	ABC transporter G family member 9	Solyc12g013630.1.1
LNC_C_12		N	4477879	524	ABC transporter G family member 9	Solyc12g013640.1.1
LNC_C_4		Max	6513493	39	ATP-binding cassette 1	Solyc04g015970.2.1
LNC_C_4		N	7068003	69	Organic anion transporter	Solyc04g016280.2.1
LNC_C_6.1	P	Max	37707208	4	Protein-P-II uridylyltransferase	Solyc06g059800.2.1
LNC_C_6.1		L	38644635	72	Nitrate transporter 1	Solyc06g060620.2.1
LNC_N_6.1		Med	38358807	142	Organic anion transporter	Solyc06g060370.2.1
LNC_N_6.1		L	38644635	117	Nitrate transporter 1.1 like	Solyc06g060620.2.1
LNC_N_6.1		Max	38791367	103	Aquaporin	Solyc06g060760.2.1
LNC_N_6.1		VL	39263684	53	Amino acid transporter	Solyc06g061260.1.1
LNC_N_6.1		L	39266520	52	Amino acid transporter	Solyc06g061270.1.1
LNC_N_6.1	P	Max	39755908	21	Exocyst complex protein exo70	Solyc06g062990.1.1
LNC_N_6.1	P	Max	40442817	111	Nitrilase 2	Solyc06g064880.2.1
LNC_N_6.1		H	40541196	124	Peptide transporter	Solyc06g065020.2.1
LNC_N_12	P	H	1144017	91	Early nodulin 93 protein	Solyc12g006680.1.1
LNC_N_12	E	VL	2237269	34	Cytokinin oxidase/dehydrogenase 2	Solyc12g008920.1.1
LNC_N_12	P	Max	2943402	112	Plant synaptotagmin	Solyc12g009700.1.1
LNC_N_12.1		L	4203735	0	Amino acid transporter	Solyc12g011370.1.1
LNC_N_12.2	E	Max	62177603	37	Aquaporin	Solyc12g056220.1.1
LNC_N_12.3	P	Med	63156111	102	Purine permease family protein	Solyc12g057090.1.1
LNC_N_12.3	P	Med	63184276	101	Purine permease family protein	Solyc12g057100.1.1
LNC_N_2		Med	37659144	0	Porin/voltage-dependent anion-selective	Solyc02g067460.2.1
					channel	
LNC_N_2		VL	37900235	28	Nitrate transporter 2	Solyc02g067790.2.1
LNC_N_2		Max	38125655	57	Voltage-gated chloride channel	Solyc02g068080.2.1
LNC_N_6.2	P	Max	40442817	57	Nitrilase 2	Solyc06g064880.2.1
LNC_N_6.2		H	40541196	70	Peptide transporter	Solyc06g065020.2.1
LNC_N_9.1		H	58658805	10	Solute carrier family 35 member F4	Solyc09g061320.2.1
LNC_N_9.2		Max	69981424	46	Peptide transporter	Solyc09g090470.2.1
LNC_N_9.2		Max	70165532	20	Ammonium transporter	Solyc09g090730.1.1
LNC_N_9.2		Max	70486380	21	Glutathione S-transferase/chloride channel	Solyc09g091140.2.1
tZ_C_5	E	Max	60994786	26	Chaperone dnaJ 3	Solyc05g050810.2.1

tZ_C_5	E	Max	60998175	25	DNAJ chaperone	Solyc05g050820.2.1
tZ_C_12		VL	2209297	47	Cytokinin oxidase/dehydrogenase 2	Solyc12g008900.1.1
tZ_C_12	E	VL	2237269	49	Cytokinin oxidase/dehydrogenase 2	Solyc12g008920.1.1
SA_N_3	.	Max	59839092	46	Hydroxycinnamoyl CoA shikimate/	Solyc03g097500.2.1
					quinate hydroxycinnamoyltransferase-like protein	
SA_N_3	.	VL	59493895	79	Cinnamoyl-CoA reductase-like protein	Solyc03g097170.2.1
SA_N_3	.	VL	59381671	93	4-coumarate CoA ligase	Solyc03g097030.2.1
SA_N_9	.	N	70787703	78	Salicylic acid carboxyl methyltransferase	Solyc09g091530.1.1
SA_N_9	.	N	70794052	79	Salicylic acid carboxyl methyltransferase	Solyc09g091540.1.1
SA_N_9	.	VL	70802564	80	Salicylic acid carboxyl methyltransferase	Solyc09g091550.2.1
SA_N_9	P	Max	69863984	39	Xenotropic and polytropic retrovirus receptor	Solyc09g090360.2.1
GA3_N_6		Max	47979516	153	Gibberellin 2-beta-dioxygenase 7	Solyc06g082030.2.1
GA3_N_10.1/2	P	N	60673746	0	Squalene synthase	Solyc10g079040.1.1
GA3_N_10.3	P	Max	63237460	1	Pyrimidine-specific ribonucleoside hydrolase	Solyc10g083430.1.1
GA3_N_12	E	H	66134936	20	ATP synthase subunit H family protein	Solyc12g098720.1.1
					(V-type proton ATPase subunit e1)	
GA3_N_12	P	Max	66630152	99	Chaperone protein dnaJ 49	Solyc12g099510.1.1
GA3_N_12	P	Max	66684250	106	Nodulin MtN21 family protein	Solyc12g099580.1.1
JA_N_4	P	Max	62755589	27	Calcium-transporting ATPase 1	Solyc04g077870.2.1
JA_N_4	P	Max	63103059	73	Cytochrome P450	Solyc04g078340.2.1
JA_N_4	P	Max	63106650	75	Cytochrome P450	Solyc04g078360.1.1
JA_N_5	P	Max	5087522	25	Sulfotransferase family protein	Solyc05g011850.1.1
JA_N_5	P	Max	5087920	24	Sulfotransferase family protein	Solyc05g011860.1.1
JA_N_5	P	Max	5179822	13	Cytochrome P450	Solyc05g011970.2.1
JA_N_8	P	Max	56633138	47	ATP-binding cassette transporter	Solyc08g067620.2.1
JA_N_9	P	Max	4826832	55	Glutathione S-transferase-like protein	Solyc09g011520.2.1
JA_N_9	P	Max	4873131	46	Glutathione S-transferase-like protein	Solyc09g011610.2.1