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# Phenolic characterization and variability in leaves, stems and roots of Micro-Tom and patio tomatoes, in response to nitrogen limitation

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ABSTRACT

Phenolics are implicated in the defence strategies of many plant species rendering their concentration increase of putative practical interest in the field of crop protection. Little attention has been given to the nature, concentration and distribution of phenolics within vegetative organs of tomato (*Solanum lycopersicum*. L) as compared to fruits. In this study, we extensively characterized the phenolics in leaves, stems and roots of nine tomato cultivars using high-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC–MS$^n$) and assessed the impact of low nitrogen (LN) availability on their accumulation. Thirty-one phenolics from the four sub-classes, hydroxycinnamoyl esters, flavonoids, anthocyanins and phenolamides were identified, five of which had not previously been reported in these tomato organs. A higher diversity and concentration of phenolics was found in leaves than in stems and roots. The qualitative distribution of these compounds between plant organs was similar for the nine cultivars with the exception of Micro-Tom because of its significantly higher phenolic concentrations in leaves and stems as compared to roots. With few exceptions, the influence of the LN treatment on the three organs of all cultivars was to increase the concentrations of hydroxycinnamoyl esters, flavonoids and anthocyanins and to decrease those of phenolamides. This impact of LN was greater in roots than in leaves and stems. Nitrogen nutrition thus appears as a means of modulating the concentration and composition of organ phenolics and their distribution within the whole plant.


*Abbreviations:* AR, apio-furanosyl-rutin; BR, brassinosteroid; C, carbon; CCoRG, cyanidin-3(coumaroyl)rutinoside-5-O-glucoside; CGT, Chlorogenate:glucarate caffeoyl transferase; CHA1–5, caffeoyl hexaric acid isomers 1–5; CMA, caffeoyl malic acid; CNB, carbon nutrient balance; CP, caffeoyl putrescine; CQA1–3, caffeoyl quinic acid isomers 1–3; DCoRG1–2, delphinidin-3(coumaroyl)rutinoside-5-O-glucoside isomers 1 and 2; DCoS, di-coumaroyl spermidine; DNP, dictionary of natural product; DW, dry weight; ESI$^{+/-}$, electro-spray ionization in positive/negative mode; FAH1–2, feruloyl hexaric acid isomers 1 and 2; FDA, factorial discriminant analysis; FQA, feruloyl quinic acid; GDBH, growth differentiation balance hypothesis; JA, jasmonic acid; JA-Ile, jasmonic acid isoleucine conjugate; FT, feruloyl tyramine; HN, high nitrogen; HPLC, high performance liquid chromatography; KR, kaempferol rutinoside; KRG, kaempferol-3-O-rutinoside-7-O-glucoside; LC–MS, liquid chromatography coupled to mass spectrometry; LN, low nitrogen; MS, mass spectrometry; MetOH, methanol; N, nitrogen; NFT, nutrient film technique; NMR, nuclear magnetic resonance; *p*CoQA1–2, *para*-coumaroyl quinic acid isomers 1 and 2; *p*CoT, *para*-coumaroyl tyramine; PCoRG, petunidin-3(coumaroyl)rutinoside-5-O-glucoside; PCRG, petunidin-3(caffeoyl)rutinoside-5-O-glucoside; PHT, putrescine hydroxycinnamoyl transferase; QRG, Quercetin-3-O-rutinoside-7-O-glucoside; R, rutin; SG, sinapoyl glucose; TCoS1-2, tri-coumaroyl spermidine isomers 1 and 2; THT, tyramine hydroxycinnamoyl transferase; UPLC, ultra performance liquid chromatography; UV, ultra-violet; [*X*], concentration of *X*.

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## 1. Introduction

Phenolic compounds (phenolics), together with terpenoids and alkaloids, represent one of the three main groups of plant secondary metabolites. In dicots, all phenolics are derived from the sole amino acid phenylalanine but the subsequent complex metabolic network leads to several phenolic sub-families, among them, flavonoids, anthocyanins, hydroxycinnamoyl esters, phenolamides and monolignols.

Phenolics contribute largely to plant–environment interactions. They are implicated in symbiotic relationships (pollination, nitrogen fixation in Fabaceas) as well as in the defence of plants against biotic and abiotic constraints. Several studies have reported that high phenolic concentrations increase plant resistance to pathogens [1–4]. Moreover, several phenolics have been identified as resistance factors to specific pests. For example chlorogenic and feruloyl quinic acids in *Chrysanthemum* and kaempferol glucoside in *Senecio* hybrids increase resistance to thrips [5,6] while chlorogenic acid and kaempferol rutinoside enhance that of potato tubers to *Pectobacterium* [7]. Phenolics consequently represent a potential means of enhancing crop protection.

The accumulation of phenolics is controlled by genetic factors [8], the type of plant organ and its developmental stage [9–12] and responds to environmental factors, notably temperature, light and nitrogen (N) availability [13–15]. N deficiency or limitation leads to phenolic accumulation in different plant parts [14,16–18] though it alters plant growth and primary metabolism [14,16–19]. In cropping systems not seeking to maximize growth and yield, it is therefore to be expected that controlling N supply combined with low pesticide input could represent a means of agronomic leverage in the prospect of designing environmentally friendly practices. A promising approach to enhance plant resistance may therefore be to control phenolic accumulation by rational N fertilization. Such investigation necessitates extensive characterization of the phenolic composition of different plant organs and the assessment of the impact of N limitation on the accumulation of individual compounds. A recent work on tomato plants highlighted the accumulation of chlorogenic acid in all vegetative organs in response to N limitation, roots being more responsive than leaves and stems [17]. Although chlorogenic acid has been reported as the main phenolic in tomato vegetative organs, the presence of minor phenolics might also be important for plant defence. There is therefore a need to investigate their response to N limitation.

Metabolomics, using mass spectrometry (MS) and/or nuclear magnetic resonance (NMR), allow the extensive characterization of phenolic composition and content in specific plant organs or tissues. It has been widely used to characterize in detail the phenolic composition and content of edible organs in crops like grapevine [20,21], potato [22,23], cabbage [24], sweet potato [25] or medicinal plants [26–28], since it is proposed that phenolics provide a health protective effect because of their antioxidant properties [29]. Much less is known, however, about non-edible plant organs, despite the fact that (i) they are potential sites for pathogen infection or parasite attack and (ii) they constitute valuable by-products for the cosmetic and pharmaceutical industries [30]. This is especially the case for tomato, the third most important vegetable grown in the world. For this crop, the phenolic composition of the fruit and its distribution within this organ, have been extensively documented [31–35]. Most studies on vegetative organs (leaves, stems and roots), however, have only been concerned with major compounds such as chlorogenic acid, rutin and kaempferol rutinoside [36,37] or a limited subset of molecules responding to specific treatments [38,39]. As a result, the phenolic composition of these organs remains largely unknown.

The objectives of the present work were, firstly, to characterize the phenolic composition of the vegetative organs (leaves, stems and roots) of tomato plants and, secondly, to assess the impact of nitrogen limitation on the accumulation of the characterized compounds in the different organs. The study took into account the genetic variability of the phenolic composition and its response to N limitation, by comparing six determinate and three indeterminate tomato genotypes (Table 1). The plants were grown hydroponically in a greenhouse under two contrasting nitrogen fertilization regimes, one limiting for plant growth (low nitrogen: LN), while the other adequate (high nitrogen: HN) [17]. Phenolic composition and concentration in vegetative organs of 28-day-old plants were determined using liquid chromatography combined with mass spectrometry analyses.

## 2. Materials and methods

### 2.1. Standards and chemicals

Chlorogenic acid, rutin, kaempferol rutinoside, ferulic, *p*-coumaric, sinapic and caffeic acids were purchased from Sigma (Steinheim, Germany). Malvidin-3glucoside was purchased from Extrasynthese (Genay, France). Caffeoyl putrescine, dicoumaroyl spermidine and tricoumaroyl spermidine were kindly provided by Dr. Werck-Reichhart (IBMP, Strasbourg, France).

### 2.2. Plant growth and harvest

Ten-day-old plantlets from six determinate (coded A–F, Table 1) and three indeterminate (coded G–I, Table 1) patio tomato (*Solanum lycopersicum* L.) cultivars were grown in a glasshouse in Avignon (43°56′58″ N, 4°48′32″ E) from April 17th to May 5th 2009. Details of the growth conditions and harvests are provided in detail in [17]. In summary, tomato plants were grown in fully randomized blocks with full nutrient solutions imposing two N regimes, one limiting plant growth (LN) and the other with high N availability (HN). Plants were grown using an NFT system with a $[NO_3^-]$ concentration of 3 mM for the HN treatment whereas $[NO_3^-]$ supply to the LN plants was periodically modified (from 10 to 30 μM) in order to maintain a $NO_3^-$ uptake corresponding to 1/3 of that of the HN uptake. $[NO_3^-]$ and pH were corrected hourly using a computer-controlled setup (the Totomatix system) described previously [40]. Determinate and indeterminate cultivars were grown in eight and four separate blocks, respectively. In total, 120 plants were analyzed, comprising eight and four replicates respectively for determinate and indeterminate cultivars, in each of the two N regimes.

The plants were harvested after 28-days of growth. Morphological traits were measured prior to separating leaves, stems and roots. Roots were rinsed in deionised water and spin-dried (2 min at 2800 × *g*). The plant organs were weighed, frozen in liquid $N_2$ and stored at −80 °C until freeze-drying. Dry samples were weighed, ground to a fine powder and stored under dry air in a desiccator at room temperature.

### 2.3. Extraction procedure

Phenolics were extracted from dried leaf, stem and root powder as described in [17].

### 2.4. Qualitative HPLC-DAD-$MS^n$ analysis of phenolics

Qualitative analysis of phenolics from the cv A was carried out using a HPLC-MS system (ThermoFisher Scientific, San Jose, CA, USA) consisting in a binary solvent delivery pump connected to a photodiode array detector (PDA) and a LTQ Orbitrap hybrid mass spectrometer equipped with an atmospheric pressure ionization interface operating in electrospray mode (ESI). Twenty μL of