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ORIGINAL ARTICLE

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ABSTRACT

Phenolic compounds are secondary metabolites involved in plant's innate chemical defenses against pests and diseases. Their concentration is variable between plant tissues and depends also on genetic and environmental factors, such as the availability of nutrient resources. This study examines the specific effects of low (LN) compared to high (HN) nitrogen supply on organ (root, stem and leaf) growth and accumulation of major phenolics (chlorogenic acid – CGA; rutin; kaempferol rutinoside – KR) in 9 hydroponically-grown tomato cultivars. LN limited shoot growth but did not affect that of roots. LN increased the concentration of each individual phenolic in all organs. The strength of the response was organ ~~dependent~~dependent, roots being more responsive than leaves and stems, respectively. Significant differences were observed between genotypes. Nitrogen limitation did not change the phenolic content in shoots whereas it stimulated accumulation in roots. The results show that this trade-off between growth and defense in LN environment can be discussed within the framework of the growth differentiation balance hypothesis (*i.e.* GDBH), but they point out the need to integrate all plant organs in future modeling approaches regarding the impact of nitrogen limitation on primary and secondary metabolisms.

INTRODUCTION

Plant secondary metabolites represent a large range of molecules mainly involved in (plant × environment) interactions. Among them, phenolic compounds constitute a major class widely distributed in the plant kingdom. Even though all their biological functions are not yet fully understood, phenolics are reported to participate in several aspects of (plant × environment) interactions notably in the innate chemical defense strategy against pathogens (Dixon and Paiva, 1995; Treutter, 2006). Defense-wise, these quantitative metabolites appear to be dosage dependent *i.e.* their activity relies on their local concentration. From these findings, it may be inferred that all practices optimizing their accumulation in plants~~,~~ may also provide new agronomic leverage for integrated pest management strategies seeking lower pesticide use in crop protection.

Phenolic concentrations in plant tissues are affected by both genotypes (Hanson et al., 2004) and environmental factors (i.e., light, nutrient availability, temperature, see: Larsson et al., 1986; Wilkens et al., 1996 a,b; Koricheva et al., 1998; Løvdal et al., 2010). Changing growth conditions, in particular nitrogen (N) availability, have been shown to affect phenolic concentrations in plant tissues. Indeed, N limitation enhances leaf phenolics (Lea et al., 2007; Bénard et al., 2009; Le Bot et al., 2009) and promotes resistance to specific pathogens (Hoffland et al., 2000; Leser and Treutter, 2005; Matros et al., 2006). At a broader scale, increased ~~N~~ nutrient use efficiency is a key-point in the concept of ecological intensification (for a review, see Doré et al., 2011). Limiting the use of N may have environmental benefits through decreasing greenhouse gas emission, reducing agriculture dependency on fossil fuels, preventing health and environmental disorders without decreasing productivity.~~Limiting the use of N participates in sound environmental issues, as well as decreasing greenhouse gas emission, reducing agriculture dependency on fossil fuels, preventing health and~~

~~environmental disorders without decreasing productivity.~~ In this framework, the enhancement of plant phenolic concentration *via* reduced N fertilization could benefit the agrosystems conducted under integrated pests management (IPM) strategies to lower pesticide use in crop protection. However its feasibility remains to be assessed, because N limitation rapidly alters crop yield and affects plant primary metabolism (Urbanczyk-Wochniak and Fernie, 2005). A more comprehensive understanding of the relationships between growth (primary metabolism) and defense (secondary metabolism), accounting for the impacts of environment and genetics, is required for the design of sustainable production systems less harmful to the environment and saving on N inputs (Jarvis, 1992; Brown, 2002).

Regarding the impact of environmental conditions, in particular nutrient availability, several plant defense hypotheses have been proposed to explain the changes of secondary compound concentrations in plant tissues. The most famous one is the growth-differentiation balance hypothesis (GDBH, Loomis 1932; Herms and Mattson, 1992). GDBH is based on a trade-off for allocation of plant resources to primary metabolism (accounting for plant growth) and secondary metabolite production (beneficial to defense). According to GDBH, any resource that restricts plant growth more than carbon fixation (photosynthesis) favors consequently the accumulation of secondary metabolites. Considering N availability, most experimental results on phenolic compound concentrations corroborate the GDBH, higher concentrations being measured under low N supplies (Stout et al., 1998; Stewart et al., 2001; Glynn et al., 2007). However, it must be noted that almost all studies focused on leaves, data on roots and stems being scarce. Nitrogen availability has different effects on organ development. For example, low N availability reduces much more the growth of shoots than that of roots (Adamowicz and Le Bot., 2008). Thus, from the GDBH viewpoint, one could expect plants to express specific phenolic accumulation profiles in each organ rather than a generic response to N, accounting for the observed spatial variability of metabolites location in tissues.

The genetic variability of tomato fruit phenolic composition has been recently reviewed (Slimestad and Verheul, 2009) but the study ignored the vegetative organs. To our knowledge, there is no study reporting on the impact of (genotype × nitrogen) interactions on tomato growth and phenolic accumulation at the organ level, although such information is important to rank cultivar (cv) performances under low N nutrition. Furthermore, this knowledge is essential to develop integrated crop management (ICM) strategies for growing tomato, since the selection of suitable genotypes is one of the simplest methods for reducing the management procedures and the negative environmental impacts during the growing season.

Amongst the difficulties to compare cultivars, plant physiologists are particularly concerned with the capacity of growing plants at high densities, on reduced space and over short time periods. Patio tomatoes, in particular cultivar Micro-Tom, have the small size required for a suitable biological model and they are increasingly used in molecular biology and physiology studies on tomato plants (Marti et al., 2006). Micro-Tom possesses distinctive mutations (*i.e.* dwarf, brassinosteroid-related and self-pruning) rendering debatable its status of "model system" but Campos et al. (2010) recently argued that they do not interfere with normal behaviours. They took advantage of this cultivar to study plant-pest interactions (Campos et al., 2009). Nevertheless, due to its extremely compact size, studies dealing with (growth x nitrogen) interactions are likely to exhibit low responses. Therefore, there is an important need for a comparative study with other dwarf cultivars to explore the genetic variability of these "model systems" in their responses to nitrogen nutrition.

The main objective of our work was to test the hypothesis that N availability impacts differently the relationship between growth and phenolic compounds accumulation at organ level. The second objective was to characterize the broadness of this response using 9 "patio tomato" cultivars, 6 being determinate (growth stopping after fruiting) with a large range of plant size and 3 indeterminate (continuous growth), in order to rank the cultivars and choose

an appropriate “model system” for future studies. The tomato plants were raised from seeds and grown hydroponically in a greenhouse on full nutrient solutions containing two tightly controlled N concentrations known to limit (LN) or not (HN) the growth of commercial tomato plants (Adamowicz and Le Bot, 2008). Growth and phenolics were measured in the three main vegetative organs (leaves, stems and roots) of 28 day-old plants. The analyses focused on three major phenolics reported or suspected to contribute to tomato plant defense: a caffeic acid derivative, chlorogenic acid (CGA, Ikonen et al., 2001) and two flavonoids, rutin (Baidez et al., 2007) and kaempferol rutinoside (KR, Mirnezhad et al., 2009).

MATERIALS AND METHODS

Plant material and growth conditions

Six growth-determinate (det, coded A-F, Table 1) and 3 growth-indeterminate (ind, coded G-I, Table 1) patio tomato (*Solanum lycopersicum* L.) cultivars (cvs) were sown in a NFT (nutrient film technique) system set in a growth room with the following constant day-night conditions: air temperature (T) 20°C, humidity (H) 80%, photoperiod 12h. The six determinate patio tomato were chosen in order to cover a large range of plant sizes (Tab. 1).

Plantlets were transferred to a glasshouse located in Avignon (France, 43°56′58″N; 4°48′32″E) on April 17th 2009, 10 days after sowing (DAS), with the following conditions: heating when T ≤ 18°C, ridge opening when T ≥ 25°C, mist spraying when H ≤ 55%. The glasshouse was whitewashed to ease T control. Plantlets were selected for homogeneity according to the length of their first true leaf. Roots were then rinsed with a nitrate-free solution prior to plant transfer to a NFT system. Plants were arranged in fully randomized blocks with two N regimes, limiting N nutrition (LN) and high N nutrition (HN). Because we expected large differences in plant development and N demand, determinate and

indeterminate cultivars were planted in separate blocks (8 and 4 blocks, respectively) with larger plant spacing for indeterminate cultivars.

Two plants per cultivar and nutrition treatment were randomly allocated to blocks in order to perform a harvest before fruiting and another at fruit maturity. Only the 1st harvest was analyzed and reported in this paper, since the development of some cultivars provoked mutual shading with artifactual growth effects rendering the 2nd harvest not exploitable.

Hydroponic setup

Separate solution tanks were used for determinate and indeterminate cultivars. They were located in an underground laboratory where automatic devices maintained 1) solution temperatures at 25°C; 2) solution volumes (optical level sensors LLE 102000; Honeywell, Morristown, USA) at 0.5 m^3 (LN det), 0.3 m^3 (LN ind and HN det) and 0.08 m^3 (HN ind) by additions of deionized water; 3) pH at 5.0 by automatic additions of H_2SO_4; 4) [NO_3^-] by automatic injection (precision syringe drive PSD2, Hamilton company, Reno Nevada) of a stock solution containing (mol m^{-3}) 1000 NO_3^-, 408 K^+, 204 Ca^{2+} and 92 Mg^{2+}, thus ensuring major cation repletion. Nitrate concentration was measured by automatic on-line UV absorption spectrometry (double-beam UVmc2; SAFAS, Monaco) derived from the method of Vercambre and Adamowicz (1996). Volume, pH and [NO_3^-] analyses and corrections were performed hourly ~~and [NO_3^-]~~ using the Totomatix system described previously (Adamowicz et al., 2011). [NO_3^-] never drifted more than 5% from set values. Periodic phosphate analyses were performed manually (vanadomolybdate colorimetry) and the set concentration was restored by addition of potassium phosphate buffer (pH 5.0).

The NO_3 uptake rate (U in mmol N h^{-1} per plant) was calculated hourly between times t and t+1 by the automatic laboratory using the following variables: C, measured [NO_3^-] (mol m^{-3}); I, injected stock NO_3^- (mol per tank); V, volume (m^3 per tank); n, number of plants.

$$U = \frac{V_t \times C_t - V_{t+1} \times C_{t+1} + I_t}{n} \times 10^3$$

V was calculated as the difference between the set value and the water volume added to restore the tank level.

Nutrition and treatments

The nutrient solutions were made up with deionized water and pure salts. Major ions were at the following concentrations (mol m^{-3}): K^+, 3; Ca^{2+}, 3.5; Mg^{2+}, 1.5; $H_2PO_4^-$, 1; we used sulfate to balance nitrate charges so that $[NO_3^-] + 2 \times [SO_4^{2-}] = 12$ mol m^{-3}. Trace elements were given as Kanieltra (formula 6 Fe, Hydro Azote, France) 0.1 L m^{-3} and EDTA-Fe 43 mmol m^{-3}. $[NO_3^-]$ were 0.3 mol m^{-3} (germination) and 3.0 mol m^{-3} (HN) which is non-limiting for tomato growth in NFT (Adamowicz and Le Bot, 2008). LN $[NO_3^-]$ was adjusted periodically in order to maintain $U_{LN}/U_{HN} \approx 1/3$. Thus, initially (10 DAS), LN $[NO_3^-]$ was set to 0.005 mol m^{-3} and from 16 to 28 DAS it ranged from 0.007 to 0.030 (det) and from 0.005 to 0.040 (ind). At harvest, the cumulative NO_3^- uptake of LN plants was 33% (det) and 36% (ind) that of HN plants. During the whole period, U_{LN} was never null (Fig. 1) and we did not observe any symptom of N deficiency.

Harvests and sample processing

Plants were harvested 28 DAS on a per block basis from 8.30 AM to 5.00 PM and stored in a dark cold room (15 °C) during sample processing. Storage at 15 °C never exceeded 1h for a given plant.

Morphological traits measurements included the plastochron index (Coleman & Greyson, 1976) base 2 cm, number of flowers per plant, stem height, and epicotyl diameter.

Roots, stems and leaves were separated and leaf area was measured (area meter LI-3000A, Li-Cor, Lincoln, NE, USA). Roots were rinsed in deionized water and spin-dried (2 min at 2800 g). Plant parts were weighed, frozen in liquid N_2 and stored at -80°C until freeze-drying

(Lyovac GT-2, Steris, Germany). Dry samples were weighed (model AE 100S, Mettler Toledo, Columbus, OH, USA), ground to a fine powder (ball mill MM200, Retsch, Haan, Germany) and stored under dry air in a desiccator at room temperature.

Plant analyses

Extraction of phenolics was adapted from the procedure described in Le Bot et al. (2009). All steps were carried out at 4 °C either in a cold chamber or on ice. The dry powder (50 mg) was extracted once with 2 ml of 70% aqueous ethanol. Taxifolin solution (50 µl of a stock at 2 mg ml^{-1} methanol) was added as an internal standard. The mixture was blended for 1 min and homogenized for 30 min. After centrifugation (8 min, 12000 g), the supernatant was collected and evaporated to dryness under vacuum. The residue was dissolved in 1 ml of 70% methanol and centrifuged (10 min, 12000 g). The supernatant was collected and analyzed (50 µl) for phenolic content and composition by HPLC according to Bénard et al. (2009). Samples were analyzed on a HPLC system (LC20AD, Shimadzu Corporation, Japan) equipped with a diode array detector (200-400 nm) and a Lichrospher RP-18 end-capped column (4 × 250 mm, 5 µm, Merck, Darmstadt, Germany) fitted with a Lichrospher RP-18 guard column (5 µm, Merck). The mobile phase consisted of a binary solvent system of (A) water adjusted to pH 2.6 with orthophosphoric acid and (B) methanol. The gradient (from 3% to 60% of B in 180 min) was eluted at a flow rate of 0.5 ml min^{-1} at room temperature. The good separation of the compounds allowed quantifying rutin, chlorogenic acid, and kaempferol-rutinoside from peak area calibrated against standards (rutin and CGA from Sigma, Saint Quentin-Fallavier, France; KR and taxifolin from Extrasynthèse, Lyon, France).

C and N concentration in plant tissues were determined according to the Dumas method with an elemental auto-analyser (Flash EA 1112 series, Thermo Fisher Scientific, Courtaboeuf, France).

Statistical analysis

Determinate and indeterminate cultivars were separately compared by three-way analysis of variance (cultivar and nutrition as fixed factors, blocks as random). The respective degrees of freedom for determinate and indeterminate cultivars were: cultivar = 5 and 2, nutrition = 1 and 1, cultivar x nutrition = 5 and 2, blocks = 7 and 3, residuals = 77 and 15. Box-plots, Q-Q plots and correlation between variance and mean assessed the data distribution and homoscedasticity. The Log-transformation was necessary for homoscedasticity of some variables (leaf area, dry biomasses, stem height and number of flowers, compound contents). When the cultivar x nutrition interaction was not significant, the Tukey's test was used for mean comparisons. Otherwise, the Student's test assessed the nutrition effect on each cultivar. Computations were performed using the procedure lm in R software (R Project for Statistical Computing, http://www.R-project.org) and statistical significance was set at $p < 0.05$.

RESULTS

Nitrogen effects on organ N content and growth

Organ N concentration significantly decreased under LN ($p < 10^{-9}$) in all tomato cultivars and organs, on average from 5.05 to 3.06 % DW in leaves, from 3.62 to 1.65 % DW in stems and from 4.76 to 2.73 % DW in roots (not shown).

Regarding growth, LN significantly decreased total DW of all determinate ($p < 10^{-9}$) and indeterminate ($p < 10^{-6}$) cultivars (Fig. 2 A, Tab. S1). The reduction was around 30-50%, except for the cultivar C that was less affected (17%). Among the organs, leaves and stems (Fig. 2 B-C, Tab. S1) showed this same response, but LN did not affect significantly root DW (Fig. 2 D, Tab. S1) ($p > 0.08$). Furthermore, LN decreased the plastochron index ($p < 10^{-3}$), total leaf area ($p < 10^{-15}$ det, $p < 10^{-8}$ ind), stem height ($p < 10^{-3}$ det, $p < 10^{-4}$ ind), epicotyl diameter ($p < 10^{-15}$ det, $p < 10^{-8}$ ind), leaf area ratio ($p < 10^{-15}$ det, $p < 10^{-3}$ ind) and specific

leaf area ($p < 10^{-8}$ det, $p < 0.1$ ind), whereas the number of flower was not affected ($p > 0.1$) (Tab. S2). The root : shoot ratio was higher under LN than HN ($p < 10^{-15}$ det, $p < 10^{-11}$ ind).

In each N regime, there were large and significant ($p < 10^{-7}$ det and $p < 10^{-4}$ ind) DW differences between cultivars. For all organs, determinate cvs A and B yielded the smallest DW and indeterminate cvs H and I the highest (Fig. 2).

Comparison of organ phenolics composition

All cultivars exhibited similar phenolic profiles for a given organ. However the phenolic composition was organ dependent (Fig. 3). Based on the phenolic profiles recorded at 330 nm, leaves (Fig. 3 A) showed a more complex composition and phenolics were more concentrated than in stems and roots (Fig. 3 B-C respectively). Whereas CGA was detected in all organs, rutin was absent from roots and KR occurred only in leaves.

In leaves, concentrations of CGA (Fig 4 A, Tab. S3) and rutin (Fig. 4 B, Tab. S3) were in most cases higher than in stems (Fig. 4 C-D, Tab. S3). These differed significantly between genotypes ($p < 10^{-4}$) except for leaf rutin concentration in indeterminate cvs (G-I, $p > 0.08$). CGA was the unique major phenolic in roots (Fig. 3; 4 E, Tab. S3) where its concentration differed only between determinate cvs ($p < 10^{-10}$). Leaf concentration of KR (Fig. 4 F, Tab. S3) differed significantly between cvs ($p < 10^{-4}$).

Ranking cultivars for phenolics depended on organs and on the particular molecule considered, but indeterminate cvs (G-I) were generally poor in phenolics (Fig. 4 A-F, Tab. S3). Cultivar A behaved peculiarly since CGA concentration was higher in stems than in leaves while stem rutin concentration almost equalled that in leaves.

Nitrogen effect on phenolic acid concentrations and contents in tomato

LN increased significantly the concentration of all major phenolics (p from 10^{-15} to 0.02) but the amplitude of the changes varied according to organs and molecules (Fig. 4, Tab. S3).

Compared to HN, LN increased leaf CGA concentration by a factor of 1.5 (cvs A, H) to 2.3 (cv E). This gain was less prominent in stems, *i.e.* from null (cv D) up to twofold (cv E). Among organs, roots were the most sensitive to N nutrition, as CGA concentration markedly rose from a factor of 2.3 (cv G) to 5.2 (cv C). As a consequence, the roots, which were the poorest organs in phenolic concentration under HN, became the richest under LN. There was a significant (cv × N) interaction for stem and root CGA concentration (both $p<10^{-2}$) only for determinate cultivars. Leaf rutin concentration increased in LN by a factor of 1.6 (cv A) to 3.2 (cv E) and in the stems, by a factor of 1.3 (cv A) to 2.8 (cv G). KR was detected in leaves only, where its concentration rose in LN by a factor of 2.1 (cvs A, D) to 3.3 (cv E). There was a significant (cv × N) interaction for leaf rutin and KR concentrations ($p<10^{-2}$ and $p<10^{-4}$ respectively) for determinate cultivars only.

From figure 4, it appears that cultivar E under LN exhibited the highest concentrations of CGA, rutin and KR in leaves, whereas in stems, cultivar A was the most concentrated in CGA and rutin. For all cultivars but A and E, roots of LN plants had the highest CGA concentration among organs.

The effect of nitrogen availability was also determined on phenolic content (mg per plant, Fig. 5). Leaf CGA content (Fig. 5 A, Tab. S4) was insensitive to N nutrition in all cultivars ($p > 0.7$ det; $p > 0.5$ ind). The same held true for stems (Fig. 5 B, Tab. S4), except for cv D ($p<10^{-5}$) and F ($p<10^{-2}$). In contrast, LN significantly enhanced root CGA in all cultivars (Fig. 5 C, Tab. S1) by a factor of 2.3 (cv G) to 5.7 (cv C). N nutrition affected differently the whole plant CGA content of cultivars: LN increased CGA significantly in cvs C, E and I but not in the other cvs (Fig. 5 D, Tab. S4 and S5).

Leaf rutin content (Fig. 6 A, Tab. S4) significantly increased under LN in cvs B, C, E and I (Tab. S5), all others being insensitive to N. In stems (Fig. 6 B, Tab. S4), LN increased significantly the rutin content in cv G (Tab. S5). The whole plant rutin content (Fig. 6 C, Tab.

S4) was higher under LN than HN in cvs B, C, E, H and I, other cultivars being insensitive to N.

Leaf KR content (Fig. 7, Tab. S4) was significantly higher under LN than HN in the determinate cvs B, C, E and in the indeterminate cvs H-I (Tab. S5).

Organ specific response of CGA and N status

Organ CGA concentration was depressed under HN compared to LN (Fig. 5, Tab. S4). At the same time, organ N concentration was depressed under LN compared to HN. In figure 8, we plotted the relative changes in the concentration of CGA (*i.e.* $0 < \text{HN/LN ratios} \leq 1$) versus the relative changes in that of N for each tissue (*i.e.* $0 < \text{LN/HN ratios} \leq 1$). For all other data points, Fig. 8 shows 3 ordered clusters corresponding to organ classes that were regressed through the maximum (1,1) taken as the absolute reference (*i.e.* HN = LN). For each cluster, the slope of the regression indicates the mean organ sensitivity of changing CGA concentration to changing N status. The roots exhibited the highest sensitivity and the stems the lowest.

DISCUSSION

This study was conducted on determinate and indeterminate patio tomato cultivars that share the characteristic of a small size and growth compared to commercial accessions. Despite a 3-4 fold range diversity in foliar phenolic concentration between cultivars the average concentrations (CGA, rutin, KR) were in the same order of magnitude than generally found in other conventional accessions (Hoffland et al., 2000; Stewart et al., 2000; Niggeweg et al., 2004; Millar et al., 2007; Le Bot et al., 2009). As a consequence, we may consider this set of patio tomatoes and their response to N limitation as representative of what may occur for commercial cultivars. In stems and roots, comparisons are more difficult as little information is available in the literature. The lower rutin and CGA concentrations found in stems

compared to leaves are in agreement with statements on other plants (Cirak et al., 2007; Koncic et al., 2010). Moreover, the average root CGA concentration, which was shown to be the major soluble phenolic in roots, matched the total soluble phenolic concentration reported by Le Floch et al. (2005) in their study on tomato roots.

We intended to assess the effect of N nutrition on phenolics at two contrasted growth stages: vegetative stage whose results are presented here and at fruiting stage. The latter has not been examined because the development of the plants induced a competition for light between plants (shading) prior to harvest. Earlier studies, particularly our work published recently indicate that the patterns of response of vegetative parts to N limitation were independent of growth stage, whereas the phenolic composition of tomato fruits was not greatly influenced by N availability (Bénard *et al*., 2009).

All cultivars exhibited the same qualitative response to N limitation characterized by (*i*) a lower shoot growth, while root growth was not affected (*ii*) a higher phenolic concentration at the plant level. Those effects reinforce previous studies made on a large range of plants (Scheible et al., 1997; Stewart et al., 2001; Fritz et al., 2006; N'Guyen and Niemeyer, 2008; Le Bot et al., 2009). Regarding growth and development parameters, the amplitude of the response to LN was almost the same for all genotypes, as illustrated by the ranking of cultivars for plant DW that was not affected by N nutrition (except for cv C, Fig. 2 A). In contrast, phenolic concentrations in all organs, but that of rutin in stem, revealed significant genotype × nitrogen interactions for determinate cultivars. This indicates that different tomato genotypes react specifically to N limitation and that a generic response cannot be drawn from studies based on a few ~~number of~~ cultivars. We cannot conclude yet for indeterminate cultivars as the limited panel of indeterminate accessions may explain the absence of interactions. From a plant defense viewpoint, recent literature reported that ~~a~~ two- and four-fold increases of leaf CGA and rutin concentrations allowed higher plant resistance

303 respectively to bacteria and insects in solanacea (Niggeweg et al., 2004; Misra et al., 2010),
304 whereas KR was identified as a detrimental compound to thrips invasion in *Senecio* and
305 Chrysantemum (Leiss et al., 2009 a, b). Under LN, the phenolic (CGA, rutin, KR)
306 concentrations in shoots were increased by a factor 1.5 to 3 and that of CGA in roots by a
307 factor of 2.5 to 4.5 suggesting that plant defense against several pathogens could have been
308 reinforced under LN. This assumption needs to be assessed by specific experiments testing
309 parasite behavior under contrasted N regimes inducing differential phenolic concentrations.

310 Root CGA and other phenolics are allelochemicals (Kanchan and Jayachandra, 1980; Abdul-
311 Rahman and Habib, 1989), released in the rhizosphere when nutrient deficiencies occur (Uren
312 and Reisenauer, 1988). Root phenolic exudation is stimulated in response to P and N
313 deficiency in bean (Juszczuk et al., 2004), as well as in Fe-deficient pigeon peas (*Cajanus*
314 *cajan* L., Ae et al., 1990). The functionality of root-released phenolics is through their
315 contribution to plant adaptation to nutrient deficiency by (i) solubilizing nutrients from
316 inaccessible sources (Dakora and Phillips, 2002), (ii) favoring mycorhization (Antunes et al.,
317 2006) thus improving plant foraging for nutrients. CGA has also been shown to mediate
318 lateral root growth in lettuce (Narukawa et al., 2009).

319 In our experiment, organ concentrations of CGA and N were inversely correlated and this
320 relationship was organ-specific. Plants are known to optimize N allocation to leaves, whereas
321 root~~s~~ is~~are~~ the organ by which N is absorbed from the nutrient solution and is translocated to
322 other plant organs.

323 Whereas concentrations of all the phenolics increased under LN in all the organs, their
324 contents did not evolve the same way. Indeed they depend on the organ, the molecule and the
325 cv tested. Leaf and stem phenolic contents were not or only slightly affected by LN (Fig 5 A-
326 B, Fig 6 A-B, Fig 7), meaning that their observed concentration increase resulted mainly from
327 the reduced leaf and stem growth. This observation is consistent with the conclusions of a

recent model describing carbon allocation to primary and secondary metabolism in young tomato leaves (Le Bot et al. 2009) suggesting that the secondary metabolite concentration declines at high N availability owing to a dilution process by primary metabolites and not necessarily to a lesser rate of secondary metabolism. However, the weak but significant increase of leaf flavonoid (rutin, KR) contents in several cultivars (B, C, E, H, I, Tab. S6) under LN suggests that a specific regulation of the phenolic pathway could occur depending on the cultivar. The higher root CGA concentrations under LN resulted from a higher phenolic content, since root growth proved insensitive to N nutrition. It remains unclear whether this increase resulted from a local regulation of the biosynthetic pathway in roots (biosynthesis increase/degradation decrease) or from changes in transports from shoots or even ~~to~~ from both. Regarding CGA content at the whole plant level, we identified two kinds of cultivars: those (C, E and I) that accumulated more CGA under LN (Fig. 5 D) implying that N limitation induced an up regulation of its biosynthesis and those~~.~~ ~~A~~(a majority of cultivars) that did not accumulate more CGA under LN at the whole plant level but at the root level, which may be interpreted as a relocation of CGA from shoots to roots. Both mechanisms have experimental support in the literature. Indeed, on one hand, Joet et al. (2010) highlighted a high correlation between CGA accumulation in coffee seeds (*Coffea arabica*) and gene expression upstream and downstream CGA biosynthesis, meaning that transcriptional control alone could explain a large part of CGA accumulation. On the other hand, Narukawa et al. (2009) showed that roots from decapitated lettuce contained less CGA compared with intact plants and Mondolot et al. (2006) that coffee phloem cells contained CGA, thus rendering consistent the hypothesis of CGA transport from shoots to roots. A more comprehensive study, involving transcriptional studies, flux analyses and isotopic labeling, is needed to understand the underlying mechanism of root CGA accumulation under LN.

In conclusion, concentrations of phenolics increased in all organs under low nitrogen and a significant genotype × nitrogen effect was observed. For all cultivars, the stimulation of CGA concentration by the nitrogen limitation was more important in roots than in leaves and stems. Nitrogen limitation did not change CGA content in shoots, whereas it stimulated accumulation in roots. The organ dependent response to N limitation points out the need to integrate all plant organs when considering plant responses to nutrient limitation and the trade-off of resource share between growth and secondary metabolism.

ABBREVIATIONS

[X], concentration of X

CGA, chlorogenic acid

KR, kaempferol rutinoside

det., determinate

ind., indeterminate

N, nitrogen

HN, high nitrogen

LN, low nitrogen

GDBH, growth differentiation balance hypothesis

IPM, integrated pest management

ICM, integrated crop management

NFT, nutrient film technique

DW, dry weight

cv/cvs, cultivar/cultivars

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Table

Table 1: Patio tomato cultivars with codes used in text and figures. The coding followed increasing whole plant dry biomass accumulation according to breeder technical resources.

	Code	Cultivar	Average plant size (from breeder resources)	Breeder
Determinate	A	Micro-Tom	15-20 cm	University of Florida, Bradenton
	B	Red Robin	20-30 cm	Burpee/seminis
	C	Tiny Tim	25-35 cm	University of New Hampshire, Durham
	D	Florida Basket	30-45 cm	University of Florida, Bradenton
	E	Pixie II (F1)	45 cm	Burpee/seminis
	F	Totem (F1)	60 cm	Floranova/Vegetalis
Indeterminate	G	Husky Cherry Gold (F1)	90-120 cm	Petoseed
	H	Husky Cherry Red (F1)	90-120 cm	Petoseed
	I	Better Bush	90-120 cm	Park seed

Figure legends

Fig. 1: Cumulative nitrate uptake of determinate (thin lines) and indeterminate (thick lines) tomato cultivars under HN (solid lines) and LN (dashed lines) nutrition. Nitrate uptake was calculated hourly, each solution tank feeding 96 (determinate) or 24 (indeterminate) plants until harvest, half these numbers after harvest. Irregularities in the traces result from day/night cycles and natural climate instability.

Fig. 2: Dry biomass (g per plant) of HN tomato cultivars plotted against LN: (A) whole plant DW, (B) leaf DW, (C) stem DW, (D) ~~leaf~~ root DW. Coded symbols are the means of 8 (A-F, determinate cvs) and 4 (G-I, indeterminate cvs) replicates with SE bars. The diagonals (straight lines) indicate where LN = HN. It follows that data above the diagonal are depressed by LN, whereas below the line, they are enhanced by LN.

Fig. 3: Typical HPLC chromatogram of soluble phenolic extracts from leaves (A), stems (B) and roots (C) of tomato plants under HN. The profiles were recorded at 330 nm and are expressed in arbitrary unit (AU). The labelled molecules correspond to chlorogenic acid (CGA) (1), taxifolin (2), rutin (3) and kaempferol rutinoside (KR) (4). Taxifolin is an internal control added during the extraction.

Fig. 4: Phenolic concentration (mg g^{-1} DW) of HN tomato cultivars plotted against LN: chlorogenic acid (CGA) in leaves (A), stems (C) and roots (E); rutin in leaves (B) and stems (D); kaempferol rutinoside (KR) in leaves (F). Symbols and lines as in Fig. 2.

Fig. 5: Chlorogenic acid (CGA) content (mg per plant) of HN tomato cultivars plotted against LN: (A) leaves, (B) stems, (C) roots and (D) whole plants. Symbols and lines as in Fig. 2.

Fig. 6: Rutin content (mg per plant) of HN tomato cultivars plotted against LN: (A) leaves, (B) stems and (C) whole plants. Symbols and lines as in Fig. 2.

Fig. 7: Leaf kaempferol rutinoside (KR) content (mg per plant) of HN tomato cultivars plotted against LN. Symbols and lines as in Fig. 2.

Fig. 8: Relative changes in [CGA] ($0 < HN/LN$ ratios ≤ 1) plotted versus the relative changes in tissue [N] ($0 < LN/HN$ ratios ≤ 1) in leaves (black), stems (red) and roots (blue). Coded symbols as in Fig. 2. Lines are regressions forced through the maximum (1,1) taken as the reference where HN=LN: leaves (black) $y = 1.051\ x - 0.051$ ($R^2 = 0.97$); stems (red) $y = 0.677\ x + 0.323$ ($R^2 = 0.95$); roots (blue) $y = 1.643\ x - 0.643$ ($R^2 = 0.99$).

Figure 3

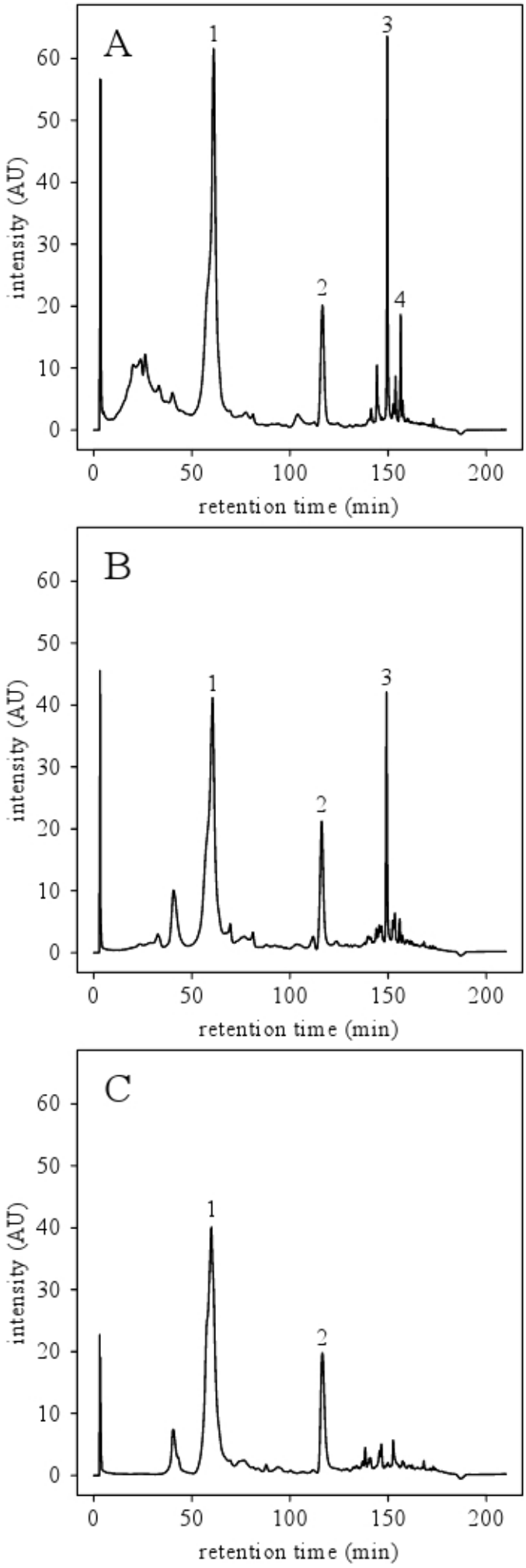
A
B
C
1
2
3
4
intensity (AU)
retention time (min)
0
10
20
30
40
50
60
0
50
100
150
200

Figure 4

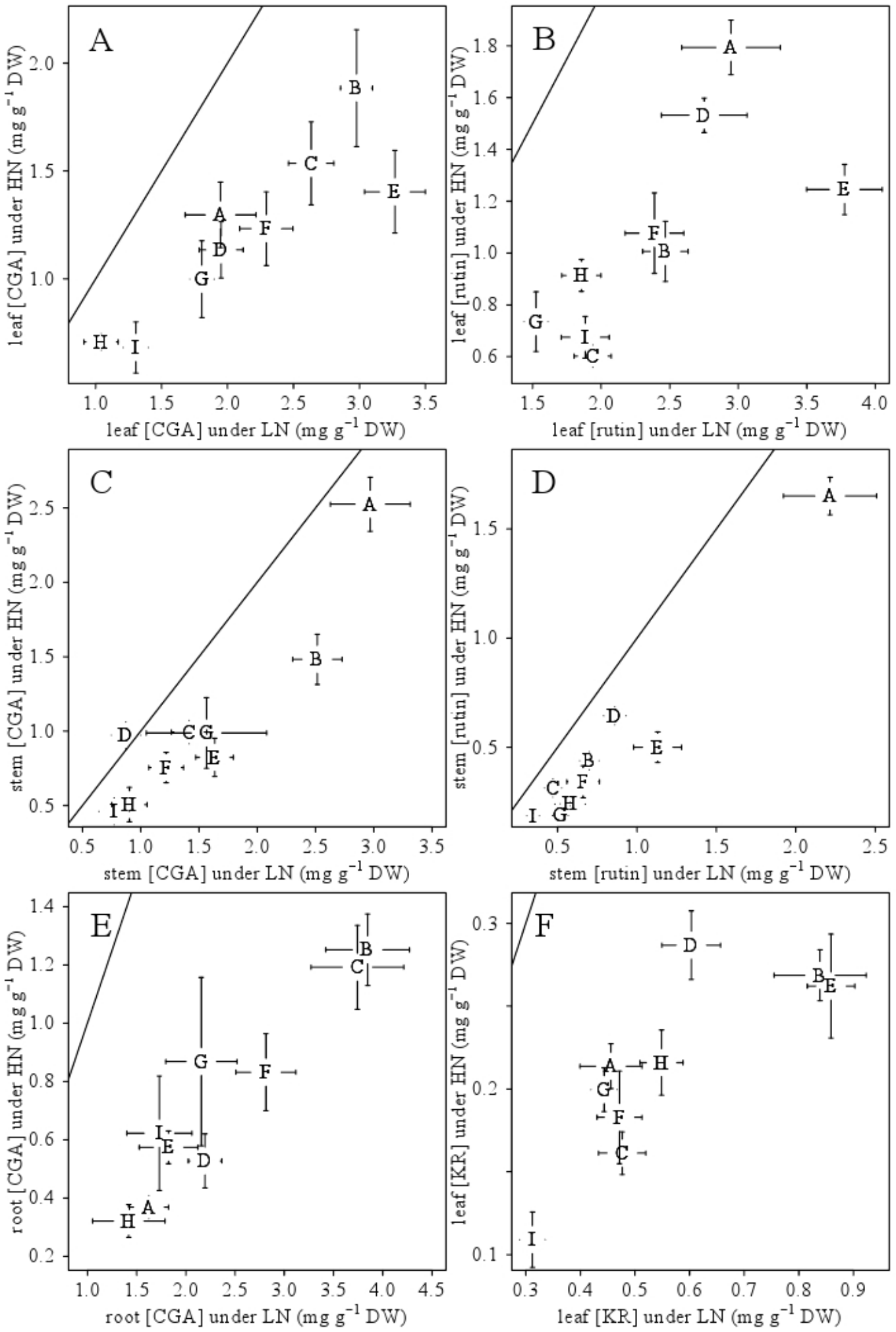
A
leaf [CGA] under HN (mg g^{-1} DW)
leaf [CGA] under LN (mg g^{-1} DW)
B
leaf [rutin] under HN (mg g^{-1} DW)
leaf [rutin] under LN (mg g^{-1} DW)
C
stem [CGA] under HN (mg g^{-1} DW)
stem [CGA] under LN (mg g^{-1} DW)
D
stem [rutin] under HN (mg g^{-1} DW)
stem [rutin] under LN (mg g^{-1} DW)
E
root [CGA] under HN (mg g^{-1} DW)
root [CGA] under LN (mg g^{-1} DW)
F
leaf [KR] under HN (mg g^{-1} DW)
leaf [KR] under LN (mg g^{-1} DW)

Figure 5

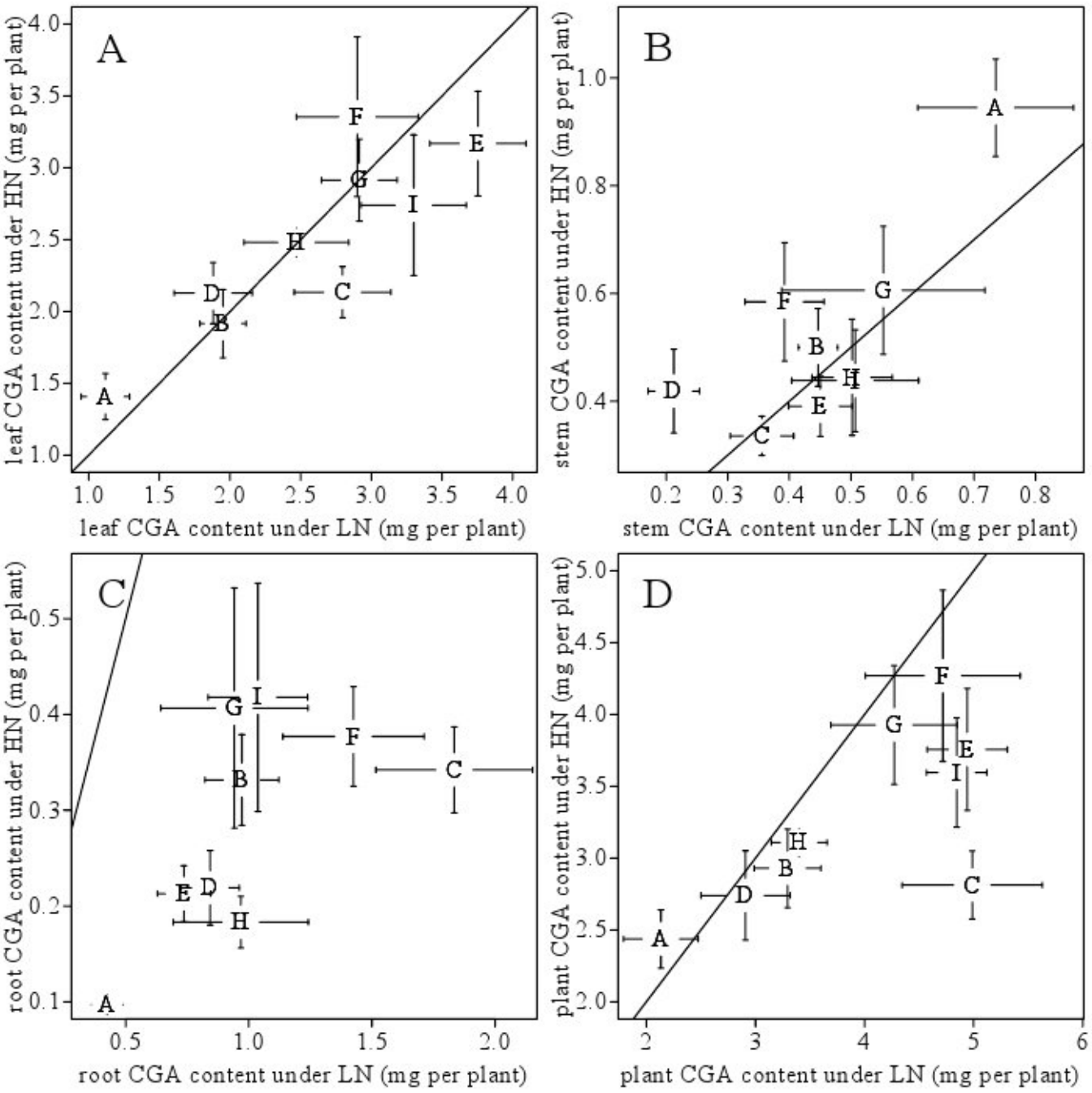
A
leaf CGA content under HN (mg per plant)
leaf CGA content under LN (mg per plant)
B
stem CGA content under HN (mg per plant)
stem CGA content under LN (mg per plant)
C
root CGA content under HN (mg per plant)
root CGA content under LN (mg per plant)
D
plant CGA content under HN (mg per plant)
plant CGA content under LN (mg per plant)

Figure 6

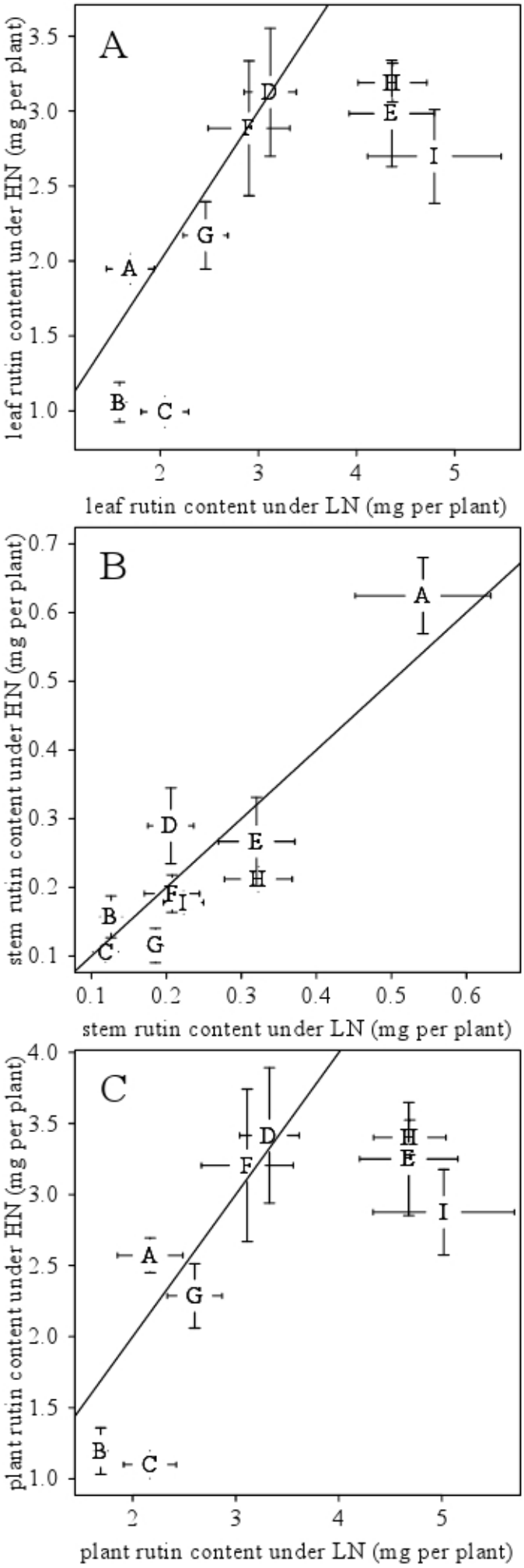
A
leaf rutin content under HN (mg per plant)
leaf rutin content under LN (mg per plant)
B
stem rutin content under HN (mg per plant)
stem rutin content under LN (mg per plant)
C
plant rutin content under HN (mg per plant)
plant rutin content under LN (mg per plant)

Figure 7

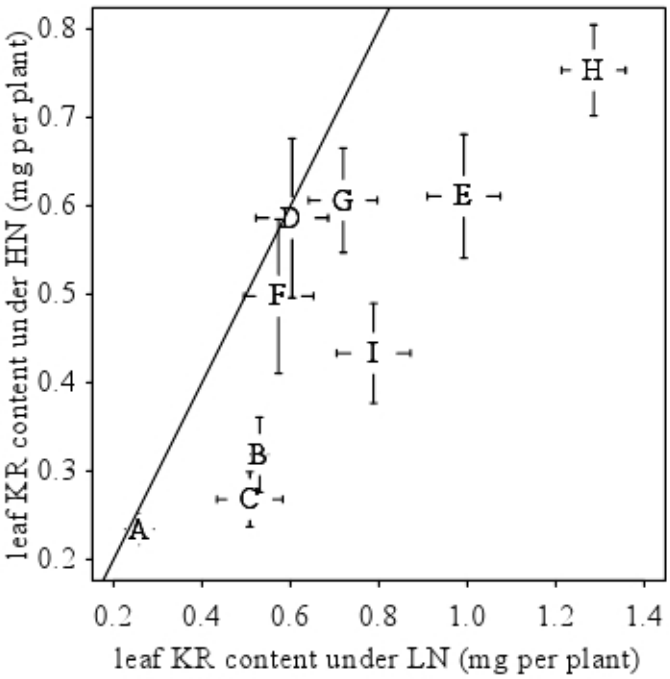
leaf KR content under HN (mg per plant)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
A
B
C
D
E
F
G
H
I
0.2
0.4
0.6
0.8
1.0
1.2
1.4
leaf KR content under LN (mg per plant)

Figure 8

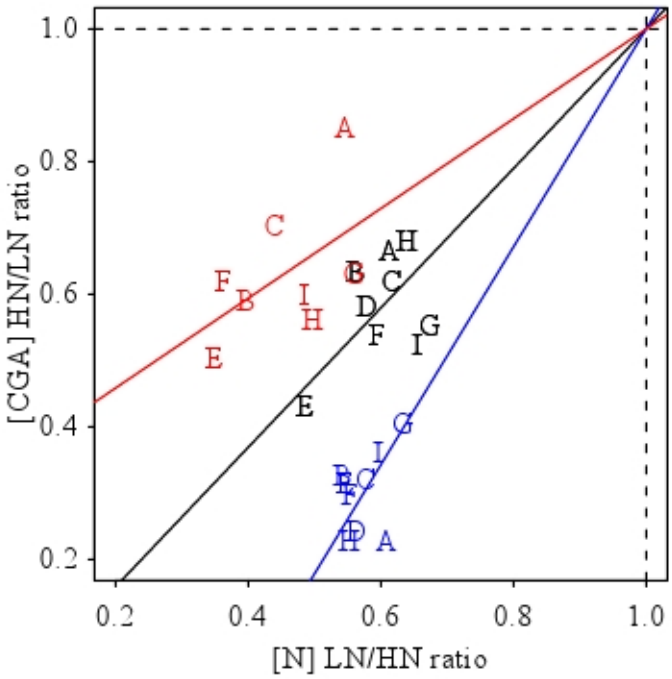

Figure 1

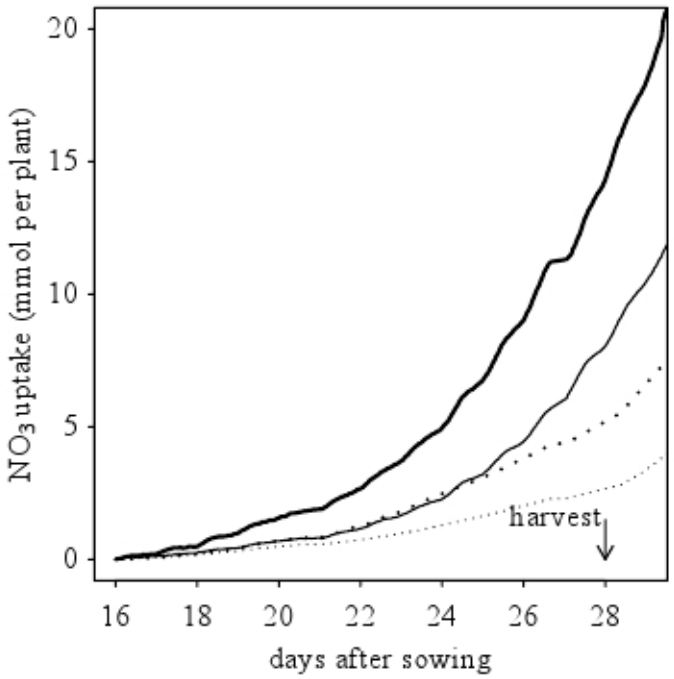
20
15
10
5
0
NO_3 uptake (mmol per plant)
harvest
16
18
20
22
24
26
28
days after sowing

Figure 2

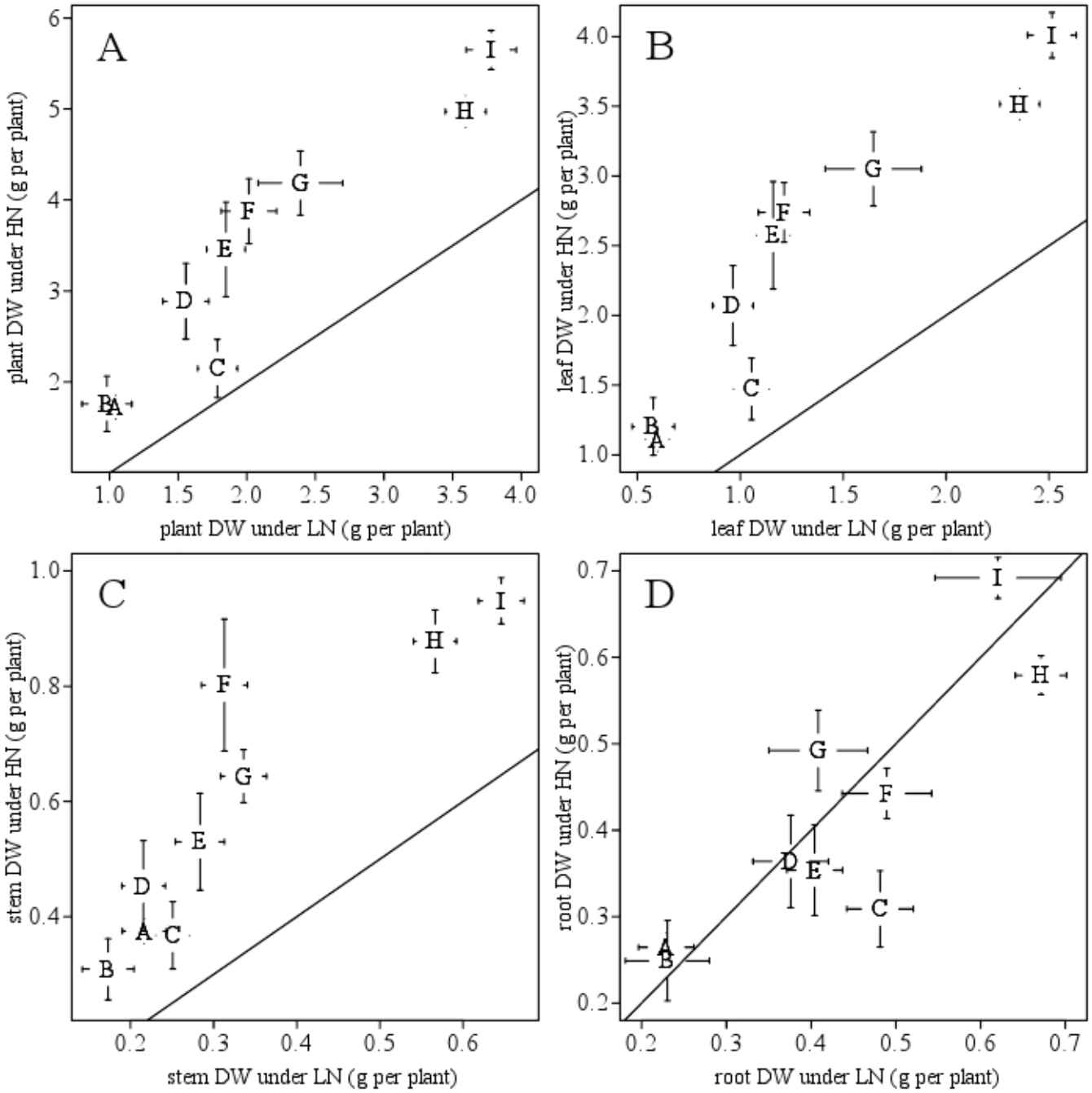
A
plant DW under HN (g per plant)
plant DW under LN (g per plant)
B
leaf DW under HN (g per plant)
leaf DW under LN (g per plant)
C
stem DW under HN (g per plant)
stem DW under LN (g per plant)
D
root DW under HN (g per plant)
root DW under LN (g per plant)

Table S1: Average organs and plant DW for the nine tomato cultivars as a function of the N nutrition. Means and standard errors are calculated from the analysis of 8 and 4 replicates for determinate and indeterminate cultivars respectively.

Cv	Nutrition	Organ/Plant DW (g)			
		Leaf	Stem	Root	Plant
A	HN	1.10 ± 0.08	0.37 ± 0.02	0.26 ± 0.02	1.72 ± 0.13
	LN	0.60 ± 0.06	0.22 ± 0.03	0.23 ± 0.03	1.04 ± 0.11
B	HN	1.20 ± 0.21	0.31 ± 0.05	0.25 ± 0.05	1.76 ± 0.30
	LN	0.58 ± 0.10	0.17 ± 0.03	0.23 ± 0.05	0.98 ± 0.18
C	HN	1.47 ± 0.22	0.37 ± 0.06	0.31 ± 0.04	2.15 ± 0.32
	LN	1.05 ± 0.09	0.25 ± 0.02	0.48 ± 0.04	1.79 ± 0.14
D	HN	2.07 ± 0.29	0.45 ± 0.08	0.36 ± 0.05	2.89 ± 0.41
	LN	0.96 ± 0.10	0.22 ± 0.03	0.38 ± 0.04	1.56 ± 0.17
E	HN	2.57 ± 0.38	0.53 ± 0.08	0.35 ± 0.05	3.46 ± 0.52
	LN	1.16 ± 0.08	0.28 ± 0.03	0.40 ± 0.03	1.85 ± 0.14
F	HN	2.74 ± 0.21	0.80 ± 0.11	0.44 ± 0.03	3.88 ± 0.36
	LN	1.21 ± 0.12	0.31 ± 0.03	0.49 ± 0.05	2.01 ± 0.20
G	HN	3.05 ± 0.27	0.64 ± 0.05	0.49 ± 0.05	4.19 ± 0.35
	LN	1.65 ± 0.23	0.34 ± 0.03	0.41 ± 0.06	2.39 ± 0.31
H	HN	3.51 ± 0.11	0.88 ± 0.05	0.58 ± 0.02	4.97 ± 0.17
	LN	2.36 ± 0.10	0.57 ± 0.03	0.67 ± 0.03	3.59 ± 0.15
I	HN	4.01 ± 0.16	0.95 ± 0.04	0.69 ± 0.02	5.65 ± 0.21
	LN	2.51 ± 0.12	0.65 ± 0.03	0.62 ± 0.07	3.78 ± 0.18

Table S2: Average values of some morphological traits for the nine tomato cultivars as a function of the N nutrition. Means and standard errors are calculated from the analysis of 8 and 4 replicates for determinate and indeterminate cultivars respectively. The morphological traits analysed were the plastochron index (Plast. Ind.), flower number (Flower Numb.), epicotyl diameter (Epicot. Diam.), stem height, total leaf area, leaf area ratio (LAR), specific leaf area (SLA) and root shoot ratio (RSR).

Cv	Nutrition	Plast.Ind.	Flower Numb.	Epicot. Diam.	Stem height	Total Leaf Area	LAR	SLA	RSR
A	HN	8.3 ± 0.2	40 ± 5	6.1 ± 0.2	11.2 ± 0.3	222 ± 12	129 ± 4	202 ± 7	0.18 ± 0.01
	LN	7.9 ± 0.4	30 ± 4	4.8 ± 0.2	10.4 ± 0.4	114 ± 11	113 ± 6	192 ± 6	0.29 ± 0.01
B	HN	8.0 ± 0.3	11 ± 2	8.3 ± 0.4	11.5 ± 0.5	564 ± 83	195 ± 2	270 ± 4	0.14 ± 0.01
	LN	7.8 ± 0.3	10 ± 1	6.0 ± 0.4	10.0 ± 0.5	235 ± 23	153 ± 5	245 ± 9	0.31 ± 0.01
C	HN	9.4 ± 0.4	8 ± 1	9.6 ± 0.6	14.0 ± 0.8	610 ± 93	176 ± 4	236 ± 6	0.12 ± 0.01
	LN	8.4 ± 0.2	10 ± 1	7.3 ± 0.2	12.9 ± 0.4	253 ± 22	136 ± 3	217 ± 5	0.28 ± 0.01
D	HN	8.9 ± 0.2	17 ± 4	7.6 ± 0.5	12.0 ± 0.8	399 ± 62	184 ± 3	269 ± 4	0.17 ± 0.01
	LN	8.3 ± 0.2	19 ± 4	6.0 ± 0.1	11.7 ± 0.4	256 ± 20	144 ± 4	244 ± 7	0.37 ± 0.01
E	HN	8.2 ± 0.6	9 ± 1	7.4 ± 0.6	8.7 ± 0.4	303 ± 53	171 ± 3	255 ± 7	0.15 ± 0.01
	LN	7.4 ± 0.4	7 ± 1	5.3 ± 0.5	8.4 ± 0.5	135 ± 25	140 ± 3	235 ± 6	0.33 ± 0.01
F	HN	9.7 ± 0.4	9 ± 2	10.8 ± 0.3	15.2 ± 0.9	673 ± 52	168 ± 3	244 ± 6	0.13 ± 0.01
	LN	8.5 ± 0.4	8 ± 1	7.5 ± 0.3	12.6 ± 0.4	275 ± 24	139 ± 4	232 ± 8	0.32 ± 0.01
G	HN	10.7 ± 0.3	6 ± 0	9.9 ± 0.2	16.2 ± 0.6	732 ± 28	147 ± 2	208 ± 2	0.13 ± 0.01
	LN	9.6 ± 0.4	6 ± 1	8.4 ± 0.1	14.1 ± 0.5	469 ± 30	131 ± 7	199 ± 10	0.23 ± 0.01
H	HN	9.2 ± 0.2	3 ± 0	11.1 ± 0.2	15.4 ± 0.4	793 ± 15	141 ± 6	199 ± 9	0.13 ± 0.01
	LN	8.5 ± 0.2	3 ± 0	9.9 ± 0.2	13.4 ± 0.2	499 ± 15	133 ± 3	199 ± 4	0.22 ± 0.01
I	HN	9. ± 0.2	6 ± 1	9.3 ± 0.1	12.6 ± 0.7	634 ± 69	151 ± 4	207 ± 5	0.13 ± 0.01
	LN	8.4 ± 0.3	6 ± 1	7.4 ± 0.2	10.8 ± 0.6	303 ± 19	129 ± 8	189 ± 14	0.23 ± 0.01

Table S3: Average phenolics (CGA, rutin, KR) concentration in organs of the nine tomato cultivars as a function of the N nutrition. Means and standard errors are calculated from the analysis of 8 and 4 replicates for determinate and indeterminate cultivars respectively. Concentrations are given in $mg.g^{-1}DW$.

Cv	Nutrition	[CGA]			[Rutin]		[KR]
		Leaf	Stem	Root	Leaf	Stem	Leaf
A	HN	1.29 ± 0.15	2.52 ± 0.18	0.37 ± 0.04	1.79 ± 0.10	1.65 ± 0.09	0.21 ± 0.01
	LN	1.95 ± 0.27	2.97 ± 0.34	1.62 ± 0.20	2.95 ± 0.36	2.22 ± 0.29	0.46 ± 0.06
B	HN	1.88 ± 0.27	1.48 ± 0.17	1.25 ± 0.12	1.01 ± 0.12	0.44 ± 0.04	0.27 ± 0.01
	LN	2.98 ± 0.12	2.51 ± 0.21	3.85 ± 0.42	2.47 ± 0.17	0.70 ± 0.06	0.84 ± 0.08
C	HN	1.54 ± 0.19	0.99 ± 0.08	1.19 ± 0.14	0.60 ± 0.04	0.31 ± 0.04	0.16 ± 0.01
	LN	2.63 ± 0.17	1.42 ± 0.15	3.74 ± 0.47	1.94 ± 0.13	0.47 ± 0.05	0.48 ± 0.04
D	HN	1.13 ± 0.13	0.97 ± 0.09	0.53 ± 0.09	1.53 ± 0.07	0.64 ± 0.04	0.29 ± 0.02
	LN	1.95 ± 0.17	0.87 ± 0.13	2.19 ± 0.17	2.75 ± 0.31	0.86 ± 0.07	0.60 ± 0.05
E	HN	1.40 ± 0.19	0.82 ± 0.13	0.57 ± 0.06	1.24 ± 0.10	0.50 ± 0.07	0.26 ± 0.03
	LN	3.27 ± 0.23	1.63 ± 0.16	1.82 ± 0.30	3.78 ± 0.28	1.13 ± 0.15	0.86 ± 0.04
F	HN	1.23 ± 0.17	0.75 ± 0.10	0.83 ± 0.13	1.08 ± 0.15	0.34 ± 0.07	0.18 ± 0.03
	LN	2.29 ± 0.20	1.22 ± 0.15	2.81 ± 0.30	2.38 ± 0.21	0.66 ± 0.10	0.47 ± 0.04
G	HN	0.99 ± 0.18	0.99 ± 0.24	0.87 ± 0.29	0.73 ± 0.11	0.19 ± 0.05	0.20 ±0.01
	LN	1.81 ± 0.09	1.56 ± 0.52	2.16 ± 0.36	1.52 ± 0.09	0.52 ± 0.05	0.44 ± 0.02
H	HN	0.71 ± 0.04	0.51 ± 0.11	0.32 ± 0.06	0.91 ± 0.06	0.24 ± 0.01	0.22 ± 0.02
	LN	1.04 ± 0.13	0.90 ± 0.15	1.42 ± 0.37	1.86 ± 0.14	0.58 ± 0.10	0.55 ± 0.04
I	HN	0.68 ± 0.12	0.46 ± 0.09	0.62 ± 0.20	0.67 ± 0.08	0.19 ± 0.01	0.11 ± 0.02
	LN	1.30 ± 0.09	0.77 ± 0.13	1.73 ± 0.33	1.88 ± 0.17	0.34 ± 0.04	0.31 ± 0.02

Table S4: Average phenolics (CGA, rutin, KR) contents in organs of the nine tomato cultivars as a function of the N nutrition. Means and standard errors are calculated from the analysis of 8 and 4 replicates for determinate and indeterminate cultivars respectively. Phenolic content are given in mg.

Cv	Nutrition	CGA content				Rutin content			KR content
		Leaf	Stem	Root	Plant	Leaf	Stem	Plant	Leaf/Plant
A	HN	1.41 ± 0.16	0.94 ± 0.09	0.10 ± 0.01	2.44 ± 0.20	1.95 ± 0.10	0.62 ± 0.06	2.57 ± 0.12	0.23 ± 0.02
	LN	1.12 ± 0.17	0.73 ± 0.13	0.42 ± 0.06	2.13 ± 0.34	1.70 ± 0.24	0.54 ± 0.09	2.17 ± 0.32	0.26 ± 0.03
B	HN	1.91 ± 0.24	0.50 ± 0.07	0.33 ± 0.05	2.93 ± 0.27	1.06 ± 0.13	0.16 ± 0.03	1.19 ± 0.16	0.32 ± 0.04
	LN	1.95 ± 0.16	0.45 ± 0.03	0.97 ± 0.15	3.29 ± 0.31	1.59 ± 0.07	0.13 ± 0.01	1.69 ± 0.07	0.53 ±0.02
C	HN	2.13 ± 0.18	0.34 ± 0.04	0.34 ± 0.04	2.81 ± 0.24	0.99 ± 0.10	0.11 ± 0.01	1.10 ± 0.10	0.27 ± 0.03
	LN	2.80 ± 0.34	0.35 ± 0.05	1.83 ± 0.32	4.99 ± 0.64	2.05 ± 0.24	0.12 ± 0.01	2.17 ± 0.25	0.51 ± 0.07
D	HN	2.13 ± 0.21	0.42 ± 0.08	0.22 ± 0.04	2.74 ± 0.31	3.13 ± 0.43	0.29 ± 0.05	3.42 ± 0.48	0.59 ± 0.09
	LN	1.88 ± 0.28	0.21 ± 0.04	0.84 ± 0.12	2.90 ± 0.41	3.12 ± 0.27	0.21 ± 0.03	3.33 ± 0.29	0.60 ± 0.08
E	HN	3.17 ± 0.36	0.39 ± 0.06	0.21 ± 0.03	3.76 ± 0.42	2.98 ± 0.35	0.27 ± 0.06	3.25 ± 0.40	0.61 ± 0.07
	LN	3.75 ± 0.34	0.45 ± 0.05	0.74 ± 0.11	4.94 ± 0.37	4.36 ± 0.43	0.32 ± 0.05	4.68 ± 0.48	0.99 ± 0.08
F	HN	3.35 ± 0.55	0.58 ± 0.11	0.38 ± 0.05	4.27 ± 0.60	2.89 ± 0.45	0.19 ± 0.03	3.20 ± 0.54	0.49 ± 0.09
	LN	2.90 ± 0.43	0.39 ± 0.06	1.42 ± 0.29	4.72 ± 0.71	2.90 ± 0.42	0.21 ± 0.04	3.11 ± 0.44	0.57 ± 0.08
G	HN	2.91 ± 0.28	0.61 ± 0.12	0.41 ± 0.12	3.93 ± 0.41	2.17 ± 0.22	0.11 ± 0.02	2.29 ± 0.23	0.61 ± 0.06
	LN	2.91 ± 0.27	0.55 ± 0.16	0.94 ± 0.30	4.27 ± 0.58	2.46 ± 0.23	0.18 ± 0.01	2.60 ± 0.27	0.72 ± 0.08
H	HN	2.48 ± 0.10	0.44 ± 0.11	0.18 ± 0.03	3.11 ± 0.10	3.19 ± 0.13	0.21 ± 0.02	3.40 ± 0.12	0.75 ± 0.05
	LN	2.47 ± 0.37	0.50 ± 0.06	0.97 ± 0.27	3.40 ± 0.26	4.36 ± 0.35	0.32 ± 0.04	4.69 ± 0.35	1.29 ± 0.07
I	HN	2.74 ± 0.49	0.44 ± 0.09	0.42 ± 0.12	3.60 ± 0.38	2.70 ± 0.31	0.18 ± 0.02	2.88 ± 0.30	0.43 ± 0.06
	LN	3.30 ± 0.37	0.51 ± 0.10	1.04 ± 0.20	4.84 ± 0.28	4.79 ± 0.68	0.22 ± 0.03	5.02 ± 0.68	0.79 ± 0.08

Table S5: Significance of LN effect on phenolic content in vegetative organs and plant of the nine tomato cultivars analysed. ns means non significant.

	CGA				Rutin			KR
Cv	Leaf	Stem	Root	Plant	Leaf	Stem	Plant	Leaf
A	ns	ns	$p<10^{-12}$	ns	ns	ns	ns	ns
B	ns	ns	$p<10^{-9}$	ns	$p<10^{-3}$	ns	$p<10^{-3}$	$p<10^{-4}$
C	ns	ns	$p<10^{-15}$	$p<10^{-5}$	$p<10^{-7}$	ns	$p<10^{-6}$	$p<10^{-5}$
D	ns	$p<10^{-5}$	$p<10^{-12}$	ns	ns	ns	ns	ns
E	ns	ns	$p<10^{-9}$	$p<10^{-2}$	$p<10^{-3}$	ns	$p<10^{-2}$	$p<10^{-3}$
F	ns	$p<10^{-2}$	$p<10^{-10}$	ns	ns	ns	ns	ns
G	ns	ns	$p<10^{-2}$	ns	ns	$p<10^{-2}$	ns	ns
H	ns	ns	$p<10^{-4}$	ns	ns	ns	$p<10^{-2}$	$p<10^{-3}$
I	ns	ns	$p<10^{-2}$	$p<10^{-2}$	$p<10^{-4}$	ns	$p<10^{-4}$	$p<10^{-4}$